

BREEDING FOR INTERCROPPING

EDITED BY: Martin Weih, Niels P. R. Anten, Jérôme Enjalbert,
Pierre Hohmann and Diego Rubiales
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BREEDING FOR INTERCROPPING

Topic Editors:

Martin Weih, Swedish University of Agricultural Sciences, Sweden

Niels P. R. Anten, Wageningen University and Research, Netherlands

Jérôme Enjalbert, Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), France

Pierre Hohmann, Research Institute of Organic Agriculture (FiBL), Switzerland

Diego Rubiales, Institute for Sustainable Agriculture, Spanish National Research Council (CSIC), Spain

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EDITED AND REVIEWED BY

Valerio Hoyos-Villegas,
McGill University, Canada

*CORRESPONDENCE

Diego Rubiales

✉ diego.rubiales@ias.csic.es

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Editorial: Breeding for intercropping

Diego Rubiales^{1*}, Jérôme Enjalbert², Pierre Hohmann³,
Niels P.R. Anten⁴ and Martin Weih⁵

¹Institute for Sustainable Agriculture, CSIC, Cordoba, Spain, ²AgroParisTech, UMR GQE-Le Moulon, Institut National de Recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Gif-sur-Yvette, France, ³BETA Technological Center, University of Vic, Vic, Spain, ⁴Centre for Crop Systems Analysis, Wageningen University and Research, Wageningen, Netherlands, ⁵Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

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Editorial on the Research Topic

Breeding for intercropping

Intercropping, also known as mixed cropping, consists on simultaneously growing more than one species on a field. It has a great potential for enhancing water- and nutrient-use efficiency and improving plant productivity, yield stability and resilience to biotic and abiotic stress, including those triggered by climate change. Despite their manifold benefits, the practice of intercropping has not risen above its niche status in many regions of the world. The selection of varieties specifically adapted to intercropping remains a major practical challenge to its widespread deployment. This Research Topic hosted at Frontiers in Plant Sciences entitled “Breeding for intercropping” gathers a series of articles covering new insights in the areas of quantitative genetics, ecology, ecophysiology and agronomy integrating theoretical, experimental as well as participatory approaches.

Why is specific breeding needed for intercrops?

Moutier et al. showed that the performance of genotypes grown in pure stand as monocrops is not necessarily a good indicator for their performance grown in intercrops. In this research performed in France, eight wheat genotypes and five legume testers (three pea and two faba bean varieties) were field-grown as monocrops and in all possible binary intercrops in nine contrasting environments for three years. The mixing abilities of the varieties investigated was evaluated in terms of their ability to maintain or exceed their monocrop yield when grown in intercropping (producer effect); and their ability to benefit the yield of the companion crop (associate effect). Mixing abilities varied greatly between the investigated varieties, both for the wheat and the legume testers, implying that the choice of the legume tester is important for better discriminating the producer or associate effects of the wheat genotype that it is intercropped with. The authors conclude that both the wheat varietal choice and the identity of the legume tester variety are key issues in the breeding for intercropping. They also note that the breeding should consider the mixing ability in terms of both the producer and the associate effects.

In their review, [Moore et al.](#) provide an overview of three case studies, identifying relevant considerations for plant breeding efforts. Forage mixtures are the most mature among the cropping systems discussed. However, there is a need to accelerate efforts to breed for mixture systems, e.g., through genomic selection and/or selection of both component species. Breeding for perennial groundcover systems and winter oilseed systems is much less developed. In both cases, there is an opportunity to design a breeding pipeline that incorporates intercropping systems as one of its primary goals. Although nascent, breeding for intercropping systems holds great potential for improving intercropping systems and realizing the potential of this crop diversification strategy for addressing sustainability challenges.

[Bourke et al.](#) highlight the need for re-designing breeding programs to accommodate inter-specific interactions, as genotypes bred for monoculture are not the best adapted to intercropping systems. They summarize how to decipher plant interactions in intercropping, studying trait plasticity or plant-microbiome interactions, or exploring its ecophysiological basis using a functional structural plant model (FSPM). They then identify two general breeding strategies, either i) ideotype-driven (i.e., “trait-based” breeding) or ii) quantitative genetics-driven (i.e., “product-based” breeding), and they highlight the interest of the theoretical framework of direct genetic effect (DGE, equivalent to producer effect) and indirect genetic effects (IGE, equivalent to associate effect). They propose a “Powerful Troika”, combining the two strategies, for example coupling FSPM modelling with genomic-assisted selection and analysis of indirect genetic effects.

Current breeding programs do not select for enhanced general mixing ability (GMA) and neglect biological interactions within species mixtures. To address this issue, [Haug et al.](#) proposed a model framework for general and specific mixing ability (GMA and SMA). Incomplete factorial designs show the potential to drastically improve genetic gain by providing similar estimates for GMA and SMA variances compared with a two commonly used full factorial designs that employ the same amount of resources. This model was extended to the producer and associate concept to exploit information on fraction yields and allowed to characterise genotypes for their contribution to total mixture yield. Correlations between Producer/Associate effects and plant traits allowed to describe biological interaction functions (BIF) such as commensalism, competition and others. BIF can be used to optimize species ratios at harvest as well as to extend our understanding of competitive and facilitative interactions in a mixed plant community. This study provides an integrative methodological framework to promote breeding for mixed cropping.

[Timaues et al.](#) evaluated inter- and intraspecific diversity intercropping 15 wheat cultivars with one winter pea cultivar under organic conditions. Mixtures increased cereal grain quality, weed suppression, resource-use efficiency, yield gain, and reduced lodging. Under higher nutrient availability, entry-based variation was reduced in both systems, and pea was suppressed. Heterogeneous populations were more stable than line cultivars. Trait analysis revealed a possible link between harvest index and reduced competition in mixture, which can increase yield performance in specific line cultivars. They conclude that while

cultivar breeding for mixtures can be successful in monocultures, high environmental variation highlights the necessity of evaluating cultivars in mixtures. In addition, use of intraspecific diversity within interspecific mixed cropping systems can be a valuable addition to further improve mixture performance and its stability under increasing environmental stresses due to climate change.

Which suits of traits are most important in the breeding for intercropping?

The primary focus of the work by [Kiær et al.](#) is the importance of the end use in the evaluation of potential beneficial effects of intercrops. Thus, this perspective paper evaluated breeding targets for genotypes to be grown in intercropping in a supply chain perspective, using three case studies of intercropped legume and cereal species for human consumption to identify crop traits that could be desirable for different actors along the corresponding supply chains. The authors concluded that the widespread adoption and integration of intercrops will only be successful if all supply chain actors are included and collaborate; if the breeding approach takes into account the relative complexity of intercrop supply chains; and if diversification strategies are implemented in every process from field to fork.

Morphological and functional plant traits involved in species interactions were addressed by [Peng et al.](#), who evaluated the effects of intercropping on the medicinal plant *Atractylodes lancea* on various morphological traits including growth and volatile oil content. In their field study carried out in a subtropical environment in China, the authors have grown *A. lancea* plants in monocrop and intercropped with *Zea mays*, *Tagetes erecta*, *Calendula officinalis*, *Glycine max*, or *Polygonum hydropiper* as mixing component. Significantly enhanced growth and accumulation of some volatile oils was found especially when *A. lancea* was mixed with *Z. mays*, *T. erecta* or *C. officinalis*. However, large and significant variation in all measured traits was found also between the two years of this study, and the effects of the mixing treatments on the assessed traits partly varied greatly between the two years; suggesting strong management (here mixing partner) by environment interaction.

[Kammoun et al.](#) hypothesized that the grain yield achieved by a cultivar in low nitrogen input durum wheat–grain legume intercrops could be estimated using a few simple variables: (i) the yield of the wheat cultivar at full density in monocrop, (ii) the yield of the legume cultivar at half density in monocrop, and (iii) an indicator of legume cultivar response to interspecific competition that reveals cultivars’ competitive abilities and tolerance to competition. Such a competition index appeared less predictable for the legume than for the durum wheat. Further studies on more diverse genotypes and growing conditions are needed to improve the predictive quality of the model. Moreover, further mechanistic understanding is required to better evaluate the links between the tolerance to interspecific interactions and the plant phenotype characteristics (traits).

Land Equivalent Ratio (LER), is a classical agronomical index used for comparing the performance of species when intercropped, taking as reference their yields in monocrops. [Tavoletti and](#)

Merletti, proposed to use LER to identify best performing varieties. They explored the yield response to intercropping of durum wheat (*Triticum turgidum* ssp. *durum*) and faba bean (*Vicia faba*), using, respectively, 13 and 3 varieties of the two species. They focussed on a factorial design of 24 mixtures (12 wheat x 2 faba varieties), recording yield in a field trial performed over two years. They observed contrasting performances between the two years, with LER significantly higher than 1 only in the first year. To better discriminate the varietal performances in intercropping, the authors performed principal component and cluster analyses for total yield, LER_{total}, i.e. LER_w + LER_{fb}, and ln(LER_{ratio}), i.e. (LER_w/LER_{fb}). This multivariate analysis provides a way to identify the best variety combinations, while the authors propose to use principal component scores as indices of selection within breeding programs aimed to simultaneously improve intercropped species.

Demie et al. reviewed how the performance of cereal/legume intercropping depends on the genotypes used. Over 69 publications analysed, a subset of 35 of them reported land equivalent ratio (LER), with a mean LER of 1.26. Genotype x cropping system (monocrop/intercrop) interactions were tested in 71% of the 69 publications, and reported significant in 75%, of the studies. Interestingly, the different species analysed exhibited different land-use efficiencies in the different design types with finger millet having the highest land-use efficiency for cereals. In most of the studies, the link between traits and intercropping performance were not properly addressed, even if some key traits for intercropping performance, such as earliness, plant height, or growth habit, were also critical in intercropping. The lack of data on traits and genotypes effects on intercropping performance calls for additional experimental efforts, including more genotypes, to improve breeding and blending designs for intercropping systems.

Can crop growth models and quantitative predictions assist breeding for intercropping?

In a perspective paper, Weih et al. revisited the challenges associated with breeding for intercropping, and gave an outlook on the application of crop growth models to assist breeding for intercropping. Previous approaches using crop models to assist plant breeding were mostly based on the performance and properties of monocrops. For models to be effective in assisting breeding for intercropping, they need to (i) incorporate the relevant plant features and mechanisms driving interspecific plant–plant interactions in the model; (ii) rely on parameters that are closely linked to the traits that breeders would select for; and (iii) be calibrated and validated with field data that are assessed in intercrops. In addition, due to their lower complexity and much reduced parameter requirement, the authors consider minimalist crop growth models to be more likely to incorporate the above elements than comprehensive and parameter-rich crop growth models.

Firmat and Litrico, point out that obtaining reliable community level quantitative predictions for diverse crop systems empirically is

limited by the size and complexity of experiments that would be needed. Breeding strategies should instead be compared using theoretically informed qualitative predictions. To this end, they reviewed different approaches arising from the field of evolutionary ecology focusing on: (i) the community heritability approach, (ii) the joint-phenotype approach and (iii) the community trait genetic gradient approach. They suggest research strategies related to each of these approaches.

To explore the interest of genomic selection for intercrop breeding, Bančić et al. proposed an elegant study based on stochastic simulation, where they compared four breeding programs implementing genomic selection and one breeding program based on phenotype only. The different breeding schemes were sized according to a constant budget, using realistic steps as double-haploid production, or intercropping evaluation using testers. Three different genetic correlations (0.4, 0.7, and 0.9) between monocrop and intercrop grain yield were assumed, and only GMA was simulated. Under these three scenarios, all four simulated breeding programs using genomic selection produced significantly more intercrop genetic gain than the phenotypic selection program (~1.3–2.5 times), but at the cost of genetic variance. Under low genetic correlations, the Grid-GS program, which employed an incomplete factorial instead of using testers, was the most efficient. Authors suggest a genomic selection strategy which combines monocrop and intercrop trait information, using a selection index that includes economic weights, in order to increase selection accuracy.

Annicchiarico et al. studied efficiency of several phenotypic or genomic selection strategies in pea breeding for intercropping with cereals. The efficiency of an indirect selection index including onset of flowering, plant height, and grain yield in monocrops was comparable to that of pea yield selection in intercrops. Genomic selection for pea yield in monocrop displayed an efficiency close to that of phenotypic selection for pea yield in intercrop, and nearly two-fold greater efficiency when also taking into account its shorter selection cycle and smaller evaluation cost.

Instead of breeding to improve monoculture yield of single crops in isolation, Wolfe et al. propose optimizing multiple interacting species and genotypes by enabling joint-selection to improve the performance of the cropping system across time and space. Genomic and phenomic prediction poses an exciting opportunity to develop a multi-tiered selection scheme. There are multiple levels or “tiers” of selection, which when considered jointly enact agroecosystem improvement. The objective at Tier 1 is intraspecific population improvement, which is addressed simultaneously across each species to affect co-adaptation of the germplasm pools. At Tier 2, selection is focused on predictions of performance of the combination over space and time.

The practice of wheat variety mixtures is spreading. However, there are few blending rules to design variety mixtures, and not any based on plant architecture. As the high dimensionality of trait combinations in intercropping is hardly compatible with field experiments, Blanc et al. proposed to use the FSPM WALTER to simulate wheat cultivar mixtures and try to better understand how key traits driving the aerial architecture can influence mixture performance. However, most FSPM are slow to run and do not allow to explore the combinatorics of their numerous parameters.

Hence the authors combined two original methods: i) they used a metamodel of WALTER, i.e. an approximation of the FSPM outputs, to speed up computation, and ii) they then performed a sensitivity analyses based on both mean and differences in architectural trait values of the mixed components (binary and balanced mixtures). These analyses highlighted the impact of the leaf dimensions and the tillering capability on the performance of the simulated mixtures. Identifying the best performing mixtures revealed original combinations of ideotypes with contrasting tillering abilities and leaf dimensions, asking for experimental confirmations.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Advances in Breeding for Mixed Cropping – Incomplete Factorials and the Producer/Associate Concept

Benedikt Haug^{1,2}, Monika M. Messmer¹, Jérôme Enjalbert², Isabelle Goldringer², Emma Forst³, Timothée Flutre², Tristan Mary-Huard^{2,4} and Pierre Hohmann^{1*}

¹ Department of Crop Sciences, Research Institute of Organic Agriculture FIBL, Frick, Switzerland, ² Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE – Le Moulon, Gif-sur-Yvette, France, ³ Dipartimento di Scienze Agrarie, Alimentari ed Ambientali (D3A), Università Politecnica delle Marche, Ancona, Italy, ⁴ UMR MIA-Paris, AgroParisTech, INRA, Université Paris-Saclay, Paris, France

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Thomas Miedaner,
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Biodiversity International, Italy

*Correspondence:

Pierre Hohmann
pierre.hohmann@fibl.org

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Mixed cropping has been suggested as a resource-efficient approach to meet high produce demands while maintaining biodiversity and minimizing environmental impact. Current breeding programs do not select for enhanced general mixing ability (GMA) and neglect biological interactions within species mixtures. Clear concepts and efficient experimental designs, adapted to breeding for mixed cropping and encoded into appropriate statistical models, are lacking. Thus, a model framework for GMA and SMA (specific mixing ability) was established. Results of a simulation study showed that an incomplete factorial design combines advantages of two commonly used full factorials, and enables to estimate GMA, SMA, and their variances in a resource-efficient way. This model was extended to the Producer (Pr) and Associate (As) concept to exploit additional information based on fraction yields. It was shown that the Pr/As concept allows to characterize genotypes for their contribution to total mixture yield, and, when relating to plant traits, allows to describe biological interaction functions (BIF) in a mixed crop. Incomplete factorial designs show the potential to drastically improve genetic gain by testing an increased number of genotypes using the same amount of resources. The Pr/As concept can further be employed to maximize GMA in an informed and efficient way. The BIF of a trait can be used to optimize species ratios at harvest as well as to extend our understanding of competitive and facilitative interactions in a mixed plant community. This study provides an integrative methodological framework to promote breeding for mixed cropping.

Keywords: mixed cropping, intercropping, breeding, general mixing ability, producer/associate concept, incomplete factorial design, biological interaction, simulations

INTRODUCTION

Climate change, such as rising global temperatures and climatic volatility are predicted to jeopardize future agricultural productivity (Rahmstorf and Coumou, 2011). The current strategies to produce stable and high yields, e.g., by the application of mineral fertilizer, are of limited future use since they themselves are a contributor to these changing climatic parameters (Thompson et al., 2019). Thus, alternative approaches to achieve high and stable yields while maintaining biodiversity and minimizing environmental impact have to be developed. Mixed cropping is the simultaneous

cultivation of two or more crops on the same field. Especially legume/non-legume species mixtures have been proposed to achieve a higher per area production and profitability and higher yield stability with less or no external inputs (Bedoussac et al., 2015; Raseduzzaman and Jensen, 2017; Wendling et al., 2017; Viguier et al., 2018). Good pairs of complementary species have already been identified, such as combinations of corn (*Zea mays* L.) with cowpea (*Vigna unguiculata* L., Ofori and Stern, 1986), with common bean (*Phaseolus vulgaris* L., Hoppe, 2016; Starke, 2018), and with faba bean (*Vicia faba* L., Li et al., 2020), as well as small grain cereals such as barley (*Hordeum vulgare* L., Hauggaard-Nielsen et al., 2001) with pea (*Pisum sativum* L.) or wheat (*Triticum aestivum* L.) with faba bean (Agegnehu et al., 2006) or with lentil (*Lens culinaris* MEDIK., Viguier et al., 2018).

The choice of the genotypes best suited to mixing within each species is not straight-forward, and a robust strategy for evaluating and identifying the right mixing partners from among a large number of candidates is essential to selecting the components of mixtures and improving the mixing ability of each species. Selection efficiency for mixed cropping yield under pure stand has been reported to be moderate or low highlighting the value dedicated breeding efforts for mixed cropping (de Oliveira Zimmermann, 1996; O'Leary and Smith, 2004; Annicchiarico et al., 2019). Well performing genotypes should display a high general mixing ability (GMA), i.e., lead to a high total mixture yield performance across several potential mixing partners, and a low variance in specific mixing ability (SMA), i.e., little or no specific interaction with individual mixing partners in that respect. In order to develop efficient breeding strategies for crop mixtures of two species, trial designs must be developed that allow precise estimation of GMA and SMA variance.

Current trial designs apply a factorial setup, combining m genotypes of species one and n genotypes of species two. With increasing numbers of genotypes of both mixing partners, factorial designs quickly result in an unfeasibly high number of experimental plots. Therefore, often specific dimensions of both crop species are used: depending on the question to be addressed either (i) factorials of equal (or similar) dimensions of m and n with a small to medium number of genotypes (Annicchiarico and Piano, 1994; Hauggaard-Nielsen and Jensen, 2001) or (ii) factorials of different dimensions for m and n (Annicchiarico, 2003; Hoppe, 2016; Starke, 2018) are employed. The former allows GMA and SMA estimations of both crop species involved, the latter emphasizes one species over the other and is comparable to the topcross designs, used in hybrid breeding. With advances in mixed modeling statistical software (such as with the GNU R packages “lme4” or “SOMMER”), analyses of largely incomplete datasets are possible (Bates et al., 2015; Covarrubias-Pazarán, 2016; R Core Team, 2019). Incomplete factorial designs have been suggested to mitigate the limitations of (i) and (ii) by expanding the numbers of m and n while maintaining a feasible number of experimental plots. Previously, they have been applied to assess GMA and SMA effects in wheat (*Triticum aestivum* L.) cultivar mixtures (Forst et al., 2019) and found recent application in genomic prediction in corn (*Zea mays* L.) hybrid breeding (Seye et al., 2020).

Another important area to advance breeding for mixed cropping focuses on exploiting information that is contained in the fraction yields of mixed crops via the application of the producer (Pr) and associate (As) concept (Wright, 1985; Goldringer et al., 1994; Annicchiarico et al., 2019). In the mixed cropping context, the Pr effect, sometimes also referred to as direct effect, is the capacity of a genotype to influence its own yield in a mixture, while the As effect is its capacity to influence the yield of its companion crop or variety (Annicchiarico et al., 2019). As laid out by Wright (1985), Forst (2018), and Sampoux et al. (2020), the Pr and As effects of a given genotype sum up to its GMA effect. It has been applied to single row experiments in breeding nurseries (Goldringer et al., 1994) or wheat cultivar mixtures (Forst, 2018), and to the mixed cropping context (Wright, 1985; Sampoux et al., 2020). Separated yield data enables either uni- or bivariate (or multivariate) analysis, i.e., joint analysis of the two (or multiple) fraction yields. In clinical psychology as well as in livestock breeding, multivariate analysis procedures have successfully been applied in situations where traits were correlated, e.g., due to pleiotropy, and yielded higher precision for QTL detection than univariate approaches (Sørensen et al., 2003; Meier et al., 2015). In mixed cropping conditions, it can be assumed that errors of measurements are generally negatively correlated between the two crops, e.g., via compensation effects. Often in mixed cropping, one of the species is at a competitive disadvantage and genotypes of the species that is very non-competitive generally express a low GMA in mixtures (Annicchiarico and Piano, 1994; Corre-Hellou et al., 2006). However, the competitive ability of genotypes is obscured when only whole mixture yield is observed (Annicchiarico et al., 2019). The assessment of fraction yields is not only important to identify these competitive abilities, but can also be applied to optimize a mixture toward a specific ratio, e.g., for feed nutrition or legume subsidies reasons, as is for example the case in Switzerland (Bundesamt für Landwirtschaft BLW, 2019). Thus, shaping community performance and composition via traits can be of interest in the breeding process. Furthermore, high genetic correlations between certain traits and mixture yield would allow indirect selection based on most important key traits for Pr and As effects.

The choice of an efficient trial design, the choice of an efficient analysis method and the assessment of yield proportions are interrelated topics. They provide the potential to be combined in an integrated approach to promote breeding for mixed cropping. While some published work focuses on the parallel genetic improvement of two species (Sampoux et al., 2020), many publications rely on the improvement of one species at a time and do not take the potential of analyzing separated yield data into account. Thus, the objectives of this study were to (i) develop a model to estimate GMA and SMA variances of binary species mixtures, and to compare different experimental designs for their usefulness in estimating these parameters, (ii) subdivide GMA into Pr and As effects in order to categorize cultivars' influence on mixture yield and to compare the precision of a uni- versus a bivariate approach in estimating Pr, As, and error (co)variances, and (iii) establish a concept to link plant traits to biological interactions between involved species in mixture.

MATERIALS AND METHODS

To exemplify the case of a mixed crop, a hypothetical binary mixture of a legume species (pea) and a non-legume species (barley) will be used in this study.

The GMA Model of Total Mixture Yield

Mixture yield can be expressed with the following model:

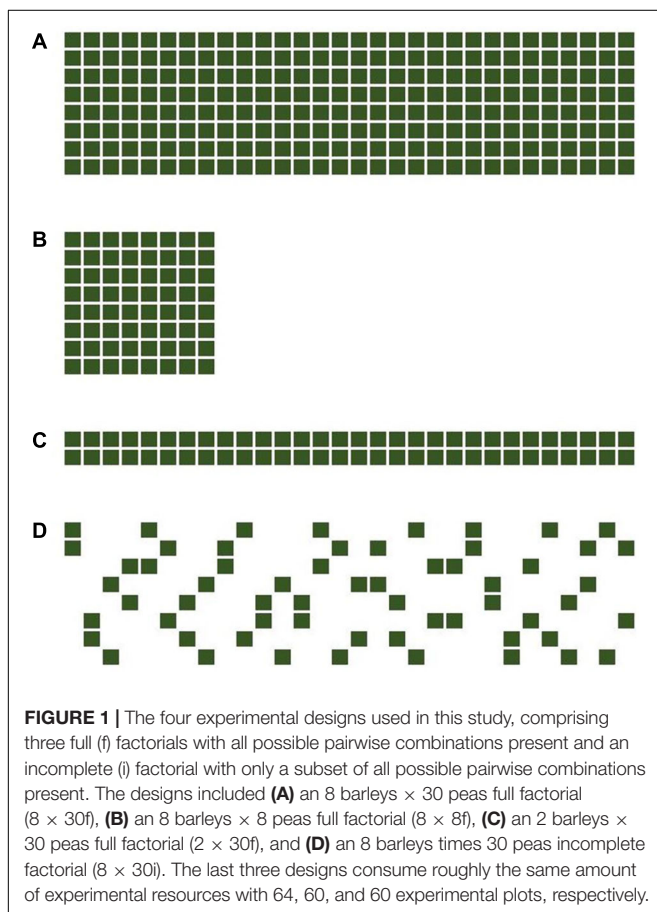
$$y_{ijk} = \mu + r_k + G_{p_i} + G_{b_j} + S_{ij} + E_{ijk} \quad (1)$$

with y_{ijk} the total mixture yield of the i -th pea cultivar mixed with the j -th barley cultivar in the k -th block, μ the intercept of mixture yields, r_k the effect of the k -th block (replication), G_{p_i} and G_{b_j} the GMA effects the i -th pea cultivar and the j -th barley cultivar, respectively, S_{ij} the SMA effect, i.e., interaction, of the i -th pea cultivar with the j -th barley cultivar and E_{ijk} the error term.

This model-framework was used to compare four different trial designs (A–D), comprising three full (“f”) factorials, with all possible pairwise combinations present, and an incomplete (“i”) factorial with only a subset of all possible pairwise combinations present (Figure 1). In the following, m is the number of barley cultivars and n the number of pea cultivars, used in the design. Design A, using 240 plots per replicate ($m = 8$ and $n = 30$), is the most resource-expensive design. Designs B, C, and D were using

only approximately 25% of the resources of A with, respectively, 64 ($m = 8$, $n = 8$), 60 ($m = 8$, $n = 30$), and 60 ($m = 2$, $n = 30$) plots per replicate, while sharing commonalities with design A. Designs B and C are both also full factorials (with equal and unequal dimensions of m and n) and design D shares the same size of m and n with design A while being an incomplete factorial. Design C (2×30 f) full factorial has similarities with a top cross design, used in early stages of a hybrid breeding program. Design D (8×30 i) was constructed using four independently randomized 8×8 latin squares of which only two “entries” were used for the mixtures. This ensured that (a) every barley was combined with eight pea cultivars, and every pea was combined with two different barley cultivars and (b) confounding, i.e., two or more pea cultivars sharing the same two barley cultivars was minimized.

For the comparison of the four designs, datasets with total mixture yield-data (“total yield setting”) were simulated with the following models for a “SMA present” and a “SMA absent” simulation, respectively. For the SMA absent simulation, S_{ij} was set to zero in the model in formula (1). Simulations were performed according to the following procedure, using parameter settings in the same order of magnitude as empirical values from preliminary trials (Haug et al., in preparation) to produce data that is as close to empirical data as possible. The intercept of mixture yield was set to 38.8 dt/ha. For each of the following parameters, the corresponding effects were drawn from their respective probability distributions: block effects r_k were drawn from a normal distribution with a mean of 0 and a variance of 2, i.e., $N(0, 2)$. Pea GMA effects G_{p_i} were drawn from $N(3, 0)$, barley GMA effects G_{b_j} were drawn from $N(0, 5)$ and SMA effects S_{ij} were drawn from $N(0, 5)$ for the SMA present simulation and from $N(0, 0)$ for the SMA absent simulation, i.e., the effect size was set to zero. Errors e_{ijk} were drawn from $N(0, 5)$. For each simulation run, effects were drawn anew. “True” values (i.e., values used to simulate data) of each effect were saved after each simulation run for later comparison with the estimated parameter values, e.g., the true GMA effects of the pea cultivars, were stored for later comparison with GMA effects estimated by the best linear unbiased predictors (BLUPs) received by the mixed model to analyze the simulated data. Design D normally would have 64 pairwise combinations. The realistic case of missing/unusable genotypes was assumed, by excluding two pea lines in our case, thus resulting in 60 pea-barley combinations. For each of the four trial designs $n = 1,000$ data sets were simulated, for the SMA present, as well as the SMA absent simulation, i.e., $8 \times 1,000$ simulated data-sets in total. Each dataset comprised two replicates (blocks). All simulations and subsequent analyses were done using GNU R (R Core Team, 2019). The R-code used for simulation and analysis is publically available (Haug, 2020a).



The Pr/As Model of Fraction Yield of Each Species

Effects on separated yield data, i.e., pea and barley fraction yields, can be described by a model, containing Pr and As effects:

$$y_{p_{ijk}} = \mu_p + r_{p_k} + P_{p_i} + A_{b_j} + E_{p_{ijk}} \quad (2)$$

$$y_{bijk} = \mu_b + r_{b_k} + P_{b_j} + A_{p_i} + E_{bijk} \quad (3)$$

with y_{pijk} the fraction yield of pea from the combination of the i -th pea cultivar with the j -th barley cultivar in the k -th block, μ_p the intercept of pea fraction yields, P_{p_i} the effects of the k -th block on pea fraction yield, P_{p_i} the Pr effects and A_{b_j} the As effects of the i -th pea and the j -th barley cultivar, respectively, and E_{pijk} the error for the fraction yield of the i -th pea with the j -th barley in the k -th block. Each dataset comprised two replicates (blocks). Parameters apply in analogy for barley fraction yields in formula 3. Interactions between Pr and As effects are ignored.

Since total mixture yield y_{ijk} decomposes into $(y_{pijk} + y_{bijk})$, also the other parameters can be decomposed: μ into $(\mu_p + \mu_b)$, r_k into $(r_{p_k} + r_{b_k})$, G_p into $(P_p + A_p)$, G_b into $(P_b + A_b)$, and E_{ijk} into $(E_{pijk} + E_{bijk})$. Hence, formula 1 (without S_{ij}) can be rewritten as in formula 4.

$$y_{pijk} + y_{bijk} = \mu_p + \mu_b + r_{p_k} + r_{b_k} + P_{p_i} + A_{p_i} + P_{b_j} + A_{b_j} + E_{pijk} + E_{bijk} \quad (4)$$

$$\begin{bmatrix} P_{p_i} \\ A_{p_i} \end{bmatrix} \sim N_2 \left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 6.1 & -4.1 \\ -4.1 & 7.5 \end{bmatrix} \right) \quad (5)$$

$$\begin{bmatrix} P_{b_j} \\ A_{b_j} \end{bmatrix} \sim N_2 \left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 6.1 & -2.7 \\ -2.7 & 1.5 \end{bmatrix} \right) \quad (6)$$

$$\begin{bmatrix} P_{b_j} \\ A_{b_j} \end{bmatrix} \sim N_2 \left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 6.1 & 3.6 \\ 3.6 & 8.4 \end{bmatrix} \right) \quad (7)$$

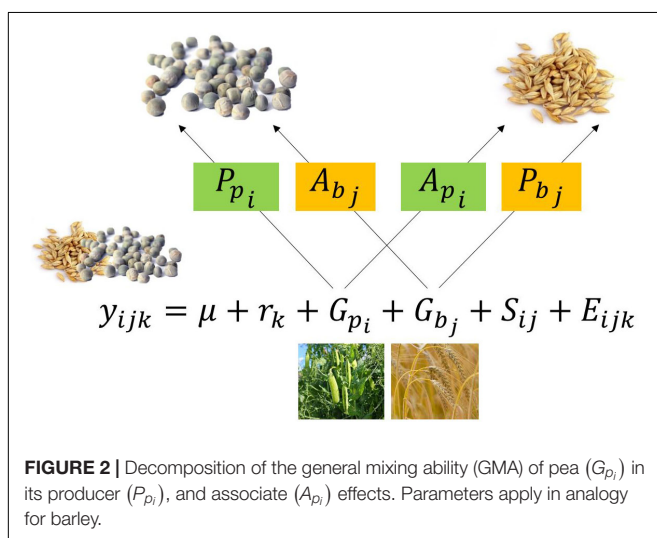
Decomposition of GMA into Pr and As effects is illustrated in **Figure 2**. Separated yield data (“fraction yield setting”) were simulated to compare the precision of a univariate versus a bivariate analysis approach for design D (**Figure 1**). As in the total yield setting, two blocks per data set were simulated. In contrast to the total yield setting, for each “plot” the separated yields of pea and barley were simulated with a mean pea yield of 18.3 dt/ha

and a mean barley yield of 19.1 dt/ha, according to previously mentioned preliminary experimental data. The following settings were used: block effects r_{p_k} and r_{b_k} were both drawn from $N(0, 2)$ as in the total yield setting. Pea Pr effects P_{p_i} were drawn from $N(0, 6.1)$ and As effects A_{b_j} from $N(0, 7.5)$. Barley Pr effects P_{b_j} were drawn from $N(0, 6.1)$ and As effects A_{p_i} from $N(0, 1.5)$, errors E_{pijk} and E_{bijk} were drawn for pea yield from $N(0, 8.4)$ and for barley yield from $N(0, 6.1)$. Correlations of pea Pr effects with pea As effects were set to -0.61 , and correlation of barley Pr with barley As effects were set to -0.81 . Within-plot error-correlations, i.e., the correlation of errors of barley yields with the errors of the corresponding pea yields in the same plot, were set to 0, -0.5 , and -0.9 , respectively, to create three different error-correlation scenarios. Within-plot error-correlations and Pr/As correlations were translated into co-variances. The variance-covariance matrices of Pr/As effects of pea, barley and the errors are shown in formulas 5, 6, and 7. Pr and As effects as well as errors were drawn from a distribution that follows the law of a multivariate normal distribution, using the function “mvrnorm” from the R-package “MASS” (Venables and Ripley, 2002), and the covariances shown in formulas 5–7. In total, three data sets for each of the three different error correlation settings with 1,000 simulations were created.

Simulated data from the fraction yield setting was analyzed using (i) a univariate approach with models equal to those used to simulate the data (formulas 2, 3) and (ii) a bivariate approach (formula 4) in which the two dependent variables were analyzed jointly (Covarrubias-Pazarán, 2018). In addition to the parameters estimated by the univariate approach, the bivariate approach also estimates the before mentioned covariances. Both approaches were done as mixed models where block-effects were considered as fixed and all other effects as random, assuming independent and identically distributed random variables. The uni- and the bivariate analyses were done with the “mmer” function of the R-package “SOMMER” (Covarrubias-Pazarán, 2016, 2018). Estimates of the model parameters, e.g., estimated GMA or Pr variances of pea, and BLUPs for the genetic effects, e.g., BLUPs of GMA effects, were saved for later analysis for each of the 1,000 datasets per setting. Depending on the analysis approach, each dataset yielded a different set of BLUPs for the pea and barley Pr and associate effects. For $n = 1,000$ analyses, Pearson correlations between the two sets of BLUPs and the true value were computed, Fisher-z-transformed, averaged for each coefficient and transformed back. T-tests between the mean correlation of the univariate and the bivariate approach within each parameter were conducted to compare the approaches for their accuracy to estimate the true effect values. The R-code used for simulation and analysis is publically available (Haug, 2020b).

Trait versus GMA/Pr/As Analyses for the Characterization of Biological Interaction Functions (BIFs)

Beyond the purely statistical treatment of the data described above, the relationships between a fictive explanatory trait and



the GMA/Pr/As variables were investigated. This explanatory trait was set into relation with the GMA, Pr, and As effects of equally fictive genotypes. Nine possible scenarios of trait-GMA, trait-Pr effect, and trait As-effect relationships were investigated, allowing the categorization of traits according to their biological interaction function in a mixed-crop plant community. Exemplary scatter plots with $n = 100$ simulated genotypes and positive, null and negative relationships between trait and GMA, trait and Pr, and trait and As effects were created to suggest a simple visual analysis of these relationships. Potential symbiotic trait functions were associated to the corresponding functions relationships.

RESULTS

Incomplete Design Yields Comparable Estimates to Full Factorial Designs

Four experimental designs were compared for their ability to estimate GMA and SMA variances as well as estimating genotypic effects (BLUPs) correctly in two different simulations, a “SMA-present” and a “SMA-absent” simulation.

Over both scenarios, the precision of the estimates increased with experimental resource input (Table 1). With design A ($8 \times 30f$), utilizing 240 experimental plots per replicate, the narrowest CIs among the four designs were received, more narrow than the ones of designs B ($8 \times 8f$), C ($2 \times 8f$), and D ($8 \times 30i$), which were using only 64, 60, and 60 experimental plots per replicate, respectively (Table 1). Among the latter, only minor differences in CIs were observed (except lower reliability on GMA variance of barley in design C and on GMA variance of pea in design B in the SMA-absent simulation). Besides GMA variance of barley of design C in the SMA-present simulation and the GMA variance of pea of design B in the SMA-absent simulation, certain parameters were estimated similarly well with designs B, C, and D as with the benchmark design A. Barley GMA variance of designs B and D showed similar CIs compared to design A for both the SMA-present and the SMA-absent simulation, whereas design C, which only uses two instead of eight barley cultivars, estimated barley GMA variance less precisely (CI of ± 0.43 in both scenarios). In addition, pea GMA variance of designs C and D of the SMA-absent simulation were similarly precisely estimated compared with design A, whereas design B estimated this parameter with lower precision (CI of ± 0.11).

Besides the variation of estimates, a check of the correct estimation of the size of the parameter itself revealed that for the SMA-present simulation (Table 1), all four experimental designs accurately estimated GMA, SMA, and error variances, with all confidence intervals (CIs) of means overlapping the true values, except for the SMA variance of design C.

For the SMA-absent simulation, pea and barley GMA variances were mostly accurately estimated in the four designs with significant but small underestimations of pea GMA variance by designs C and D. SMA variances in this simulation were significantly overestimated and error variances were significantly underestimated for all four designs. Compared with the mean SMA variance of the benchmark design A of 0.14, in this

TABLE 1 | Results on general and specific mixing ability (GMA and SMA) from simulated data for four different trial designs (A–D).

Simulation	Trial design ¹	Plots per replicate	Pea GMA			Barley GMA			SMA			Error
			Means of variance estimates (truth: 3.0) ²	Means of correlations BLUPs vs. true effects ³	Means of variance estimates (truth: 5.0)	Means of correlations BLUPs vs. true effects	Means of variance estimates (truth: 5.0/0.0)	Means of correlations BLUPs vs. true effects	Means of variance estimates (truth: 5.0/0.0)	Means of correlations BLUPs vs. true effects	Means of variance estimates (truth: 5.0)	
SMA-present simulation	A ($8 \times 30f$)	240	2.97 $\pm$ 0.06	0.88 ^c	4.98 $\pm$ 0.17	0.98 ^c	5.00 $\pm$ 0.05	0.77 ^d	5.02 $\pm$ 0.03		5.02 $\pm$ 0.03	
	B ($8 \times 8f$)	64	2.90 $\pm$ 0.13	0.89 ^d	4.98 $\pm$ 0.19	0.93 ^b	5.03 $\pm$ 0.10	0.74 ^c	5.02 $\pm$ 0.06		5.02 $\pm$ 0.06	
	C ($2 \times 30f$)	60	3.06 $\pm$ 0.12	0.68 ^b	4.64 $\pm$ 0.43	– ⁴	4.85 $\pm$ 0.12	0.70 ^b	5.03 $\pm$ 0.00		5.03 $\pm$ 0.00	
	D ($8 \times 30i$)	60	3.01 $\pm$ 0.13	0.65 ^a	5.13 $\pm$ 0.21	0.91 ^a	4.95 $\pm$ 0.13	0.67 ^a	5.02 $\pm$ 0.06		5.02 $\pm$ 0.06	
SMA-absent simulation	A ($8 \times 30f$)	240	2.98 $\pm$ 0.05	0.95 ^c	4.94 $\pm$ 0.17	0.99 ^c	0.14 $\pm$ 0.01	– ⁵	4.88 $\pm$ 0.02		4.88 $\pm$ 0.02	
	B ($8 \times 8f$)	64	2.90 $\pm$ 0.11	0.96 ^d	4.94 $\pm$ 0.18	0.98 ^b	0.27 $\pm$ 0.02	–	4.76 $\pm$ 0.04		4.76 $\pm$ 0.04	
	C ($2 \times 30f$)	60	2.89 $\pm$ 0.07	0.84 ^b	4.64 $\pm$ 0.43	– ⁴	0.35 $\pm$ 0.03	–	4.76 $\pm$ 0.05		4.76 $\pm$ 0.05	
	D ($8 \times 30i$)	60	2.84 $\pm$ 0.07	0.83 ^a	4.89 $\pm$ 0.18	0.96 ^a	0.36 $\pm$ 0.03	–	4.80 $\pm$ 0.05		4.80 $\pm$ 0.05	

The four designs employ different amounts of resources in terms of experimental plots per replicate, with design A being the benchmark. Numbers after $\pm$ denote the 95% confidence intervals for the mean of 1,000 variance estimates. Different letters following correlation values between best linear unbiased predictors (BLUPs) and true effects indicate significant differences (Tukey-HSD; $p < 0.05$).

¹the different designs as described in Figure 1 ²means of 1000 estimates per design and 95% confidence intervals ³means of rho-z-rho transformed Pearson correlations, see “Materials and Methods,” letters a–d indicate significant differences with $p < 0.001$ ⁴correlation of only two data-points is by definition one ⁵correlations were not computed in scenario two, for the true SMA-effects all being 0 by definition.

simulation designs B, C, and D showed significantly higher SMA variances with 0.27, 0.35, and 0.36, respectively. Similarly, the mean error variance of design A was with 4.88 significantly higher (and thus closer to the truth of 5.0) than the error variances of designs B, C, and D, with means between 4.76 and 4.80.

When comparing the four designs for their correlation of BLUPs with the truth value, (Table 1) the correlation coefficients of benchmark design A were among the highest across all estimated BLUPs. However, correlations of BLUPs of pea GMA effects with the true effects of design B were similar compared with the benchmark design A for both simulations. For the correlations of BLUPs for pea GMA with their true values of designs C and D showed significantly lower correlation coefficients compared with design A and B for both SMA simulation models. However, this difference was less apparent in the SMA-absent simulation with correlation coefficients of 0.84 and 0.83 of designs C and D compared with 0.95 and 0.96 of designs A and B, respectively. All correlation coefficients differed significantly from each other ($p < 0.001$). For barley GMA, design B and D showed with 0.93 and 0.91 high mean correlation coefficients that were similar to the mean of design A (0.98). Correlation coefficients for SMA effects were in the range of the correlation coefficients for pea GMA effects and barley GMA effects, with values between 0.67 (design D) and 0.77 (design A).

The Pr/As Concept Allows to Characterize Cultivars' Contribution to Mixture Yield

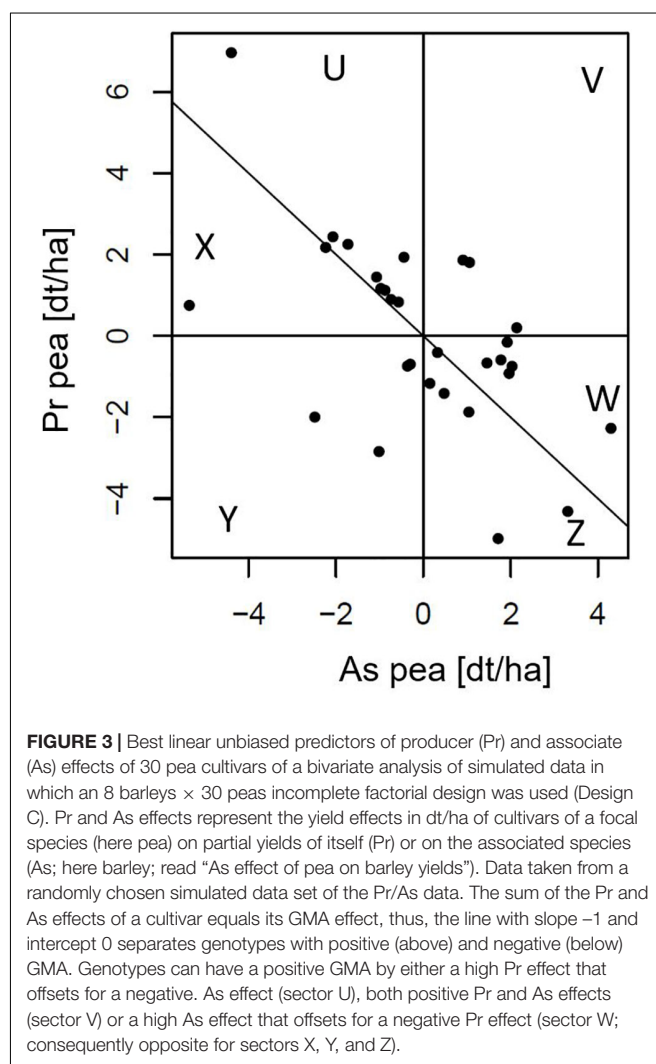
In Figure 3, thirty simulated cultivars with their Pr and As effects are shown. Pr effects range from -5.0 to $+7.0$ and As effects range from -5.4 to $+4.3$. As effect has to be read as the effect of the species (e.g., pea) on the yield (or any other trait) of its companion species (e.g., barley). Since the Pr and As effects of a cultivar sum up to its GMA, cultivars that lie on the line with slope -1 and intercept 0 have a GMA of zero, those above this line have a positive and below a negative GMA. The cultivars thus can be grouped into six sectors, U, V, W, X, Y, and Z, with U–W having positive GMA due to a high Pr effect that offsets a negative As effect (sector U), both a positive Pr and As effect (sector V) and a positive As effect that makes up for a negative Pr effect (sector W). On the other hand, cultivars below the identity line have a negative GMA with a positive Pr effect that does not compensate for a negative As effect (sector X), both negative Pr and As effects (sector Y) and a negative Pr effect which is not offset by their positive As effect (sector Z). These six sectors allow to characterize and differentiate the mixing ability of the pea cultivars.

Comparing the uni- and bivariate approach using the data from the fraction yield setting, correlation of errors of 0 , -0.5 , and -0.9 resulted in very similar parameter estimates (Supplementary Table 1). The “error correlation of -0.5 scenario” was used to analyze separated fraction yield data of both models in more detail. Both analysis approaches, uni- and bivariate, produced unbiased results of parameter estimates, i.e., all 95% confidence intervals of the estimates contained the true values for both approaches (see Supplementary Table 2).

Estimates did not differ significantly between the univariate and the bivariate analysis approach. CIs, used as a measure for precision, differed only by 0.01 or not at all. However, only the bivariate model allows to estimate the correlation between Pr and As effects.

Pr/As-Trait Relationships Uncover Biological Interaction Functions (BIFs) of Traits

The GMA, Pr, and As effects on total or fraction yield do not reveal the underlying biological processes or traits that influence the mixing ability. Yet, the examination of relationships between a fictive explanatory trait and Pr/As effects on fraction yield fills this lack by defining nine potential biological interaction functions (BIFs) of a given trait that underlie the GMA-trait pattern (Figure 3). This GMA-trait relationship can be positive (+), absent (0) or negative (−). However, the GMA-trait relationship is subdivided in its underpinned three possible Pr-trait/As-trait relationships. These can then be interpreted in terms of BIFs: commensalism (Pr+/As0, Pr0/As+, i.e., trait



will profit only one species), mutualism ($Pr+/As+$, i.e., trait promotes both species), antagonism ($Pr+/As-$, $Pr-/As+$, i.e., trait promotes one species but hampers second species), neutralism ($Pr0/As0$, i.e., trait does not affect any of the two species), amensalism ($Pr0/As-$, $Pr-/As0$, i.e., trait is hampering only one species), and competition ($Pr-/As-$, i.e., trait is hampering both species). This more detailed correlations will allow to identify key traits that are important for good mixing ability for a crop and can contribute to indirect selection.

DISCUSSION

The overall goal of this study was to develop a novel framework for breeding for mixed cropping by (i) formulating models for mixed cropping, suggesting experimental designs and analysis methods for mixed cropping experiments, (ii) proposing extensions to the use of the Pr/As concept, and (iii) linking the latter to traits in order to uncover the biological interaction function (BIF) of traits.

Incomplete Designs to Increase Selection Intensities

With all four designs, GMA, SMA, and error variances were overall correctly estimated, i.e., with little or no bias. As expected, the low-resource designs B, C, and D showed a slightly lower precision (i.e., higher CIs). The comparison of the three low-resource designs, incomplete factorial design (D) versus the two full factorial designs B and C, revealed similar estimations of GMA, SMA, and error variances. Design C (with two barley genotypes) does not allow a meaningful estimation of GMA variance. Thus, for an estimation of both species' GMA variance and SMA variance, designs B or D are preferred, which is in line with the suggestion of Annicchiarico et al. (2019). Seye et al. (2020) used an incomplete factorial design for hybrid testing that was created by crossing one inbred line of one pool with one inbred line from the opposite other pool and compared to a classical topcross design where all inbred lines of pool 1 were crossed with the same line of pool 2. In the incomplete design, estimates for general combining ability (GCA) for both parents of a hybrid cannot be disentangled and are identical. Nonetheless, they emphasize, that if one only considers selection among the tested lines of the training set, the incomplete factorial (similar to design D of this study) always outperformed a topcross design (similar to design C) in terms of genetic gain, since twice the amount of genotypes (similar to barley in our case) could be tested and consequently selection intensity could be twice as high.

Correlations of true with estimated pea GMA values are lower in the incomplete design D compared with design B. In the absence of SMA, however, correlations come close to those of design B and even high-resource design A. This suggests that incomplete factorials can combine the advantages and applications of both designs with equal or similar dimensions of m and n and of designs with unequal dimensions of m and n , the latter being similar to topcross designs in hybrid breeding. At similar resource requirements, incomplete factorials allow

more genotypes to be tested without a substantial loss of GMA precision and accuracy. This will allow to increase selection intensity similar to the example shown in maize hybrid breeding (Seye et al., 2020). Moreover, testing larger sets of genotypes allow to exploit larger genetic variance of a given species. Since selection gain depends on both intensity and genetic variance, incomplete designs have a great potential to increase selection gain when breeding for mixed cropping.

Incomplete Designs for Early and Later Stages of Breeding for Mixed Cropping

Besides having been suggested for calibration of genomic prediction models for hybrid breeding (Seye et al., 2020), incomplete designs have been used to estimate GMA and SMA effects in wheat cultivar mixtures (Forst et al., 2019). The findings of Forst et al. (2019) could be applied to hybrid breeding as well as to breeding for mixed cropping: in the early development of a hybrid selection scheme for a crop, a broad range of genotypes could be tested in an incomplete diallel, similar to the one in Forst et al. (2019) that identified suitable material to form "pools" in cultivar mixtures. In mixed cropping, in early stages of breeding, where the size of the GMA variances of the two species are yet unknown and both species are of equal interest, an incomplete factorial with equal sizes of m and n would be advisable to subsequently design a breeding scheme based on the results. In later stages of both hybrid breeding (heterotic pools have been formed) and breeding for mixed cropping (focal species has been chosen), an incomplete factorial, e.g., in the form of design D, could be applied to both pools (hybrid breeding) or the focal species (mixed cropping). Only little literature has been published on actual experiments for breeding for mixed cropping. Some authors focus on the stepwise approach, first conducting a topcross design (similar to design C) to identify most promising genotypes for mixtures, followed by a full factorial to identify best combinations (similar to design B) for the development of two components of a mixture, such as species mixtures of maize (Hoppe, 2016) or common bean (Starke, 2018). The results presented in this study suggest such stepwise experiments could have been combined to a single one by the application of an incomplete design, thus speeding up the selection process. Incomplete designs can be applied to similar problems where factorial experimental designs are used, notably hybrid breeding, animal breeding.

Pr/As Concept to Select Genotypes According to Their Species-Specific Mixing Ability

The Pr/As concept allows to cluster genotypes into groups of particular "mixing-behaviors" within positive or negative GMA. Therefore, depending on the desired proportion of fraction yield by farmers, in our example a larger ratio of pea to barley, pea genotypes can be selected from either sectors U or V of **Figure 3**, while pea genotypes of sector W would support a higher proportion of barley. The Pr/As concept also allows to select for GMA maximization via a regression of the Pr effects on the As effects, i.e., fit a regression to the **Figure 3**

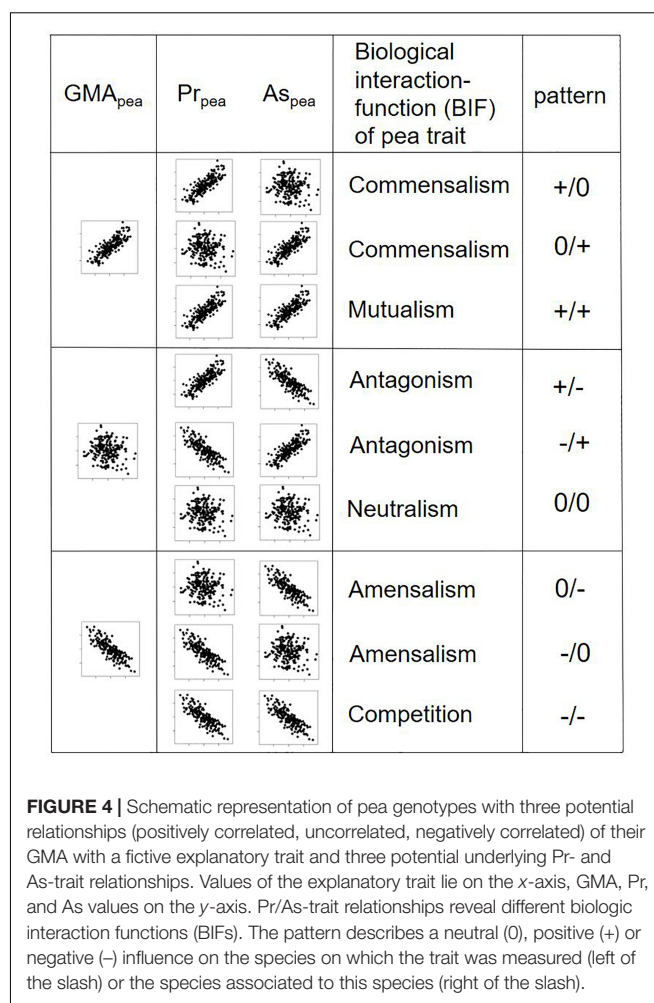
dataset. For instance, a regression with a slope strictly steeper than -1 (e.g., -1.5) indicates total yield can be increased by more competitive cultivars of the focal species, thus, GMA is maximized via the selection toward higher Pr or lower As effects.

The Pr/As concept can be seen as the genetic correspondence to the replacement series as, for example, described by Wendling et al. (2017). They compared four different crop species in pairwise combinations for their biomass yield under mixed cropping. Both the Pr/As concept and replacement series describe levels of competitiveness between two species under varying competitive conditions within the mixture, conveyed by different sowing ratios in the replacement series, and by genetic differences in competitiveness in the Pr/As concept. The low and high sowing ratios of a species in the replacement series would then correspond to low (positive As effects) or high competitive (negative As effects) genotypes, respectively. Wendling et al. (2017) observed linear relationships between mixed crop species only in two out of twelve replacement series. For all other scenarios, local maxima with transgressive overyielding were identified, i.e., mixture biomass yield exceeded the pure-stand biomass yield of each species. Due to the resemblance of the two concepts, it is quite possible, that similar local maxima for mixture yield occur in a Pr/As context. In this case, instead of a linear regression, bi-, polynomial, local, or non-parametric regressions could be applied, in order to find a target interval for As values to maximize mixture yield.

Pr and As effects are correctly estimated without being biased by different levels of correlated errors. A bivariate model is provided, able to take such a correlation into account. This model is an original analysis approach and the canonical way to treat paired variables that are presenting obvious inter-dependencies (yield of two crops cultivated on the same plot). However, this bivariate approach, with our design chosen to ensure balance and avoid confounding as much as possible, did not yield an improvement in terms of precision of estimates compared with the univariate approach. This is against our expectation and suggesting literature (Sørensen et al., 2003; Meier et al., 2015) but the focus of this study was on parameter estimation, whereas strengths of bivariate approaches might rather lie in other applications, such as hypothesis testing and outcome prediction. Even though the precision of estimates could not be improved with it, the multivariate approach can be used to estimate genetic correlations between traits (Meyer, 1991), which is fundamental for the use of indirect selection methods in mixed crops as suggested by Annicchiarico et al. (2019).

Pr/As-Trait Relationships to Shape Species Mixtures

The Pr/As concept can be seen as an extension of the concept of competitive effect and response (Goldberg and Fleetwood, 1987). There, a relationship or effect of trait A (e.g., early vigor), measured in species one (competitive effect) on a different trait B (e.g., yield), measured in species two (competitive response) is assumed. In the Pr/As context, however, trait



A in species one (e.g., early vigor) can have an effect on a trait B that is common in both species (e.g., yield of species one and two, i.e., Pr/As effects), as visualized in **Figure 4**. By combining the Pr/As concept with trait measurements, the BIF of a trait can be determined. This bears the potential for further systematic investigation and categorization of trait functions in mixed cropping and community ecology, where it might serve to discover, which trait categories prevail in successful plant or other organismic communities, and shape the functioning – or non-functioning – coexistence of these. The identification of BIFs is therefore very important for breeding for crop mixture, which is not possible, if only the trait-GMA relationship (i.e., total yield) is being looked at. Nevertheless, analyzing correlations between GMA and traits can still be of interest to identify key traits that influence total mixture performance like in forage crops but not the performance of individual species.

CONCLUSION

A GMA/SMA model could be applied to compare different experimental designs for their capacity to provide meaningful

information for breeders engaging in mixed cropping. Based on our findings, we recommend to use an incomplete factorial design in early stages of breeding for mixed cropping, since it allows to extend the number of tested cultivars at equal levels of experimental resources. Breeding programs can be sped up by the possibility to merge otherwise stepwise full factorial experiments into one single step. The Pr/As concept applied to an incomplete factorial design was shown to be an adequate tool to optimize mixture yield. It enables (i) to select genotypes with a suitable GMA-type thus optimizing mixture composition and (ii) to identify competitive optima for yield maximization in mixed cropping. It further allows to characterize the function of traits within species mixtures by their BIF and thus gain knowledge about their role in the biological interactions between species in a plant community. For ease of comprehension, the current study does not take genotype $\times$ environment ($G \times E$) interactions into account. Future research should address these interactions, and the models and methodology provided here can be expanded to integrate this interaction. This study provides an integrative methodological approach for the emerging field of breeding for mixed cropping of arable crops.

DATA AVAILABILITY STATEMENT

The R-codes used for the simulations and analyses supporting the conclusions of this article were made available by the authors under (see also Haug, 2020a,b).

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AUTHOR CONTRIBUTIONS

BH wrote the manuscript. The simulation experiments were designed and its statistical framework was established by all authors. IG, JE, TMH, EF, MM, PH, and BH developed the incomplete design idea. TF, TMH, and BH wrote the R-scripts for the analyses. All authors reviewed the final manuscript.

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Modeling Illustrates That Genomic Selection Provides New Opportunities for Intercrop Breeding

Jon Bančič^{1,2*}, Christian R. Werner^{1*}, R. Chris Gaynor¹, Gregor Gorjanc¹, Damaris A. Odeny³, Henry F. Ojulong³, Ian K. Dawson², Stephen P. Hoad² and John M. Hickey¹

¹ Royal (Dick) School of Veterinary Studies, The Roslin Institute, University of Edinburgh, Easter Bush Research Centre, Midlothian, United Kingdom, ² Scotland's Rural College (SRUC), Edinburgh, United Kingdom, ³ International Crops Research Institute for the Semi-Arid Tropics, ICRAF House, Nairobi, Kenya

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Jérôme Enjalbert,
Institut National de Recherche pour
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Reviewed by:

Lizhen Zhang,
China Agricultural University, China
Andrés Ricardo Schwember,
Pontificia Universidad Católica
de Chile, Chile

*Correspondence:

Jon Bančič
jon.bancic@roslin.ed.ac.uk;
jon.bancic@gmail.com
Christian R. Werner
c.werner@cgiar.org

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Intercrop breeding programs using genomic selection can produce faster genetic gain than intercrop breeding programs using phenotypic selection. Intercropping is an agricultural practice in which two or more component crops are grown together. It can lead to enhanced soil structure and fertility, improved weed suppression, and better control of pests and diseases. Especially in subsistence agriculture, intercropping has great potential to optimize farming and increase profitability. However, breeding for intercrop varieties is complex as it requires simultaneous improvement of two or more component crops that combine well in the field. We hypothesize that genomic selection can significantly simplify and accelerate the process of breeding crops for intercropping. Therefore, we used stochastic simulation to compare four different intercrop breeding programs implementing genomic selection and an intercrop breeding program entirely based on phenotypic selection. We assumed three different levels of genetic correlation between monocrop grain yield and intercrop grain yield to investigate how the different breeding strategies are impacted by this factor. We found that all four simulated breeding programs using genomic selection produced significantly more intercrop genetic gain than the phenotypic selection program regardless of the genetic correlation with monocrop yield. We suggest a genomic selection strategy which combines monocrop and intercrop trait information to predict general intercropping ability to increase selection accuracy in the early stages of a breeding program and to minimize the generation interval.

Keywords: sustainable agriculture, intercrop breeding program designs, genomic selection, intercropping ability, stochastic simulation

INTRODUCTION

Intercropping is an agricultural practice in which two or more component crops are grown together (Vandermeer, 1989). A common combination is a cereal with a legume, such as maize with beans in Latin America (Zimmermann, 1996), and millet/maize/sorghum with pigeon pea in India and East Africa (Dass and Sudhishri, 2010; Kiwira et al., 2019). Intercropping can lead to enhanced soil structure and fertility, the conservation of soil moisture, improved weed suppression, and better

control of pests and diseases, enabling greater yields and higher profitability (Brooker et al., 2015; Litrico and Violle, 2015). Recent meta-analysis showed low- and high- input intercropping systems on average delivered 16 to 29% more grain per hectare while using 19 to 36% less fertilizer per unit output than in conventional monocrop production, respectively (Li et al., 2020). Intercropping also allows for simultaneous cultivation of crops with different nutritional profiles, which can contribute to improving diets (Dawson et al., 2019a) and to increasing the stability and resilience of food systems (Himmelstein et al., 2017; Raseduzzaman and Jensen, 2017). Due to these characteristics, intercropping has great potential to optimize farming, especially in subsistence agricultural systems, which has recently led to an increased interest in the development and evaluation of efficient intercrop production (Dawson et al., 2019b).

Despite the potential benefits of intercropping, intercrop breeding has received only very little attention to date, with varieties specifically bred for intercrop production being unavailable (Brooker et al., 2015; Litrico and Violle, 2015). This lack of attention is due to in large part two reasons:

- (i) In advanced economies, major global crop species are predominantly grown as monocrops (Leff et al., 2004) and the majority of breeding programs are focused on generating varieties adapted to monocrop production (Acquaah, 2012).
- (ii) Intercrop breeding is more complex than monocrop breeding. Breeding for intercrop production requires the optimization of two or more component crops simultaneously (Francis, 1981; Wright, 1985); intercrop varieties ideally exhibit both a high *per se* performance and combine well with the other component crop(s) (Davis and Woolley, 1993).

As a result, the literature on intercrop breeding methodology is rare (Hamblin et al., 1976; Wright, 1985; Hill, 1996) and almost no progress in approaches has been made over the last few decades, with one recent exception (Sampoux et al., 2020). The crop varieties currently used for intercropping have typically been bred for monocrop production, and most often their performance in intercropping has not even been evaluated in advance (Brooker et al., 2015), strongly restricting the potential benefits of this practice.

Genomic selection offers many opportunities to address the complexity of intercrop breeding programs and aid the simultaneous improvement of two or more component crops that combine well in the field. Genomic selection uses associations between genome-wide markers and phenotypic performance to predict the value of genotypes based on their genomic markers (Meuwissen et al., 2001; Lorenz et al., 2011; Hickey et al., 2017). In the context of an intercrop breeding program, genomic selection could be used in several ways to increase the rate of genetic gain:

- (i) Selection accuracy can be increased for individual performance and combined performance of the component crops in an intercrop.

- (ii) The generation interval can be reduced, since new crossing parents can be selected based on their genomic predicted values as soon as they are genotyped.
- (iii) Selection intensity can be increased, since thousands of potential intercrop combinations could be evaluated without testing all of them in the field.

This study aimed to test whether genomic selection can speed up and better facilitate the process of breeding for intercropping. To test this, we compared four different intercrop breeding programs which implemented genomic selection to an intercrop breeding program using only phenotypic selection, using a stochastic simulations approach. We assumed three different levels of genetic correlation between monocrop grain yield and intercrop grain yield to investigate how the different breeding strategies are affected. We found all four breeding programs using genomic selection produced significantly more intercrop genetic gain than the phenotypic selection program, regardless of the genetic correlation. The combination of monocrop and intercrop trait information in genomic models to predict general intercropping ability seems to be the best strategy to both increase selection accuracy in the early stages of a breeding program and to minimize the generation interval.

MATERIALS AND METHODS

Stochastic simulations were used to evaluate the potential of genomic selection for intercrop breeding. We compared four different intercrop breeding programs implementing genomic selection and an intercrop breeding program using phenotypic selection for long-term efficacy for maximizing intercrop grain yield. Below, we have subdivided Material and Methods into three sections that describe: first, the simulation of the founder genotype population; second, the simulation of the recent (burn-in) breeding phase using a phenotypic selection breeding program; and third, the simulation of the future breeding phase to compare four different genomic selection breeding programs to the phenotypic selection breeding program. These topics are briefly reviewed below before detail is provided.

Simulation of the founder genotype population:

- (i) Genome simulation: a genome sequence was simulated for two hypothetical component crops in intercrop production.
- (ii) Simulation of founder genotypes: the simulated genome sequences were used to generate a base population of 100 founder genotypes for each of the two component crops.
- (iii) Simulation of genetic values: for each of the two component crops, two traits were simulated, representing monocrop grain yield and intercrop grain yield. Genetic values for the two traits were calculated by summing the additive genetic effects for both traits at 10,000 quantitative trait nucleotides and three different genetic correlations (0.4, 0.7, and 0.9) were simulated.
- (iv) Simulation of phenotypes: phenotypes were simulated for monocrop grain yield and intercrop grain yield.

Phenotypes representing monocrop grain yield were generated by adding random error to the genetic values for monocrop grain yield. Phenotypes representing intercrop grain yield were generated by adding random error to the mean genetic values for intercrop grain yield of two genotypes from both component crops.

Simulation of the recent (burn-in) breeding phase: A phenotypic selection breeding program was simulated for 20 years (burn-in) to provide a common starting point for the comparison of the different intercrop breeding programs during the future breeding phase.

Simulation of the future breeding phase: Four different genomic selection breeding programs were simulated and compared to the phenotypic selection breeding program for an additional 20 years of future breeding. The four genomic selection breeding programs included three variations of a Conventional genomic selection breeding program and a Grid genomic selection breeding program.

Simulation of the Founder Genotype Population

Genome Simulation

Genome sequences were simulated for two hypothetical component crops used in intercropping. For modeling purposes, the two crops' genomes were assumed to have the same characteristics. Each genome sequence consisted of 10 chromosome pairs. Each chromosome had a genetic length of 1.43 Morgans and a physical length of 8×10^8 base pairs. The chromosome sequences were generated using the Markovian coalescent simulator (MaCS, Chen et al., 2009) implemented in AlphaSimR (R Core Team, 2019; Gaynor et al., 2020). Recombination rate was derived as the ratio between genetic length and physical length (i.e., 1.43 Morgans/ 8×10^8 base pairs = 1.8×10^{-9} per base pair). The per-site mutation rate was set to 2×10^{-9} per base pair. Effective population size was set to 50, with linear piecewise increases up to 32,000 at 100,000 generations ago, as described by Gaynor et al. (2017).

Simulation of Founder Genotypes

The simulated genome sequences were used to generate a base population of 100 founder genotypes in Hardy-Weinberg equilibrium, for each of the two component crop species. These genotypes were formed by randomly sampling 10 chromosome pairs per genotype. A set of 1,000 bi-allelic quantitative trait nucleotides (QTNs) and 2,000 single nucleotide polymorphisms (SNPs) were randomly selected along each chromosome. This was done to simulate the structure of a quantitative trait that was controlled by 10,000 QTN and a SNP marker array with 20,000 genome-wide SNP markers. The founder genotypes were converted to doubled haploids (DH) and served as initial parents in the burn-in phase.

Simulation of Genetic Values

For each of the two component crops, two traits were simulated:

- (i) Monocrop grain yield, representing the yield of a genotype under monocrop production.
- (ii) Intercrop grain yield, representing the total yield of two genotypes, each from one of the two component crops, under intercrop production.

Genetic values for the two traits were calculated by summing the additive genetic effects for both traits across all 10,000 QTN. Additive effects were sampled from a standard normal distribution and scaled to obtain an additive variance of $\sigma_A^2 = 1$ in the founder population, as described in detail in the vignette of the AlphaSimR package (Gaynor et al., 2020).

Three different genetic correlations (0.4, 0.7, and 0.9) were simulated to represent different degrees of genotype-by-cropping interaction (Davis and Woolley, 1993).

Simulation of Phenotypes

Phenotypes were simulated for monocrop grain yield and for intercrop grain yield. Phenotypes for monocrop grain yield were generated by adding random error to the genetic values for monocrop grain yield. The random error was sampled from a normal distribution with mean zero and error variance σ_e^2 , defined by the target level of heritability at each stage of a breeding program. Entry-mean values for narrow-sense heritability (h^2) in the founder population were set to 0.1 in the doubled haploid stage and 0.33 in the preliminary yield trial stage. Narrow-sense heritabilities in later breeding stages increased as a result of an increased number of replicates (r) per genotype (Tables 1, 2).

Phenotypes for intercrop grain yield were generated by adding random error to the mean genetic values for intercrop grain yield of two genotypes from the two component crops. The following equation was used to calculate intercrop grain yield:

$$y_{i,j}^{ic} = \frac{a_{A,i}^{ic} + a_{B,j}^{ic}}{2} + e_{i,j},$$

where $y_{i,j}^{ic}$ is the intercrop grain yield, $a_{A,i}^{ic}$ is the genetic value for intercrop grain yield of genotype i from component crop A, $a_{B,j}^{ic}$ is the genetic value for intercrop grain yield of genotype j from component crop B, and $e_{i,j}$ is the random error. The random error for intercrop grain yield was also sampled from a normal distribution with mean zero and error variance defined by the target level of narrow-sense heritability at each stage of a breeding program.

Narrow-sense heritabilities for monocrop grain yield and intercrop grain yield were calculated as (Bernardo, 2010):

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_e^2/r}.$$

Simulation of the Recent (Burn-in) Breeding Phase

An intercrop breeding program using phenotypic selection was simulated to provide a common starting point (burn-in) for the comparison of the five intercrop breeding programs during the future breeding phase. The simulation modeled 20 years of

TABLE 1 | Summary of *per stage* parameters and annual operational costs for four 'medium' breeding programs.

Year	Stage	Reps	h^2	Pheno	Baseline-GS	PYT-GS	DH-GS
1	Cross			100 × 50 DH	100 × 47 DH	100 × 47 DH	40 × 80 DH
2	DH	1	0.10	5,000	4,700	4,700	3,200
3	PYT	4	0.33	500	500	500	500
4	GIA 1	4	0.33	50 ¹	50 ¹	50 ¹	50 ¹
5	GIA 2	24	0.50	13 ²	13 ²	13 ²	13 ²
6	SIA 1	16	0.67	9*	9*	9*	9*
7	SIA 2	32	0.80	3*	3*	3*	3*
Cost (United States \$)				493,200	492,200	492,200	495,200

Cost is set at approximately United States \$500K. The cost breakdown is shown for the Phenotypic selection breeding program (Pheno) and the three Conventional genomic selection breeding programs.

Baseline-GS, the Baseline genomic selection breeding program; PYT-GS, the Preliminary yield trial genomic selection breeding program; DH-GS, the Doubled haploid genomic selection breeding program; Reps, the effective number of replications (i.e., locations); h^2 , narrow-sense heritability; DH, the doubled haploid stage; PYT, the preliminary yield trial stage; GIA 1 and 2, the general intercropping ability stages 1 and 2; SIA 1 and 2, the specific intercropping ability stages 1 and 2. ¹ denotes testing with one probe variety; ² denotes testing with three probe varieties; *number of specific intercrop combinations.

phenotypic selection to reflect prior breeding that has taken place in the two component crops.

In brief, the four key features of the phenotypic selection breeding program (**Figure 1**) were:

- A crossing block of 80 DH lines was used to develop 100 bi-parental populations each year for each of the two component crops.
- New DH lines were developed from each bi-parental cross.
- A 2-year monocrop testing phase, in which monocrop grain yield was evaluated for each of the two component crop species.
- A 4-year intercrop testing phase, in which intercrop grain yield was evaluated for each intercrop combination of two genotypes from the two component crops. New parents were selected in the second year of the intercrop testing phase, giving a generation interval of 5 years.

The time from crossing to the release of a pair of component crop varieties for intercrop production was 7 years.

In what follows, the four key features of the phenotypic selection breeding program are explained in more detail. Simulation parameters, including heritability, the number of trial replications, and the number of tested genotypes at each stage

of the breeding program, are shown in **Table 1**. In the context of the phenotypic selection breeding program for intercropping varieties, we introduce the following terms:

- Probe variety: represents a genotype of one of the two component crops that has good intercropping ability with genotypes from the other component crop. It is comparable to a tester in a hybrid breeding program, which is used to evaluate the general combining ability of genotypes from one heterotic pool with another heterotic pool.
- General intercropping ability (GIA): the average intercrop grain yield of a genotype from one component crop grown with genotypes from the other component crop. It is evaluated using one or several probe genotypes from the other component crop.
- Specific intercropping ability (SIA): the intercrop grain yield of a specific intercrop combination of two genotypes from the two component crops.

Crossing Block (Year 1)

Each year, for each crop, a crossing block of 80 DH lines was used to produce 100 bi-parental crosses (**Table 1**). Parental combinations were chosen at random from all 3,160 possible pairwise combinations.

Development of Doubled Haploids (Year 2)

From each bi-parental cross, 50 DH lines were produced for each of the two component crops. The resulting 5,000 DH lines per crop were advanced to the monocrop testing phase and tested in the same year.

Monocrop Testing Phase (Years 2 and 3)

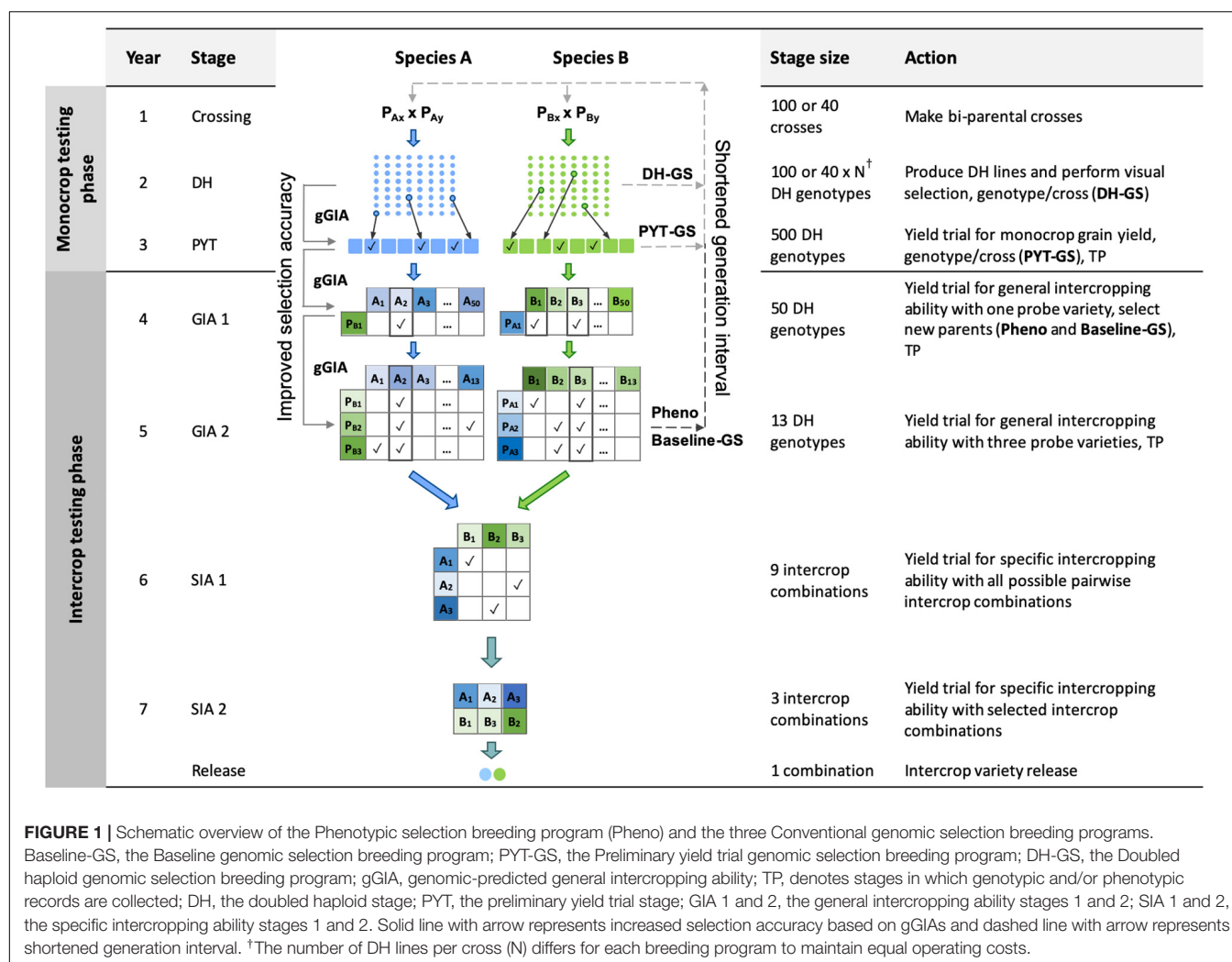
The monocrop testing phase spanned 2 years. Performance was evaluated as monocrop grain yield. Monocrop testing included the doubled haploid stage (DH stage, year 2) and the preliminary yield trial stage (PYT stage, year 3). In the DH stage, seed was increased and phenotypic selection was based on single plants within each bi-parental cross, to ensure there was variation in the later stages. In the PYT stage, phenotypic selection was based on multi-location trial plots (**Table 1**).

TABLE 2 | Summary of *per stage* parameters and annual operational costs for the 'medium' Grid genomic selection breeding program (Grid-GS).

Year	Stage	Reps	h^2	Grid-GS
1	Cross			40 × 90
2	DH	1	0.10	3,600
3	Grid	1	0.10	900/250,000*
4	SIA1	16	0.67	50*
5	SIA2	32	0.80	8*
Cost (United States \$)				493,800

Cost is set at approximately United States \$500K.

Reps, the effective number of replications (i.e., locations); h^2 , narrow-sense heritability; DH, the doubled haploid stage; Grid, the Grid stage; SIA 1 and 2, the specific intercropping ability stages 1 and 2; *number of specific intercrop combinations.



Intercrop Testing Phase (Years 4 to 7)

The intercrop testing phase spanned 4 years. Performance was evaluated as intercrop grain yield, i.e., the total yield from both simultaneously grown genotypes of the two component crops. The intercrop testing phase included two general intercropping ability testing stages (GIA1, year 4; and GIA2, year 5) and two specific intercropping ability testing stages (SIA1, year 6; and SIA2, year 7).

In the GIA1 stage, phenotypic selection was based on general intercropping ability in a yield trial with one probe variety. In the GIA2 stage, phenotypic selection was based on general intercropping ability using three probe varieties. Each year, the four best performing genotypes from the GIA2 stage replaced the probe varieties from the previous year. New parents were selected in the GIA1 stage. Each year, the 20 best performing genotypes from the GIA1 stage were used to replace the 20 oldest parents in the crossing block. Hence, every genotype stayed in the crossing block for four crossing cycles. The generation interval was 5 years.

In the SIA1 stage, all possible pairwise combinations of the selected lines were tested in a yield trial. In the SIA2 stage, the best combinations from the SIA1 stage were tested in a multi-location

trial (Table 1). The highest yielding intercrop combination was then released as an intercrop variety combination.

Simulation of the Future Breeding Phase

The future breeding phase was used to evaluate the phenotypic selection breeding program and the four genomic selection breeding programs for an additional 20 years of breeding. The genomic selection breeding programs included three variations of a Conventional genomic selection breeding program and a Grid-GS breeding program. The three Conventional genomic selection breeding programs replaced phenotypic selection by genomic selection at different stages of the phenotypic selection breeding program (Figure 1). They comprised a Baseline-GS, a PYT-GS and a DH-GS breeding program. The Grid-GS breeding program reorganized the phenotypic selection breeding program to enable the evaluation of a greater number of specific intercrop combinations using genomic selection. Details on all programs are given below.

In order to obtain approximately equal annual operating costs, the number of doubled haploids per bi-parental cross was reduced in the genomic selection breeding programs to

compensate for additional costs due to genotyping. **Table 1** shows the resources used for the phenotypic selection breeding program and the three Conventional genomic selection breeding programs. **Table 2** shows the resources used for the Grid-GS breeding program. Estimated applied costs in calculations were \$20 per monocrop test plot, \$50 per intercrop test plot, \$35 for producing a doubled haploid line and \$20 for producing a single genotype by array genotyping. The former two values were based on consultations with breeders at ICRISAT and the latter two values were previously used by Gaynor et al. (2017).

The Baseline Genomic Selection Breeding Program (Baseline-GS)

In the Baseline-GS breeding program, genomic selection was used to replace phenotypic selection in the PYT stage and in the GIA1 stage. Each year, the best 20 genotypes from the GIA1 stage were selected as new parents using genomic selection to replace the oldest 20 parents in the crossing block. As for the phenotypic selection breeding program, the generation interval was 5 years.

The Preliminary Yield Trial Genomic Selection Breeding Program (PYT-GS)

In the PYT-GS breeding program, genomic selection was used to replace phenotypic selection in the PYT stage and in the GIA1 stage. Each year, the best 80 genotypes from the PYT stage and last year's crossing block were selected as new parents using genomic selection.

The Doubled Haploid Genomic Selection Breeding Program (DH-GS)

In the DH-GS breeding program, genomic selection was used to replace phenotypic selection in the DH stage, the PYT stage and the GIA1 stage. Each year, 80 genotypes from the DH stage were selected as new parents to replace the entire last year's crossing block. As preliminary results showed a rapid decrease in genetic variance when the best 80 genotypes were selected, we implemented a maximum avoidance crossing scheme using genomic selection to reduce the rate of decrease (Kimura and Crow, 1963).

Genomic Selection Model for the Three Conventional Genomic Selection Breeding Programs

The three Conventional genomic selection breeding programs (above) used a multivariate ridge regression genomic selection model (RR-BLUP) to obtain genomic predictions of general intercropping abilities (gGIA) for each component crop separately.

In this model, monocrop grain yield from the PYT stage and mean intercrop grain yield with one or three probes, respectively, from the GIA1 and the GIA2 stage were fitted simultaneously. Genomic predictions of general intercropping ability can be directly calculated using intercrop grain yield from the GIA1 and the GIA2 stage as phenotypic information. In addition, the multivariate model uses information on monocrop grain yield, which was included as a correlated trait.

The following model was used:

$$y = Xb + Za + e,$$

expanded in matrix form as:

$$\begin{bmatrix} y_m \\ y_{ic1} \\ y_{ic2} \end{bmatrix} = \begin{bmatrix} X_m & 0 & 0 \\ 0 & X_{ic1} & 0 \\ 0 & 0 & X_{ic2} \end{bmatrix} \begin{bmatrix} b_m \\ b_{ic1} \\ b_{ic2} \end{bmatrix} + \begin{bmatrix} Z_m & 0 \\ 0 & Z_{ic1} \\ 0 & Z_{ic2} \end{bmatrix} \begin{bmatrix} a_m \\ a_{ic} \end{bmatrix} + \begin{bmatrix} e_m \\ e_{ic1} \\ e_{ic2} \end{bmatrix},$$

where y_m , y_{ic1} and y_{ic2} respectively denote the vectors of monocrop grain yield from the PYT stage, and mean intercrop grain yield with one or three probes from the GIA1 and the GIA2 stage; b_m , b_{ic1} and b_{ic2} respectively denote the vectors for the fixed effects of year and stage for PYT, GIA1, and GIA2; a_m and a_{ic} respectively denote the vectors of the marker effects for monocrop grain yield and intercrop grain yield; X_m , X_{ic1} , X_{ic2} , Z_m , Z_{ic1} and Z_{ic2} denote the corresponding incidence matrices; and e_m , e_{ic1} and e_{ic2} denote the corresponding vectors of residuals.

Additive genetic (G) and residual (R) variance-covariance matrices were:

$$G = \text{var} \begin{bmatrix} a_m \\ a_{ic} \end{bmatrix} = \begin{bmatrix} \sigma_{A_m}^2 & \sigma_{A_m,ic} \\ \sigma_{A_m,ic} & \sigma_{A_{ic}}^2 \end{bmatrix},$$

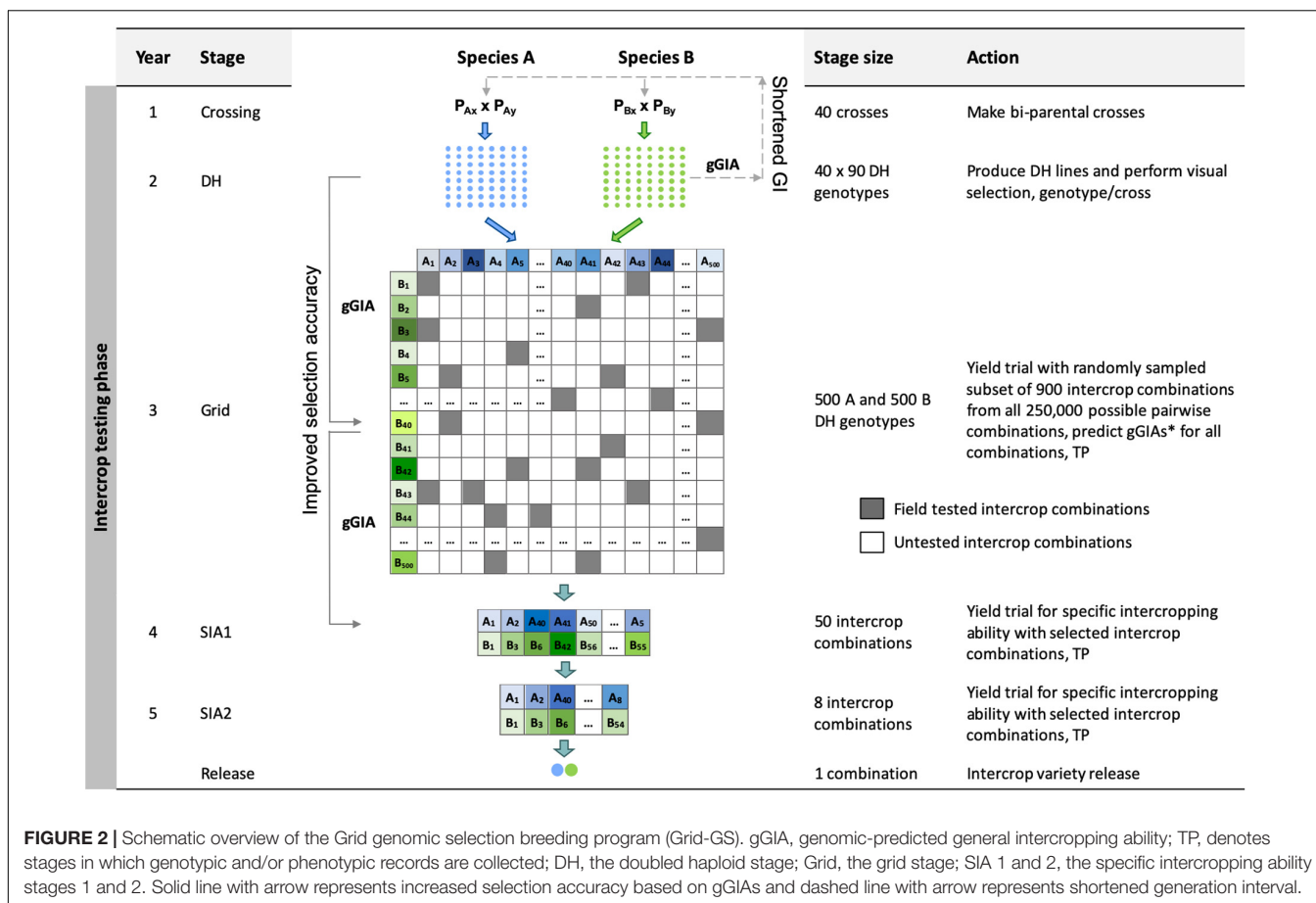
$$R = \text{var} \begin{bmatrix} e_m \\ e_{ic1} \\ e_{ic2} \end{bmatrix} = \begin{bmatrix} \sigma_e^2/r_m & 0 & 0 \\ 0 & \sigma_e^2/r_{ic1} & 0 \\ 0 & 0 & \sigma_e^2/r_{ic2} \end{bmatrix},$$

where $\sigma_{A_m}^2$ and $\sigma_{A_{ic}}^2$ respectively denote the additive genetic variances for monocrop grain yield and intercrop grain yield, and $\sigma_{A_m,ic}$ denotes the additive genetic covariance between the two traits; and σ_e^2 denotes the residual variance. **R** modeled heterogeneous residual variances by weighting σ_e^2 for the effective number of replications (r) in a particular stage (**Table 2**). To reduce computation time, additive genetic variances were assumed known and calculated each year using the true additive genetic effects.

The initial training population at the start of the future breeding phase consisted of all genotypes from the PYT stage of the last 5 years of the burn-in phase. This training population consisted of 2,500 genotypes and 2,739 phenotypic records from the PYT, the GIA1 and the GIA2 stages. In every year of the future breeding phase, 500 new genotypes from the PYT stage were added to the training population, as well as 500, 50 and 13 new phenotypic records from the PYT, the GIA1 and the GIA2 stages, respectively. The training population was updated using a 5-year sliding window approach, in which it always contained the most recent 5 years of training data.

Grid Genomic Selection Breeding Program (Grid-GS)

The Grid genomic selection breeding program reorganized the phenotypic selection breeding program to enable the evaluation of a greater number of specific intercrop combinations using genomic selection (**Figure 2**). To achieve this, the PYT, the GIA1 and GIA2 stages were replaced by a single 'grid' stage.



The reorganized program design also tested an increased number of specific intercrop combinations at the SIA1 and the SIA2 stages (Table 2).

The grid stage involved field testing of 900 intercrop combinations. At first, genomic prediction of general intercropping ability (gGIA) was used at the DH stage to select the best 500 DH lines from each component crop. From all 250,000 possible intercrop combinations between the 500 DH lines from each component crop, 900 were randomly sampled for field testing at a single location (Table 2).

Genomic predictions of general intercropping ability were calculated for all 250,000 intercrop combinations using the following equation:

$$gGIA_{i,j}^{ic} = \frac{gGIA_{A,i} + gGIA_{B,j}}{2},$$

where $gGIA_{i,j}^{ic}$ is the mean genomic-predicted general intercropping ability; and $gGIA_{A,i}$ and $gGIA_{B,i}$ are respectively the genomic-predicted general intercropping abilities of the i -th and j -th genotypes of component crops A and B. The best 50 predicted intercrop combinations were then advanced to the SIA1 stage (compared to nine intercrop combinations in our four other breeding programs).

Each year, 80 genotypes from the DH stage were selected as new parents to replace the entire last year's crossing block. As

preliminary results showed a rapid decrease in genetic variance when the best 80 genotypes were selected, we implemented a maximum avoidance crossing scheme with genomic selection to reduce the rate of decrease (Kimura and Crow, 1963). The generation interval was 2 years and the total length of the breeding program from initial crosses to the release of the intercrop variety pair was 5 years.

Genomic Selection Model for the Grid-GS Breeding Program

Genomic predictions of general intercropping ability were calculated using a ridge regression model (RR-BLUP) which predicted marker effects for both component crops simultaneously based on intercrop grain yield.

The following model was used:

$$y = Xb + Z_A a_A + Z_B a_B + e,$$

expanded in matrix form as:

$$y_{ic} = Xb + \begin{bmatrix} Z_A & 0 \\ 0 & Z_B \end{bmatrix} \begin{bmatrix} a_A \\ a_B \end{bmatrix} + e_{ic},$$

where y_{ic} denotes the vector of intercrop grain yield from the grid, SIA1 and SIA2 stages; b denotes the vector for fixed effects of year and stage for grid, SIA1, and SIA2; a_A and a_B respectively

denote the vectors of the marker effects for intercrop grain yield in component crops A and B; $\mathbf{X}$, $\mathbf{Z}_A$ and $\mathbf{Z}_B$ denote the incidence matrices; and $\mathbf{e}_{ic}$ denotes the vector of residual effects.

The residual ($\mathbf{R}$) variance-covariance matrix modeled heterogeneous residual variances by weighting σ_e^2 for the effective number of replications (r) in a particular stage (**Supplementary Table 4**), as described for the genomic selection model used in the Conventional genomic selection breeding programs. To reduce computation time, additive genetic variances were assumed known and calculated each year using the true additive genetic effects.

To initialize the training population, the grid stage was already simulated during the last 5 years of the burn-in breeding phase. For each of the 5 years, the best 23 genotypes at the PYT stage for each component crop were selected based on their genomic-predicted general intercropping abilities. These selected genotypes were then used to generate all 529 possible intercrop combinations, which were then tested in the field. At the beginning of the future breeding phase, the initial training population thus consisted of 115 genotypes from each component crop and 2,645 intercrop grain yield phenotypes (5×529 different intercrop combinations). In every year of the future breeding phase, 500 new genotypes from each component crop were added to the training population, as well as 900, 50, and 8 intercrop grain yield records, respectively, from the grid, SIA1 and SIA2 stages. The training population was updated using a 5-year sliding window approach, in which the training population always contained the most recent 5 years of data. This training population contained a total of 2,500 genotypes and 4,790 phenotypic records.

Comparison of the Intercrop Breeding Program Designs

The performance of the five intercrop breeding programs (the four using genomic selection and the phenotype-alone comparison) was evaluated by measuring the mean intercrop genetic value over time in the DH stage of both component crops as follows:

$$\bar{a}_{A,B}^{ic} = \frac{\bar{a}_A^{ic} + \bar{a}_B^{ic}}{2},$$

with $\bar{a}_A^{ic}$ and $\bar{a}_B^{ic}$ being the mean intercrop genetic values of the genotypes in the DH stage from component crops A and B, respectively. Mean intercrop genetic values of the two component crops were centered at 0 for the last year of the burn-in breeding phase. Intercrop genetic variance was measured as variance of the mean intercrop genetic values. Direct comparisons between breeding program designs for intercrop genetic gain and intercrop genetic variance were reported as ratios. These were calculated by performing a paired t -test (Welch) on log-transformed values from the 30 simulation replicates; the log-transformed differences from the t -test were then back-transformed to obtain ratios (**Supplementary Table 5**; Gaynor et al., 2017).

Prediction accuracy was evaluated as the correlation coefficient between the true and predicted performance at the DH stage. In the phenotypic selection breeding program,

the phenotype served as a predictor of intercropping ability. Prediction accuracy was evaluated as the correlation between the phenotypic value (i.e., monocrop grain yield) and true intercrop genetic value. In all four genomic selection breeding programs, prediction accuracy was measured as the correlation between the genomic-predicted general intercropping ability and the true intercrop genetic value of the doubled haploids.

Comparisons of the five breeding programs were done under three different levels of annual operating budget (see the start of Simulation of the Future Breeding Phase): (i) a 'large' budget (United States \$1M); (ii) a 'medium' budget (United States \$500K); and (iii) a 'small' budget (United States \$250K). Since the results for all our breeding programs showed similar rankings across these budgets, the methods presented above and the results presented below are described only for the medium budget scenario (**Tables 1, 2**). The parameters applied for breeding program designs and the results of the simulations at other budget levels are presented in the **Supplementary Materials (Supplementary Tables 1–4 and Supplementary Figures 1, 2)**.

RESULTS

Our results show that intercrop breeding programs using genomic selection can produce faster genetic gain than an intercrop breeding program using only phenotypic selection. All four breeding programs using genomic selection produced more intercrop genetic gain than the phenotypic selection breeding program (~ 1.3 – 2.5 times), regardless of the genetic correlation between monocrop grain yield and intercrop grain yield. However, the three Conventional genomic selection breeding programs produced increasingly more genetic gain when the genetic correlation between monocrop grain yield and intercrop grain yield increased, while the Grid-GS breeding program produced slightly less genetic gain when the genetic correlation increased. The DH-GS breeding program always produced the most genetic gain among the three Conventional genomic selection breeding programs (2.1 and 2.5 times the gain of the phenotypic selection breeding program at correlations of 0.4 and 0.9, respectively). Intercrop breeding using genomic selection also gave a faster reduction in genetic variance than intercrop breeding with phenotypic selection, regardless of the genetic correlation between monocrop yield and intercrop yield. Selection accuracy for intercropping ability was higher when genomic selection was compared to phenotypic selection. Selection accuracy in the three Conventional genomic selection breeding programs and the phenotypic selection breeding program increased when the genetic correlation between monocrop grain yield and intercrop grain yield increased, while selection accuracy in the Grid-GS breeding program was similar under different levels of genetic correlation. The general trends and rankings observed under the medium annual budget were representative of the trends observed under low and high annual budgets. Our findings are discussed in more detail below in terms of gain, genetic variance and selection accuracy.

Intercrop Genetic Gain

Intercrop breeding using genomic selection produced faster genetic gain than intercrop breeding with phenotypic selection. This is shown in **Figure 3**, which plots intercrop genetic gain as mean intercrop genetic value in the DH stage for the entire future breeding phase. The three panels show intercrop genetic gain for the five simulated breeding programs under different levels of genetic correlation between monocrop grain yield and intercrop grain yield. All four breeding programs using genomic selection produced significantly more intercrop genetic gain than the phenotypic selection program under all three levels of genetic correlation between monocrop yield and intercrop yield.

Figure 3 also shows that the three Conventional genomic selection breeding programs produced increasingly more genetic gain when the genetic correlation between monocrop grain yield and intercrop grain yield increased, while the Grid-GS breeding program produced slightly less genetic gain when the genetic correlation increased. As a result, the ranking of the four genomic selection breeding programs for genetic gain was dependent on the level of genetic correlation. When the genetic correlation was low (0.4), the Grid-GS breeding program produced the most genetic gain over time, closely followed by the DH-GS breeding program. Both breeding programs produced more than twice the genetic gain of the phenotypic selection breeding program. However, when the genetic correlation was high (0.9), the Grid-GS breeding program produced less genetic gain than all three Conventional genomic selection breeding programs. It generated 1.3 times the gain of the phenotypic selection breeding program,

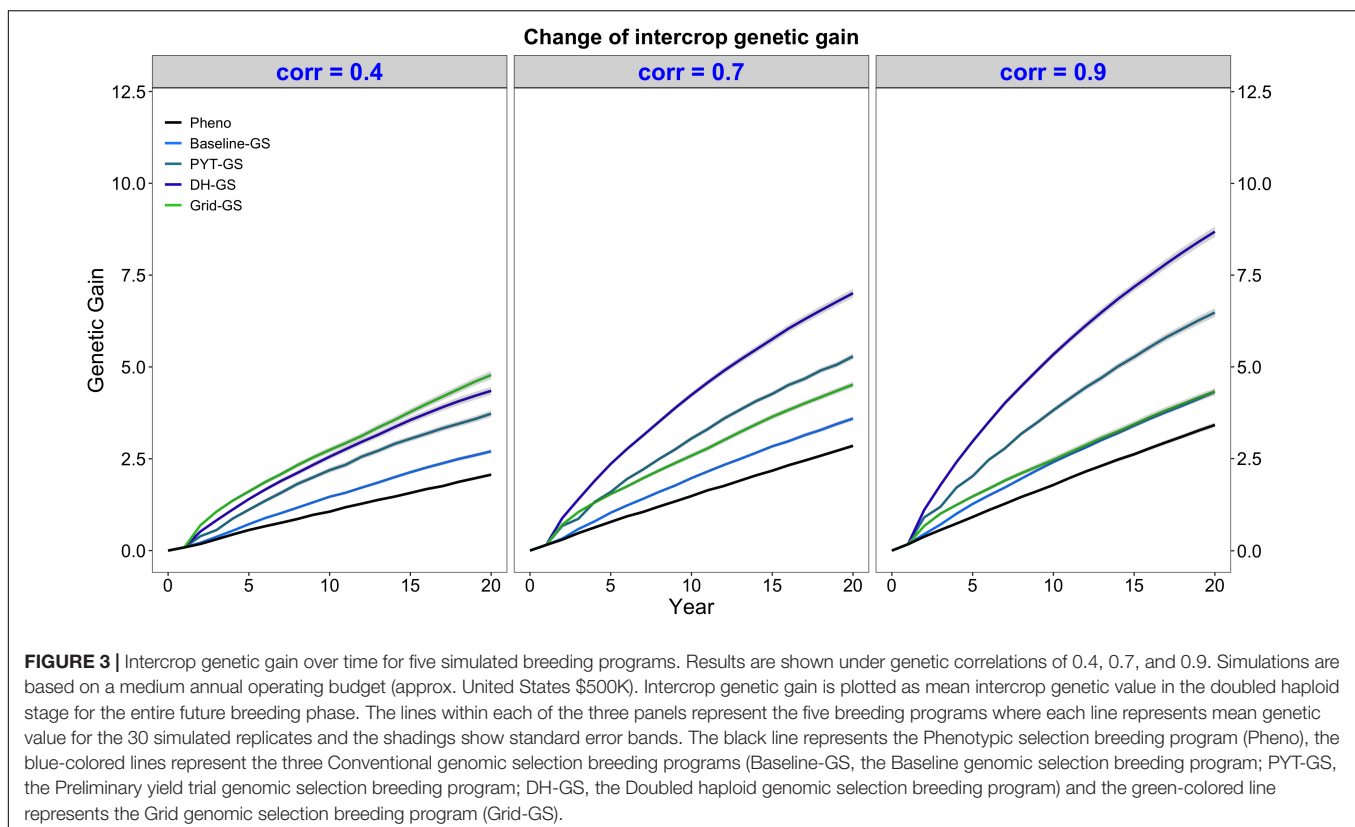
while the DH-GS breeding program produced 2.5 times the genetic gain of the phenotypic selection breeding program.

Figure 3 also shows that the DH-GS breeding program always produced the most genetic gain of the three Conventional genomic selection breeding programs, followed by PYT-GS and Baseline-GS breeding programs. The relative performance of the DH-GS breeding program compared to the other two Conventional genomic selection breeding programs increased when the genetic correlation between monocrop grain yield and intercrop grain yield increased. When the genetic correlation was low (0.4), the DH-GS breeding program generated 1.2 times the genetic gain of the PYT-GS breeding program and 1.6 times the gain of the Baseline-GS breeding program. When the genetic correlation was high (0.9), it generated 1.3 times the genetic gain of the PYT-GS breeding program and twice the gain of the Baseline-GS breeding program.

All breeding programs produced more genetic gain when the annual operating budget was high (**Supplementary Table 5** and **Supplementary Figure 2a**) and less genetic gain when the annual operating costs were low (**Supplementary Table 5** and **Supplementary Figure 1a**). The general trends and rankings observed under the medium annual budget were however representative of the trends observed under low and high annual budgets.

Intercrop Genetic Variance

Intercrop breeding using genomic selection gave a faster reduction in genetic variance than intercrop breeding with



phenotypic selection. This is shown in **Figure 4**, which plots the genetic variance of the intercrop genetic values in the DH stage for the entire future breeding phase. All four breeding programs using genomic selection gave a faster reduction in genetic variance than the phenotypic selection breeding program under all three levels of genetic correlation between monocrop yield and intercrop yield.

Figure 4 also shows that the Grid-GS breeding program gave the fastest reduction in genetic variance at the end of the future breeding phase under all three levels of genetic correlation. The Baseline-GS breeding program gave the slowest reduction in genetic variance among the four breeding programs using genomic selection. The DH-GS and the PYT-GS breeding programs always showed a similar reduction in genetic variance and ranked between the other two breeding programs using genomic selection. However, these two breeding programs became more similar to the Grid-GS breeding program as the genetic correlation increased. When the genetic correlation was high (0.9), the Grid-GS, the PYT-GS and the DH-GS breeding programs all showed a similar reduction in genetic variance at the end of the future breeding phase.

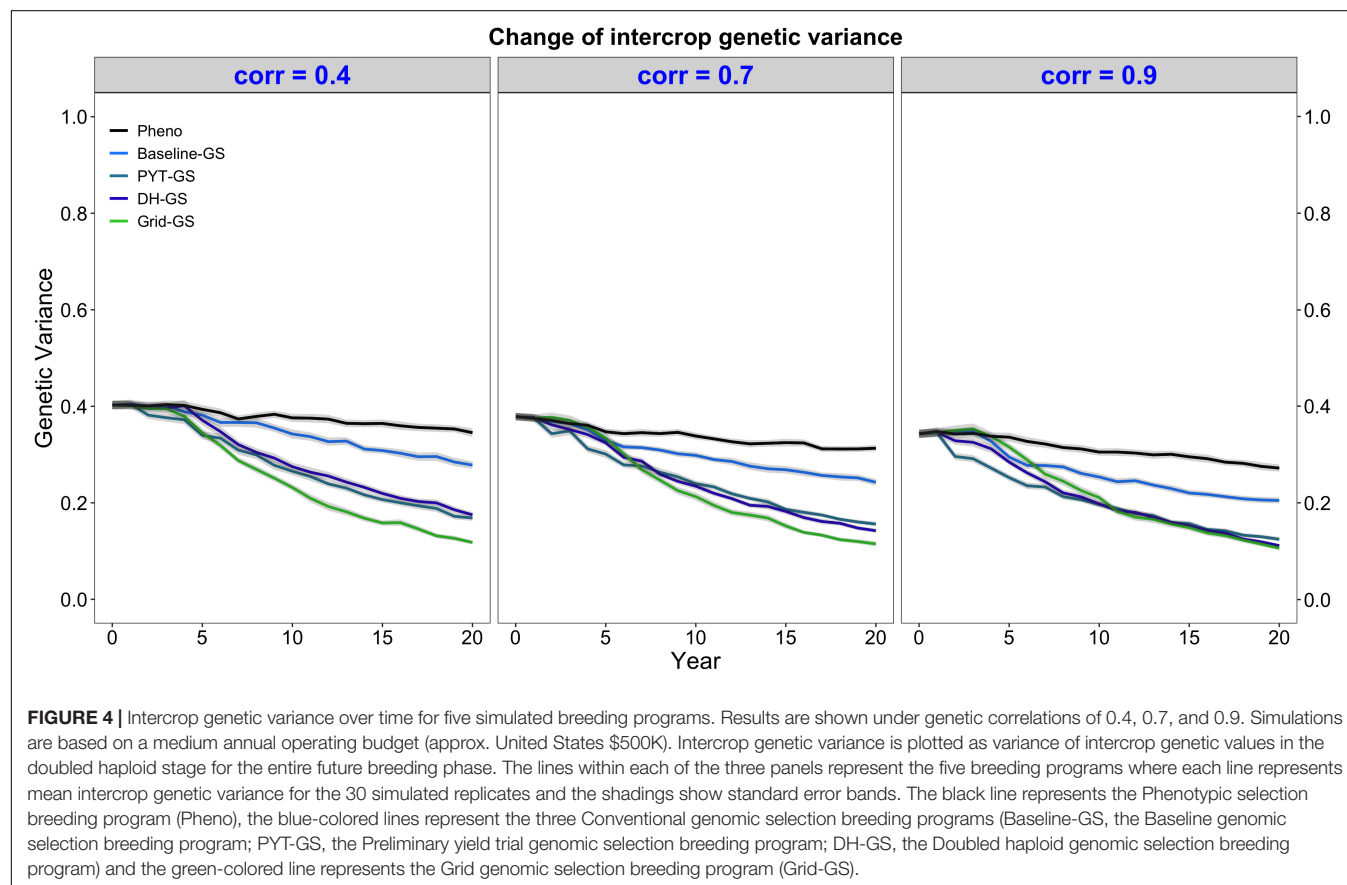
All breeding programs gave a faster reduction in genetic variance when the annual operating budget was high (**Supplementary Figure 1b**) and a slower reduction in genetic variance when annual operating costs were low (**Supplementary Figure 2b**). The general trends observed under the medium

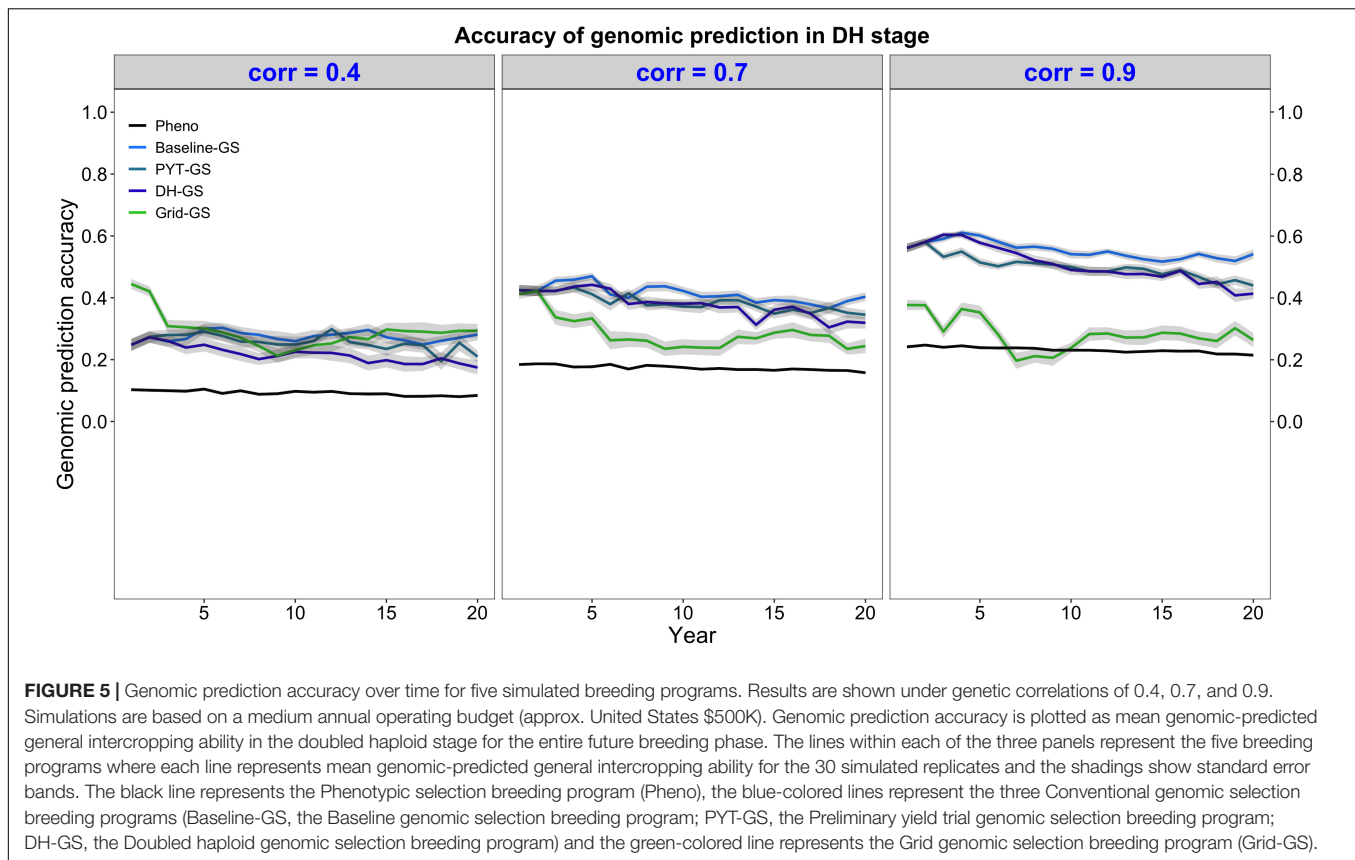
annual budget were however representative of the trends observed under low and high annual budgets.

Genomic Selection Accuracy

Genomic selection for intercropping ability was more accurate than phenotypic selection for intercropping ability. This is shown in **Figure 5**, which plots the mean selection accuracy for general intercropping ability in the DH stage for the entire future breeding phase. All four breeding programs using genomic selection showed on average higher accuracy than the phenotypic selection breeding program under all three levels of correlation between monocrop yield and intercrop yield.

Figure 5 also shows that selection accuracy for intercropping ability in the three Conventional genomic selection breeding programs and the phenotypic selection breeding program increased when the genetic correlation between monocrop grain yield and intercrop grain yield increased. Selection accuracy in the Grid-GS breeding program, on the other hand, was on average similar under all three levels of genetic correlation. When the genetic correlation was low (0.4), all four breeding programs using genomic selection showed on average a relatively similar selection accuracy. However, when the genetic correlation was high (0.9), the three Conventional genomic selection breeding programs showed a significantly higher selection accuracy than the Grid-GS breeding program. During most of the future breeding phase, the selection accuracy of the Grid-GS breeding





program was even lower than the selection accuracy of the phenotypic selection breeding program.

The four breeding programs using genomic selection showed a higher selection accuracy when the annual operating budget was high (Supplementary Table 5 and Supplementary Figure 1a) and a lower selection accuracy when the annual operating costs were low (Supplementary Table 5 and Supplementary Figure 2a). The general trends observed under the medium annual budget were however representative of the trends observed under low and high annual budgets.

DISCUSSION

High-performance intercrop production systems require more efficient intercrop breeding approaches that make use of advances in breeding (Dawson et al., 2019b). While it offers potential advantages, genomic selection also incurs additional costs, so it is necessary to understand the balance between benefits and costs. Stochastic simulations are becoming widely used to explore the efficiency of genomic selection in monoculture breeding (e.g., Gaynor et al., 2017; Gorjanc et al., 2018; Muleta et al., 2019), but to our knowledge our use of simulations to explore genomic selection's value for intercrop breeding is unique. Through simulations, we have shown that intercrop breeding programs using genomic selection can produce faster genetic gain than intercrop breeding programs which only use phenotypic

selection, working to a common cost basis that reflects the resources available for a mediumly invested breeding initiative.

To discuss our results, we first examine the value of genomic selection to increase selection accuracy and reduce the generation interval in breeding crops for intercrop production. We also explain that maximizing the rate of genetic gain using genomic selection can significantly increase genetic gain in the short term, but may impair long-term genetic gain due to rapid depletion of genetic variance. We then describe the value of strategies which reduce the loss of genetic variance to solve this problem, such as maximum avoidance crossing schemes or optimal contribution selection. We explain why the performance of the different genomic selection breeding programs was dependent on the genetic correlation between monocrop grain yield and intercrop grain yield, and we conclude that the DH-GS breeding program should be used in intercrop breeding unless the genetic correlation between the two traits is known to be low. We finish by discussing the most important limitations of our simulations and explain why we believe that our results are still valid in the context of real-world intercrop breeding programs.

Genomic Selection Increases Intercrop Genetic Gain

In phenotypic selection breeding programs, new crossing parents are usually selected after several years of intensive testing in multiple environments. This enables high selection accuracies but also results in long generations intervals, substantially restricting

the rate of genetic gain. Replacing phenotypic selection by genomic selection increases selection accuracy in early testing stages and thereby allows for selection of new parents based on their genomic predicted performance as soon as they can be genotyped. Our observations showed that all the intercrop breeding programs using genomic selection that we tested produced faster genetic gain than the phenotypic selection breeding program. This was observed regardless of the genetic correlation between monocrop grain yield and intercrop grain yield, and under three operating budgets. We observed that the major drivers of increased genetic gain were both an increased selection accuracy in early selection stages and a reduction of the generation interval. Our results were consistent with those of Gaynor et al. (2017) who used stochastic simulations to evaluate genomic selection strategies in plant breeding programs for developing inbred lines. We refer the reader to this study for a more detailed analysis of the relationship between genetic gain, the generation interval and prediction accuracy. As a consequence of increased selection accuracy and the reduced generation interval, all four genomic selection breeding programs also showed a faster reduction in genetic variance over time compared to the phenotypic selection breeding program. We discuss particular features of our findings in more detail below.

Genomic Selection Accelerates the Reduction of Intercrop Genetic Variance Over Time

We found that all intercrop breeding programs using genomic selection showed a faster reduction of genetic variance than the phenotypic selection breeding program. As genomic selection improved the conversion of genetic variance into genetic gain, the accelerated reduction of intercrop genetic variance was a direct outcome of the increased selection accuracy and the reduced generation interval. While maximizing this conversion will significantly increase the rate of genetic gain in the short term, the long-term genetic gain may be impaired due to a rapid depletion of genetic variance. To solve this problem, maximum avoidance crossing schemes can be used, which maintain genetic variation by selecting the best genotypes within families while ensuring that each family equally contributes to the next generation (Kimura and Crow, 1963). In this way, an over-representation of the top families in future generations is prevented.

We experimented with this approach by applying a maximum avoidance crossing scheme in the DH-GS and the Grid-GS breeding programs, as initial simulations using truncation selection to select new parents resulted in rapid exhaustion of genetic variance. The approach was successful, but a downside of maximum avoidance crossing schemes is that they require a closed population with a constant number of families and a minimum number of progeny per family to ensure the least related crosses are made each generation. While these requirements can be easily met within a simulation framework, practical application of a maximum avoidance crossing scheme may be more challenging, as breeders might introduce new genetic material to their breeding population, and not every crossing event might produce seed. Other, more complex, strategies might be more suitable to reduce the loss of genetic variation in real-world breeding programs, such as optimal

contribution selection and crossing (Meuwissen, 1997; Sonesson et al., 2012; Akdemir and Sánchez, 2016; Gorjanc et al., 2018), and exploring these could be a feature of future work.

The DH-GS Breeding Program Produces the Most Genetic Gain When the Genetic Correlation Between Monocrop Yield and Intercrop Yield Is Medium to High

In our simulations, the DH-GS breeding program produced approximately two times the genetic gain of the Grid-GS breeding program and approximately 2.5 times the genetic gain of the phenotypic selection breeding program when the genetic correlation between monocrop yield and intercrop yield was medium to high (0.7 and 0.9). The DH-GS scheme benefited from a short generation interval and an increased selection accuracy in the DH, PYT and GIA1 stages.

To obtain genomic predictions of general intercropping abilities for each component crop, the DH-GS breeding program used a multivariate genomic selection model which fitted monocrop grain yield and intercrop grain yield simultaneously. While phenotypic information on intercrop yield came from the GIA1 and the GIA2 stages, the multivariate model enabled us to extract additional information from monocrop yield phenotypes due to the genetic correlation between monocrop yield and intercrop yield. This additional information resulted in increased selection accuracy when the correlation was medium to high. While novel in the context of intercrop breeding, the use of correlated traits in multivariate genomic models is a well-known approach to improve prediction accuracy with wide application in plant and animal breeding (Calus and Veerkamp, 2011; Jia and Jannink, 2012).

The same multivariate genomic selection model was also used in the Baseline-GS and the PYT-GS breeding programs. These two breeding programs also outperformed the phenotypic selection breeding program regardless of the genetic correlation between monocrop yield and intercrop yield, but produced less genetic gain than the DH-GS breeding program. The PYT-GS breeding program benefited from an increased selection accuracy and a reduced generation interval compared to the phenotypic selection breeding program. The Baseline-GS breeding program did not reduce the generation interval. It was used to demonstrate the increase in selection accuracy when genomic selection is used compared to phenotypic selection.

The Grid-GS Breeding Program Is Advantageous When Genetic Correlation Is Low

In our modeling, the Grid-GS breeding program produced approximately 1.2 times the genetic gain of the DH-GS breeding program and approximately 2.3 times the genetic gain of the phenotypic selection breeding program when the genetic correlation between monocrop yield and intercrop yield was low (0.4). Our findings can be explained by the fact that the Grid-GS genomic selection model did not consider monocrop yield records, so it is unaffected by the genetic correlations between monocrop yield and intercrop yield, and prediction accuracies are similar under all correlations. When the genetic correlation was low, it therefore outperformed the DH-GS

breeding program. However, under a high genetic correlation, the training population size of the DH-GS breeding program was effectively increased by including phenotypic information from monocrop stages, while the training population in the grid stage of the Grid-GS program was not affected by the level of genetic correlation. We hypothesize that a larger training population and an optimized sampling strategy (rather than random sampling) of intercrop combinations in the grid stage would further increase the predictive ability of the genomic model and performance of the Grid-GS breeding program. Sampling strategies such as data-mining tools that exploit both genomic relationships and phenotypic variation to obtain the most representative subset as the training population from factorial design have already been discussed in the context of hybrid breeding and the existing theory could be extended to our Grid-GS design (Guo et al., 2019).

Unless the Genetic Correlations Between Monocrop and Intercrop Yield Are Known to Be Low, the DH-GS Breeding Program Should Be Used

Unless the genetic correlation between monocrop yield and intercrop yield was low, the DH-GS breeding program produced the most genetic gain. Even when the genetic correlation was 0.4, it was only slightly outperformed by the Grid-GS breeding program. These results indicate that the DH-GS breeding program has great potential to improve breeding for intercrop production.

In practical intercrop breeding programs, the genetic correlation between monocrop traits and intercrop traits will most likely be unknown and can change over time. The estimation of these parameters is difficult and requires large and costly experimental designs (Hamblin et al., 1976; Wright, 1985; Hill, 1996). When data for a precise decision-making process is not available, a strategy is required that delivers consistent performance across a wide parameter space. The DH-GS breeding program achieved substantially higher genetic gains than the phenotypic selection breeding program under all simulated correlations. Hence, we recommend that it is suitable for prompt implementation without prior knowledge about the level of genetic correlation.

A further advantage of the DH-GS breeding program is that it is a relatively simple way to implement genomic selection on top of a phenotypic selection intercrop breeding program, as it only requires minor resource re-allocations to compensate for the extra cost of genotyping. The Grid-GS breeding program, on the other hand, requires extensive restructuring of the breeding program, which might be harder to realize, particularly in low- and middle-income countries with limited resources.

The genomic selection models employed in this study should not be considered the ideal models to use in practice. These models were chosen to provide a reasonable estimate of the performance of genomic selection in an intercrop breeding program. In practice, the choice of a model should be guided by data and models that assess each of the actual component crops that are being considered. These models were not considered in our simulations, because the assumptions of the simulations made them unnecessary. Specifically, our simulations assumed

no interaction between component crops, and that the genetic variance and heritability was the same for each component crop. These assumptions are unlikely to be met in reality.

Limitations of Applying Stochastic Simulations for Intercrop Breeding Program Design

Our simulations have revealed the value of applying genomic selection in intercrop breeding. However, they are based on various simplified assumptions and do not model the full complexity of an actual intercrop breeding program. In this section, we discuss the most important limitations of our simulations and explain why we believe that our results still remain valid for real-world intercrop breeding. In the below we will discuss in turn assumptions which impact genomic selection accuracy, assumptions about making crosses and seed production, assumptions about the complexity of the breeding goal, and assumptions about the absence of genotype-by-genotype interaction between the two component crops.

Assumptions Which Impact Genomic Selection Accuracy

The intercrop prediction accuracies obtained in our simulations are likely to be higher than those realized under real-world conditions. Our simulations may be inflated because: the variance components provided to genomic models were estimated directly from the true simulation parameters; there were no genotyping or phenotyping errors in our data set; and we assumed additive gene action only without non-additive effects (i.e., dominance and epistasis) and genotype-by-environment interaction (Vitezica et al., 2013; Forneris et al., 2017; Jarquín et al., 2017). Also, the population structure of both component crops was much simpler than that found in real breeding programs which may further affect the prediction accuracy (Guo et al., 2014; Isidro et al., 2015).

These factors should affect all genomic selection breeding programs and we do not expect their relative performance to change much under real-world conditions. Inclusion of non-additive effects in more complex genomic selection models, however, may also only provide very low (or negligible) improvements in genetic gain or the prediction accuracy (Hill et al., 2008; Varona et al., 2018); while dominance does not even apply in our simulations since we were dealing with inbred lines. Gaynor et al. (2017) observed similar rankings of breeding programs even when using simulated genotype-by-environment interaction. This suggests that the DH-GS breeding program would still show overall best performance compared to other genomic selection breeding programs. However, the performance of the phenotypic selection breeding program relative to the genomic selection breeding programs could change. If this was to occur, the magnitude of the difference between the best genomic selection breeding program and the phenotypic selection breeding program we have observed leads us to believe that under real-world conditions the genomic selection program would still outperform the phenotypic selection breeding program. Lastly, the population structure might be an issue at the beginning of an intercrop breeding

program when extensive phenotypic and genetic variation will be required for various interaction traits (e.g., days to flowering and plant height) (Litrico and Violle, 2015). However, in our simulations, genomic selection was only implemented after 20 years of phenotypic selection (burn-in), when populations were already more uniform and a sufficient number of training records was available. Such gradual transition is likely to happen also in reality.

Assumptions About Making Crosses and Seed Production

To minimize complexity, in our simulated breeding programs we assumed no differences in flowering time between crossing parents and that all crosses produce sufficient amounts of seed for immediate next step implementation. In real-world breeding, differences in maturity between potential crossing parents might reduce the number of possible crosses, while some crosses may not immediately produce enough seed, with additional seed multiplication steps required that prolong the breeding process. If dealing with a self-pollinating and an outcrossing component crop simultaneously, these issues might present the most significant challenge (Hamblin and Zimmermann, 1986). As Hamblin et al. (1976) indicate, two self-pollinating crops with large seed production may be the simplest case for intercrop breeding. Breeding programs that use either phenotypic or genomic selection would be similarly affected by these seed production issues. We thus assume that the relative performance of the different breeding programs would be similar under more realistic crossing scenarios.

Assumptions About the Complexity of the Breeding Goal

In our analysis, comparisons between breeding programs were based on a single quantitative trait representing intercrop grain yield. We also assumed that both component crops equally contributed to intercrop grain yield and its economic value. Real-world breeding programs, however, have to consider multiple quantitative and qualitative traits simultaneously to maximize agronomic performance. Furthermore, it is unlikely that both component crops produce comparable amounts of yield and that both component traits have a similar market value.

In fact, the contribution of each component crop to the total economic value of the combined product will depend on various factors. These include: the cultivation environment (i.e., biological, economic and cultural) and management practices (Francis, 1981; Mead and Riley, 1981); the *per se* yield potential and economic value of each component crop (Hamblin et al., 1976; Francis, 1981; Wright, 1985); and the intended use of the products, especially whether for subsistence use or market (Mead and Riley, 1981).

In theory, a selection index could be developed to enable selection of the best intercrop combinations by combining several key traits and through considering the above factors. Selection indices can allow the assignment of customized economic weights to the component crops, thereby optimizing

their individual yield gains to maximize the market value of the combined crop product. In real-world breeding programs, estimation of the relative (economic) weights for traits of interest is not a trivial exercise, and weights may also need to be changed over time (Mead and Riley, 1981). Moreover, the selection index is likely to differ between different intercrop combinations and should be determined by both the data and breeding objectives. However, in the context of a simulation, the simulated trait also can be considered as the total economic value resulting from a linear selection index (e.g., Smith-Hazel index). We assume that we would observe similar trends for our simulated breeding programs if we were to include multiple traits in an index.

Assumptions About the Absence of Genotype-by-Genotype Interaction Between the Two Component Crops

In our simulations, we ignored the possible effects of genotype-by-genotype interactions between component crops. In practical intercrop production, these interactions play an important role in determining their productivity, ecosystem service provision and resilience (Dawson et al., 2019a). Although strategies have been outlined through which genetic variants underlying mutualisms between pairs of plant species in natural ecosystems can be characterized, studies reporting genotype-by-genotype interactions are currently relatively scarce (Subrahmaniam et al., 2018). We expect the effect of genotype-by-genotype interactions to be most significant at the start of breeding activities, when material is unadapted to a particular growing system and when they could potentially result in re-ranking of our breeding programs. We expect that through continuous recurrent selection these interactions may become minimal, as the competition component is minimized through continuously improved coexistence between two component crops (Hill, 1996).

CONCLUSION

Our results show that genomic selection shows great promise in breeding crops for intercrop production. We have demonstrated that genomic selection can significantly increase the rate of genetic gain in intercrop breeding. In particular, the DH-GS breeding strategy provides a simple solution to implement genomic selection on top of an existing phenotypic selection breeding program, without major rearrangements and regardless of the genetic correlation between monocrop yield and intercrop yield. Clearly, the practical challenges of the implementation of genomic selection strategies differ between breeding programs, but we believe that our results will aid breeders in optimizing the implementation process. Overall, the current study can be considered as an initial piece that future modeling work can build on. In our further work we are exploring the utility of different design approaches for crop combinations such as finger millet and groundnut that could be optimized as an important intercrop

for reaching multiple human and environmental health benefits in East Africa (Dawson et al., 2019b).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JB, JH, and RG conceived and designed the study. JB developed the plant breeding program simulations. DO and HO provided information on existing breeding programs. JB, CW, and ID wrote the manuscript, with input from all authors. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article, including scripts to run simulations, can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.605172/full#supplementary-material>

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Multi-Species Genomics-Enabled Selection for Improving Agroecosystems Across Space and Time

Marnin D. Wolfe^{1*}, Jean-Luc Jannink^{1,2}, Michael B. Kantar³ and Nicholas Santantonio^{4*}

¹ Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, United States,

² United States Department of Agriculture – Agriculture Research Service, Ithaca, NY, United States, ³ Department of Tropical Plant and Soil Science, University of Hawai'i at Mānoa, Honolulu, HI, United States, ⁴ School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, United States

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Sher-e-Kashmir University
of Agricultural Sciences
and Technology, India

*Correspondence:

Marnin D. Wolfe
wolfermd@gmail.com
Nicholas Santantonio
nsant@vt.edu

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Plant breeding has been central to global increases in crop yields. Breeding deserves praise for helping to establish better food security, but also shares the responsibility of unintended consequences. Much work has been done describing alternative agricultural systems that seek to alleviate these externalities, however, breeding methods and breeding programs have largely not focused on these systems. Here we explore breeding and selection strategies that better align with these more diverse spatial and temporal agricultural systems.

Keywords: genomic selection, agroecosystems, intercropping, polyculture, breeding, ecosystem services

INTRODUCTION

Climate change and human population growth are continually increasing demand for food and services from agroecosystems. To meet these demands sustainably, food production must be intensified. These challenges require innovation and diversification in agroecological-systems design and management (Runck et al., 2014; Litrico and Violle, 2015; Henkhaus et al., 2020). Today the dominant form of agriculture across the globe consists of large acreages of monoculture production (Crews et al., 2018). Monocultures provide uniformity in plant architecture and maturation, facilitating efficient mechanical harvesting and minimizing human labor.

The combination of new crop types, synthetic fertilizers, and irrigation has dramatically increased crop production per unit area while simultaneously sparing land for natural ecosystems (Burney et al., 2010). This has come at an environmental cost. Increases in water and nutrient pollution, vast new energy and fossil fuel requirements to produce fertilizers, and steady losses of crop diversity. Maintaining or intensifying production while decreasing external inputs and soil disturbance (i.e., tillage) requires cropping systems that are more spatially (intercrops, polycultures) and temporally (rotations, relays) diverse, and in many cases include longer-lived (i.e., perennial) species (Lovell and Taylor, 2013).

Modern plant and animal breeding is a predictive, data-driven, multi-disciplinary science. Statistical prediction methods that leverage genomic and phenomic data (e.g., drone-based hyperspectral imaging) are greatly accelerating the rate of population genetic improvement (Jannink et al., 2010; Hickey et al., 2017; Voss-Fels et al., 2019; Krause et al., 2020). Decision support tools based on these technologies are now available to large-acreage monoculture systems.

Transitions to new agricultural practices are expensive and require agronomy and operations research. Nevertheless, state-of-the-art breeding is largely focused on individual species and the development of single genotypes, for their single-season monoculture performance.

Indeed, breeding and agronomy typically operate on vastly different scales of genetic variation. Breeders evaluate hundreds or thousands of genotypes in only limited combinations of management, environmental and cropping system variations. Agronomists and agroecologists, in contrast, test diverse cropping and management practices, but against relatively few, “representative,” cultivars of each species.

A sustainable future for food is a *highly* multi-objective optimization problem. At the landscape level there is incredible heterogeneity, comparable in magnitude to variability in yearly climate patterns. Therefore creating sustainable landscapes that serve multiple functions requires combining food and non-food crops as well direct and *indirect* services from landscapes. Diversified agroecosystems are expected to exhibit better sustained productivity and multifunctionality over long time periods, borne out in theory from economics (Goerner et al., 2009; Paut et al., 2020), ecology (Holling, 1973), and agriculture (Schipanski et al., 2016). The productivity-diversity relationship is expected to depend on the degree of resource-use niche complementarity vs. redundancy and the nature of interspecific interactions (Brooker et al., 2015; Bowles et al., 2020; Tamburini et al., 2020). However, these robust results have yet to be widely adopted in the breeding industry and when they are, they rarely use state-of-the-art tools. Despite strong evidence for the benefits of cropping-system diversification (Tamburini et al., 2020) and calls in the literature (Brooker et al., 2015; Litrico and Violle, 2015; Sompoux et al., 2020), the improvement of complex multi-species, multi-genotype systems has not been a priority.

Instead of breeding to improve monoculture yield of single crops in isolation, we propose optimizing multiple interacting species and genotypes. We seek to enable joint-selection to improve the performance of the cropping system across time and space. We argue that the largely disparate literature on diversification and agroecological intensification, genomics and phenomics-enabled selection collectively indicate the advantage of developing prediction and selection strategies to tackle the multiple outputs of cropping systems and their responses to environmental changes. This represents an important frontier in agriculture and strategies need to be devised for maintaining and enhancing beneficial interactions while reducing or avoiding negative ones.

JOINT-SEARCH OF MULTIPLE GENE POOLS FOR ADAPTIVE INTERSPECIFIC INTERACTIONS WITH GENOMIC PREDICTION

Investigating all possible combinations of genotypes between any diverse set of germplasm from one species (or population),

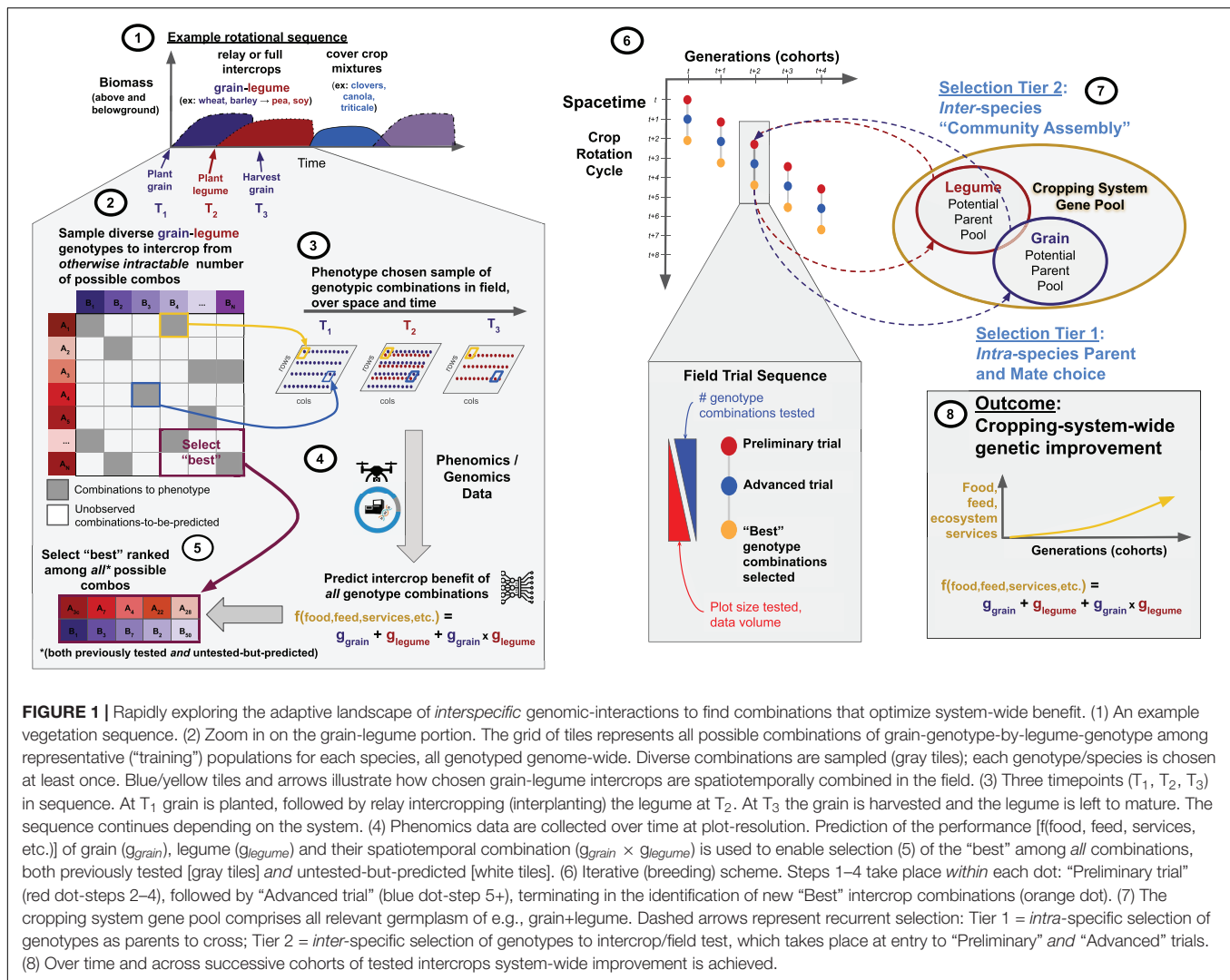
and a diverse set of another interacting species (or population), is intractable. Borrowing methodology from maize hybrid breeding [reciprocal recurrent selection (Comstock et al., 1949)], (Wright, 1985) developed an interspecies selection scheme, which partitions plot-level performance into main effects for each species (general mixing ability; GMA) and an interaction (specific mixing ability; SMA) (Federer, 1993; Forst et al., 2019; Sompoux et al., 2020; Haug et al., 2021). We note that a GMA is estimated for each genotype of each single crop, but that these GMAs refer to emergent plot-level properties (e.g., erosion protection) that can only be measured on crop combinations. The intractably large genotype-by-genotype *interspecific* interaction landscape can be enumerated and the “best” *interspecific* genotypic combinations can be identified using numerical optimization and genomic prediction. Rather than attempting to test all possible combinations, accessions-to-be-phenotyped should be algorithmically chosen, similar to modern approaches in hybrid breeding (Zhao et al., 2015) such that genetic variation in each species is tested against a representative sampling of variation in the other species.

The application of genomic prediction to unobserved intercrop combinations has recently been suggested (Annicchiarico et al., 2019; Bančič et al., 2020). Genomic prediction has not been applied using these models. Empirical estimates of GMA/SMA are scarce and have only occasionally detected statistically significant SMA (Collins and Rhodes, 1989; Knott and Mundt, 1990; Federer, 1993; Holland and Brummer, 1999; Lopez and Mundt, 2000; Forst et al., 2019; Haug et al., 2021). Approaches to date have been constrained to individual species productivity in the immediate environment of the other species rather than accounting for total agroecosystem productivity through time.

Genomic and phenomic prediction poses an exciting opportunity to develop what we describe below as a multi-tiered selection scheme. **Figure 1** shows an example of how this can be operationalized, using a no-till grain-legume sequence example and an experimental design that develops a profile of phenomic and genomic variation within- and among-species across space and time. The iterative field evaluation procedure has the potential to enable directed co-improvement of all species and their interaction for overall system performance.

MULTI-TIERED SELECTION: GENES TO CROPPING SYSTEMS

Consider that the phenotype of any individual is the response to an environment that is shaped by the other organisms present in that environment, both current and past. This highlights that, from the perspective of prediction, genome-by-genome interactions ($G \times G$) are a special case of the genotype-by-environment ($G \times E$) interaction where the covariance of the environment is the interacting species' genotypic covariance. Typically, phenotypic evaluation is done at particular locations under targeted management conditions in an effort to “control” the environment under which focal species are observed and for which they are selected. In the



general case, the objective function, $f[G_{ij} | E(G_{i'j'}, t, s)]$, assigns a genetic value to j th individual, of the i th species (G_{ij}) conditional on the environment, E , which is itself a function of other species ($G_{i'j'}$) in the system, space (s) and time (t). In the classic case, $G_{i'j'}$, t and s are all held constant or partitioned to the error term and single season yield is the objective function to be optimized. When the other species in the system, space and time are simultaneously taken into account, we develop a generalized agroecosystem selection scheme.

Prediction and selection strategies that leverage genomic/phenomic tools to address more than single-species, single-season, monoculture evaluation should be a major frontier for future research and development. We highlight that there are multiple levels or “tiers” of selection, which when considered jointly enact agroecosystem improvement. Importantly before selection begins, the goals must be defined (Table 1). The objective at Tier 1 is *intraspecific* population improvement, which is addressed simultaneously across each

species to effect co-adaptation of the germplasm pools. Tier 1 evaluation identifies promising parents and matings. At Tier 2, selection is focused on predictions of performance of the combination over space and time (e.g., of the intercrop overall). The objective at Tier 2 is to select the “best” *inter-specific* (or *intra-specific*) genotype combinations to assemble in space over time, i.e., to release to farmers that maximize farm profit and ecosystem function.

Determining the selection goals for Tiers 1 (breeding decisions) and 2 (intercropping decisions) are the landscape-scale, cropping-system wide properties, considered over multiple seasons, species and performance indicators, which are community- and market-defined. Thus, while Tier 1 can be viewed as effecting co-adaptation of crops to an overall diversified cropping sequence, Tier 2 includes optimization of and potential specific decisions about the sequence of cropping in space and time. This framework can thus be adapted to both generally and specifically diversified spatiotemporal configurations (e.g., cropping sequences, planting densities)

TABLE 1 | Potential cropping system applications, their associated interactions and agroecosystem objectives.

Cropping system / Agroecosystem	Interactions	Objectives
Temporal rotations E.g., following corn with soy.	Indirect. Legacy effects. Current (past) crops condition (esp. soil) environment for future crops.	Minimize or Reduce: loss of soil N, non-prod. time, non-target / weed species, runoff, nutrient input, herbicide pesticide
Relay intercrops E.g., soy planted between rows of maturing barley without tillage.	Direct and Indirect effects. Maturing crops influence microenvironment (weed suppression, shade, soil moisture, architectural support) to young crops <i>plus</i> legacy effects on subsequent crops.	Maximize or Increase: retention of soil C, net primary productivity, germination
Full intercrops Planted together. Harvested separately, or separated at harvest. E.g., three-sisters (corn, beans, squash), maize-peanut, guava/mango/cowpea, banana and root crops (sweet potato, yam, cassava), sugarcane-sweet potato, orchard and agroforestry alleys		↑↓
Species mixtures / polycultures Harvested together. E.g., mixtures of grasses, legumes and mustards used as cover crop mixtures / green manures, biofuel / biomass crops, perennial plantings to reduce erosion/runoff		Max avg. profit, Min var. Profit

for any potential product profile of the agroecosystem that is to be considered.

BROADER IMPLICATIONS FOR CHANGING THE LANDSCAPE

There are many important potential applications, which this framework can address. Each of these represent different multi-objective optimization problems with respect to competition and interactions, which need to be defined and have been, in some cases, reviewed elsewhere (Picasso et al., 2008; Brooker et al., 2015; Kantar et al., 2016; Ryan et al., 2018; Duchene et al., 2019). **Table 1** provides brief examples of applications, the types of interactions to improve and potential benefits.

Theory and agronomic knowledge are available to help understand how different crop species should interact, but optimal multi-species selection strategies have not been developed. Selection and optimization strategies need to balance positive effects against potentially negative ones including financial, human health and environmental costs of managing such systems. While farmers already practice crop rotation, they do not have access to varieties explicitly adapted to one another beyond their ability to meet the basic phenology and management requirements. Identification of the cropping systems and selection indices that support stated multi-species system-level goals is critical and will need careful consideration. We suggest that involvement of farmers and other stakeholders through participatory breeding approaches will be an important component for success (Runck et al., 2014; Lammerts van Bueren et al., 2018). Stakeholder and policy support throughout the process is essential to ensure resources

and acreage are not overspent and that cropping system selection indices are constructed in such a way that the agricultural products that are developed perform verifiable services that are collectively desirable.

The framework described here aims to facilitate the design, development and marketing of co-cultivars. These seed “packages” would consist of combinations of varieties selected to optimize the agroecosystem over the long-term, for objectives beyond single-season, single-crop yield.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MW led the writing and developed the figure and table. MK contributed substantially to writing. MW, J-LJ, MK, and NS contributed to the conceptual development, read, commented, and approved the manuscript for submission. NS supervised the development of the article. All authors contributed to the article and approved the submitted version.

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Diverse Intercropping Patterns Enhance the Productivity and Volatile Oil Yield of *Atractylodes lancea* (Thunb.) DC.

Zheng Peng^{1,2†}, Yan Zhang^{1†}, Binbin Yan¹, Zhilai Zhan¹, Xiulian Chi¹, Yang Xu^{1,2}, Xiuzhi Guo¹, Xinpeng Cui¹, Tielin Wang¹, Sheng Wang¹, Chuanzhi Kang¹, Xiufu Wan¹, Kai Sun^{1*}, Luqi Huang^{1*} and Lanping Guo^{1*}

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University of Rostock, Germany

*Correspondence:

Kai Sun
sun-kai@pku.edu.cn
Luqi Huang
huangluqi01@126.com
Lanping Guo
glp01@126.com

[†]These authors share first authorship

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¹ State Key Laboratory of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China, ² Institute of Healthcare China Academy of Chinese Medical Sciences, Nanchang, China

Commercial cultivation of the medicinal plant *Atractylodes lancea* is significantly restricted by low survival rates and reduced yields. Intercropping can reasonably coordinate interspecific interactions, effectively utilize environmental resources, and increase survival and yield. We conducted a field experiment from 2014 to 2016 to analyze the advantages and effects of intercropping on *A. lancea* survival, growth traits, individual volatile oil content, and total volatile oil content. In addition to *A. lancea* monoculture (AL), five intercropping combinations were planted: *Zea mays* L. (ZM) + *A. lancea*, *Tagetes erecta* L. (TE) + *A. lancea*, *Calendula officinalis* L. (CO) + *A. lancea*, *Glycine max* (Linn.) Merr. (GM) + *A. lancea*, and *Polygonum hydropiper* L. (PH) + *A. lancea*. The survival and average rhizome weight of *A. lancea* was higher in the ZM, CO, and TE treatments than in the monoculture treatment, and the average plant height was higher in all intercropping treatments than in the monoculture. The volatile oil content of *A. lancea* from the ZM and CO treatments was significantly improved relative to that of monoculture plants. The volatile oil harvest was higher in the ZM, CO, and TE treatments than in the monoculture. We conclude that intercropping is an effective way to increase the survival and yield of *A. lancea*. Furthermore, intercropping with ZM, CO, and TE increases the harvest of four volatile oils from *A. lancea*.

Keywords: *Atractylodes lancea* (Thunb.) DC., intercropping, survival, production, volatile oil

INTRODUCTION

The rhizomes of *Atractylodes lancea* (Thunb.) DC. (Chinese: Cangzhu) are commonly used in traditional Chinese medicine as a remedy for rheumatic diseases, digestive disorders, night blindness, and influenza (Oseko et al., 2005). In the past few decades, the demand for *A. lancea* has been increasing, as the use of its active compounds in the pharmaceutical industry has grown substantially. During the 2020 novel coronavirus pneumonia outbreak, *A. lancea* was one of the

main traditional Chinese medicinal materials used for the prevention of COVID-19 infection (Yang et al., 2020; Zhao et al., 2020). *A. lancea* is a perennial plant that is typically harvested from the field after 2–3 years of cultivation. Because wild *A. lancea* resources are increasingly endangered, the market depends heavily on artificial cultivation. However, industrial *A. lancea* monoculture systems face great hazards associated with continuous cropping, including the suppression of soil fertility, reduced productivity, and increased pest and disease damage. The disease incidence rate on *A. lancea*, especially that of root rot disease, can reach up to 80%, causing serious reductions in growth and productivity (Wang et al., 2016). Guo et al. (2005) have previously reported that autotoxicity may be another negative effect of the continuous cropping of single cultivars.

Compared with the planting of single cultivars, intercropping has significant agro-ecological advantages (Power, 1989; Khan et al., 1997; Brooker et al., 2015). The disease problems associated with continuously cropping patchouli can be ameliorated by intercropping with turmeric and ginger (Zeng et al., 2020). Maize/soybean intercropping suppressed the occurrence of soybean red crown rot (Gao et al., 2014), and maize/pepper intercropping can reduce disease levels of soil-borne *Phytophthora* on pepper (Yang et al., 2015). Intercropping Chinese chive cultivars with banana can reduce the incidence of Panama disease (Li Z. et al., 2020). Traditional intercropping usually aims at the improvement of crop yields (Li C. et al., 2020), which consist mainly of primary metabolites. By contrast, the aim of medicinal plant cultivation is usually the production of more secondary metabolites. Previous research has reported that intercropping may lead to changes in plant accumulation of secondary metabolites (Maffei and Mucciarelli, 2003; Ngwene et al., 2017; Zeng et al., 2020).

In this study, we carried out two years of field experimentation to determine the effects of intercropping on *A. lancea*. The objectives of the study were: (i) to compare important growth indicators in different intercropping systems; (ii) to compare plant yield and the accumulation of secondary metabolites in different intercropping systems; and (iii) to investigate the different accumulation patterns of major active components under five intercropping systems.

MATERIALS AND METHODS

Experimental Site

Field experiments were conducted on newly developed terraces in Huadun village, Laibang Town, Yuexi County, Anhui Province (30°56'7.15"N, 116°1'40.43"E, altitude 620 m) in 2015 and 2016. This site is located in the north subtropical humid monsoon climate area, and its frost-free period is 220 days. The mean annual temperature is 17°C, the mean annual ground temperature is 17°C, the average annual precipitation is 2434.6 mm, and the average sunshine duration is 2070.5 h. Meteorological data from 2015 and 2016 were collected by automatic weather stations near the test site.

Experiment Design and Field Management

In this study, intercropping partners were selected on the basis of their functions. Selected species are all common native and agricultural species. The gramineous roots of *Zea mays* L. (ZM) can activate soil microbial flora (Abbott and Robson, 1991), and its aboveground parts are tall and dense, providing a degree of shade. Both marigold and calendula are from the *Asteraceae* family, and their above- and belowground parts contain volatile oils that provide resistance to pests and diseases (Mansoor and Mashkoor, 1988; Natarajan et al., 2006). It has been shown that *Calendula officinalis* L. (CO) can be used for pest control, and the calendula oil contained therein can be used as a repellent to prevent egg laying by flies (Pudasaini et al., 2008; Riaz et al., 2009). Extracts of *Tagetes erecta* L. (TE), leaves, and roots are toxic to the nematode that is closely associated with root rot (Wang et al., 2003; Hooks et al., 2010). The *Glycine max* (Linn.) Merr. (GM) root system harbors nitrogen-fixing rhizobia and has the effect of enhancing soil fertility. Finally, *Polygonum hydropiper* L. (PH) is the natural companion species of *A. lancea*.

Seedlings of *A. lancea* were derived from *A. lancea* rhizomes growing in Huoshan, Anhui Province, and seedlings of similar size were used for the experiment. Seeds of maize, soybean, marigold, calendula, and *P. hydropiper* were those of commercial cultivars.

Five intercropping treatments and an *A. lancea* monoculture treatment were used in the experiment: *A. lancea* alone, *A. lancea* + *Zea mays* L. (ZM), *A. lancea* + *Glycine max* (Linn.) Merr. (GM), *A. lancea* + *Tagetes erecta* L. (TE), *A. lancea* + *Calendula officinalis* L. (CO), and *A. lancea* + *Polygonum hydropiper* L. (PH). The row spacing between *A. lancea* and *A. lancea* is 20 × 30 cm, and the row spacing between *A. lancea* and partner plants is 20 × 30 cm, and the spacing between partner plants and partner plants is also 20 × 30 cm. The experiment used a randomized complete block design with four replications, and each experimental plot was 10 m² (2 m × 5 m). Therefore, there are 187 *A. lancea* in AL treatment and 99 *A. lancea* in intercropping treatment.

All plants were planted by hand. *A. lancea* was only planted in December 2014 and has been grown in the field for two years. And the partner plants were planted for the first time in April 2015 and the second planted in April 2016. *A. lancea* was planted by rhizome propagation after sterilization (soaking for 30 min at room temperature in 50% carbendazim diluted 800–1000 times), and rhizomes were buried 1–2 cm underground. Intercropping plants were grown from seed sown 1–2 cm deep. The plantings were weeded in March, June, and November of each year, and no pesticides or fertilizers were used throughout the experimental period.

Measurement Parameters and Methods

Plant Biomass and Yield

At the end of November 2015 and 2016, ten *A. lancea* were selected from each experimental plot for biomass and yield

analysis, including both their above- and belowground parts. Measurements included plant height, number of branches, number of apical and lateral buds on the rhizome, and rhizome fresh weight.

Collection and Analysis of Volatiles

Atractylodes lancea rhizomes were collected and dried in a 40°C oven for one week to constant weight, then crushed to <0.3 mm. A 500-mg sample of the resulting powder was placed into a 50-ml centrifuge tube, 25 ml of n-hexane was added, and the mixture was first shaken (250 min⁻¹, 15 min) and then centrifuged at 3000 rpm for 10 min. The supernatant was removed for subsequent use, 20 ml of n-hexane was added, and the above process was repeated. Both supernatants were combined in a volumetric flask, 1.0 ml of the internal standard solution was added, the sample was diluted to 50 ml, and a 1-ml sample was injected into the gas chromatograph/mass spectrometer (GC/MS) (Trace 1310 gas chromatograph and TSQ 8000 mass spectrometer).

The contents of four volatile active ingredients, atractylon, hinesol, β -camphor (β -eudesmol), and atractylodin, were measured using the method of Li (2018) with an Agilent DB-5ms series column (0.25 mm id $\times$ 30 m, 0.25 μ m). The carrier gas was helium (flow rate: 1 ml/min), the injection mode was split (proportion 50:1), the injection volume was 1 ml, and the inlet temperature was 240°C. The column temperature was 120°C for the first 2 min; then the temperature was programmed to rise to 240°C at 5°C/min and was maintained at 240°C for 5 min. The MSD ionization mode had the following parameters: ionization voltage (EI) 70 V, ion source 230°C, and quadrupole 150°C. The MSD data acquisition mode was scanning (40–500 AMU). As shown in Figure 1, the method is accurate, fast, and reproducible.

Data Analysis

Tukey's HSD test was used to test for differences among intercropping treatments. Pearson's correlation coefficient was used to analyze the correlations among *A. lancea* growth and biochemical indices under different intercropping treatments. Statistics and correlation analysis were performed using SPSS v16.0 (SPSS Inc., Chicago, United States) and Microsoft Excel 2003.

RESULTS

Effects of Different Intercropping Treatments on the Survival of *A. lancea*

The survival percentage of *A. lancea* from different treatments was investigated two years after planting (Figure 2). Plant survival was significantly higher in the TE treatment ($69 \pm 4.2\%$) than in the monoculture ($52 \pm 8.3\%$), followed by the CO ($60 \pm 7.2\%$) and ZM ($59 \pm 1.6\%$) treatments. By contrast, the survival of plants in the PH ($49 \pm 5.7\%$) and GM ($37 \pm 1.8\%$) treatments was significantly lower than that of monoculture plants.

Effects of Different Intercropping Treatments on Growth and Yield of *A. lancea*

In 2015, the average plant height was significantly greater in the ZM and TE treatments (both ~ 34 cm) than in the monoculture (26 ± 1.6 cm) (Figure 3). Likewise, the average rhizome weight was significantly greater in the ZM and TE treatments (109 ± 8.8 g and 96 ± 11.3 g, respectively) than in the monoculture (72 ± 7.5 g) (Figure 3). In 2016, the average plant height was significantly greater in all the intercropping treatments than in the monoculture (Figure 3). Similarly, the average rhizome weights in the ZM, TE, and CO treatments were 141 ± 13.0 g, 150 ± 10.9 g, and 161 ± 19.2 g, all significantly greater than that in the monoculture (90 ± 8.7 g) (Figure 3).

Effects of Different Intercropping Treatments on the Concentrations of Volatile Oils in *A. lancea*

The concentrations of the four main volatile oils in *A. lancea* were analyzed in 2015 and 2016 (Figure 4). The concentration is the ratio of the mass of volatile oil to the mass of *A. lancea* rhizome. In 2015, the atractylon concentration of *A. lancea* was lowest in the GM treatment, the hinesol concentration of *A. lancea* was lowest in the TE treatment, and the atractylodin concentration of *A. lancea* was lowest in the CO treatment and the monoculture. In 2016, the hinesol and β -eudesmol concentrations of *A. lancea* were significantly lower in the TE treatment than in any other treatment. There were no other significant differences in concentrations of the four volatile oils among the treatments in 2015 and 2016.

The concentrations of the four volatile oils increased significantly from 2015 to 2016 in a few treatments. The hinesol concentration of *A. lancea* in the monoculture, the β -eudesmol concentration of *A. lancea* in the CO and PH treatments, and the atractylodin concentration of *A. lancea* in the CO treatment all increased significantly from 2015 to 2016. However, the hinesol concentration of *A. lancea* decreased from 2015 to 2016 in the GM treatment.

Effects of Different Intercropping Treatments on Volatile Oil Content of *A. lancea*

The content indicates the quality of volatile oil from *A. lancea*. Based on the analysis of the average rhizome weight and the concentrations of the four main volatile oils in *A. lancea*, the content of the four volatile oils was calculated for individual plants. In 2015, the atractylon content of *A. lancea* was significantly higher in the CO treatment than in the GM, TE, and PH treatments. The hinesol content of *A. lancea* was significantly higher in the ZM and GM treatments than in other treatments. The β -eudesmol content was significantly higher in the ZM treatment than in the TE and PH treatments, and the atractylodin content was significantly higher in the ZM treatment than in the other treatments. In 2016, the atractylon content was significantly higher in the TE, ZM, and CO treatments than in the other

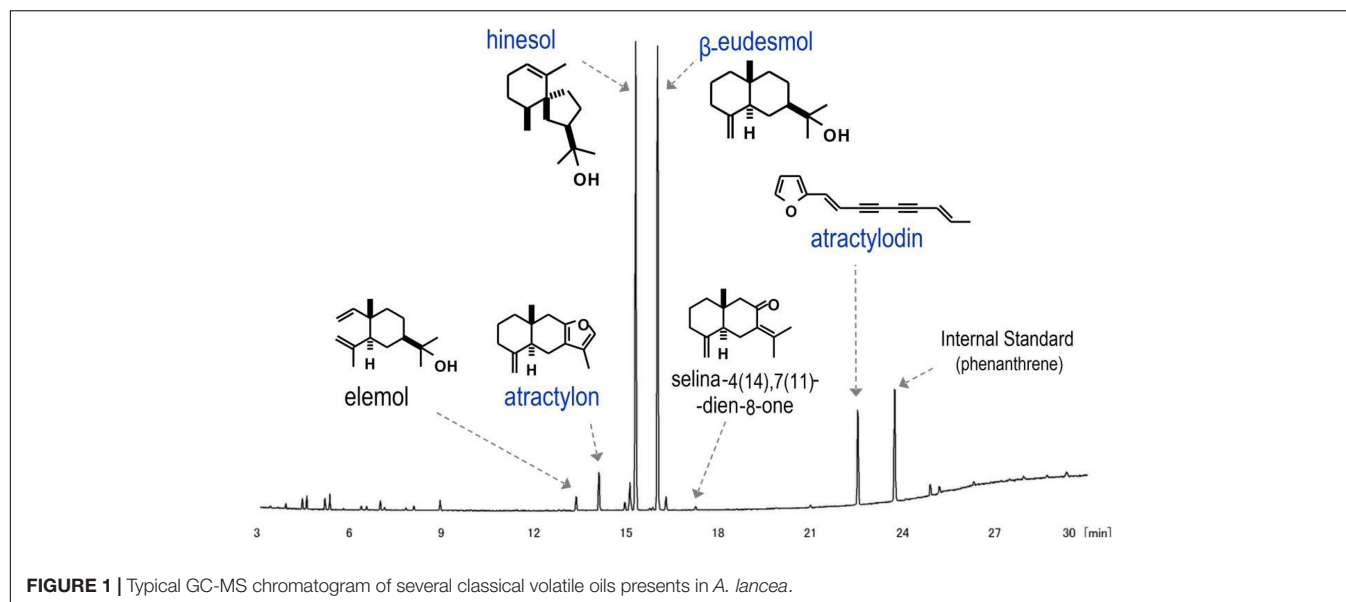


FIGURE 1 | Typical GC-MS chromatogram of several classical volatile oils presents in *A. lancea*.

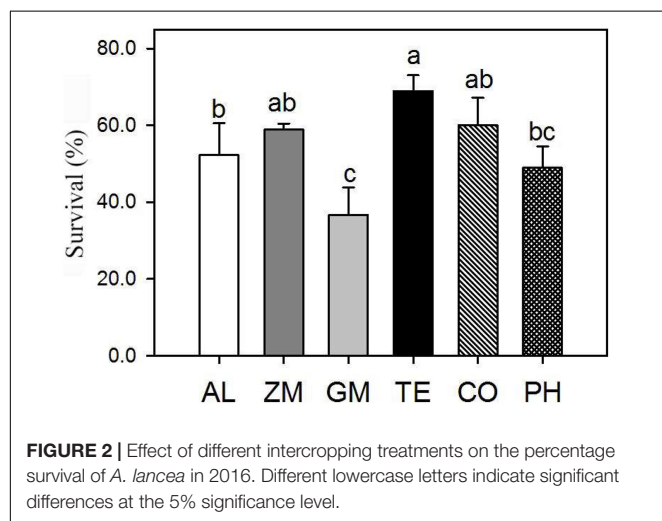


FIGURE 2 | Effect of different intercropping treatments on the percentage survival of *A. lancea* in 2016. Different lowercase letters indicate significant differences at the 5% significance level.

Effects of Different Intercropping Treatments on the Accumulation of Volatile Oils in *A. lancea*

The proportion of different volatile oils is an important characteristic of Daodi herbs (Guo et al., 2005). The proportion of individual volatile oil concentrations to the total concentration of all four volatile oils changed markedly from 2015 to 2016 in all treatments (Figure 6, Appendix A.). The total volatile oil concentration increased significantly from 2015 to 2016 in only the AL and PH treatments. The relative concentration of atractylon was higher in 2015 than in 2016 in the AL and CO treatments; the opposite pattern was found in other treatments, particularly for GM, in which the atractylon concentration was significantly higher in 2016. The relative concentration of hinesol was significantly higher in 2015 than in 2016 for the ZM, GM, and TE treatments, and this difference was significant for GM. With the exception of the PH treatment, the relative concentration of β-eudesmol was higher in 2016 than in 2015 for all treatments. The relative concentration of atractylodin did not change from 2015 to 2016 in the GM and TE treatments, whereas its relative concentration was lower in 2016 than in 2015 for the other treatments.

As biomass increased through time, the total volatile oil content increased in all treatments (Figure 6(II)) and increased significantly in the AL, ZM, TE, CO, and PH treatments. Total volatile oil content was highest in the CO treatment, followed by the ZM treatment; it was lowest in the GM treatment. Although the increase in content of individual volatile oils was slight in some treatments, the contents of all four individual volatile oils increased with time in all treatments (with the exception of hinesol in the GM treatment). Contents of individual volatile oils also differed among the treatments. For example, the AL and CO treatments showed clear increases in contents of all four volatile oils, whereas the ZM treatment showed increases primarily in atractylon and β-eudesmol content. The TE treatment showed

treatments. The hinesol content was significantly higher in the CO treatment than in the monoculture, and the hinesol content was significantly lower in the GM and TE treatments than in the monoculture. The β-eudesmol content was significantly higher in the ZM and CO treatments than in the monoculture, and the β-eudesmol content was significantly lower in the GM and PH treatments than in the monoculture. The atractylodin content was significantly higher in the CO, ZM, and TE treatments than in the monoculture.

The contents of individual volatile oils increased significantly from 2015 to 2016 in most of the intercropping systems (Figure 5). The atractylon content was significantly higher in 2016 than in 2015 in the TE, ZM, CO, and PH treatments. Likewise, the contents of hinesol (except in the monoculture), β-eudesmol, and atractylodin (except in the ZM treatment) were significantly higher in all treatments in 2016 than in 2015.

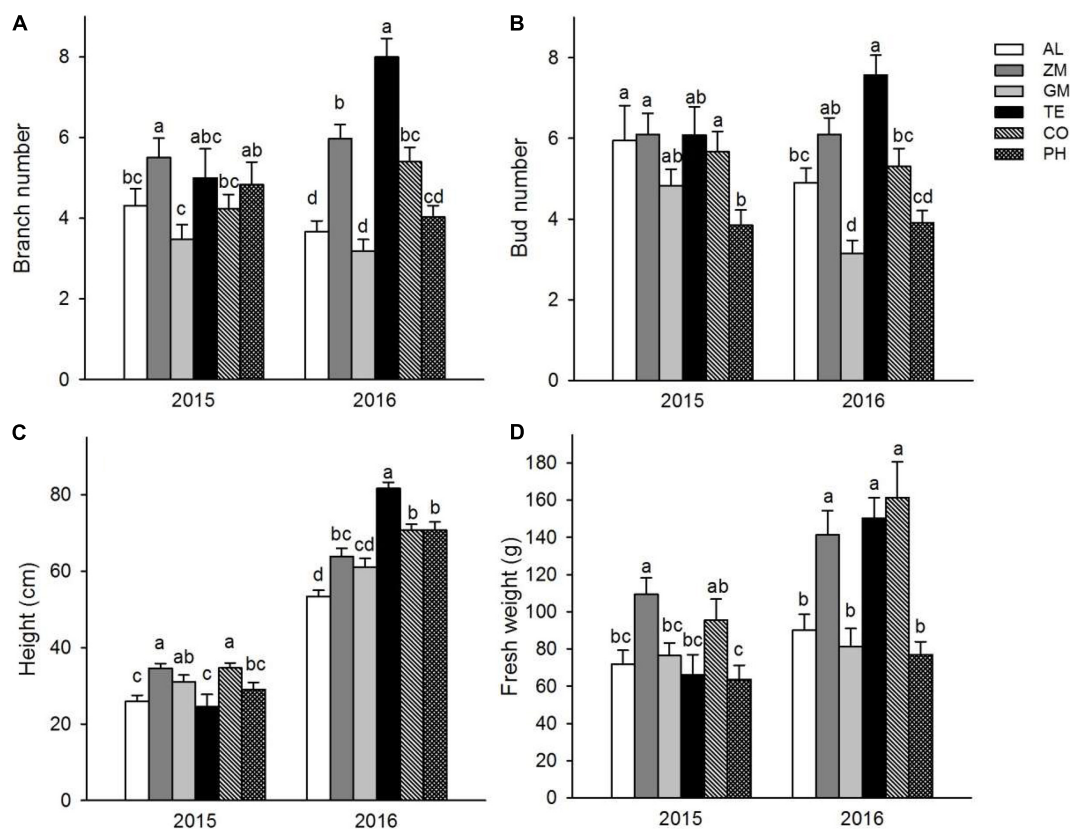


FIGURE 3 | Effect of different intercropping treatments on branch number (A), bud number (B), height (C), and fresh weight (D) of *A. lancea* in 2015 and 2016. Different lowercase letters indicate significant differences at the 5% significance level.

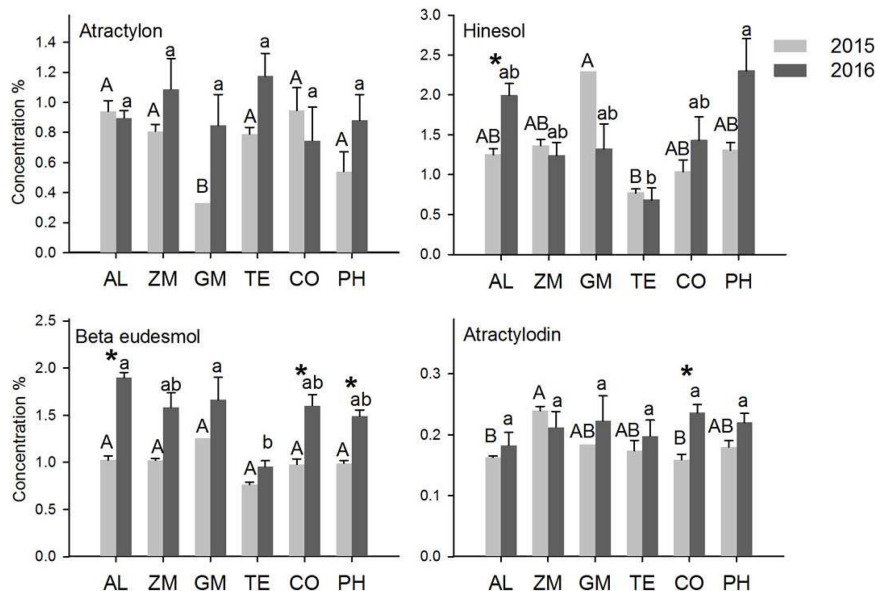


FIGURE 4 | The effect of different intercropping treatments on the concentrations of four volatile oils from *A. lancea* in 2015 and 2016. Different capital (2015) and lowercase (2016) letters indicate significant differences at the 5% significance level, and asterisks indicate significant differences between 2015 and 2016 within individual treatments.

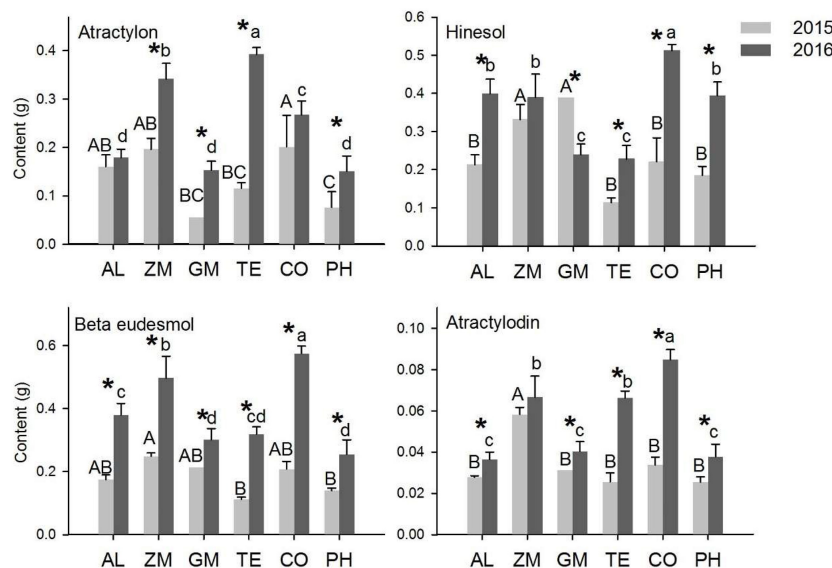


FIGURE 5 | The effect of different intercropping treatments on the contents of four volatile oils in 2015 and 2016. Different capital (2015) and lowercase (2016) letters indicate a significant difference at the 5% significance level, and asterisks indicate significant differences between 2015 and 2016 within individual treatments.

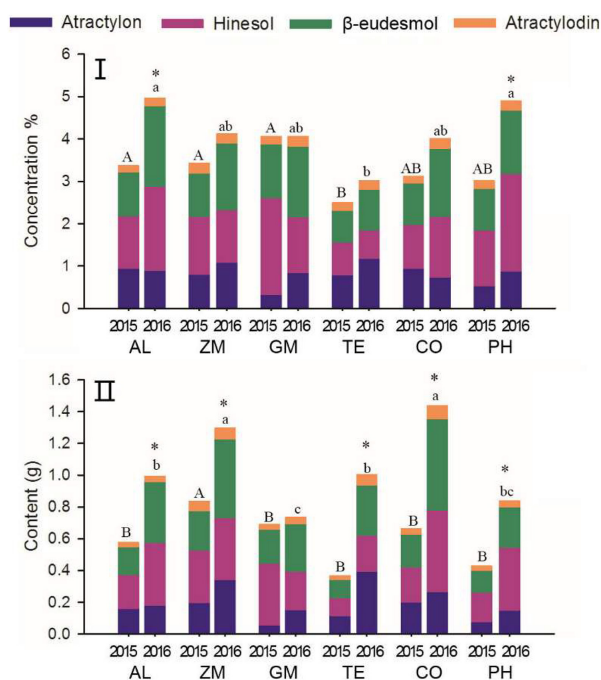


FIGURE 6 | The effect of different intercropping treatments on concentration (I) and content (II) of total volatile oils in 2015 and 2016. Different capital (2015) and lowercase (2016) letters indicate significant differences at the 5% significance level, and asterisks indicate significant differences between 2015 and 2016 within individual treatments.

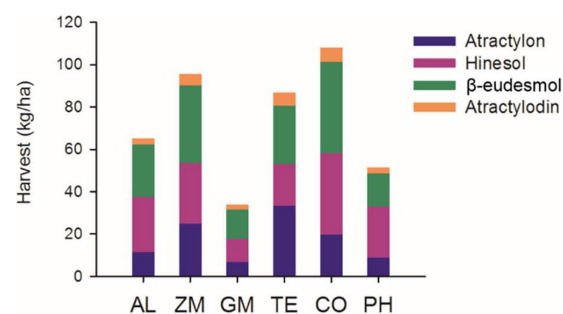


FIGURE 7 | The harvest of total volatile oils from different intercropping treatments in 2016.

Based on plant survival, rhizome weight, and the number of plants per hectare, we calculated the total harvest of the four volatile oils in 2016 (Figure 7). The total volatile oil harvest was higher in the CO, ZM, and TE treatments than in the AL monoculture, whereas that of other treatments was lower than in the AL monoculture.

DISCUSSION

Improved Biomass of *A. lancea* Under Intercropping

Complementary patterns of root distribution and plant phenology are important mechanisms by which intercropping improves yield (Keating and Carberry, 1993; Martin-Guay et al., 2018). Intercropping a deeply rooted plant with a more shallowly rooted plant efficiently utilizes belowground space and reduces root competition (Li et al., 2001; Chapagain et al., 2018;

increases mainly in atractylon and β -eudesmol content, and the PH treatment showed increases mainly in atractylon, β -eudesmol and hinesol content.

Zhang et al., 2020). Phenological complementation of different species can also reduce nutrient competition and increase resource use efficiency. In this study, ZM, TE, and CO treatments markedly improved biomass and the accumulation of four volatile oils in *A. lancea*. In the ZM treatment, *A. lancea* has a shallow root system, whereas ZM has a deep root system. In addition, the phenological phases of ZM, TE, and CO differ from those of *A. lancea*, thereby potentially providing suitable environmental conditions compared with the monoculture.

Effect of Root Exudates on Survival of *A. lancea*

Root exudates play an important role in plant health (Bais et al., 2006; Sasse et al., 2018; Olanrewaju et al., 2019). In the second year of intercropping, plant survival was higher in the ZM, TE, and CO treatments than in the other treatments (Figure 2), perhaps related to the growth-promoting effect of their root exudates. For example, maize root exudates can enrich plant growth-promoting rhizobacteria and enhance the metabolic capacity of soil bacteria (Baudoin et al., 2002; Benizri et al., 2002; Mendes et al., 2013; Vejan et al., 2016). Moreover, studies have confirmed that the root exudates of CO can inhibit the occurrence of pests and diseases, and it is widely used for this purpose in the field (Ploeg, 2000). *Tagetes erecta* L. has a similar inhibitory effect on root-knot nematodes (Steiner, 1941) and can effectively reduce the density of harmful nematodes (Akhtar and Mashkooor Alam, 1992; Reynolds et al., 2000).

Effects of Shading on the Accumulation of Four Volatile Oils in *A. lancea*

The accumulation of secondary metabolites is an important index for the evaluation of medicinal materials (Zofou et al., 2013; Song et al., 2014). Secondary metabolites are small molecular organic substances that can assist plants in adapting to the external environment (Kong et al., 2016). In our previous experiments, we found that shading increased the biomass and the content of four volatile oils in *A. lancea* in the short term (Li, 2018). We speculate that the shading effect of the maize plant is responsible for the increased volatile oil content of *A. lancea*. Maize was the tallest plant in this study, and the first year's results showed that the atractylodin content was significantly higher in the ZM treatment than in the monoculture. Likewise, in 2016, the total volatile oil harvest of *A. lancea* was significantly higher in the ZM treatment

than in the monoculture and was the second highest among all the treatments.

CONCLUSION

Five plant species were chosen as intercropping partners for *A. lancea*, and the growth traits, survival, and volatile oil production of *A. lancea* were analyzed to evaluate each intercropping combination. Compared with the monoculture, intercropping with ZM, CO, and TE significantly increased the survival and rhizome weight of *A. lancea*. Two years after planting, *A. lancea* intercropped with ZM, TE, and CO showed a great advantage in total volatile oil harvest. The underlying mechanisms of plant interaction in these systems remain to be explored in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

ZP: writing – original draft and data curation. YZ: investigation, methodology, project administration, and funding acquisition. BY, YX, XG, ZZ, XLC, XpC, and XW: data curation. TW, SW, and CK: data curation and methodology. LH: conceptualization and supervision. LG: investigation, conceptualization, supervision, project administration, and funding acquisition. KS: sample collection, conceptualization, methodology, data curation, and writing – review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX A

The relative proportion of individual volatile oils to all four volatile oils in *A. lancea* in 2015 and 2016.

Treatment	Atractylon (%)		Hinesol (%)		β-eudesmol (%)		Atractylodin (%)	
	2015	2016	2015	2016	2015	2016	2015	2016
AL	27.87	18.02	36.99	40.11	30.32	38.19	4.83	3.67
ZM	23.54	26.39	39.71	30.07	29.76	38.40	6.98	5.15
GM	8.11	20.89	56.36	32.58	30.99	41.03	4.54	5.50
TE	31.59	39.05	30.92	22.66	30.53	31.71	6.96	6.58
CO	30.32	18.58	33.23	35.61	31.35	39.91	5.10	5.89
PH	17.90	18.01	43.33	47.02	32.81	30.47	5.96	4.50



Pea Breeding for Intercropping With Cereals: Variation for Competitive Ability and Associated Traits, and Assessment of Phenotypic and Genomic Selection Strategies

Paolo Annicchiarico*, Nelson Nazzicari, Tommaso Notario, Cristina Monterrubio Martin, Massimo Romani, Barbara Ferrari and Luciano Pecetti

Council for Agricultural Research and Economics (CREA), Research Center for Animal Production and Aquaculture, Lodi, Italy

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Aleksandar M. Mikich,
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Vaijayanthi PV,
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*Correspondence:

Paolo Annicchiarico
paolo.annicchiarico@crea.gov.it

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Annicchiarico P, Nazzicari N, Notario T, Monterrubio Martin C, Romani M, Ferrari B and Pecetti L (2021) Pea Breeding for Intercropping With Cereals: Variation for Competitive Ability and Associated Traits, and Assessment of Phenotypic and Genomic Selection Strategies. *Front. Plant Sci.* 12:731949. doi: 10.3389/fpls.2021.731949

Mixed stand (MS) cropping of pea with small-grain cereals can produce more productive and environment-friendly grain crops relative to pure stand (PS) crops but may require selection to alleviate the pea competitive disadvantage. This study aimed to assess the pea variation for competitive ability and its associated traits and the efficiency of four phenotypic or genomic selection strategies. A set of 138 semi-leafless, semi-dwarf pea lines belonging to six recombinant inbred line populations and six parent lines were genotyped using genotyping-by-sequencing and grown in PS and in MS simultaneously with one barley and one bread wheat cultivar in two autumn-sown trials in Northern Italy. Cereal companions were selected in a preliminary study that highlighted the paucity of cultivars with sufficient earliness for association. Pea was severely outcompeted in both years albeit with variation for pea proportion ranging from nearly complete suppression (<3%) to values approaching a balanced mixture. Greater pea proportion in MS was associated with greater total yield of the mixture ($r \geq 0.46$). The genetic correlation for pea yield across MS and PS conditions slightly exceeded 0.40 in both years. Later onset of flowering and taller plant height at flowering onset displayed a definite correlation with pea yield in MS ($r \geq 0.46$) but not in PS, whereas tolerance to ascochyta blight exhibited the opposite pattern. Comparisons of phenotypic selection strategies within or across populations based on predicted or actual yield gains for independent years indicated an efficiency of 52–64% for indirect selection based on pea yield in PS relative to pea yield selection in MS. The efficiency of an indirect selection index including onset of flowering, plant height, and grain yield in PS was comparable to that of pea yield selection in MS. A genome-wide association study based on 5,909 SNP markers revealed the substantial diversity of genomic areas associated with pea yield in MS and PS. Genomic selection for pea yield in MS displayed an efficiency close to that of phenotypic selection for pea yield in MS, and nearly two-fold greater efficiency when also taking into account its shorter selection cycle and smaller evaluation cost.

Keywords: genomics, GWAS, morphophysiological traits, phenology, *Pisum sativum*, plant competition dynamics, selection efficiency, selection index

INTRODUCTION

Intercropping, i.e., the simultaneous cultivation of two or more crop species in the same field, provides agronomic benefits that have long since been noticed (e.g., Darwin, 1859). This technique has largely been adopted in traditional subsistence agriculture (Vandermeer, 1989; Altieri, 2004), while remaining widespread in modern agriculture only for some perennial forages, e.g., white clover-grass mixtures (Haynes, 1980). However, the association of annual legumes with cereals may become a cornerstone of the necessary agroecological transition of modern agriculture, to exploit plant functional diversity for a sustainable intensification aimed to raise crop yields, yield stability, and/or crop quality while simultaneously enhancing ecosystem services and reducing adverse environmental impacts. Indeed, meta-analysis studies encompassing different interspecific mixtures and cropping regions indicated that intercropping, compared with the mean value of the sole crops of its component species, displayed an average yield advantage of 22–30% (Yu et al., 2015; Himmelstein et al., 2017; Martin-Guay et al., 2018) along with distinctly greater crop yield stability (Raseduzzaman and Jensen, 2017). The main reason for these advantages lies in more efficient utilization of light, water, or nutrients by a complementary plant foraging pattern that implies lower interspecific competition than intraspecific competition (Lithourgidis et al., 2011; Brooker et al., 2015). The intercropping of grain legumes with cereals, which accounts for the large majority of the scientific reports for annual crops (Yu et al., 2015; Raseduzzaman and Jensen, 2017), can exploit species complementarity effects for nitrogen use (atmospheric N₂ for legumes and soil N for cereals) (Schmidtke et al., 2004; Bedoussac et al., 2015; Rodriguez et al., 2020) that allow to reduce crop N fertilization and, thereby, greenhouse gas emissions, energy consumption, and N leaching into fresh water (Jensen et al., 2020). While increasing and stabilizing crop yields in both high- and low-input systems (Li et al., 2020), these mixtures under low soil N availability (as it may be in organic systems) also lead to greater cereal protein content (Gooding et al., 2007; Bedoussac and Justes, 2010). Additional advantages of grain legume-cereal intercrops relative to sole crops may include the reduction of pests and diseases caused by dilution of the host density (Boudreau, 2013), better control of weeds (Liebman and Dyck, 1993; Corre-Hellou et al., 2011), and the ability of some species to chemically mobilize and make available for the companion species one or more limiting soil nutrients such as phosphorus, iron, zinc, or manganese (Zhang and Li, 2003; Li et al., 2014). The increasing awareness of all these advantages is leading to increasing intercropping of grain legume-cool season cereals in Europe, particularly in organic systems (Schneider et al., 2015).

While offering several opportunities, grain legume-cereal intercropping also poses various technical challenges that hinder its adoption by farmers, among which the development of suitable cultivars and better mechanical implementation stand out for importance (Martin-Guay et al., 2018). A balanced competition between component species is required for the display of agroecological benefits (Corre-Hellou et al., 2006) and, when relevant, the achievement of certain crop quality

characteristics (e.g., protein content). However, asymmetrical competition leading to a competitive advantage of cereals has frequently been reported as a consequence of weaker competitive ability by legumes. This emerged for pea in different mixtures and cropping regions (Jensen, 1996; Corre-Hellou et al., 2006; Lithourgidis et al., 2011; Annicchiarico et al., 2017), with the exception of one experiment whose management (adoption of a relatively weak competitor such as barley associated with lack of N fertilization) limited the cereal aggressiveness (Hauggaard-Nielsen and Jensen, 2001). Competitive disadvantage was also reported for other cool-season annual legumes associated with small-grain cereals, such as lentil (Schmidtke et al., 2004), white lupin (Mariotti et al., 2009) and vetches (Annicchiarico et al., 2017), and warm-season legumes such as common bean, cowpea, soybean, pigeonpea, or groundnut intercropped with maize or sorghum (Ofori and Stern, 1987; Santalla et al., 2001; Boukar et al., 2015).

The size of the genetic correlation between pure stand (PS) and mixed stand (MS) conditions for performance of a reasonably large genotype sample of a focus species describes synthetically the intrinsic consistency of genotype response across growing conditions and contributes crucially to assess the predicted efficiency of different phenotypic selection strategies aimed to improve the species performance in MS (Annicchiarico et al., 2019a). These strategies may encompass direct selection for yield in MS, indirect selection in PS for yield (which implies lower cost than MS because there is no need for separation or proportion assessment of the focus species), and indirect selection in PS based on morphophysiological traits associated with yield and competitive ability in MS (Annicchiarico et al., 2019a). By the third strategy, traits that are not highly correlated to each other and that feature high correlation with performance in MS, low evaluation cost, and moderately high broad-sense heritability and repeatability across locations and/or cropping years are pooled into a selection index applied to material evaluated in PS (Annicchiarico, 2003). Breeding for intercropping was studied on large genotype numbers, and produced documented improvements, only for perennial legumes, especially white clover (e.g., Annicchiarico and Proietti, 2010). In contrast, investigations on grain legumes were usually based on small numbers of cultivars or breeding lines, thereby producing data that may support selection strategies by revealing genotype variation in competitive ability and different top-performing genotypes across PS and MS conditions [as in Baxevanos et al. (2017) for pea in MS with oat] but could hardly be used to compare breeding strategies in terms of selection efficiency. Likewise, traits associated with competitive ability, whose mechanisms may also contribute to complementarity of the associated species (Litrico and Violle, 2015), were poorly investigated in grain legumes (Annicchiarico et al., 2019a).

Breeding for intercropping is challenged by the commercial interest of selecting for a range of possible companion species and varieties in a cost-efficient manner. Results for perennial legumes indicated that general-compatibility effects (which express consistent yield responses across different associations) are definitely larger than specific-compatibility effects (which express association-specific yield responses) (Holland and Brummer,

1999; Maamouri et al., 2017), and that the latter effects are affected by the difference in competitive ability more than by the species of the associated partner (Annicchiarico and Piano, 1994). Because of that, selection in one MS condition in which the associated partner was represented by a few highly competing genotypes of different grass species sown together as a pooled tester in Annicchiarico (2003) proved to be a low-cost means to select white clover for general compatibility, as indicated by improvements of clover yield and competitive ability expressed consistently across a set of different species and varieties (Annicchiarico and Proietti, 2010). A recent study indicated that general-compatibility effects are much larger than specific-compatibility effects also for pea-barley associations (Haug et al., 2020). For breeding and cultivation of annual legumes to be intercropped for grain production, a further challenge is the identification of cereal companions whose maturity date is sufficiently close to that of the legume cultivar to be selected or grown.

Genomic selection was indicated as a priority theme for research aimed to define new breeding strategies for intercropping, because of the costs and complexity of phenotypic selection in MS conditions (Annicchiarico et al., 2019a). Genomic selection, which implies the construction of a statistical model based on phenotyping and genotyping data of a germplasm sample representative of the target genetic base and its subsequent application to predict breeding values of a large set of independent genotyped individuals (Heffner et al., 2009; Lorenz et al., 2011), aims to reduce selection costs by partly substituting for phenotypic selection. Its cost-efficient application to plant breeding has greatly been enhanced by recent sequencing techniques, such as genotyping-by-sequencing (GBS; Elshire et al., 2011), that allow large germplasm sets to be genotyped by thousands of single nucleotide polymorphism (SNP) markers at a relatively low cost. Pioneer studies for pea suggested greater genetic gain per unit time of genomic over phenotypic selection for improving grain yield under PS conditions in moisture-favorable (Annicchiarico et al., 2019b) and severely drought-prone target regions (Annicchiarico et al., 2020). Genomic selection out-performed phenotypic selection in breeding for intercropping in a study based on stochastic simulation data (Bančić et al., 2021), but no experimental assessment of the value of genomic selection for intercropping is available.

This study focused on 144 pea inbred lines, of which 138 were randomly sorted out in equal proportions from six recombinant inbred line (RIL) populations issued by crosses between elite semi-dwarf, semi-leafless cultivars and six parent lines. This material was genotyped by GBS and was grown in PS and MS in Northern Italy in two cropping years. MS implied the simultaneous association of pea with one barley and one bread wheat cultivar selected by a prior phenology assessment study. The main objectives of this study were (a) to investigate the pattern and extent of pea genetic variation for competitive ability against cereals, (b) to assess the consistency of pea grain yield responses across MS and PS conditions according to estimates of genetic correlation and information on genomic regions associated with yield responses in a genome-wide association study (GWAS), (c) to identify traits associated

with pea competitive ability, and (d) to compare four selection strategies for pea performance in intercropping, namely, direct phenotypic selection for grain yield in MS, indirect phenotypic selection based on grain yield in PS, indirect selection based on an index of traits associated with pea competitive ability assessed in PS, and genomic selection for grain yield in MS, in terms of predicted or actual yield gains.

MATERIALS AND METHODS

Definition of Cereal Cultivars With Acceptable Maturity Date for Use as Testers

All experiments were carried out under field conditions in Lodi, Northern Italy (45°19' N, 9°30' E, 81 m a.s.l.), which features sub-continental climate and sandy-loam soils with pH around 6.5. Pea intercropping was foreseen with barley or triticale for feed production, and with bread or durum wheat mainly for food production. A preliminary experiment was set up to assess the heading and maturity dates and the plant height at heading of 14 cultivars of bread wheat, three of barley, two of triticale and one of durum wheat, in relation to onset of flowering and maturity dates and plant height at onset of flowering of a reference set of 14 pea genotypes that were concurrently evaluated (**Supplementary Table 1**). The set of bread wheat genotypes included nine recent commercial varieties grown in Italy, three breeding lines (A208, A210, and F426) chosen among the earliest-maturing ones bred by INRAE's UMR Génétique Quantitative et Évolution of Le Moulon (France), and the historical Italian cultivars San Pastore bred in 1929 (still adopted by Italian organic farmers) and Spada bred in 1985 because of their known outstanding earliness. The set of cereal material was completed by three elite modern varieties of barley, and recent commercial varieties of triticale or durum wheat that were described as very early. The pea genotypes included the commercial varieties Alliance, Attika, Dove, Guifilo, Isard, and Kaspera, which acted as parent lines of the six RIL populations that provided the genetic base for subsequent experiment work, and eight breeding lines that expressed the range of variation for phenology and plant height observed in the prior multi-locational testing by Annicchiarico et al. (2019b) of 306 lines issued by three connected crosses among the varieties Attika, Isard, and Kaspera. All pea genotypes were semi-dwarf, semi-leafless plant types.

The genotypes were evaluated as single rows 2 m long and 0.37 m apart, according to a group balanced block design (Gomez and Gomez, 1984) with three replications, of which cereal and pea material represented the groups. The sowing rate was 260 seeds/m² for bread wheat, 222 seeds/m² for durum wheat and triticale, 186 seeds/m² for barley, and 55 seeds/m² for pea. The experiment was sown at the end of October 2017. The total rainfall during the growing period (November–June) was 622 mm. The number of frost days was 57, with a minimum absolute temperature of −11.6°C.

The experimental data underwent an analysis of variance (ANOVA) holding the fixed factors group and genotype within group and the random factor block aimed to compare cereal

vs. pea germplasm groups, and separate ANOVAs aimed to assess the variation within cereal and pea germplasm groups. The results assisted the selection of the cereal cultivars used as testers in the following work.

Evaluation of Pea Inbred Lines in Pure Stand and Mixed Stand

A set of 144 semi-leafless, semi-dwarf inbred lines was evaluated under PS and MS in Lodi during the cropping seasons 2018–19 and 2019–20. The set included 23 lines randomly sorted from each of six RIL populations, and the six parent lines of the populations. The populations originated from the following crosses: (a) Attika × Isard, (b) Kaspas × Attika, (c) Kaspas × Isard, (d) Dove × Attika, (e) Attika × Guifilo, (f) Alliance × Isard. The parent lines were selected within a large number of international cultivars because of their high and stable grain yield and only moderate phenological differences across the environments of northern and southern Italy (Annicchiarico, 2005; Annicchiarico and Iannucci, 2008). The large use of Attika as a parent in these crosses was due to its high competitive ability against weeds under organic management (Annicchiarico and Filippi, 2007), which may relate to competitive ability under intercropping (Annicchiarico et al., 2019a).

The first cropping season adopted an early sowing (October 25), a cereal tester represented by the mixture of the barley cultivar Atlante with the tall early wheat cultivar San Pastore, and a pre-sowing fertilization of 50 kg/ha of N along with 75 kg/ha P₂O₅ and 100 kg/ha K₂O. In order to widen the environmental variation between test environments, the second cropping year adopted crop establishment conditions that were expected to be more favorable for pea growth in MS relative to the first year, namely, a late sowing (December 10), a cereal tester represented by the mixture of the barley cultivar Atlante with the short early wheat cultivar Spada, and a pre-sowing fertilization including 25 kg/ha of N along with 75 kg/ha P₂O₅ and 100 kg/ha K₂O. Each experiment was laid out as a split-plot with three replications holding growing condition (MS or PS) on main plots and pea lines on subplots. The seed rate of pea in MS was half of that adopted in PS (40 vs. 80 seeds/m²). The cereal seed rates in MS were 75 seeds/m² for barley and 100 seeds/m² for wheat, corresponding to 25% of the ordinary rate in the region for each species (which implied a halved seed rate for the whole of the cereal tester in MS relative to the ordinary rate in PS). MS plots were 2.0 m long and 1.36 m wide, and PS plots were 1.0 m long and 1.36 m wide, to keep constant the number of pea test seeds in each condition. All plots included 6 rows, blending pea and cereal seeds on each row in MS as done ordinarily by local farmers for pea-cereal intercrops. Seedbed preparation by plowing and harrowing was the same for MS and PS, whereas chemical weed control [Stomp® 330 E (a.i. Pendimethalin at 307 g/L) at 4.5 L/ha] was applied only to PS to limit the relatively large growth of weeds expected in this condition. The first cropping year, compared with the second year, featured greater winter cold stress (61 vs. 53 frost days; absolute minimum temperature of −12.0 vs. −10.9°C) and more rainfall, especially in late spring (April–May rainfall of 233 mm vs. 66 mm).

The following traits of pea lines were recorded on PS plots: (a) winter plant survival, based on plant counts at the onset and the end of winter; (b) onset of flowering, as number of days from April 1 to when 50% of plants in the plot had at least one fully open flower; (c) mean plant (canopy) height at onset of flowering; (d) susceptibility to the ascochyta blight disease complex (*Didymella* spp.), assessed in spring on a visual 9-level scale ranging from 1 (no damage) to 9 (plant mortality > 20%) (recorded in the first year, the only year that featured a sizeable disease incidence); (e) crop maturity (as the number of days from April 1); (f) plant height at crop maturity, measured on two random outstretched plants; (g) dry grain yield, after combine-harvesting of the plots at crop maturity (PS) and assessment of seed moisture on a random sample of 100 seeds oven-dried at 90°C for 4 days; (h) dry individual seed weight, assessed on the seed sample used for seed moisture determination. The traits recorded on MS plots included the dry grain yield of pea and of the pooled cereal tester, the total (pea + cereal) dry yield of the mixture and the proportion of pea dry yield on total yield, computed after harvesting the plot fresh seed and using a seed sample of 100 g for separation and dry weight assessment of the relative proportion of pea and cereal components. Onset of flowering, mean plant height at onset of flowering, and dry individual seed weight were recorded on MS plots only in the second year, to assess their consistency across MS and PS conditions in one test year. The ratio between pea yield in MS and pea yield in PS, defined for MS plots by imputing the mean yield in PS of each line, provided an additional variable aimed to highlight genotype × growing condition interaction responses leading to relatively better response in MS. Pea yield in MS was doubled prior to ratio computation, in order to express the ratio with respect to the same growing area (thus, assuming a halved area for pea in MS relative to pea in PS).

Statistical Analysis of Phenotypic Data of Pea Inbred Lines in Pure Stand and Mixed Stand

A preliminary analysis of variance (ANOVA) including the factors pea line and block was performed on data of separate growing conditions (PS or MS) and cropping years to verify the occurrence of genetic variation among lines for each trait. A second ANOVA including the factors pea line, growing condition, and block was performed on the data of separate cropping years according to the split-plot lay-out to verify the variation between conditions and the occurrence of genotype × condition interaction for traits recorded in both conditions. A third ANOVA including the factors pea line, cropping year, and block within year was performed separately for data recorded in PS or MS in both years to verify the variation between years and the occurrence of genotype × year interaction. This ANOVA was also applied to a composite trait represented by a selection index including traits observed in PS. One last ANOVA including the factors pea line, growing condition, cropping year, and block within year was performed on pea grain yield data mainly to verify first- and second-order interactions of the genotype factor (while testing the variation for condition and condition × year

interaction using condition $\times$ block within year as the error term). Variance components were estimated by a Restricted Maximum Likelihood (REML) method for the same ANOVA with respect to genotype (considered as a random factor) and its interactions with growing condition and year.

Relationships between traits were investigated by simple correlation analysis of genotype values. Statistical differences between correlation coefficients between PS and MS conditions were assessed by the u test described by Dagnelie (1975).

An index of indirect selection for pea yield in MS was defined from pea characters observed in PS, using line values previously averaged across cropping years. The weights of the variables in the index were estimated from partial regression coefficients as reported in Wricke and Weber (1986). The choice of the best index was based on the significance of partial regression coefficients within a stepwise multiple regression approach, allowing for the inclusion of an additional trait in the index when the trait featured $P < 0.05$ significance and increased the regression R^2 by at least 0.02.

Three pea selection strategies for pea yield in MS, namely, direct selection in MS, indirect selection in PS based on pea yield, and indirect selection in PS based on the selection index, were first compared according to predicted yield gains estimated separately from the data of each cropping year. Recalling that the genetic parameters for a selection index can be estimated in the same manner as those for individual traits (Lin, 1978), the relative efficiency E_R of indirect selection in PS vs. direct selection in MS, expressed in percentage, was estimated by the following equation (Falconer, 1989):

$$E_R = [(H_{PS}/H_{MS})r_g] \times 100$$

where H_{PS} and H_{MS} are the square root of the broad-sense heritability on a line mean basis (H^2) for the relevant selection criteria in PS and MS, respectively, and r_g is the genetic correlation between the two criteria. Heritability values were computed from genotypic (s_g^2) and experiment error (s_e^2) components of variance estimated by a REML method and n number of line replicates per condition by the equation: $H^2 = s_g^2 / (s_g^2 + s_e^2 / n)$. An approximate standard error was computed as reported in Uddin et al. (1994). The genetic correlation was estimated as described by Robertson (1959) for traits assessed in different experiment units. The consistency of pea line response across conditions as described by the genetic correlation was also estimated for the three morphophysiological traits of pea recorded in both conditions in the second cropping year.

The described comparison of selection criteria was limited to single-year results without taking into account the possible differences among criteria for the extent of genotype $\times$ year (GY) interaction. We verified the significance of this interaction for each selection criterion by ANOVA and assessed the extent of the interaction by the genetic correlation for pea line response across cropping years according to Itoh and Yamada (1990) for one trait assessed in different environments. Broad-sense heritability values taking also account of GY interaction were computed from genotypic (s_g^2), GY interaction (s_{gy}^2), and experiment error (s_e^2) components of variance estimated by a REML method, y

cropping years, and n number of line replicates per condition by the equation:

$$H^2 = s_g^2 / (s_g^2 + s_{gy}^2 / y + s_e^2 / y n).$$

A comparison of selection strategies based on predicted genetic gains that accounted for GY interaction effects was based on the view of each selection criterion in a given year (including that based on yield in MS) as an indirect selection criterion for the target trait represented by pea yield in MS in the other year. In this context, the size of the phenotypic correlation between pea line values for a given criterion in one year and pea line yields in MS in the other year is proportional to the expected genetic gain for the target trait provided by the relevant criterion (Cooper et al., 1996). We estimated phenotypic correlations using by turns one cropping year as the selection environment and the other year as the target environment, and expressed the relative efficiency E_R of selection in PS vs. selection in MS as a function of the average correlation across years for selection in PS based on the relevant criterion (r_{PS}) and selection based on pea yield in MS (r_{MS}) by the following equation:

$$E_R = (r_{PS}/r_{MS}) \times 100.$$

One last comparison of phenotypic selection strategies was based on actual yield gains when adopting one year for selection of two lines out of 23 for each of the 6 RIL populations and the other year for estimation of yield gains obtained by the selected material over the mean value of the six parent lines of the RIL populations, using by turns one year for selection and the other year for yield gain assessment. The relative efficiency E_R of selection in PS vs. selection in MS was estimated by the following equation:

$$E_R = (G_{SC}/G_{MS}) \times 100$$

where G_{SC} and G_{MS} are yield gains for the relevant criterion for PS selection and the selection based on yield in MS, respectively.

All analyses of phenotypic data were carried out using SAS/STAT[®] software (SAS Institute, 2011).

DNA Isolation, GBS Library Construction, Sequencing, and SNP Calling

Pea leaf green tissues for DNA extraction were collected, flash frozen in liquid nitrogen, and stored at -80°C before analyses. Genomic DNA was extracted from 6 bulked plants per genotype using the DNeasy Plant Mini Kit (Qiagen) and checked for integrity on 1% agarose gel. DNA quantitation was performed by means of the Quant-iTTM PicoGreen dsDNA assay kit (Life Technologies, P7589). The GBS data were generated by the Elshire Group Ltd. according to the protocol described by Elshire et al. (2011) with the following modifications: 100 ng of genomic DNA were used, 3.6 ng of total adapters were used, the genomic DNAs were restricted with *ApeKI* enzyme, and the library was amplified with 14 PCR cycles. Library sequencing was performed using the Illumina HiSeq X platform and paired-end runs (2×150 bp).

The SNP calling was performed using the dDocent pipeline (Puritz et al., 2014), aligning reads on the pea reference genome

(Kreplak et al., 2019) release v1a as downloaded from <https://urgi.versailles.inra.fr/download/pea/>. The resulting vcf file was filtered for quality using *vcftools* (Danecek et al., 2011) with options—remove-indels—minQ 30 —non-ref-af 0.001—max-non-ref-af 0.9999—max-missing 0.3. The resulting filtered file was transformed in a 012 SNP matrix and further filtered for minor allele frequency (MAF) >5% and several levels of maximum missing rate per marker (1, 3, 5, 10%) and per genotype (10, 25, 50%). Markers with heterozygosity ratio >95% were discarded as well. Missing data points in the resulting SNP matrices were imputed according to the k-nearest neighbors imputation (KNNI) method (Nazzicari et al., 2016).

Genome-Enabled Predictions and Comparison of Genomic vs. Phenotypic Selection Strategies

Genomic selection models were constructed from phenotypic data represented by best linear unbiased prediction (BLUP) values of pea grain yield in MS calculated as described in DeLacy et al. (1996). We considered various genomic regression models either capable of accepting SNP matrices as input, such as Ridge regression BLUP, Bayes A, Bayes C π and Bayesian Lasso, or requiring a kinship matrix, such as Genomic best linear unbiased prediction (G-BLUP) and Reproducing Kernel Hilbert Space (Lorenz et al., 2011; Wang et al., 2018). The kinship matrix was computed according to Astle and Balding (2009). No extra covariates were used. All regression models were implemented using the GROAN R package (Nazzicari and Biscarini, 2018).

Predictive ability was assessed as Pearson's correlation between observed and genomically predicted phenotypes according to inter-environment predictions based on model training in one test year and model validation in the other year. Inter-environment predictions were relative to three scenarios, namely, intra-RIL population predictions, inter-RIL population predictions, and predictions relative to the entire set of material (i.e., without distinction among populations). Intra-population inter-environment predictions were also used for a two-stage process of model tuning, in which the first stage aimed to select the thresholds of missing rate per marker and per genotype according to predictive ability values issued by Ridge regression BLUP, and the second aimed to select the statistical model on the ground of model predictive abilities for the selected configuration of marker and genotype missing rates. Intra-population predictions, and predictions for the whole genetic base, adopted a five-fold stratified cross validation scheme with modifications. In particular, model training was based on yield data of a random set of nearly 80% of the lines belonging to each of the six RIL populations (namely, 18 lines out of 23), using yield data in the other year of the remaining 20% of lines (5 lines) of each population for predictive ability assessment. The six parent lines were always added to the training set. This cross validation process was repeated 100 times by ensuring that each line from each population was included in the validation set a constant number of times, averaging the results across repetitions and repeating the whole analysis for each training year. This analysis was also used to assess actual yield gains derived from genomic

selection by selecting two top-yielding lines per population according to genome-enabled breeding values averaged across repetitions and assessing the gains as yield difference in the other test year of the selected material relative to the mean value of six parent lines, using by turns one year for selection and the other for yield gain assessment. The relative efficiency E_R of genomic selection was estimated from gains for the relevant genomic selection criterion G_{SC} and for phenotypic selection for yield in MS (G_{MS}) according to the following formula:

$$E_R = (G_{SC}/G_{MS}) \times 100.$$

Inter-population inter-environment predictions assumed model training based on data in one year of all lines of five non-target RIL populations and the set of parent lines, and model validation based on data in the other year of all lines of the target population. This assessment (which implied no need for cross validation) was repeated for each possible target population and training year.

Genome-Wide Association Study

A GWAS was carried out for pea yield in MS and in PS using line values averaged across the two cropping years. We used the same levels of filtering for the genotype matrix that optimized genome-enabled predictions. The association study was implemented using the statgenGWAS R package (van Rossum and Kruijer, 2020), including genomic control and the RIL population incidence matrix as a covariate. Significance level thresholds for multiple testing were established *via* Bonferroni method. Non-aligning markers were placed on a fictitious chromosome 99 for display purposes.

RESULTS

Definition of Cereal Cultivars With Acceptable Maturity Date for Use as Testers

On average, cereal material headed about 4 days earlier than pea mean onset of flowering, and exhibited nearly 14 dd later maturity and 17 cm taller plant stature than pea germplasm ($P < 0.01$; **Supplementary Table 1**). Barley tended to be earlier-maturing than the other cereal species, but all cereal genotypes displayed at least 4-day later maturity than the mean maturity of pea material (**Supplementary Table 1**). The earliest genotypes, namely the barley cultivar Atlante and the bread wheat cultivars Spada and San Pastore, were selected as testers, because their maturity time (albeit suboptimal) did not exceed one week relative to the pea mean maturity. Atlante featured fairly high plant stature (92 cm), Spada short stature (69 cm), and San Pastore tall stature (102 cm). As anticipated, the seed mixture of Atlante and San Pastore acted as cereal tester in the first cropping year, and that of Atlante and Spada (expected to exert somewhat lower competitive ability on pea) acted as cereal tester in the second year. Pea cultivar and breeding line groups displayed similar phenology, along with fairly large within-group variation for onset of flowering and plant height and modest variation for maturity date (**Supplementary Table 1**).

TABLE 1 | Mean and range values of pea traits in pure stand (PS) and pea and associated cereal traits in mixed stand (MS) for 144 pea inbred lines grown in two cropping years.

Trait ^a	Condition ^b	2018–19 ^c				2019–20 ^c			
		Mean ^d		Min.	Max.	Mean ^d		Min.	Max.
Pea grain yield (t/ha)	PS	6.223	a A	1.571	9.286	4.686	b A	2.282	6.904
Pea grain yield (t/ha)	MS	1.000	a B	0.174	2.126	1.071	a B	0.092	2.688
Associated cereal grain yield (t/ha)	MS	5.494	a	4.103	6.584	3.719	b	2.436	5.523
Total (pea + cereal) yield (t/ha)	MS	6.494	a	5.378	7.580	4.790	b	3.158	6.970
Pea proportion	MS	0.152	a	0.029	0.297	0.214	a	0.029	0.458
Pea MS/PS grain yield ratio	MS	0.345	a	0.058	0.902	0.478	a	0.042	1.224
Pea onset of flowering (dd from Apr 1)	PS	12.7	b	1.0	24.7	27.0	a A	23.0	31.0
Pea onset of flowering (dd from Apr 1)	MS	–		–	–	27.8	A	24.3	31.0
Pea plant height at onset of flowering (cm)	PS	62.0	a	27.7	99.0	46.1	b A	32.6	60.0
Pea plant height at onset of flowering (cm)	MS	–		–	–	50.1	A	33.3	68.3
Pea individual seed weight (g)	PS	0.146	b	0.101	0.213	0.198	a A	0.148	0.292
Pea individual seed weight (g)	MS	–		–	–	0.194	A	0.137	0.276
Pea maturity date (dd from Apr 1)	PS	64.5	a	59.0	68.5	61.9	b	59.0	65.1
Pea plant height at maturity (cm)	PS	120.4	a	77.5	162.3	54.8	b	34.3	79.6
Pea winter plant survival (proportion)	PS	0.978		0.849	1.000	–		–	–
Pea susceptibility to ascochyta blight (scale 1–9)	PS	4.1		3.0	5.3	–		–	–

^a Line variation within experiment and growing condition always significant at $P < 0.01$, except for associated cereal yield in 2019–20 significant at $P < 0.05$.

^b Cereal tester in MS formed by mixing one barley cultivar and one bread wheat cultivar.

^c Sowing time, cereal companions and pre-sowing N fertilization expected to be more favorable for pea in MS in 2019–20 relative to 2018–19.

^d Means followed by different lower-case letter differ between cropping years in the same growing condition at $P < 0.05$; means followed by different capital letter differ between growing conditions in the same cropping year at $P < 0.05$.

Phenotypic Variation in Mixed Stand and Pure Stand and Comparison of Phenotypic Selection Strategies

The first cropping year, featuring earlier sowing and wetter spring, had over 30% greater mean yield of pea in PS and mean total (pea + cereal) yield in MS relative to the second year (Table 1). This result was associated with a prolonged reproductive stage of the crops favored by moisture-favorable conditions, as indicated by pea in PS showing slightly later mean crop maturity along with much earlier mean onset of flowering in the first year compared with the second year (Table 1). On average, the total yield of the mixed crop was about 4% higher in the first year and 2% higher in the second year relative to pea yield in PS (Table 1). On average, pea was at severe competitive disadvantage with associated cereals in both years, although the disadvantage was greater in the first year than in the second one (0.152 vs. 0.214 mean pea proportion on total grain yield) as expected from its less favorable conditions for pea growth in MS (as determined by earlier sowing, taller wheat companion, and higher N fertilization). Severe mean depression of pea yield in MS relative to PS was highlighted by the MS to PS ratio of pea yield per unit area, which fell below 0.5 in both years (Table 1) (while equalling unity in the case of no yield depression).

Pea line variation within the cropping year and growing condition was significant at $P < 0.01$ for all traits except associated cereal yield in MS in 2019–20, which achieved $P < 0.05$ significance, and pea winter plant survival and susceptibility to ascochyta blight in the second year, in which the absence

of pea line variation was associated with climatic conditions that did not favor the occurrence of winter plant mortality and foliar diseases. The range of genotype variation for winter mortality in the first year was modest albeit significant (Table 1). In contrast, large variation was observed in both years for most traits recorded in PS or MS, including pea and total yield in MS, pea competitive ability as expressed by pea proportion in MS, and the pea yield ratio between MS and PS (Table 1). The variation for pea proportion ranged from pea lines that were nearly suppressed (values $< 3\%$) to lines competitive enough to approach a balanced mixture (values close to 30% in the first year and 45% in the second year: Table 1). The poorest-competing pea material exhibited about twenty-fold reduction of grain yield per unit area in MS relative to PS (as indicated by ratio values close to 0.05), whereas the best-competing material suffered a modest or nil yield reduction in MS (ratio values close to unity) (Table 1).

The variation for pea proportion in MS was nearly coincident with that for pea yield in MS, based on the correlation close to unity of these traits (Table 2). This finding reinforced the choice of pea yield in MS as the focus trait for pea selection targeted to intercropping. The correlation of the pea MS/PS grain yield ratio with pea yield and pea proportion in MS was high although not close to unity (Table 2), as the ratio expressed genotype $\times$ growing condition interaction effects while the other two traits expressed performance in MS as derived from the combination of positive genotype $\times$ growing condition interaction effects and intrinsic yielding ability as displayed in PS. Importantly, greater pea proportion in MS was correlated with greater total yield of the mixture ($r \geq 0.46$; Table 2), revealing that greater pea yield

TABLE 2 | Phenotypic correlation of pea grain yield or pea proportion in mixed stand with cereals (MS) with pea or cereal yield traits in MS or pea yield in pure stand (PS), for 144 pea inbred lines grown in two cropping years.

Trait	Pea grain yield in MS		Pea proportion in MS	
	2018–19	2019–20	2018–19	2019–20
Pea proportion in MS	0.98**	0.96**	–	–
Associated cereal grain yield in MS	–0.31**	0.04 NS	–0.46**	–0.16*
Total (pea + cereal) grain yield in MS	0.60**	0.77**	0.46**	0.62**
Pea MS/PS grain yield ratio	0.64**	0.90**	0.64**	0.85**
Pea grain yield in PS	0.28**	0.30**	0.27**	0.35**

NS, *, **, correlation not different from zero and different from zero at $P < 0.05$ and $P < 0.01$, respectively.

and competitive ability in MS tends to produce mixtures that are not only more balanced but also more productive (albeit in the presence of some trade-off between pea and cereal yields highlighted by a low inverse correlation between these traits: **Table 2**).

Genotype $\times$ growing condition interaction for pea yield was observed in both test years ($P < 0.01$) and implied fairly low consistency of genotype yield responses across conditions, as indicated by genetic correlation values slightly above 0.40 in both test years (**Table 3**). Pea yield in PS exhibited similar broad-sense heritability as in MS, because the advantage of smaller experiment error was counterbalanced by the disadvantage of smaller genetic variation in PS relative to MS (**Table 3**). As a result, the predicted efficiency of indirect selection based on yield in PS relative to direct selection in MS was largely determined by genetic correlation values, achieving only 44% in both test years (**Table 3**).

The large impact on pea yield responses of specific adaptation to MS or PS conditions was confirmed by estimates of variance components for grain yield across cropping years. While all genotypic and genotype $\times$ environment interaction components of variance were different from zero ($P < 0.01$), the variance of genotype $\times$ growing condition interaction was nearly two-fold larger than the genotypic variance, was definitely larger than the genotype $\times$ cropping year interaction variance, and was somewhat larger than the genotype $\times$ growing condition $\times$ year interaction variance (**Supplementary Table 2**). The occurrence of interaction of genotype with the year factor reduced the ability of performance data assessed in one year to predict genotype responses in an independent year.

The correlation of pea morphophysiological characteristics (as measured in PS) with pea yield was significantly different ($P < 0.01$) across MS and PS conditions for three traits, namely, onset of flowering, plant height at onset of flowering, and susceptibility to ascochyta blight (**Table 4**). In both test years, later onset of flowering and taller plant height were associated with pea yield in MS, while being poorly associated or not associated with pea yield in PS (**Table 4**). In contrast, greater susceptibility to ascochyta blight was strongly associated with lower pea yield in PS but not in MS (**Table 4**). Accordingly, relatively better yield response in MS as indicated by greater values of the pea MS/PS grain yield ratio was correlated with later onset of flowering and taller plant height (**Table 4**). The positive correlation of the

MS/PS yield ratio with susceptibility to ascochyta blight (**Table 4**) indicated that relatively better performance in PS was associated with greater tolerance to the disease. Taller plant at onset of flowering (expected to be a key trait to compete for light), later flowering onset (contributing to maturity matching with associated cereals), and greater yield in PS were selected in this order as components of a selection index for greater pea yield in MS based on traits in PS. These traits were all significant at $P < 0.001$ in a stepwise multiple regression as a function of genotype yield in MS and jointly explained nearly 60% of the genotype variation. Their correlation to each other ($r < |0.78|$) was safely below any risk of collinearity. The selection index equation for pea yield in MS based on traits recorded in PS was:

$-1.413 + (0.0184 \times \text{pea plant height [in cm]}) + (0.0962 \times \text{pea yield [in t/ha]}) + (0.0476 \times \text{pea onset of flowering [in dd from April 1]})$.

The mean pea response for three morphophysiological traits across PS and MS conditions in the only year when it was assessed indicated non-significant trends toward delayed onset of flowering and taller plant stature in MS relative to PS (**Table 1**). Genotype $\times$ growing condition interaction was significant ($P < 0.05$) for onset of flowering and seed weight, but the consistency of genotype responses across conditions was very high for all traits according to genetic correlation ($r_g \geq 0.93$).

Genotype value according to the selection index assessed in PS exhibited high genetic correlation with genotype yield in MS ($r \geq 0.72$; **Table 3**) and somewhat higher broad-sense heritability than yield in MS (particularly in the first year, when the favorable growing conditions emphasized the genotype variation for most component traits of the index and, thereby, the genetic variation for index value: **Table 3**). As a result, the predicted efficiency of index-based selection in PS was in the range 87–96% relative to direct selection in MS (**Table 3**).

The comparison of direct vs. indirect selection strategies for predicted efficiency reported in **Table 3** was relative to independent assessments for each test year and, as such, could not take account of possible differences among selection criteria for extent of genotype $\times$ location or genotype $\times$ year interactions (which ought to be minimal for an ideal selection criterion). Indeed, the selection index exhibited the additional advantage of lower genotype $\times$ year interaction (as shown by greater genetic correlation across years for genotype values) relative to both yield-based criteria (**Table 5**). This feature and its low

TABLE 3 | Genetic (CV_g) and experiment error (CV_e) coefficient of variation and broad-sense heritability on a line mean basis (H^2) for pea direct selection for grain yield in mixed stand with cereals (MS) and pea indirect selection for yield in MS based on yield or a pea selection index in pure stand (PS), genetic correlation (r_g) between direct and indirect selection criteria, and predicted efficiency (E_R) of indirect selection criteria in PS relative to direct selection in MS, based on data of 144 pea inbred lines in each of two cropping years.

Selection criterion	CV_g (%)	CV_e (%)	$H^2 \pm SE^a$	$r_g \pm SE^b$	E_R (%) ^c
Year 2018–19					
Pea grain yield in MS	33	44	0.62 $\pm$ 0.05		100
Pea grain yield in PS	24	27	0.70 $\pm$ 0.04	0.42 $\pm$ 0.11	44
Pea selection index in PS ^d	51	27	0.91 $\pm$ 0.01	0.72 $\pm$ 0.06	87
Year 2019–20					
Pea grain yield in MS	46	51	0.71 $\pm$ 0.04		100
Pea grain yield in PS	17	18	0.72 $\pm$ 0.04	0.43 $\pm$ 0.10	44
Pea selection index in PS ^d	15	13	0.80 $\pm$ 0.03	0.90 $\pm$ 0.05	96

^a Value $\pm$ standard error.

^b Value $\pm$ standard error. Genotype $\times$ condition (MS or PS) interaction for pea yield significant at $P < 0.01$.

^c $E_R = [(H_{PS} / H_{MS}) r_g] \times 100$, where H_{PS} and H_{MS} are the square root of H^2 for the relevant selection criteria in PS and MS, respectively.

^d Selected from traits in PS associated with pea yield in MS; equal to: $-1.413 + (0.0184 \times \text{pea plant height [in cm]}) + (0.0962 \times \text{pea yield [in t/ha]}) + (0.0476 \times \text{pea onset of flowering [in dd from April 1]})$.

TABLE 4 | Phenotypic correlation of pea grain yield in mixed stand with cereals (MS) or in pure stand (PS) and ratio between MS and PS for pea grain yield with pea morphophysiological traits in PS, for 144 pea inbred lines grown in two cropping years.

Trait	Pea grain yield						MS/PS pea yield ratio	
	2018–19			2019–20			2018–19	2019–20
	MS ^a	PS ^a	u test ^b	MS ^a	PS ^a	u test ^b		
Onset of flowering	0.46**	−0.13 NS	**	0.50**	−0.03 NS	**	0.49**	0.53**
Plant height at onset of flowering	0.55**	0.12 NS	**	0.59**	0.31**	**	0.41**	0.50**
Individual seed weight	−0.08 NS	0.30**	**	−0.09 NS	0.18*	*	−0.29**	−0.16 NS
Maturity date	0.20*	0.37**	NS	−0.07 NS	−0.16 NS	NS	−0.14 NS	0.00 NS
Plant height at maturity	0.36**	0.41**	NS	0.44**	0.21*	*	−0.02 NS	0.38**
Winter plant survival	0.13 NS	0.27**	NS	–	–		−0.09 NS	–
Susceptibility to ascochyta blight	−0.22**	−0.57**	**	–	–		0.25**	–

^a NS, *, **, correlation not different from zero and different from zero at $P < 0.05$ and $P < 0.01$, respectively.

^b NS, *, **, correlation coefficients between growing conditions in the same cropping year not different and different at $P < 0.05$ and $P < 0.01$, respectively, according to u test.

TABLE 5 | Genetic correlation (r_g) across two cropping years and broad-sense heritability over years on a line mean basis (H^2) of pea yield in mixed stand with cereals (MS) and pea yield or a pea selection index in pure stand (PS), for 144 pea inbred lines across two cropping years.

Trait ^a	$r_g \pm SE$	H^2
Pea grain yield in MS	0.72 $\pm$ 0.09	0.619
Pea grain yield in PS	0.74 $\pm$ 0.09	0.581
Pea selection index in PS ^b	0.93 $\pm$ 0.03	0.696

^a Genotype $\times$ year interaction always significant at $P < 0.01$.

^b See footnote d in **Table 3** for index definition.

experiment error (**Table 3**) contributed to higher broad-sense heritability over years of this criterion relative to yield-based criteria (**Table 5**). The comparison of selection strategies for predicted efficiency based on the size of phenotypic correlations between genotype value for the relevant selection criterion in a

selection year and genotype yield in MS in another year could account for the advantage represented by lower genotype $\times$ year interaction for the selection index. This comparison revealed an average predicted efficiency advantage of 19% for this criterion relative to direct selection based on pea yield in MS (**Table 6**). The advantage of this criterion was greater for the selection year 2018–19 than for 2019–20 (**Table 6**), in coincidence with the much greater genetic variation that emerged for the selection index in the former year relative to the latter (**Table 3**). The predicted efficiency of yield-based selection in MS was about two-fold that of yield-based selection in PS according to this comparison (**Table 6**).

The alternative comparison of selection strategies based on actual yield gains performed by using by turns one year for selection and the other year for evaluation of yield gains also took account of genotype $\times$ year interaction effects. This comparison of selection criteria differed from that reported in **Table 6** not only because it was based on actual yield gains but also because the selection was performed within each individual

TABLE 6 | Phenotypic correlation (r) of pea genotype value in one year (selection year) with pea grain yield in mixed stand with cereals (MS) in another year (target environment) for three selection criteria based on MS or pure stand (PS) selection for individual selection years and averaged across selection years, and average predicted efficiency (E_R) of selection criteria in PS relative to selection in MS, based on data of 144 pea inbred lines over two cropping years.

Selection criterion	r value			E_R (%) ^a
	Selection in 2018–19	Selection in 2019–20	Average	
Pea grain yield in MS	0.475	0.475	0.475	100
Pea grain yield in PS	0.175	0.321	0.248	52
Pea selection index in PS ^b	0.663	0.472	0.568	119

^a $E_R = (r_{PS} / r_{MS}) \times 100$, where r_{PS} and r_{MS} are average r values for relevant PS and MS selection criteria, respectively.

^b See footnote d in **Table 3** for index definition.

RIL population (reporting results averaged across populations: **Table 7**) rather than across the entire set of lines. Its results, averaged across selection years, indicated the similar efficiency of the index-based selection criterion in PS and the direct selection for pea yield in MS, as well as 64% efficiency of yield-based selection in PS relative to yield-based selection in MS (**Table 7**). Also here, the selection index-based criterion exhibited greater efficiency when selecting in the first year than in the second (**Table 7**).

Genome-Enabled Predictions, Comparison of Genomic vs. Phenotypic Selection Strategies, and Genome-Wide Association Study

Next generation sequencing produced, on average, 2.2 M reads per genotype sample. The selected model configuration issued by the first step of genomic model tuning retained the thresholds of 0.05 for SNP missing data per marker and 0.50 for SNP missing data per genotype. This configuration, which was associated with 5,909 polymorphic SNP markers, was selected among those implying no loss of genotype samples because it maximized the average intra-RIL population inter-environment predictive ability for pea yield in MS (albeit with negligible difference to two configurations with lower SNP missing data per marker) while providing a reasonably high number of markers for the GWAS. More stringent thresholds of SNP missing data per genotype led to exclusion of some genotype samples without producing a substantial increase of intra-population predictive ability, as indicated by results in **Supplementary Figure 1**. This figure also showed the presence of variation among RIL populations for intra-population predictive ability. The selected configuration was adopted for the step of model tuning aimed to selection of the statistical model. Four models, i.e., Ridge Regression BLUP, Bayes A, Bayes C π and Reproducing kernel Hilbert space, displayed average intra-population predictive ability for pea yield in MS around 0.26, whereas Bayesian Lasso displayed slightly lower predictive ability (0.25). We selected the first model in view of its greater computational speed.

The predictive ability for intra-population inter-environment prediction of pea yield in MS averaged 0.267, while ranging from 0.183 (for progeny lines of Alliance $\times$ Isard) to 0.385 (for progeny lines of Kasper $\times$ Isard) (**Supplementary Table 3**). No distinct

relationship emerged between intra-population predictive ability and number of polymorphic markers or within-population phenotypic variation, although the RIL population with the highest predictive ability also displayed the highest number of polymorphic markers (**Supplementary Table 3**). The average predictive ability of populations was reduced by 27% (0.195 vs. 0.267) for the challenging scenario of inter-population inter-environment prediction (**Table 8**). In contrast, high predictive ability (0.532) was achieved for inter-environment predictions regarding the entire set of lines (considered as a unique genetic base) (**Table 8**). In all cases, model training on the data of the first year provided better predictions than training on the data of the second year (**Table 8**).

A comparison of genomic vs. phenotypic selection strategies was performed for the two main contexts envisaged by earlier comparisons of phenotypic strategies. One was relative to selection among all genotypes, with predicted efficiency estimated from the size of the correlation between genotype values for the relevant selection criterion in one selection year and genotype yields in MS in an independent year. Relevant correlation values for this scenario are given in **Table 6** for phenotypic selection criteria, and by correlations between cross validation-based genotype values issued by model training in one year and genotype yields in MS in an independent year as expressed by predictive ability values for all genotypes in **Table 8** for genomic selection. The comparison based on correlation values averaged across years revealed 12% greater predicted efficiency of genomic selection relative to direct phenotypic selection for pea yield in MS (0.532 vs. 0.475), and 6% lower predicted efficiency of genomic selection relative to phenotypic index-based selection in PS (0.532 vs. 0.568) (**Tables 6, 8**). The second context for comparison of genomic vs. phenotypic selection strategies was relative to selection within each RIL population, with relative efficiency estimated according to actual yield gains. Results averaged across test years indicated 10–12% lower efficiency of genomic selection relative to best-performing phenotypic selection strategies as represented by selection for yield in MS and index-based selection in PS (**Table 7**).

The results of the GWAS are summarized by Manhattan plots reporting marker-trait associations relative to pea yield in MS (**Figure 1A**) and in PS (**Figure 1B**). They indicated many regions of the genome that featured a slight association, with no marker reaching the Bonferroni threshold for significant ($P < 0.05$)

TABLE 7 | Mean grain yield and actual yield gain in mixed stand with cereals (MS) of pea lines selected within each of six recombinant inbred line (RIL) populations according to three phenotypic selection (PhS) criteria based on MS or pure stand (PS) selection and one genomic selection (GeS) criterion by performing PhS or GeS model training in one year and assessing yield gains of selected material in another year, and efficiency (E_R) of selection criteria relative to PhS selection in MS, based on data of 144 pea inbred lines grown in two cropping years.

Selection criterion	Selection/model training in 2018–19; yield assessment in 2019–20 ^a			Selection/model training in 2019–20; yield assessment in 2018–19 ^a			Average E_R (%)
	Mean yield (t/ha)	Yield gain (t/ha)	E_R (%) ^b	Mean yield (t/ha)	Yield gain (t/ha)	E_R (%) ^b	
PhS for pea grain yield in MS	1.310	0.476	100	1.205	0.397	100	100
PhS for pea grain yield in PS	1.105	0.271	57	1.090	0.282	71	64
PhS by a selection index in PS ^c	1.419	0.585	123	1.097	0.289	73	98
GeS for pea grain yield in MS ^d	1.282	0.448	94	1.129	0.321	81	88

^a Selection of two lines out of 23 for each RIL population; yield gain of the 12 selected lines over the mean value of six parent lines of the RIL populations.

^b $E_R = (G_{SC} / G_{MS}) \times 100$, where G_{SC} and G_{MS} are yield gains for relevant selection criterion and for PhS for pea yield in MS, respectively.

^c See footnote d in **Table 3** for index definition.

^d Selection within each population based on Ridge regression BLUP model training on 80% of the lines and estimation of breeding values for selection on the remaining 20% of the lines, rotating the folds and repeating the process 100 times to obtain stable predictions of top-yielding lines.

TABLE 8 | Predictive ability of genomic selection models for pea grain yield in mixed stand with cereals using one cropping year for model training and another year for model validation, for (a) intra-population predictions for each of six individual recombinant inbred line (RIL) populations, (b) inter-population predictions for individual RIL populations, and (c) predictions for all genotypes neglecting population strata.

Prediction	Predictive ability ^a		
	Training year 2018–19	Training year 2019–20	Average
Intra-population ^b	0.326	0.208	0.267
Inter-population ^c	0.275	0.115	0.195
All genotypes ^d	0.625	0.438	0.532

^a As correlation of predicted values according to the Ridge regression BLUP with observed values, using data of 144 pea inbred lines.

^b Averaged across 100 repetitions of five-fold stratified cross validations applied to each population; results for individual years averaged across populations.

^c Model training on all data of the non-target populations; results for individual years averaged across populations.

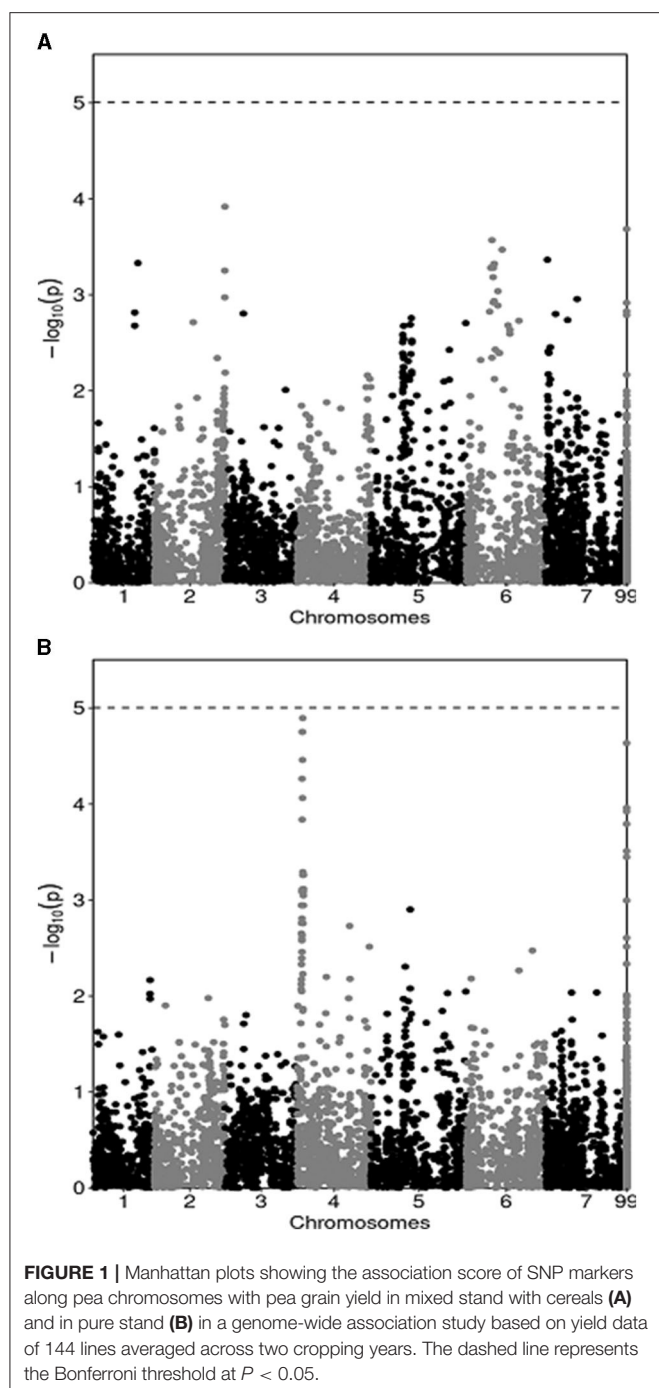
^d Averaged across 100 repetitions of five-fold stratified cross validations applied to all lines.

association. In agreement with the modest genetic correlation for pea genotype yield across MS and PS conditions, the GWAS revealed modest consistency across growing conditions for markers that tended to display some association with the yield trait. In particular, one genomic area on chromosome 4 whose association with yield in PS approached $P < 0.05$ significance (**Figure 1B**) showed no trend toward association with yield in MS (**Figure 1A**). Likewise, five genomic regions that tended toward association with yield in MS on the ground of association scores ≥ 3 (one each on chromosomes 1, 2 and 6, and two on chromosome 7: **Figure 1A**) showed no local peak for yield in PS (**Figure 1B**). Only one region on chromosome 5 tended toward association with yield in both growing conditions, albeit with a modest linkage (association score slightly below 3) (**Figure 1**).

DISCUSSION

Our preliminary study highlighted the difficulty to identify cereal companions with sufficient earliness of maturity for pea-cereal intercrops aimed to grain production. This result restricted the choice of cereal companion species and cultivars, and influenced the definition of pea traits contributing to specific adaptation to MS by promoting the advantage of a late pea phenology.

The extent of pea-cereal mismatch of maturity may depend on the specific germplasm, cropping region and sowing season. For example, pea displayed a trend toward later maturity than barley (the earliest small-grain cereal) for locally well-adapted cultivars evaluated in Switzerland under spring sowing (B. Haug, personal communication, 2021). The phenological type of the selected pea parents that originated our genetic base included spring-type (e.g., Attika), Mediterranean (e.g., Kaspia) and winter-type (e.g., Isard) material. In autumn-sown Italian environments these cultivars exhibited moderate variation for onset of flowering along with modest variation for maturity time (Annicchiarico, 2005; Annicchiarico and Iannucci, 2008) due to the combined effect of terminal drought and high temperatures. The same response was displayed by their derived lines in the current study. Later pea phenology may be searched for by growing photoperiod-sensitive germplasm selected for central Europe to enhance pea winter hardiness (Lejeune-Hénaut et al., 2008), but this material is unlikely to be adapted to the warm and dry summers of southern Europe. Therefore, the identification and/or selection of early-maturing barley and wheat companions probably is the main avenue to obtain cereal companions compatible with pea for autumn-sown intercrops in our target region.



Harper's (1977) general observation that the yield efficiency of a mixture depends mainly on the performance of its weaker partner, which was confirmed by various experimental studies (Ofori and Stern, 1987), highlighted the importance of selecting for greater competitive ability the component species that is expected to be outcompeted under ordinary cropping conditions in a target region. From a plant breeding perspective, this conclusion is supported by the fact that the genetic correlation for genotype yield responses across MS and PS conditions tends

to be lower in the presence of larger competitive stress exerted on the focus species (Annicchiarico and Piano, 1994). This study confirmed the severe competitive disadvantage reported for pea by earlier studies encompassing different cereal companions, target regions and sowing times (Jensen, 1996; Corre-Hellou et al., 2006; Lithourgidis et al., 2011; Annicchiarico et al., 2017). The value slightly above 0.40 of the genetic correlation for pea yield across MS and PS conditions was consistent across test years despite their differences for sowing time, N fertilization and cereal companions. This value was lower than the average value across studies on perennial legume-grass or annual legume-cereal intercrops in a recent review (Annicchiarico et al., 2019a). Likewise, the current predicted efficiency of indirect selection in PS relative to direct selection in MS based on results of single experiments was lower than the average one in early studies on legume-based intercrops, namely, 44% (Table 3) vs. 60% (Annicchiarico et al., 2019a). The observed increase of the genetic coefficient of variation for yield in MS relative to PS agrees with earlier results for grain (Atuahene-Amankwa and Michaels, 1997) and perennial legumes (Annicchiarico, 2003). The lack of substantially greater broad-sense heritability of MS relative to PS caused by a concurrent trend of MS toward greater experiment error than PS agrees as well with earlier findings for legume-based intercrops (Annicchiarico et al., 2019a).

The GWAS provided an unprecedented genome-based insight and justification for the modest genetic correlation for genotype yields across MS and PS conditions that emerged in a quantitative genetics framework. The presence of many genomic regions displaying a slight, non-significant association was expected for a complex, highly polygenic trait such as grain yield. The large inconsistency across growing conditions for markers that tended to display some association with the yield trait emerged clearly from the overview of association scores in Manhattan plots. In this study the GWAS did not aim to discover quantitative trait loci, given the limited practical interest of marker-assisted selection compared with genomic selection for the improvement of largely polygenic traits (Bernardo and Yu, 2007).

The only modest decrease of competitive stress exerted on pea in the second year relative to the first year suggested that pea competitive disadvantage is ordinary in the target region and is not easy to be overcome just by agronomic decisions relative to sowing time, N fertilization or cereal companion. Other considerations support the greater perspective interest of pea breeding over crop management to improve pea-cereal intercrops. While more balanced grain legume-cereal mixtures could be obtained by adopting less vigorous cereal companions, no N fertilization or increased legume sowing rate (Ofori and Stern, 1987; Yu et al., 2016), these technical choices may produce lower total yield of the mixture compared with the adoption of a legume component with increased competitive ability. This conclusion is supported by: (a) several reports highlighting the importance of N fertilization for the agronomic and economic performance of grain legume-cereal mixtures (e.g., Hauggaard-Nielsen and Jensen, 2001; Kiwia et al., 2019); (b) results for perennial crops indicating that total mixture yield tends to be maximized by pairs of components characterized by the highest

and most similar plant vigor (Zannone et al., 1986; Annicchiarico and Piano, 1994). The latter results agree with the current finding that pea lines with greater competitive ability tend to produce mixtures not only more balanced but also better yielding.

The large inconsistency across MS and PS conditions of correlations of pea morphophysiological traits with grain yield shed light on useful pea adaptive traits for intercropping. Taller plant stature at onset of flowering was the main trait in this respect according to correlation results and the selection of this trait as the first one in the stepwise regression analysis leading to definition of the selection index. Taller plant is generally associated with greater competitive ability of erect plants under moderately favorable growing conditions (Keddy, 1990), owing to its crucial importance in competition for light. Taller pea plants exhibited greater competitive ability in different pea-grain legume associations assessed by simulation (Barillot et al., 2012, 2014) and field studies (Hauggaard-Nielsen and Jensen, 2001; Annicchiarico et al., 2012, 2017). The usefulness of later onset of flowering for adaptation to MS descends from the discussed trend toward mismatched maturity of pea and associated cereals and the much narrower variation of a more relevant trait in this context such as pea maturity date. As anticipated, the relationship of phenology with yield response in MS is expected to be germplasm- and environment-specific. The selection of pea yield in PS as a third trait in the index of selection for pea yield in MS agrees with the positive genetic correlation for yield across the two conditions, which implies that a portion of the variation for intrinsic pea grain yielding ability (as indicated by PS performance) is also relevant to MS performance. The greater importance of tolerance to ascochyta blight in PS than in MS agrees with the fact that tolerance to pests and diseases is usually less important in MS, because of the dilution of host density allowed for by the associated species (Boudreau, 2013).

The assessment only in one year of morphophysiological traits across MS and PS conditions provided only preliminary indications on pea phenotypic plasticity in response to intercropping. Recalling that phenotypic plasticity is the ability of a genotype to alter its trait values in response to environmental conditions (Bradshaw, 1965), pea displayed only limited and non-significant shifts of trait mean value passing from PS to MS (albeit in the adaptively meaningful directions of delayed onset of flowering and taller plant stature). While these results concerned the mean response of pea, phenotypic plasticity responses of practical interest for breeders relate to genetic variation as revealed by genotype $\times$ growing condition interaction for a focus trait that is associated with relatively better performance in MS. For example, interaction effects relative to white clover genotypes with greater capacity of petiole elongation in MS were indicative of better phenotypic plasticity-based adaptation to intercropping with vigorous grasses (Annicchiarico, 2003), as a consequence of a phytochrome-mediated mechanism for shade avoidance that is present in white clover (Robin et al., 1992) and may affect various vegetative organs in other species (Schmitt et al., 2003). In this study, the highly consistent genotype responses across PS and MS conditions suggest quite limited variation for phenotypic plasticity of pea plant height at flowering onset or other observed traits.

The observed small difference in predictive ability among various statistical models usable for genomic selection was reported earlier for pea yield (Annicchiarico et al., 2019b) or other pea traits (Burstin et al., 2015). The good performance of Ridge regression BLUP agrees with its suitability for traits influenced by a large number of minor genes, such as grain yield (Wang et al., 2018).

The training set for genomic selection was the same for intra-population selection and for all-genotype selection (i.e., selection within the whole set of genotypes without distinction between RIL populations), always including the parent lines and 80% of the inbred lines per RIL population. The two-fold greater genome-enabled predictive ability for the latter selection scenario relative to the former (0.532 vs. 0.267; **Table 8**) descended from the possibility to also exploit the phenotypic variation due to mean differences between populations and the wider molecular variation provided by the pooled populations. The average intra-population inter-environment predictive ability for pea yield in MS was only somewhat lower than that observed for pea yield in PS across Italian environments for a subset of three of the current RIL populations, which was equal to 0.296 (Annicchiarico et al., 2019b).

The adoption of different conditions for MS testing in the two test years probably inflated the extent of genotype $\times$ growing condition $\times$ year interaction. However, such diverse conditions reflected better the diversity of possible intercropping conditions in the target region, thereby providing a more realistic (albeit more challenging) scenario for the comparison of phenotypic or genomic selection strategies based on selection in one year and assessment of yield gains in an independent year. We envisaged two main selection scenarios for these comparisons, namely (a) all-genotype selection (with comparison based on predicted yield gains), and (b) selection within each RIL population (with comparison based on actual yield gains from selection of two lines out of 23 per population). Considering direct phenotypic selection in MS as the benchmark for comparison of alternative selection strategies, our results suggested that (a) the relative efficiency of 52–64% exhibited by indirect selection based on pea yield in PS is too low to be compensated by budget savings arising from no need for pea proportion assessment; (b) the index-based selection in PS provided a valuable alternative to selection for yield in MS, particularly when selecting across RIL populations (where it displayed 19% greater predicted efficiency), when considering that the additional morphophysiological traits to be recorded beside yield, i.e., onset of flowering and plant height at flowering onset, are less expensive to record than the assessment of pea proportion in MS; (c) genomic selection for pea yield in MS has high interest for selection across or within RIL populations, because its efficiency was comparable to phenotypic selection for yield in MS and would definitely be greater when taking into account the effect on predicted or actual gains per unit time of its shorter selection cycle and smaller evaluation cost per genotype. In particular, the ability by genomic selection to perform two selection cycles per year would imply efficiency values relative to selection for yield in MS of 176% and 224% based on actual and predicted gains, respectively, per unit time. The double amount of evaluated

genotypes per year assumed for genomic selection would be supported by at least two-fold lower evaluation cost per genotype compared with phenotypic selection in MS, according to a GBS fee of about € 60 (including taxes) and an estimated cost for one-year phenotypic selection in MS of about € 120–130. We did not formally assess the relative merit of genomic selection based on inter-population inter-environment predictions, but the 27% average loss of predictive accuracy suggests that even this selection strategy may be efficient for pea selection aimed to intercropping.

In conclusion, this study highlighted the importance of pea selection for intercropping as a means to obtain more balanced and more productive pea-cereal intercrops, and indicated the high efficiency in this context of phenotypic selection for pea yield in MS, genomic selection for the same trait, and indirect phenotypic selection based on a selection index of traits related to pea competitive ability that are assessed in PS. While many studies investigated the relationship of competitive ability with morphophysiological traits in legume species, just a few provided a formal assessment of the efficiency of trait-based indirect selection relative to yield-based selection (Annicchiarico et al., 2019a). In addition, our study provided unprecedented evidence for the value of genomic selection for intercropping on the basis of experimental data. Interestingly, the about two-fold greater efficiency of genomic selection relative to phenotypic selection for yield in MS according to yield gains per unit time is close to the 2.3-fold advantage predicted for genomic selection by Bančič et al. (2021) according to simulation results for the current scenario of genetic correlation around 0.4 across MS and PS conditions. Genomic selection may display the highest efficiency but requires an initial stage of germplasm evaluation in MS for model training which can, anyway, be used for phenotypic selection purposes. A possible limitation of our findings was the limited sampling of test environments that our estimates of predicted or actual yield gains were based upon. More conclusive indications are expected from future research work aimed to compare the current selection strategies in terms of actual yield gains in MS over a larger number of test environments.

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DATA AVAILABILITY STATEMENT

The original contributions generated for the study are publicly available. This data can be found here: National Center for Biotechnology Information (NCBI) BioProject database under accession number PRJNA744476.

AUTHOR CONTRIBUTIONS

PA designed the research work and obtained financial resources. LP and PA were responsible for, and TN, MR, and CM contributed to, phenotyping experiments. BF was responsible for the extraction and preparation of DNA for outsourced analyses. PA was responsible, and CM contributed to, phenotypic data analyses. NN was responsible for molecular data analysis. PA drafted the manuscript. All authors revised and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.731949/full#supplementary-material>

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Functional–Structural Plant Modeling Highlights How Diversity in Leaf Dimensions and Tillering Capability Could Promote the Efficiency of Wheat Cultivar Mixtures

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Edited by:

Marcello Mastrorilli,
Council for Agricultural and
Economics Research (CREA), Italy

Reviewed by:

Simon Pearson,
University of Lincoln, United Kingdom
Alessandro Persiani,
Council for Agricultural and
Economics Research (CREA), Italy

*Correspondence:

Emmanuelle Blanc
emmanuelle.blanc@
univ-paris-saclay.fr
Jérôme Enjalbert
jerome.enjalbert@inrae.fr

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Emmanuelle Blanc^{1*}, Pierre Barbillon², Christian Fournier³, Christophe Lecarpentier⁴,
Christophe Pradal^{5,6} and Jérôme Enjalbert^{1*}

¹ Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE—Le Moulon, Gif-sur-Yvette, France, ² Université Paris-Saclay, AgroParisTech, INRAE, UMR MIA-Paris, 75005, Paris, France, ³ LEPSE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France, ⁴ UMR 1095 GDEC, INRAE, Université Clermont Auvergne, Clermont-Ferrand, France, ⁵ CIRAD, UMR AGAP Institut, Montpellier, France, ⁶ INRIA and LIRMM, Univ Montpellier, CNRS, Montpellier, France

Increasing the cultivated diversity has been identified as a major leverage for the agroecological transition as it can help improve the resilience of low input cropping systems. For wheat, which is the most cultivated crop worldwide in terms of harvested area, the use of cultivar mixtures is spreading in several countries, but studies have seldom focused on establishing mixing rules based on plant architecture. Yet, the aerial architecture of plants and the overall canopy structure are critical for field performance as they greatly influence light interception, plant interactions and yield. The very high number of trait combinations in wheat mixtures makes it difficult to conduct experimentations on this issue, which is why a modeling approach appears to be an appropriate solution. In this study, we used WALTER, a functional structural plant model (FSPM), to simulate wheat cultivar mixtures and try to better understand how differences between cultivars in key traits of the aerial architecture influence mixture performance. We simulated balanced binary mixtures of cultivars differing for different critical plant traits: final height, leaf dimensions, leaf insertion angle and tillering capability. Our study highlights the impact of the leaf dimensions and the tillering capability on the performance of the simulated mixtures, which suggests that traits impacting the plants' leaf area index (LAI) have more influence on the performance of the stand than traits impacting the arrangement of the leaves. Our results show that the performance of mixtures is very variable depending on the values of the explored architectural traits. In particular, the best performances were achieved by mixing cultivars with different leaf dimensions and different tillering capability, which is in agreement with numerous studies linking the diversity of functional traits in plant communities to their productivity. However, some of the worst performances were also achieved by mixing varieties differing in their aerial architecture, which suggests that

diversity is not a sufficient criterion to design efficient mixtures. Overall, these results highlight the importance of simulation-based explorations for establishing assembly rules to design efficient mixtures.

Keywords: aerial architecture, competition for light, variety mixture, sensitivity analysis, functional traits, tillering plasticity

INTRODUCTION

Increasing the cultivated diversity has been identified as a major leverage for the agroecological transition as it can help improve the resilience of low input cropping systems (Malézieux, 2012; Isbell, 2015). At the scale of the plot, diversity can be increased by mixing species or by increasing the genetic diversity within a species (varietal mixture for example). For wheat, which is the most cultivated crop worldwide in terms of harvested area (Food Agriculture Organization of the United Nations, 2018), the use of cultivar mixtures has been reported to present advantages for yield and quality as well as for diseases resistance, insect pests control, weed suppression, lodging limitation, exploitation of water and soil nutrients and yield stability (Borg et al., 2018). Moreover, the use of wheat cultivar mixtures is spreading in several countries (Faraji, 2011). In France, for example, wheat blends represented <1% of the total wheat surface in 2010 but they represented more than 11% of the total wheat surface in 2019 (FranceAgriMer, 2019). To support the spread of cultivar mixtures, assembly rules have been developed, but they're almost exclusively focused on diseases resistance (Borg et al., 2018). Studies have seldom focused on establishing mixing rules based on the plants architecture and the few existing recommendations usually advocate the association of homogeneous cultivars, but the demonstration of these guidelines seems to be lacking (Borg et al., 2018).

Yet, plant architecture and the overall canopy structure are critical for field performance, as they strongly influence light interception (Niinemets, 2010). Thus, many traits of the aerial architecture, such as height, leaf dimensions, leaf inclination and branching (i.e., tillering in cereals), have been identified for their impact on light interception. Indeed, leaf dimensions have a direct influence on the leaf area index (LAI) of the canopy, which is the most basic property affecting its light interception (Pugnaire and Valladares, 2007). Branching is also an important feature as it greatly impacts the total leaf area of the plant by changing its number of leaves (Valladares and Niinemets, 2008). On the other hand, plant height has a direct impact on the leaf area distribution in the canopy and on the overlap between neighboring leaves, which is critical for competition for light, especially in heterogeneous canopies (Givnish, 1982). As for leaf inclination, it determines the penetration of light into the canopy: the more upright the leaves are, the deeper light can penetrate into the lower layers of the canopy (Long et al., 2006). The impact of all of these traits has been considered for the definition of wheat ideotypes (i.e., ideal hypothetical plants for a given context) (Donald, 1968) and in breeding programs (Reynolds et al., 2012), but mostly in the context of single plants or pure stands. However, the impact of these architectural traits would

also be of interest for the establishment of assembly rules and the definition of mixture ideotypes (Litrico and Violle, 2015). To this end, it is necessary to better understand the interactions taking place in mixtures and the role played by architectural features in these interactions.

The very high number of trait combinations in wheat mixtures makes it difficult to conduct experimentations on this issue, which is why a modeling approach appears to be an appropriate solution. In particular, functional structural plant models (FSPM, Godin and Sinoquet, 2005; Vos et al., 2010) have the advantage of being individual-based, allowing for the explicit consideration of inter-individual variations in the stand (Zhang and De Angelis, 2020). Most importantly, FSPM explicitly represent the 3D architecture of plants and its interactions with the ecophysiological processes controlling plant development. These models are thus particularly adapted to study the impact of architectural traits on the performance of heterogeneous canopies (Evers et al., 2019; Gaudio et al., 2019). If past studies using FSPM were mainly focusing on sole crops (Sarikioti et al., 2011; Chen et al., 2014; Da Silva et al., 2014; Streit et al., 2016; Perez et al., 2018), more and more work is now addressing mixtures of different species or cultivars (Barillot et al., 2014; Munz et al., 2018; Louarn et al., 2020). For wheat cultivar mixtures, Barillot et al. (2019) have studied the impact of differences in leaf inclination during the post-anthesis period; and Vidal et al. (2018) have explored how diversity in plant height could help control rain-borne diseases. However, no FSPM study has yet focused on the impact of more than one trait on the performance of wheat cultivar mixtures.

In this study, we used an FSPM to simulate balanced binary wheat cultivar mixtures differing in four traits of their aerial architecture. Using a sensitivity analysis approach, we were able to identify the major traits impacting the performance of the simulated stands. This study improves our understanding of how differences between cultivars in key traits of the aerial architecture influence mixture performance.

MATERIALS AND METHODS

All the statistical analyses were done using the R software (R Core Team, 2018).

WALTER: A 3D Wheat Model

WALTER (Lecarpentier et al., 2019) is an FSPM that simulates the development of the aerial architecture of winter wheat (*Triticum aestivum* L.) from sowing to maturity with a daily time step. In the model, the vegetative development of the plants (initiation, emergence, elongation and senescence of organs) follows a thermal time schedule and is based on a formalism

derived from ADEL-Wheat (Fournier et al., 2003). The geometry, size, developmental kinetics and the senescence of leaves and internodes are simulated thanks to deterministic equations. However, WALTER is not a completely deterministic model, as the position of the plants, the orientation of the organs, the duration before plant emergence and the emergence of tillers are partly random.

Most importantly, WALTER simulates the competition for light between plants within the field and the resulting plasticity of tillering (i.e., the branching ability of grasses), thanks to a radiative model (CARIBU: Chelle et al., 1998). The regulation of tillering in the model is based on three simple rules. (i) Empirically fixed probabilities control the emergence of new tillers. Then, (ii) the cessation of tillering is controlled by an early neighbor perception: plants stop emitting new tillers when the surrounding Green Area Index¹ (GAI) reaches a critical value (GAI_c). Finally, (iii) some of the tillers that were emitted regress: a tiller regresses if the amount of photosynthetically active radiation (PAR) it intercepts per unit area falls below a threshold (PAR_t).

Based on this formalism, WALTER produces meaningful outputs, such as the tillering dynamics of each plant (i.e., the number of axes on a plant for each day of the simulation) and the dynamics of light interception for each organ in the stand.

Since its publication in 2019 (Lecarpentier et al.), WALTER has undergone some changes aiming at improving the performances of the simulations, enhancing its realism and improving its ability to simulate mixtures of varieties, reusing several models distributed in the OpenAlea platform (Pradal et al., 2008, 2015). Thus, in the new version of WALTER, the representation of the leaves is based on the model by Fournier and Pradal (2012). The shape of the blades is based on the equations described in Dornbusch et al. (2009) and it is now possible to represent curved leaves based on the formalism described by Perez et al. (2016). The formalism for the dimensions of the leaves remains unchanged. In addition, the discretization of the sky was optimized by the use of a TURTLE sky (den Dulk, 1989) as implemented in the Python package *Alinea.astk* [package python]. (version 2.1.0, 2019). Furthermore, the option to consider an infinite periodic canopy, which is integrated in the radiative model (Chelle et al., 1998), was implemented in WALTER. Thus, it is no longer necessary to simulate additional plants to discard border effects. The computational cost of the simulations was further reduced by removing all dispensable (i.e., non-visible) organs from the 3D representation of plants. Moreover, a project manager, that serializes parameters, inputs, and outputs for each simulation, has been designed to distribute the computation on high-performance computing infrastructure. Modifications were also implemented to WALTER to allow the simulation of complex, heterogeneous canopies, such as varietal mixtures and populations, in which plants can differ from each other in many parameters. Finally, a fitness module has been implemented to allow the computation of the total fitness of the plot (F_{tot}) for each simulation. This new output is used as a proxy of the number of kernels produced by the plot and is

computed by WALTER as the sum of the fitness of all the axes in the plot. The computation of the fitness of an axis is based on the following equation:

$$F_{axis} = \frac{PAR_{i45}}{T_{m45}}$$

where F_{axis} is the fitness of the axis (expressed in molPAR·°Cd⁻¹); PAR_{i45} is the mean daily amount of PAR intercepted by the axis during the 45 days preceding flowering (in molPAR·d⁻¹); and T_{m45} is the mean temperature during the same period (in °C).

This equation is an adaptation of the photothermal quotient (Nix, 1976), which has been demonstrated to be linearly correlated to the number of grains produced in wheat (Midmore et al., 1984; Fischer, 1985; Abbate et al., 1995; Demotes-Mainard and Jeuffroy, 2001).

WALTER is available as an open source Python package on the OpenAlea platform (<https://github.com/openalea/walter>).

Sensitivity Analysis

The impact of different architectural traits on the performance of wheat mixtures was evaluated through the simulation of balanced binary mixtures (mixtures of two varieties with 50% of each cultivar). To evaluate and hierarchize the influence of the different traits on the performance of the simulated mixtures, a sensitivity analysis was conducted with the variance-based Sobol method (Sobol, 1993). Because of the relatively high computational cost to run WALTER, a metamodeling approach was chosen to enable the computation of Sobol indices. The metamodel consists in a fast approximation of WALTER constructed from a limited number of model runs. We used a Gaussian process metamodel (Sacks et al., 1989; Currin et al., 1991), also known as Kriging metamodel, as in Marrel et al. (2009).

Three key outputs of the model were selected for the analyses to reflect the performance of each simulated plot. First, the mean number of ears per plant at maturity (N_{ears}), which is a key component of the plot's yield, was selected. Second, the proportion of incident photosynthetically active radiation (PAR) intercepted by the stand (L_{perc}), was also taken into account. For each stand, the daily L_{perc} was averaged over the whole period of use of the radiative model (from around 100 days after sowing to the maturity of the stand). Third, the total fitness of the plot (F_{tot}) was used for the analyses.

To allow the comparison of the performance of a mixture with the performance of its pure components, the overyielding was also computed for each of these three outputs, using the following equation:

$$OY_{out} = \frac{out_{mix}}{\frac{out_{pure1} + out_{pure2}}{2}}$$

where OY_{out} is the overyielding for the considered output (N_{ears}, L_{perc} or F_{tot}); out_{mix} is the value of the considered output for the mixture; and out_{pure1} and out_{pure2} are the values of the considered output for each of the two components of the mixture, when cultivated in pure stands.

¹ The GAI is the ratio of photosynthetic surface of a plant to ground area.

TABLE 1 | List of parameters for the sensitivity analysis, definitions, traits impacted by the parameter, ranges of variation explored for the mean parameter value in the stand (Ref range) and for the difference of trait value between the two varieties of the mixture (Diff range), corresponding minimum and maximum values at the plant scale and units.

Parameter	Description	Trait	Ref range	Diff range	Min value	Max value	Unit
H_{MS}	Final height of the main stem	Plant height	65; 135	−50; 50	40	160	cm
ϕ_B	Insertion angle between the blade and the stem ^a	Leaf inclination	30; 50	−40; 40	10	70	degree
	Final length of the longest blade of the main stem	Leaf dimensions	16; 27	−16; +16	8	35	cm
GAI_c	Green Area Index threshold above which the emission of tillers stops	Tillering	0.4; 0.7	−0.6; 0.6	0.1	1	–
PAR_t	PAR threshold below which a tiller does not survive	Tillering	0.3; 0.5	−0.2; 0.2	0.2	0.6	mol·cm ^{−2} ·°Cd ^{−1}

^a L_{max}^B also has an indirect impact on the final length of the other blades and on their width. Thus, at the scale of the cultivar, the higher the value of L_{max}^B , the greater the blade area. See **Supplementary Material** for details.

Five parameters associated with four key traits were considered (**Table 1**). For each of the five parameters, the impact of the differential between the two varieties in the mixture was considered (diff), as well as the impact of the mean value in the plot (ref). As a result, there were two input factors considered for each parameter, for a total of 10 input factors for the sensitivity analysis. The ranges of variation for all the input factors were selected to generate biologically realistic varieties with contrasted architectures. For example, the selected ranges allowed for the simulation of varieties with a final height between 40 and 160 cm (**Table 1**).

To build the Kriging metamodel, we used a set of WALTER runs following a 10 dimensions maximin latin hypercube sampling (LHS; McKay et al., 1979; Johnson et al., 1990) of 5,000 simulations generated with the DiceDesign R package (Dupuy et al., 2015) with the ranges detailed in **Table 1**. For each output (N_{ears} , L_{perc} and F_{tot}), a Kriging approximation was generated using the DiceKriging R package (Roustant et al., 2012). The sensitivity R package (Iooss et al., 2017) was then used to estimate the Sobol indices. To be able to compute the overyielding for every mixture, another set of WALTER runs was generated from a five dimensions maximin LHS of 1,000 simulations. This second LHS only includes simulations of pure stands (no trait differential in the stand) and the ranges explored for each input parameter corresponded to the minimum and maximum values detailed in **Table 1**. Based on this second set of simulations, a Kriging model was constructed independently for each of the three outputs (N_{ears} , L_{perc} and F_{tot}). Thus, the first design allowed the approximation of the behavior of mixtures while the second design allowed the approximation of the behavior of the corresponding pure components. Using the Kriging metamodels from both designs, it was possible to compute sensitivity indices for the overyielding of each output.

The simulated plots were composed of 110 plants sown at a density of 200 plants/m². An intermediate sowing density was chosen to allow for competition between plants while allowing plants to potentially emit and maintain several tillers. The number of plants to simulate was the object of preliminary work taking into account the impact of the stochasticity of WALTER on the model outputs (data not shown). The plants

were arranged in a grid pattern with plants equidistant from each other and the different cultivars were distributed randomly in the plot (**Figure 1**). Apart from the input factors of the sensitivity analysis described above and in **Table 1**, all genotypic parameters were identical for both varieties in the mixture and had values given in Lecarpentier et al. (2019) for the cultivar Maxwell. The climatic sequence used for the simulations was obtained by averaging the climatic sequences of five French locations over 10 years (from 2007 to 2017). The five locations, listed in **Supplementary Material**, were selected to represent contrasted climatic conditions. For the computation of light interception, only diffuse radiations were considered, according to the standard overcast sky (Moon and Spencer, 1942). Diffuse radiation was approximated using a set of 16 light sources according to a TURTLE sky (den Dulk, 1989).

Optimization

From the Kriging metamodels, it is possible to seek for combinations of architectural traits maximizing the performance of the stand. However, the uncertainty of the metamodels can affect the results. Therefore, we decided to enrich the initial LHS design with new runs of WALTER, based on an Efficient Global Optimization (EGO) algorithm (Jones et al., 1998). We decided to focus on the maximization of F_{tot} , as this output encompasses information about both N_{ears} and L_{perc} . The initial LHS design of 5,000 simulations was iteratively enriched with a new run of WALTER until 50 new runs were added. At each iteration, the parameters' values for the new run were selected based on the Expected Improvement (EI) criterion among a set of 10,000 parameter combinations sampled from a 10 dimensions latin hypercube. The EI criterion was computed with the DiceOptim R package (Roustant et al., 2012). The EI criterion is balancing the need to maximize F_{tot} with the need to reduce the uncertainty of the Kriging metamodel.

RESULTS

Outputs from the 5,000 initial simulations are highly variable (**Supplementary Material**), which indicates that at least some of the parameters varying in the LHS design have a strong impact on

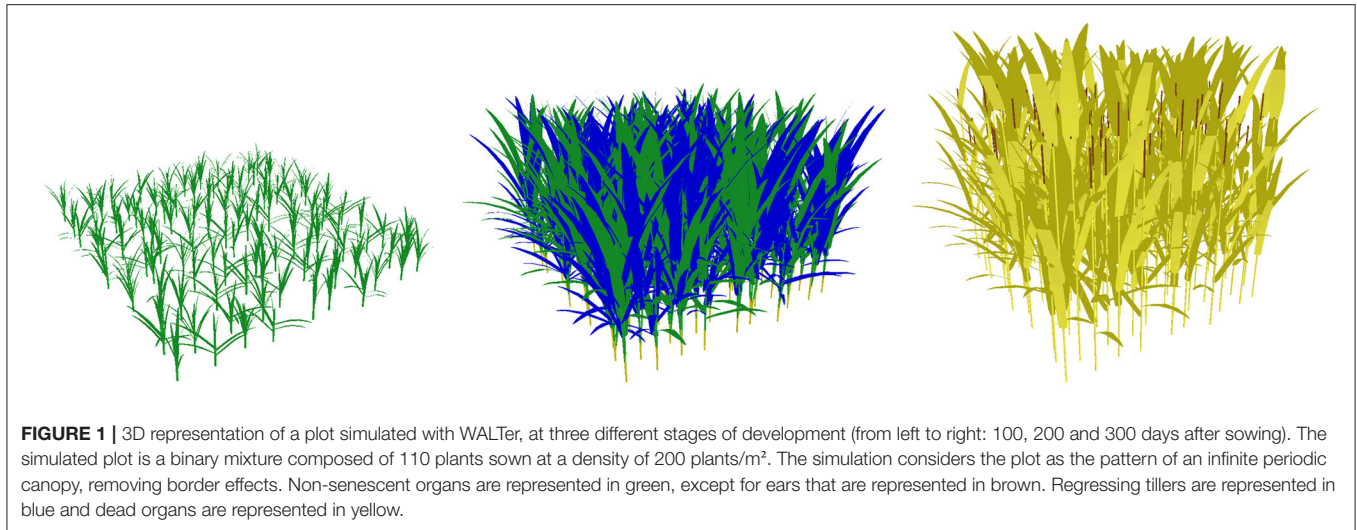


FIGURE 1 | 3D representation of a plot simulated with WALTer, at three different stages of development (from left to right: 100, 200 and 300 days after sowing). The simulated plot is a binary mixture composed of 110 plants sown at a density of 200 plants/m². The simulation considers the plot as the pattern of an infinite periodic canopy, removing border effects. Non-senescent organs are represented in green, except for ears that are represented in brown. Regressing tillers are represented in blue and dead organs are represented in yellow.

the performance of the simulated stands. However, some outputs are much more variable than others. Thus N_{ears} is the most variable output with values ranging from 1.0 to 4.3 ears per plant, even though 98% of the simulations resulted in N_{ears} values below 3.2 ears per plant. On the other hand, L_{perc} is much less variable, with values ranging from 0.76 to 0.96. As for F_{tot} , the initial simulations gave results ranging from 0.73 to 1.5 mol[∘]Cd⁻¹. For the OY indicators, simulations gave values between 0.40 and 1.6 for N_{ears} , and values between 0.67 and 1.9 for F_{tot} . Finally, the OY for L_{perc} was less variable, with values ranging from 0.97 to 1.4.

Sensitivity Analysis

The first-order sensitivity indices (MSI) provide information on the mean influence of the different inputs on each output and the total-order indices (TSI) aggregate mean influence and influence through interactions. The most important architectural traits regarding the performance of the stands are dependent on the considered output (**Figure 2**).

The mean number of ears per plant (N_{ears}) is mainly influenced by first-order effects. For this output the dimensions of the leaves is the most influential trait in the simulated plots as it explains more than 68% of the total variance of N_{ears} (taking into account the effect of the interactions). The mean value of L_{max}^B in the stand has more impact on N_{ears} than the difference of L_{max}^B between varieties (TSI = 0.41 and 0.28, respectively). The tillering capability of the plants in the stand also has an important impact on N_{ears} : $\text{GAI}_c\text{-diff}$ explains around 13% of the total variance of the output and $\text{PAR}_t\text{-ref}$ accounts for about 12%.

For L_{perc} , as for N_{ears} , the input factor with the most influence is $L_{\text{max}}^B\text{-ref}$. Indeed, the variations of $L_{\text{max}}^B\text{-ref}$ explain almost 63% of the total variance of L_{perc} . GAI_c is also an important parameter for light interception with $\text{GAI}_c\text{-ref}$ accounting for about 18% of the total variance of L_{perc} and $\text{GAI}_c\text{-diff}$ explaining more than 10% of its total variance. However, the impact of GAI_c on L_{perc} is mainly explained by interaction effects. Finally, the mean leaf insertion angle in the canopy has an important impact on L_{perc} with a total-order sensitivity index of 0.15 for $\phi_B\text{-ref}$.

All the other input factors have very little influence on the light interception efficiency.

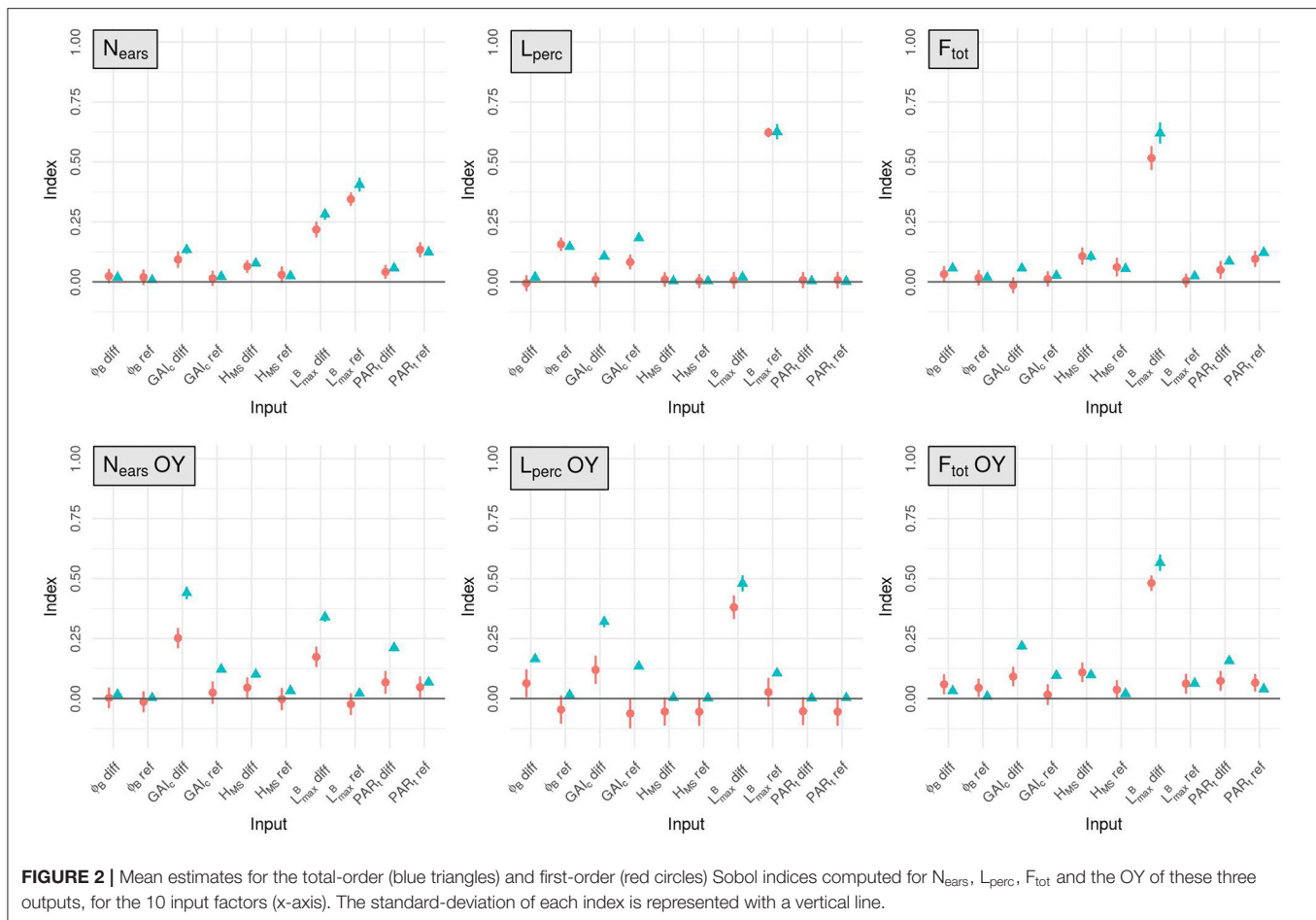
The input factor with the most impact on F_{tot} is $L_{\text{max}}^B\text{-diff}$ (TSI = 0.62). The mean value of PAR_t in the mixture ($\text{PAR}_t\text{-ref}$) and the height differential between varieties ($H_{\text{MS}}\text{-diff}$) also have a lot of influence on F_{tot} , as they explain 12 and 10% of the total variance of this output respectively.

For the OY outputs, the two most influential input factors are always $L_{\text{max}}^B\text{-diff}$ and $\text{GAI}_c\text{-diff}$, regardless of the output considered. Together, these two inputs always explain more than 77% of each OY output. Interestingly, interactions between input factors have an important impact on these outputs, particularly for the OY of N_{ears} and L_{perc} . Nevertheless, differences exist between the different OY outputs. For example, for the OY of N_{ears} , $\text{GAI}_c\text{-diff}$ has more impact than $L_{\text{max}}^B\text{-diff}$, whereas it is $L_{\text{max}}^B\text{-diff}$ that has the most influence on the OY of L_{perc} and F_{tot} .

To a lesser extent, other input factors also have a substantial influence on the OY outputs. $\text{PAR}_t\text{-diff}$, $\text{GAI}_c\text{-ref}$ and $H_{\text{MS}}\text{-diff}$ each explain more than 10% of the total variance of the OY of N_{ears} . The TSI of $\text{PAR}_t\text{-diff}$ for the OY of N_{ears} even reaches 0.21, but this effect is mainly explained by interactions as its MSI is only around 0.067. For the OY of L_{perc} , $\text{GAI}_c\text{-ref}$, $\phi_B\text{-diff}$ and $L_{\text{max}}^B\text{-ref}$ are important factors, each with a TSI >0.1. Finally, $\text{PAR}_t\text{-diff}$ explains almost 16% of the total variance of the OY of F_{tot} .

Optimization

The sensitivity indices (**Figure 2**) only provide information about the relative importance of the different input parameters for the determination of the performance of the stand. Heatmaps representing the outputs of the Kriging metamodels as a function of their most influential parameters (**Figures 3–5**) give more information on how each parameter affects each output. For these figures however, it is important to keep in mind that the less influential parameters were set to the mean value of their range of variation, thus discarding most of the interaction effect that they might have with the other inputs. Another critical point when



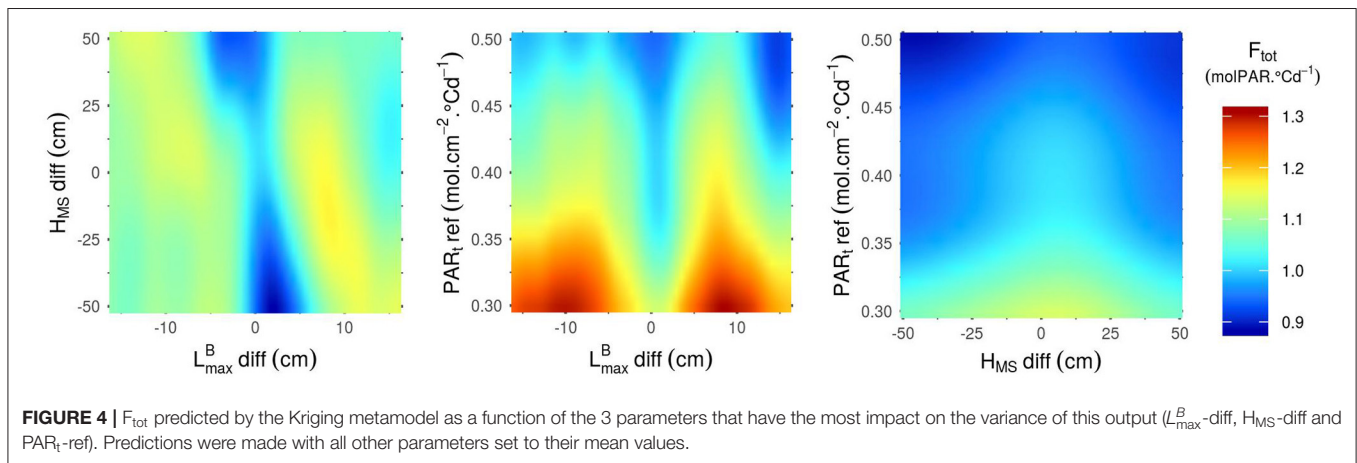
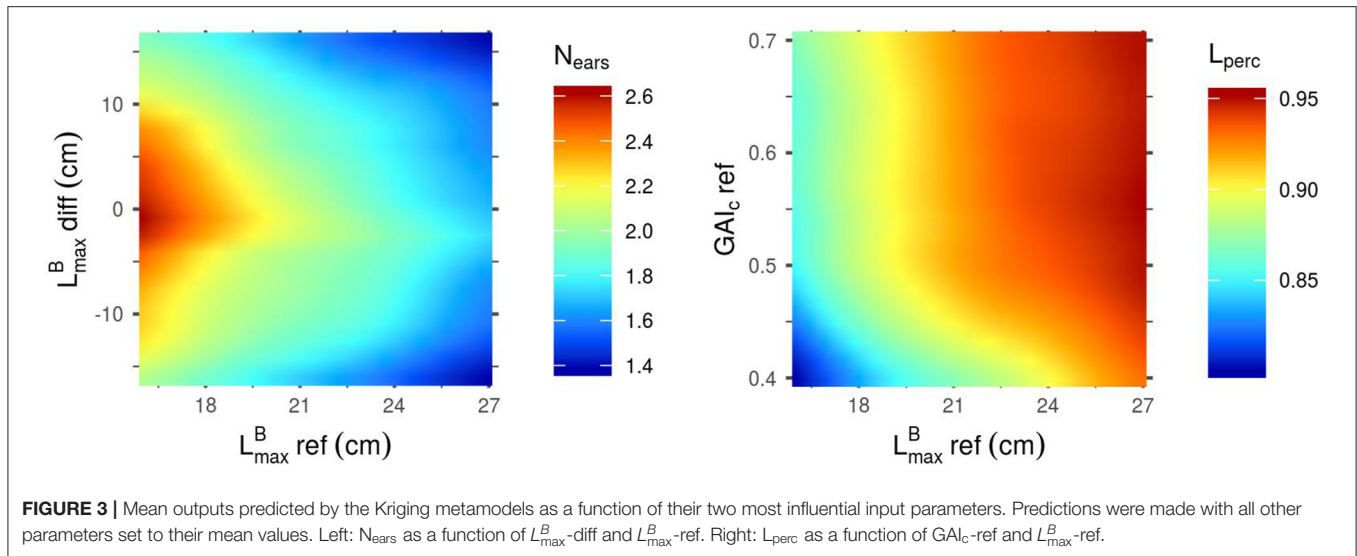
reading these heatmaps is the fact that, when the input variable is a parameter difference, it generates a plot that is symmetric on the axis $\text{diff} = 0$. And if the heatmap is a function of two parameters-diff, there is a central symmetry of the plot.

Figure 3 shows that the mean value of L_{max}^B in the stand ($L_{\text{max}}^B\text{-ref}$) is negatively correlated to N_{ears} , meaning that canopies with small blades tend to produce more spikes than stands with larger leaves. This heatmap also reveals that a differential of L_{max}^B between the cultivars of a mixture is unfavorable for the ear yield in the simulated plots. Unlike N_{ears} , L_{perc} is positively correlated to $L_{\text{max}}^B\text{-ref}$: larger leaves allow for a higher light interception efficiency. L_{perc} is also positively correlated to $\text{GAI}_c\text{-ref}$, but mainly for values of $\text{GAI}_c\text{-ref}$ lower than 0.5: above this value, the impact of $\text{GAI}_c\text{-ref}$ on L_{perc} is very low. This means that L_{perc} is higher for stands in which plants have a low sensitivity to the early competition (high GAI_c values) and can thus emit many tillers.

As for F_{tot} (**Figure 4**), unlike the results observed for N_{ears} (**Figure 3**), a differential of L_{max}^B between the cultivars of a mixture is favorable for the performance of the stand. An absolute differential of around 10 cm between the two varieties in the mixture leads to the highest values of F_{tot} . Moreover, the highest values of F_{tot} can only be reached with low values of $\text{PAR}_t\text{-ref}$.

Indeed, there is a clear negative correlation between $\text{PAR}_t\text{-ref}$ and F_{tot} , which means that stands tend to be more productive when plants in the canopy do not need much light to prevent the regression of their tillers. Finally, even though the impact of $H_{\text{MS}}\text{-diff}$ on F_{tot} is low for the combinations of parameters presented in **Figure 4**, the results show that homogeneous plant height in the canopies lead to higher values of F_{tot} than the mixture of cultivars with a height differential. Interestingly, for mixtures of varieties with a height differential, F_{tot} is higher if the tallest variety is the one with the smallest leaves.

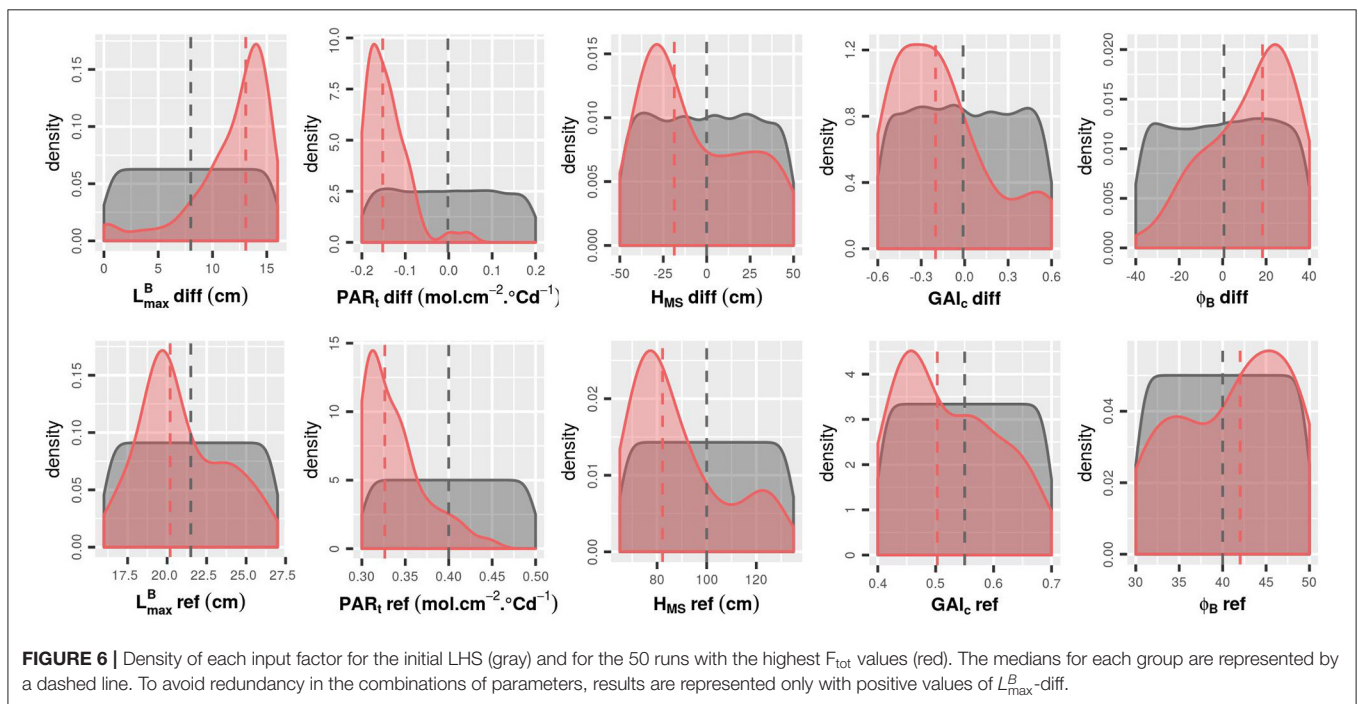
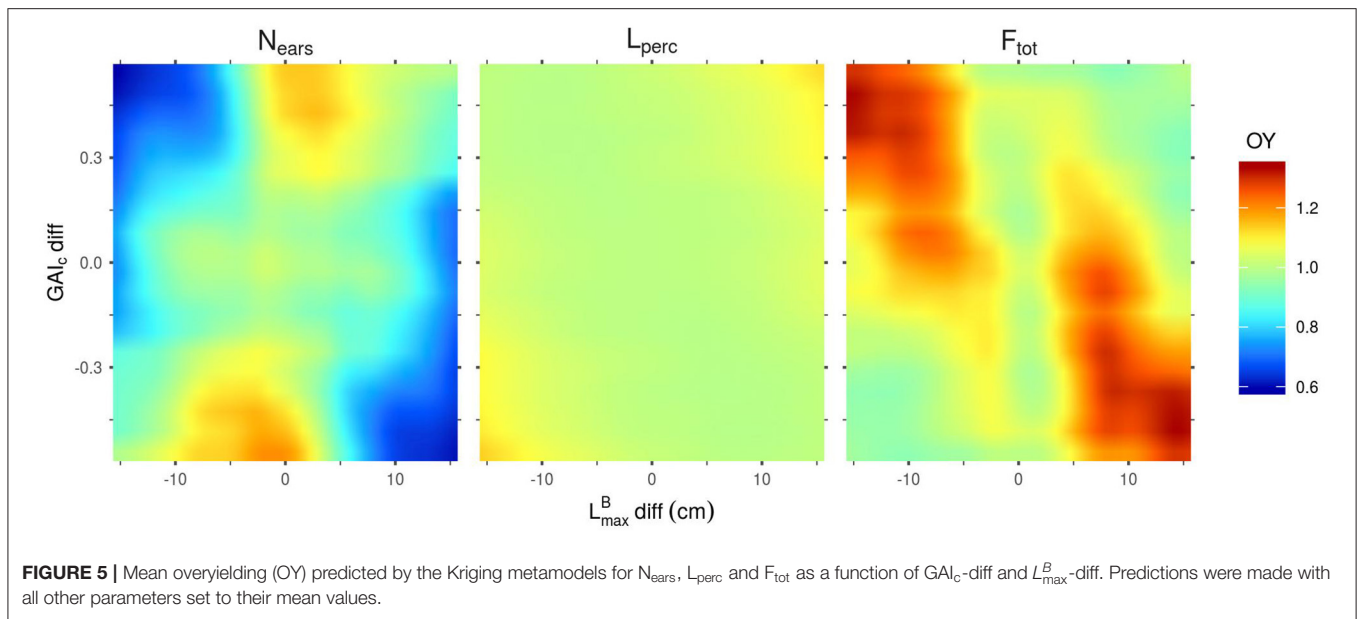
For the OY outputs, $L_{\text{max}}^B\text{-diff}$ and $\text{GAI}_c\text{-diff}$ are always the most influential inputs (**Figure 2**). However, the effect of these two inputs, when the other inputs are fixed to their mean values, are very different depending on the output considered (N_{ears} , L_{perc} or F_{tot}) (**Figure 5**). Firstly, the combinations of parameters presented in **Figure 5** lead almost exclusively to OY values ≥ 1 for F_{tot} . This means that, for these combinations of parameters, mixtures are almost always at least as performant as the mean of their pure components. Conversely, OY values for N_{ears} may be as low as 0.6 and are rarely > 1 . Finally, for L_{perc} , the OY is mostly around 1, with very little variation in its value. For this output, the highest values of OY are obtained for the extreme values of $L_{\text{max}}^B\text{-diff}$ and $\text{GAI}_c\text{-diff}$ by mixing plants with large leaves and



high values of GAI_c with plants with small leaves and low GAI_c values. For N_{ears} , the highest values of OY are also obtained for the extreme values of $\text{GAI}_c\text{-diff}$, but for values of $L_{\text{max}}^B\text{-diff}$ close to 0. For mixtures with a differential in both L_{max}^B and GAI_c , the OY of N_{ears} is more important when the cultivar with the largest leaves is also the one with the highest value of GAI_c , as is the case for the OY of L_{perc} . In contrast, the OY of F_{tot} is the highest when mixing plants with large leaves and low GAI_c values with plants with small leaves and high GAI_c values. Interestingly, despite the observed differences between the combinations of parameters leading to the highest OY for N_{ears} and F_{tot} , N_{ears} is positively correlated to F_{tot} in the overall dataset (data not shown). Finally, it is worth mentioning that the metamodells predict an OY of 1 for mixtures with no trait differential between cultivars (Figure 5), which means that they perform exactly like the corresponding pure stands. This is expected as all the plants in such canopies have identical parameter values. Thus, this result highlights the consistency of the metamodells' predictions.

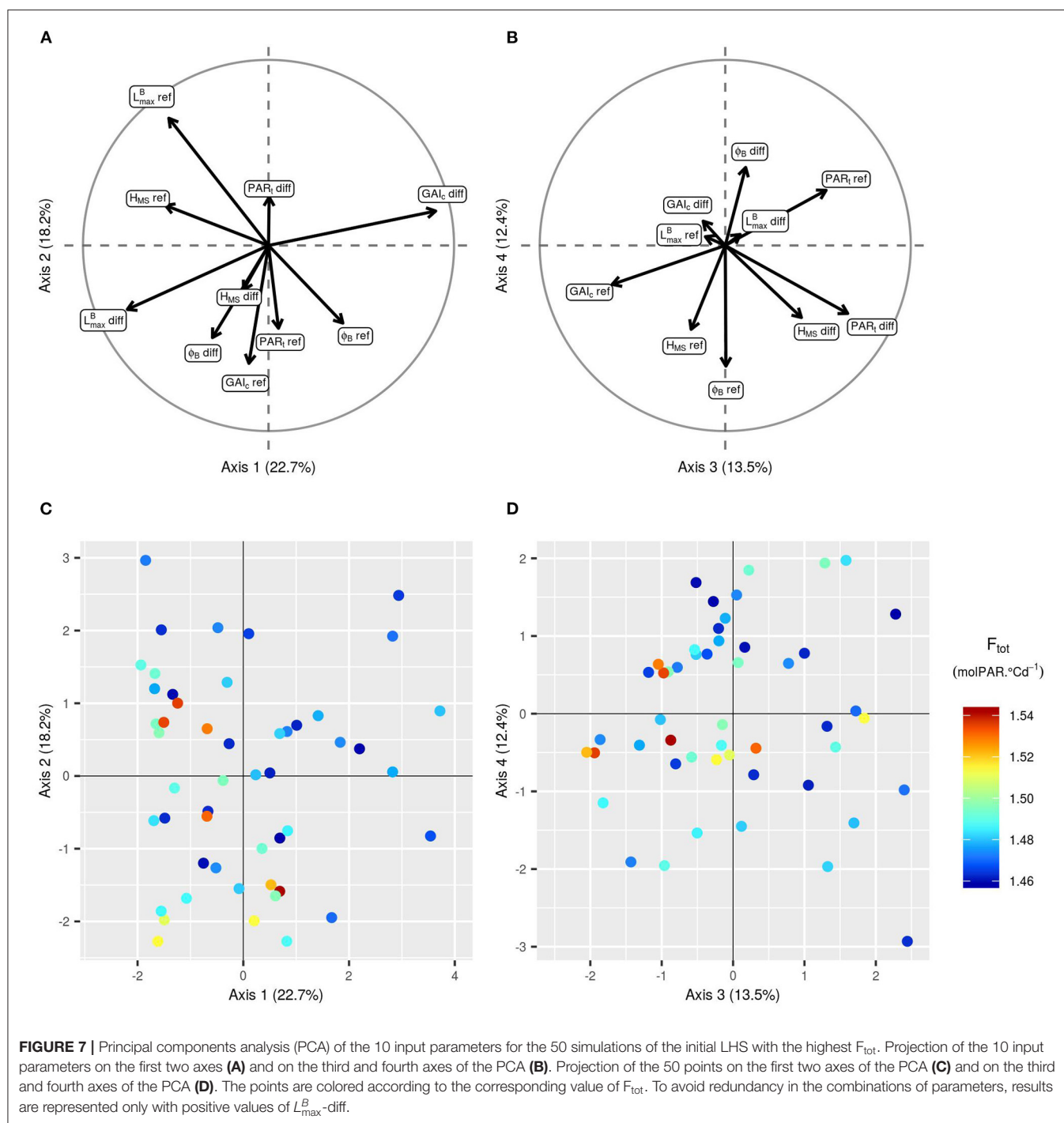
In the initial design, different combinations of parameters can describe identical mixtures (as seen in the heatmaps). For example, considering only $\phi_B\text{-diff}$ and $H_{\text{MS}}\text{-diff}$, a stand with the minimum values of these two parameters is identical to a stand with the maximum values of the two parameters. Indeed both combinations of parameters describe a mixture of tall planophile plants with short erectophile plants. To avoid this redundancy and simplify the analysis of the results, Figures 6, 7, 9 have been represented only with positive values of $L_{\text{max}}^B\text{-diff}$ (all combinations are considered, but the ones with negative values of $L_{\text{max}}^B\text{-diff}$ have been reversed).

Focusing on the 50 runs of the initial design with the highest F_{tot} values (Figures 6, 7), it is possible to identify the trait combinations leading to the best performances. Simulations with high F_{tot} values usually combine high $L_{\text{max}}^B\text{-diff}$ values with low (negative) values of $\text{PAR}_t\text{-diff}$. This combination of parameters describes mixtures in which the genotype with the largest leaves is the one with the lowest value of PAR_t , i.e., the one



less susceptible to tiller regression. However, there is a negative correlation between $L_{\text{max}}^B\text{-diff}$ and $\text{GAI}_c\text{-diff}$ (Figure 7), which means that it is preferable for the performance of the stand that the genotype with the largest leaves ceases tillering early in response to the neighboring competition. Furthermore, mixtures with a differential of leaf insertion angle are more likely to have high F_{tot} values if the genotype with the most erected leaves is the one with the shortest leaves. Finally, the mean values of H_{MS} and PAR_t in the stand should be rather low to allow for high F_{tot} values. This means that canopies of short plants with low tiller regression tend to be the most performant ones.

The addition of new runs of WALTER to the initial LHS design has confirmed the first results. In particular, for the new simulations as for the ones in the initial design, the highest values of F_{tot} are obtained for low values of $\text{PAR}_t\text{-ref}$ (low tiller regression) and for mixtures with a high diversity in leaf dimensions and in susceptibility to tiller regression (Figures 8, 9). When considering the initial design, the five simulations leading to the highest F_{tot} values had variable values of $H_{\text{MS-ref}}$ and $H_{\text{MS-diff}}$ (Figure 9). However, the five best-performing simulations within the 50 new runs added by EGO all had low $H_{\text{MS-ref}}$ values, meaning that high performing

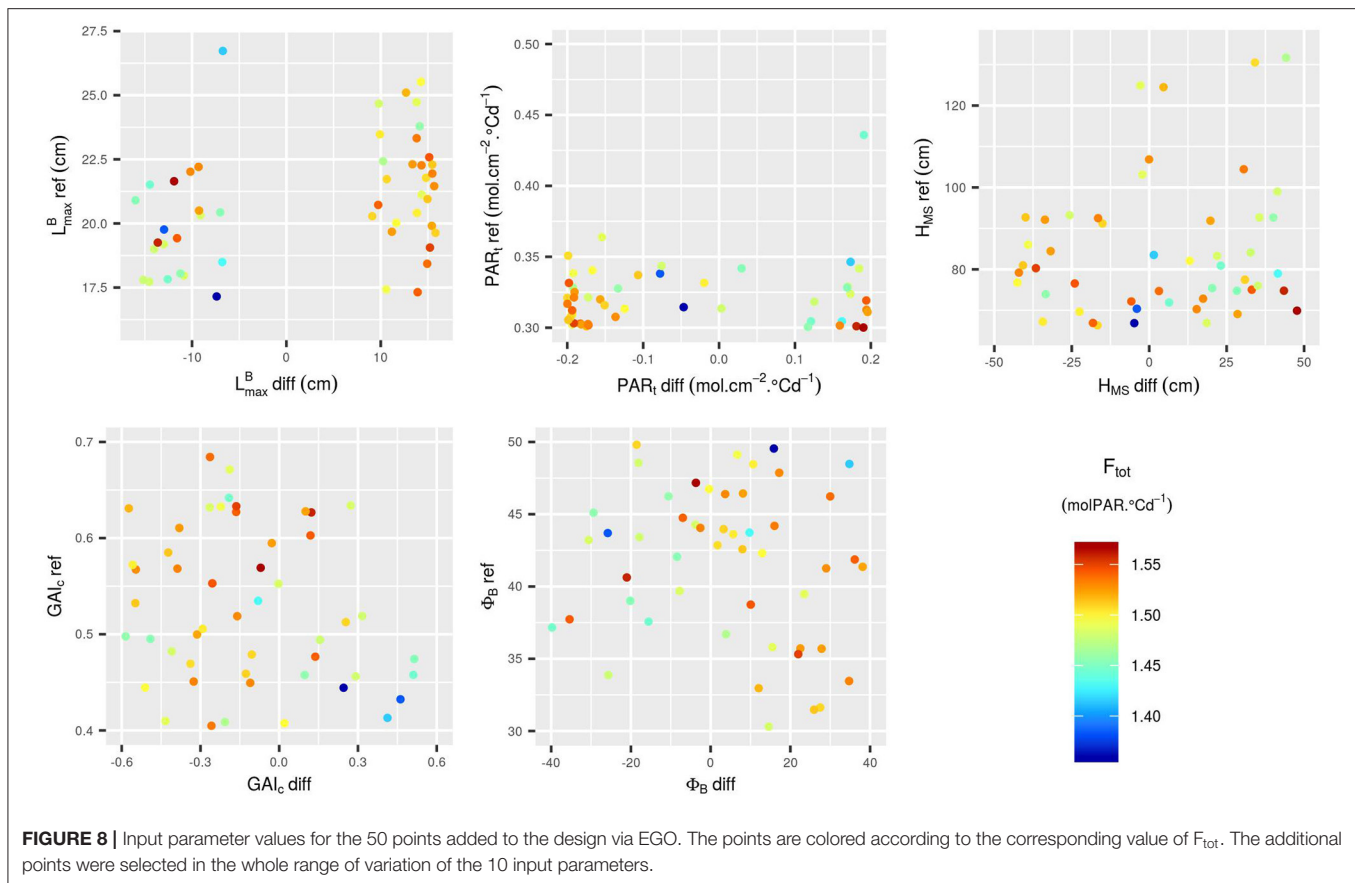


canopies are composed of short plants, as suggested by the results presented in **Figure 6**. Moreover, for the new runs of WALTER, the mixtures offering the highest F_{tot} values presented an important diversity in plant height, and the shorter genotype in the mixture was always the one with the larger leaves. This result is also in accordance with the results presented in **Figure 6**.

DISCUSSION

FSPM as a Tool to Study Heterogeneous Canopies

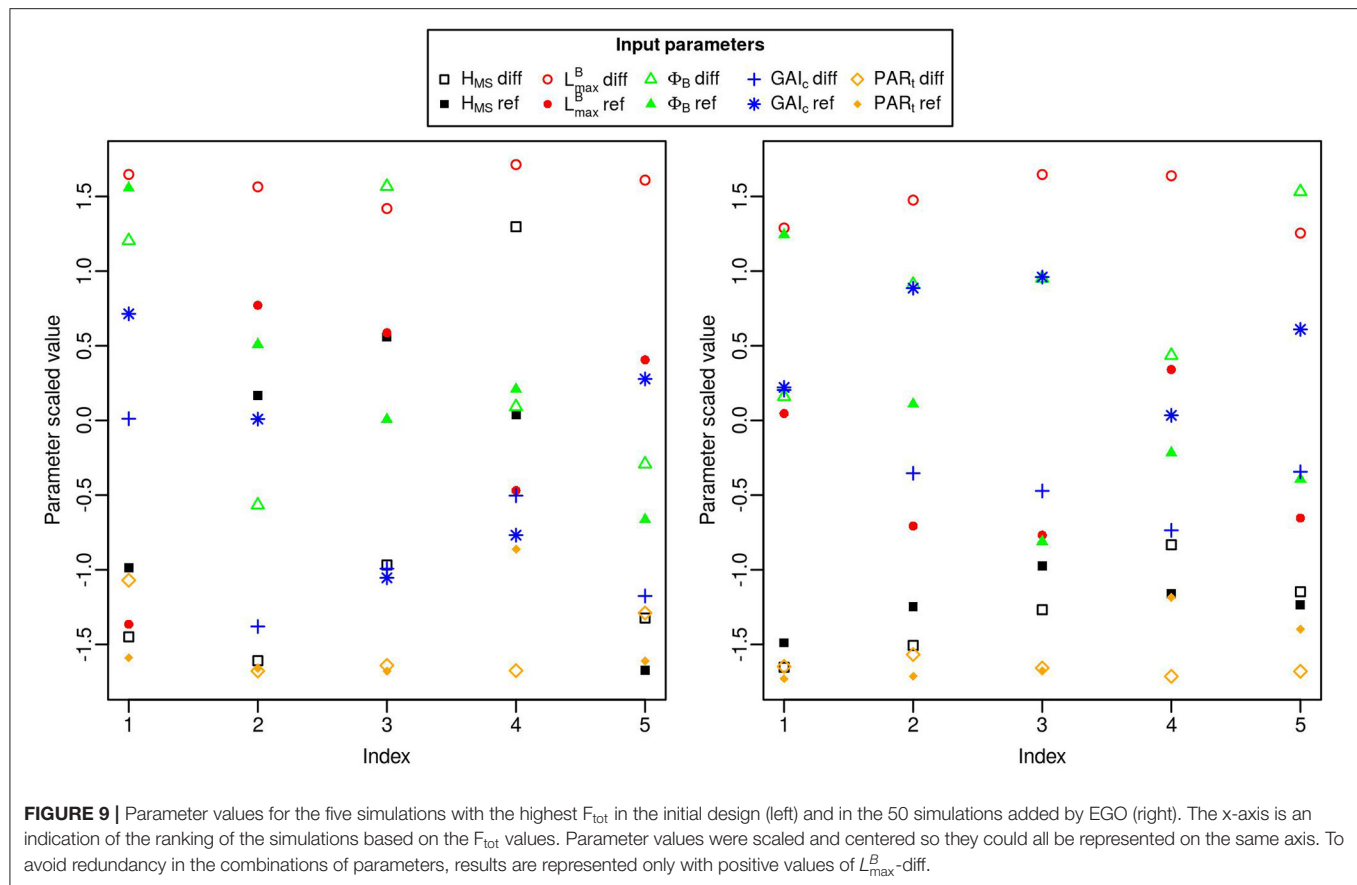
This study illustrates the interest of using FSPMs to explore the impact of architectural traits on the performance of heterogeneous canopies (Evers et al., 2019; Gaudio et al., 2019;



Muller and Martre, 2019; Louarn and Song, 2020; Stomph et al., 2020). Contrary to previous modeling work on wheat cultivar mixtures (Vidal et al., 2018; Barillot et al., 2019), this study focuses on multiple traits and on the entire development cycle of the plants. Thus, it provides a broader insight into the interactions taking place in wheat cultivar mixtures. Nevertheless, as for any modeling exercise (Passioura, 1996), our study presents some approximations that must be considered when interpreting the results. Indeed, even though WALTER allows for a realistic simulation of the tillering dynamics in response to competition for light (Lecarpentier et al., 2019; Blanc et al., 2021), some of the underlying hypotheses of the model should be mentioned. First of all, light is the only environmental factor considered in the model: water and nutrients (nitrogen, phosphorus, potassium, sulfur...) are not explicitly simulated by the model, even though these factors are known to impact the development of wheat. In particular, numerous studies have highlighted the important role of nitrogen in the regulation of tillering (Sparkes et al., 2006; Assuero and Tognetti, 2010; Dornbusch et al., 2011; Alzueta et al., 2012) and taking this factor into account would impact the results. Simulations are thus made with the hypothesis that resources other than light are not limiting. Moreover, plasticity was considered only for tillering, as it is the most plastic trait in response to competition for light in wheat (Lecarpentier, 2017). However, considering plasticity of other traits, and especially

in leaf dimensions, could lead to different results, although it would come at a higher computational cost. Furthermore, the results are also affected by the parameterization of the model. In particular, light interception was computed using only diffuse radiations, because the simulation of more realistic sky conditions (i.e., diffuse and direct radiations) would be significantly more time-consuming. Sky conditions could have an impact on our results and additional information could be provided by the comparison of our simulations with simulations using only direct radiations (Barillot et al., 2019). Nonetheless, our preliminary studies (unpublished data) showed that there were no significant differences between the results obtained with these two conditions when simulating a simple binary mixture. Finally, even though mixtures cultivated by farmers usually combine more than two cultivars, our study focuses on the simulation of binary mixtures, as these simple mixtures allow an efficient exploration of the relations between plant architecture and stand performance.

In contrast to these limitations, the strengths inherent in the modeling approach allowed us to conduct a study that would have been impossible to achieve experimentally. Contrary to experimental studies on wheat mixtures (Borg et al., 2018; Montazeaud et al., 2020), the effect of each architectural trait on the development of the stand could be isolated from any other factor. In addition, it was possible to vary the different traits independently, without including the existing



correlations between architectural traits, such as the one between leaf dimensions and leaf inclination (Ma et al., 2020), even though these biological constraints should be considered when interpreting the results. The sensitivity analysis combined with a metamodeling approach allowed us to take advantage of the complexity of an FSPM while limiting the computational time required. Thanks to the adaptive design, the uncertainty associated with the metamodeling approach was accounted for. Indeed, the addition of new runs of WALTER in areas of the parameter space that led to high values of F_{tot} improved the quality of the metamodel approximation in these areas of interest.

Leaf Dimensions and Tillering Are Major Drivers of Mixture Performance

Our study highlights the impact of the leaf dimensions and the tillering capability on the performance of the simulated mixtures. Indeed, for all the outputs considered, those two traits were the most influential ones among the four architectural traits explored. Leaf dimensions and tillering capability have an important impact on the plant's leaf area index (LAI) whereas leaf inclination and height don't have a direct impact on the LAI but rather on the spatial distribution of the leaf area and on its orientation. Thus, our results suggest that traits impacting the LAI of plants have more influence on the performance of the stand than traits impacting the arrangement of the

leaves. This result is consistent with the well-established effect of LAI on competition for light interception and productivity (Pugnaire and Valladares, 2007). Furthermore, it is in agreement with several studies using FSPMs to simulate trees (Da Silva et al., 2014; Streit et al., 2016; Perez et al., 2018), or maize intercropped with soybean (Munz et al., 2018), in which the traits related to leaf area were identified as the ones with the most influence on light interception. Finally, it is also consistent with the results of the experimental study of Montazeaud et al. (2020), in which the tillering capability was shown to have an important impact on the performance of binary wheat mixtures. The relatively smaller impact of height and leaf inclination on stand performance is congruent with the findings of Munz et al. (2018) on intercropped maize and with the study of Barillot et al. (2019) on leaf inclination in wheat cultivar mixtures. However, even though their impact on mixture performance was less important than that of tillering and leaf dimensions, plant height and leaf inclination still had an important impact on some of the performance indicators. In particular, leaf inclination had an important impact on the light interception efficiency of the simulated canopies (L_{perc}) and on its OY. As for the differential of plant height between the cultivars in mixture, it was one of the most important factors influencing F_{tot} and the OY of N_{ears} .

Contrary to studies on pure stands or on single plants (Sarikioti et al., 2011; Da Silva et al., 2014; Streit et al., 2016; Perez et al., 2018), we were able to distinguish the effect of the trait

differential between cultivars from the effect of the mean value of the trait in the stand, for each trait. Thus, for the leaf dimensions, the mean value in the canopy greatly impacts the light interception efficiency (L_{perc}) and the number of ears produced (N_{ears}), but it is mainly the differential between cultivars that influences the performance of the stand, with a major impact on all outputs except L_{perc} . As for the tillering capability, the differential between cultivars and the mean value in the stand both had an important impact on mixture performance. This is in line with the experimental study conducted by Montazeaud et al. (2020), in which mixture performance was impacted by both average trait values and trait differences. In particular, they showed an important effect of both the average tillering capability and its differential on the OY of grain yield.

Interestingly, modeling studies could guide efforts to address the lack of experimental data on intraspecific mixtures. More precisely, our results suggest that it would be interesting to design experiments mixing varieties with differences in leaf dimensions and tillering capabilities. Although it is difficult to know values of these traits in wheat varieties (due to the lack of phenotypic data and the important investment required for the measurements), this type of experiment could, in turn, help with the biological validation of the models.

Toward the Identification of Assembly Rules

Our results show that the performance of mixtures is very variable depending on the values of the explored architectural traits. In particular, the best performances were achieved by mixing cultivars with different architectures, which is in agreement with numerous studies linking the diversity of functional traits in plant communities to their productivity (Reiss and Drinkwater, 2018). However, some of the worst performances were also achieved by mixing varieties differing in their aerial architecture and the OY indicators were <1 for a significant proportion of the simulated mixtures. This suggests that diversity is not a sufficient criterion to design efficient mixtures and that random-trait assembly will not necessarily lead to better performances than pure stands, as previously stated by Louarn et al. (2020) for grassland mixtures. Overall, these results highlight the importance of establishing assembly rules to design efficient mixtures.

When focusing on the trait configurations leading to the best performances, our results converge toward a single mixture ideotype for the total fitness of the plot (F_{tot}). Indeed, even if some parameters can have variable values in well performing stands, due to their low impact on F_{tot} , there is no multimodality in the distribution of the parameters with high impact on F_{tot} for the best performing simulations. In particular, regarding the mean values of traits in the stand, the best-performing canopies are composed of short plants with low levels of tiller regression. Interestingly, the fact that stands with short plants perform better than stands with taller plants is in line with the assumption of a trade-off between vegetative development and reproduction. As a matter of fact, significant yield gains have been achieved by breeding for wheat cultivars with reduced height, as a shorter

stature limits lodging and improves the harvest index (Gale et al., 1985). In WALTER, the negative correlation between plant height and stand performance is probably due to the formalism of tiller regression. Indeed, tiller regression depends on the amount of light intercepted per unit area, and taller tillers have a larger surface of internodes and sheaths, which makes them more likely to regress.

Regarding the differential of trait values between the two varieties in the mixture, the best-performing stands are the ones with an important diversity in both leaf dimensions and tiller regression. Our results tend to encourage the association of a very competitive genotype with a genotype with poor competitive ability, as the genotype with the largest leaves must also be the one less sensitive to tiller regression in order for the stand to perform well. This conclusion must however be qualified as the cultivar with the largest leaves should also be the shorter one and the one with the earliest cessation of tillering in response to the neighboring competition. Thus, in the best-performing plots, the most productive genotype at maturity was usually not the one that produced the most tillers. Regardless, the high F_{tot} values offered by this trait combination is explained by the very important productivity gain of the cultivar with the largest leaves, which outweighs the loss of productivity of the other cultivar in the mixture. However, it is important to specify that no plant mortality is considered in WALTER and that all individuals produce at least one ear, which is not always the case in experimental settings. In the case of the best-performing configurations that were simulated with WALTER, the cultivar with the smallest leaves had a very low productivity that might have been even lower in field conditions because of potential plant death or individuals with null productivity. Under such experimental conditions, the gain of the cultivar with the largest leaves might not have compensated for the loss in productivity of the other genotype, thus calling for caution when mixing cultivars with significant differences in their competitive ability. Moreover, the results of Montazeaud et al. (2020) showed a negative effect of diversity in tillering capability on the performance of binary mixtures of spring durum wheat sown in rows, which is in contradiction with our results. However, even if the results of our simulations are not meant to be directly translated into assembly rules, they improve our understanding of the interactions taking place in mixtures.

To further improve our understanding, it would be interesting to consider phenological traits, such as the number of days to heading or to maturity, in addition to the architectural ones. Indeed, diversity in phenology could lead to temporal complementarity in addition to the spatial complementarity offered by differences in traits of the aerial architecture. Several studies have emphasized the impact of phenological traits on the performance of wheat mixtures (Borg et al., 2018; Montazeaud et al., 2020). However, these traits were not yet considered in our study because it would require the integration of a specific module to WALTER to ensure that modifications in phenology and their implication on plant development are reliably simulated. Future work could also include the exploration of the effects of some agricultural practices. We simulated balanced mixtures (with 50% of each

genotype) sown at a density of 200 plants/m². However, it is expected that both sowing density and the proportions of the two cultivars in mixture should have an impact on the results (Grace and Tilman, 1990). Moreover, the sowing density has been identified, as expected, as one of the most influential inputs in WALTER (Lecarpentier et al., 2019; Blanc et al., 2021), supporting the interest in exploring variations of this parameter. FSPMs are particularly adapted to explore these agricultural practices (Evers et al., 2019; Gaudio et al., 2019). WALTER could readily be used to consider the effects of variations in both sowing density and cultivar proportion as well as their interactions with architectural traits, albeit with an increased computational cost compared to the present study.

Finally, the trait combinations identified in this study are the ones maximizing F_{tot} , which is a proxy for the number of grains produced by the stand. However, cultivar mixtures can provide a wide variety of ecosystem services besides yield (Barot et al., 2017). Thus, it would be interesting to consider multiple objectives as exemplified by the study of Louarn et al. (2020) in which the stability of grassland mixtures over time was also considered in addition to yield. Considering the stability of the composition of a mixture over time could also be of interest for wheat, as this crop is often resown from year to year. The mixtures that we identified to maximize F_{tot} present significant differences in performance between their two cultivars, which would lead to a drastic evolution of their composition and possibly to a decrease in stand performance over time. Further work with WALTER would be of high interest to identify trait configurations leading to both high performance and mixture stability over time.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EB, JE, and PB conceived the study, designed the simulation experiments and established the statistical framework. Developments to the WALTER model were made by CF, CL, CP, and EB. EB wrote the R-scripts for the analyses and the figures with input from PB and CL. EB wrote the manuscript. All authors reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.734056/full#supplementary-material>

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Cultivar Grain Yield in Durum Wheat-Grain Legume Intercrops Could Be Estimated From Sole Crop Yields and Interspecific Interaction Index

Bochra Kammoun^{1,2}, Etienne-Pascal Journet^{1,3}, Eric Justes^{1†} and Laurent Bedoussac^{4*}

¹ AGIR, Univ Toulouse, INRAE, Castanet-Tolosan, France, ² ARVALIS—Institut du Végétal, Paris, France, ³ LIPME, Univ Toulouse, CNRS, Castanet-Tolosan, France, ⁴ AGIR, Univ Toulouse, ENSFEA, INRAE, Castanet-Tolosan, France

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Nobuhito Sekiya,
Mie University, Japan
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Mohammed VI Polytechnic
University, Morocco

*Correspondence:

Laurent Bedoussac
Laurent.Bedoussac@inrae.fr

†Present address:

Eric Justes,
CIRAD, Persyst Department,
Montpellier, France

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Ensuring food security for a world population projected to reach over nine billion by 2050 while mitigating the environmental impacts and climate change represent the major agricultural challenges. Diversification of the cropping systems using notably cereal-legume mixtures is one key pathway for such agroecological intensification. Indeed, intercropping is recognised as a practice having the potential to increase and stabilise the yields in comparison with sole crops while limiting the use of inputs notably when species exploit resources in a complementary way. However, predicting intercropped species grain yield remains a challenge because the species respond to competition through complex genotype x cropping mode interactions. Here, we hypothesised that the grain yield achieved by a cultivar in low nitrogen input durum wheat-grain legume intercropping (ICs) could be estimated using a few simple variables. The present work is based on a 2-year field experiment carried out in southwestern France using two durum wheat (*Triticum turgidum* L.), four winter pea (*Pisum sativum* L.), and four winter faba bean (*Vicia faba* L.) genotypes with contrasting characteristics, notably in terms of height and precocity, to explore a wide range of durum wheat-grain legume phenotypes combinations to generate variability in terms of yield and species proportion. The major result is that the yield of durum wheat-grain legume IC component in low nitrogen input conditions could be correctly estimated from only three variables: (i) wheat cultivar full density sole crop (SC) yield, (ii) legume cultivar half density sole crop (SC½) yield, and (iii) an indicator of legume cultivar response to interspecific competition. The latter variable, the interspecific interaction index (IE), reveals cultivars' competitive abilities and tolerance to competition. However, to propose generic IC design and management procedures, further mechanistic understanding is required to better understand the links between tolerance to interspecific competition and cultivar phenotype characteristics. In particular, a special emphasis on the grain legume is needed as their response to interspecific competition appears less predictable than that of durum wheat. Cultivar choice is

a key element to optimise the functional complementarity and subsequent IC advantages. This work proposes a simple tool to assist the design of specific breeding programs for cultivars ideotypes adapted to intercropping.

Keywords: cereal, pea (*Pisum sativum* L.), durum wheat (*Triticum turgidum* L.), Faba bean (*Vicia faba* L.), complementarity, competition, model

INTRODUCTION

Global agriculture production will have to provide enough food to a world population projected to reach over 9 billion by the year 2050 (FAO, 2010). This challenge is becoming more complex by taking into account the sustainability issues, such as ensuring the availability of resources for the next generations in the context of climate change. These increasing concerns about the environmental impacts and reduction of inputs require a transformation of current cropping systems towards improved efficiency and sustainability (Jackson and Piper, 1989; Vandermeer et al., 1998).

Improving plant diversity within agricultural systems is increasingly recognised as an important pillar of sustainable development (Davies et al., 2009; IAASTD, 2009). Including a larger proportion of legumes has been proposed as a global solution for long by many authors (e.g., Vandermeer et al., 1998; Altieri, 1999). Indeed, exploiting the leguminous symbiotic fixation of atmospheric N₂ means less nitrogen fertiliser input required (Fustec et al., 2010) contributing to reduced CO₂ emissions (Nieder and Benbi, 2008) and carbon footprints of agricultural products (Gan et al., 2011). Despite this advantage, grain legumes are less favoured now, because of their supposed low yields and instability related to several factors, such as intolerance to water stress, harvest difficulties due to lodging, diseases, sensitivity to insects, or low competition against weeds.

Intercropping is defined as the growth of two or more species in the same space at the same time (Andrew and Kassam, 1976). Among the species mixtures, the cereal–legume intercrops (ICs) appear as one of the promising levers to enhance the efficiency of the agricultural system in a context of low mineral nitrogen level (Jensen, 1996; Bedoussac and Justes, 2010a; Naudin et al., 2010) and low pesticide inputs, and most notably in organic farming to produce legumes (Malézieux et al., 2009; Lithourgidis et al., 2011; Bedoussac et al., 2015). Compared with the sole crops, intercropping is known to (i) boost crop productivity (Qin et al., 2013), (ii) improve yield stability (Raseduzzaman and Jensen, 2017), (iii) increase cereal grain protein concentration (Lithourgidis et al., 2006; Bedoussac and Justes, 2010b), (iv) favour weeds, pests, and diseases control (Altieri and Liebman, 1986), (v) provide better lodging resistance (Trenbath, 1976), (vi) improve soil conservation (Swift et al., 2004), (vii) improve the

use of soil nitrogen (Jensen et al., 2020), or (viii) emit significantly less amounts of greenhouse gases (e.g., Oelhermann et al., 2009; Naudin et al., 2014).

However, optimising the intercropping advantages needs a better understanding of the interactions between: (i) species and cultivars (Ofori and Stern, 1987; Davis and Woolley, 1993; Fukai and Trenbath, 1993; Annicchiarico et al., 2019), (ii) seeding date and density (Davis et al., 1987; Andersen et al., 2007; Barker and Dennett, 2013), (iii) nitrogen availability (Hauggaard-Nielsen, 2001; Corre-Hellou et al., 2006; Bedoussac and Justes, 2010a; Tosti and Guiducci, 2010) altogether in interaction with (iv) climatic and biotic conditions.

Regarding the choice of cultivars within each species, the competitive ability of an IC component is related to some genetic and phenotypic characteristics of cultivars, such as the height and growth dynamics (Davis and Garcia, 1983; Elmore and Jackobs, 1984; Cenpukdee and Fukai, 1992; Annicchiarico et al., 2019). However, the cultivars high yielding in the sole crop are not necessarily high yielding when intercropped due to significant interactions between the genotype and cropping mode (Francis et al., 1978; Francis, 1981; Smith and Zobel, 1991) even though Galwey et al. (1986) could show strong correlations between sorghum cultivar characters in sole crop and when intercropped with cowpea. In addition, some authors (Davis and Garcia, 1983; Elmore and Jackobs, 1984; Cenpukdee and Fukai, 1992) concluded that the intercropped cultivars should reach high yielding levels without affecting the growth of the associated species.

Therefore, specific breeding programs for intercropping are needed (Nelson and Robichaux, 1997; Hauggaard-Nielsen and Jensen, 2001; Barillot et al., 2012; Zajac et al., 2013; Annicchiarico et al., 2019). Recent theoretical developments on the relevant breeding schemes for mixed cropping have been proposed (Annicchiarico et al., 2019; Sampoux et al., 2020; Haug et al., 2021). In particular, Annicchiarico et al. (2019) concluded their review indicating that there is a need for well-focused research on the species, individual traits, and topics that have been overlooked by research. However, the identification of suited characters for intercropped cultivars seems a great challenge since multi-specific stands growth results from an unstable dynamic equilibrium depending on the mutual interaction between the species (Francis, 1981; Davis and Woolley, 1993).

Few crop models have been developed to simulate the species mixtures, such as APSIM (Keating et al., 2003) or STICS (Brisson et al., 2003). Unfortunately, according to Gaudio et al. (2019), their use remains limited notably because they are not fully taking into account the interspecific interactions and in particular the trait plasticity that could explain the

Abbreviations: SC, full density sole crop; SC½, half density sole crop; IC, intercrop; ICE, durum wheat–faba bean intercrop; ICP, durum wheat–pea intercrop; SCW, full density durum wheat sole crop; SC½W, half density durum wheat sole crop; SCF, full density faba bean sole crop; SC½F, half density faba bean sole crop; SCP, full density pea sole crop; SC½P, half density pea sole crop; IE, interspecific interaction index.

behaviour of plants in intercropping. It is also clear that we need to improve our understanding of the ecological processes and dynamical plant–plant interactions involved in the species mixtures and to identify the most relevant parameters including those related to trait plasticity (Gaudio et al., 2019). Therefore, a new toolbox is required, based on the functional ecological principles and modelling approaches before the behaviour of intercropped couples of cultivars could be predicted from their phenotype characteristics.

Our work is based on a 2-year field experiment with durum wheat (*Triticum turgidum* L.)–winter pea (*Pisum sativum* L.) and durum wheat–winter faba bean (*Vicia faba* L.) using two wheat, four pea, and four faba bean genotypes with contrasting characteristics, notably in terms of height and precocity. This allows exploring a wide range of durum wheat–grain legume phenotypes combinations to generate variability in terms of yield and species proportion. The main objective of the present work is to propose and assess a simple statistical model as a proof of concept. The model design was done to represent the interspecific interactions as a whole to estimate the grain yield achieved by each component in durum wheat–grain legume intercropping considering that it depends on: (i) the cultivars grain yield in sole cropping, (ii) the cultivars response to sowing density when it is different in IC and sole crop, and (iii) the cultivars response to interspecific competition.

MATERIALS AND METHODS

Site, Climate, and Soil

The experiments were located at the French National Research Institute for Agriculture, Food, and Environment (INRAE) experimental station in Auzeville, southwestern France (43°31'38"N, 1°30'22"E) in 2011–2012 (Exp.I) and 2012–2013 (Exp.II). Exp.I was characterised by a very unusual cold period in February (**Figure 1**) with extremum of −12°C and an average daily temperature of 1.6°C (vs. 6.6°C for the 10-year mean). The rainfall during the growing season (November to July) was (**Figure 1**) 405 and 658 mm for Exp.I and Exp.II, respectively (vs. 450 mm for the 10-year mean). The rainfall during the February–June period was 224 and 387 mm for Exp.I and Exp.II, respectively (vs. 251 mm for the 10-year mean).

The experiments were carried out on two different experimental fields separated by a dirt road with a clay loamy soil containing 39% clay, 41% silt, and 20% sand in Exp.I and 30% clay, 30% silt, and 41% sand in Exp.II. The field water capacities were 305 and 335 mm on 0–120 cm and soil water content at sowing was 259 and 230 mm for Exp.I and Exp.II, respectively. Total inorganic nitrogen at sowing was 36 and 41 kg nitrogen ha^{−1} on 0–120 cm depth for Exp.I and Exp.II, respectively. For both experiments, the previous crop was sunflower (*Helianthus annuus*).

Experimental Design

A total of ten cultivars either commercially available or under development were used and chosen within each species for their contrasting height and precocity attributes (**Table 1**): (i) two of durum wheat (W; L1823 and Scultpur), (ii) four of

winter faba bean (F; Castel, Diver, Irena, and Nordica), and (iii) four semi-leafless of winter pea (P) with determinate growth, either insensitive (AOPH10, Isard, and Lucy) or sensitive to photoperiod (Geronimo).

The species and cultivars were grown as (i) full density sole crops (SCs; sown at 336, 29, and 96 grains m^{−2} for wheat, faba bean, and pea, respectively, i.e., 120% of the targeted final plant density), (ii) half density sole crops (SC½; sown at half of the SC density), and (iii) durum wheat–grain legume substitutive ICs (with species mixed on the rows and sown at half the SC density). According to Cruz and Soussana (1997), such a design aims to distinguish and evaluate: (i) interspecific competition when comparing SC½ and IC, and (ii) intraspecific competition when comparing the SC and SC½.

Note that since the faba bean target density was low for SC½ and IC (12 plants m^{−2}), it was sown at three times higher density and controlled by manual removal after emergence to obtain a regular plant distribution pattern.

The experimental layout was a randomised split-plot design with three replicates for each combination of cropping treatment, species and cultivar. Each subplot (22.4 m²) consisted of 10 rows (14 m-long and spaced 16 cm apart). The fungicide-treated seeds were sown on 14 November 2011 (Exp.I) and 20 November 2012 (Exp.II). No fertiliser was applied while fungal diseases and pests (mainly pea weevils and aphids) were controlled with appropriate pesticides in two applications (one fungicide and one insecticide in Exp.I vs. two fungicides in Exp.II).

The plant densities were measured in each plot on a total of 3 and 10 linear metres (lm) for wheat and legumes, respectively. Aboveground plant parts from the six central rows were mechanically harvested at grain legume maturity for sole cropped legume and at wheat maturity for the IC and wheat sole crop. The samples were dried at 80°C for 48 h, and grain dry weights were determined separating those from IC into wheat and legume.

Calculations and Statistics

Interspecific Interaction Index (IE) for Yield

Interspecific interaction index (IE) allows evaluating the effect of an IC component cultivar on the second IC component by comparing the yield of the second component achieved in IC with that in SC½ (Bedoussac and Justes, 2011) as follows:

$$IE_{Wheat} = \frac{Yield_{ICWheat}}{Yield_{SC1/2Wheat}}; IE_{Pea} = \frac{Yield_{ICPea}}{Yield_{SC1/2Pea}}; IE_{Faba\ bean} = \frac{Yield_{ICFaba\ bean}}{Yield_{SC1/2Faba\ bean}}$$

where $Yield_{SC1/2}$ and $Yield_{IC}$ are the SC½ and IC grain yields per unit area. IE was calculated for each intercrop replicate using the replicate value for the numerator and the mean value over all the three replicates for the denominator to eliminate the variation in the ratio caused by SC½ yield variability.

Statistics

The statistical analyses were performed using STATGRAPHICS software (version 15.2.06, Statgraphics Technologies, Inc., VA, USA). All data were tested for normal distribution using the

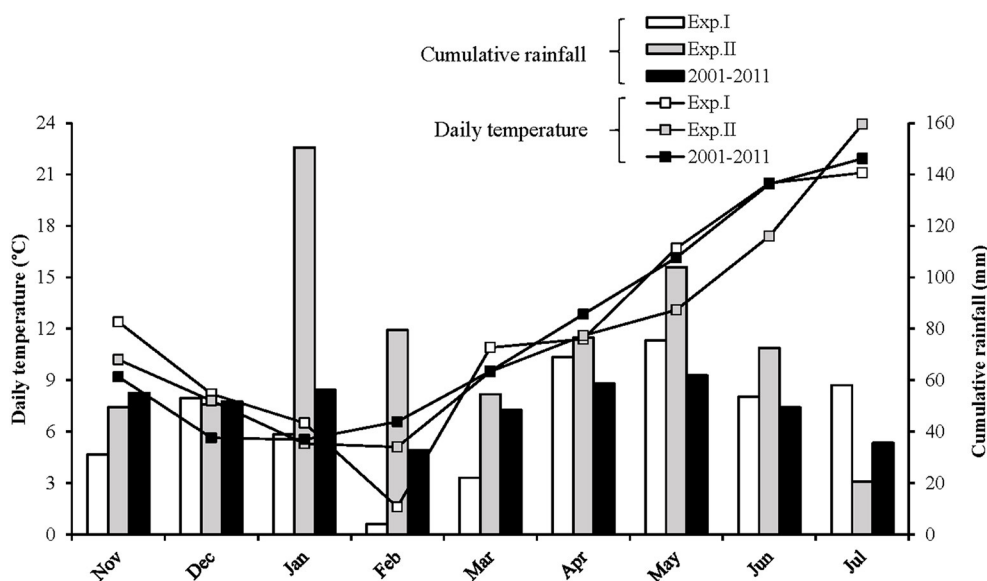


FIGURE 1 | Weather characteristics of the French National Research Institute for Agriculture, Food, and Environment (INRAE) experimental station in Auzeville, southwestern France (43°31'38"N, 1°30'22"E) in 2011–2012 (Exp. I), 2012–2013 (Exp. II), and 2001–2011 (10-year mean).

TABLE 1 | Characteristics of the cultivars used in this study.

Species	Cultivar	Breeder	Registration	Height	Precocity
Durum Wheat	L1823	INRA	Unregistered	++	++
	Sculptur	RAGT	2007	+	++
Faba Bean	Castel	SCA Epis-Sem	1987	++	++
	Diver	AgriObtentions	2008	+	+
	Irena	AgriObtentions	2002	+	+++
	Nordica	NPZ/Serasem	2010	++	+
	AOPH10	AgriObtentions	Unregistered	+++	++
Winter Pea	Geronimo	Serasem/RAGT	2011	++	+
	Isard	INRA/AgriObtentions	2005	+	+++
	Lucy	GAE/Serasem	2000	+	++

Class assignment (+: low/late; ++: medium; +++: high/early) for cultivar height at the end of flowering for legumes and the end of heading for wheat and precocity (timing of flowering initiation stage for legumes and of heading initiation stage for wheat).

Shapiro–Wilk test and the pairwise comparisons were performed with the least significant difference test (LSD) at a threshold of $p = 0.05$ (Gomez and Gomez, 1984) to compare grain yields among the species, cultivars, and cropping mode. One-tailed t -test was applied to compare the means of IE to 1. The prediction interval ellipses were used to describe the area in which a single new observation can be expected to fall with a probability of $p = 0.90$, given that the new observation comes from a bivariate normal distribution with the parameters (means, SDs, and covariance) as estimated from the observed points shown in the plot for ICs (Batschelet, 1981).

RESULTS AND DISCUSSION

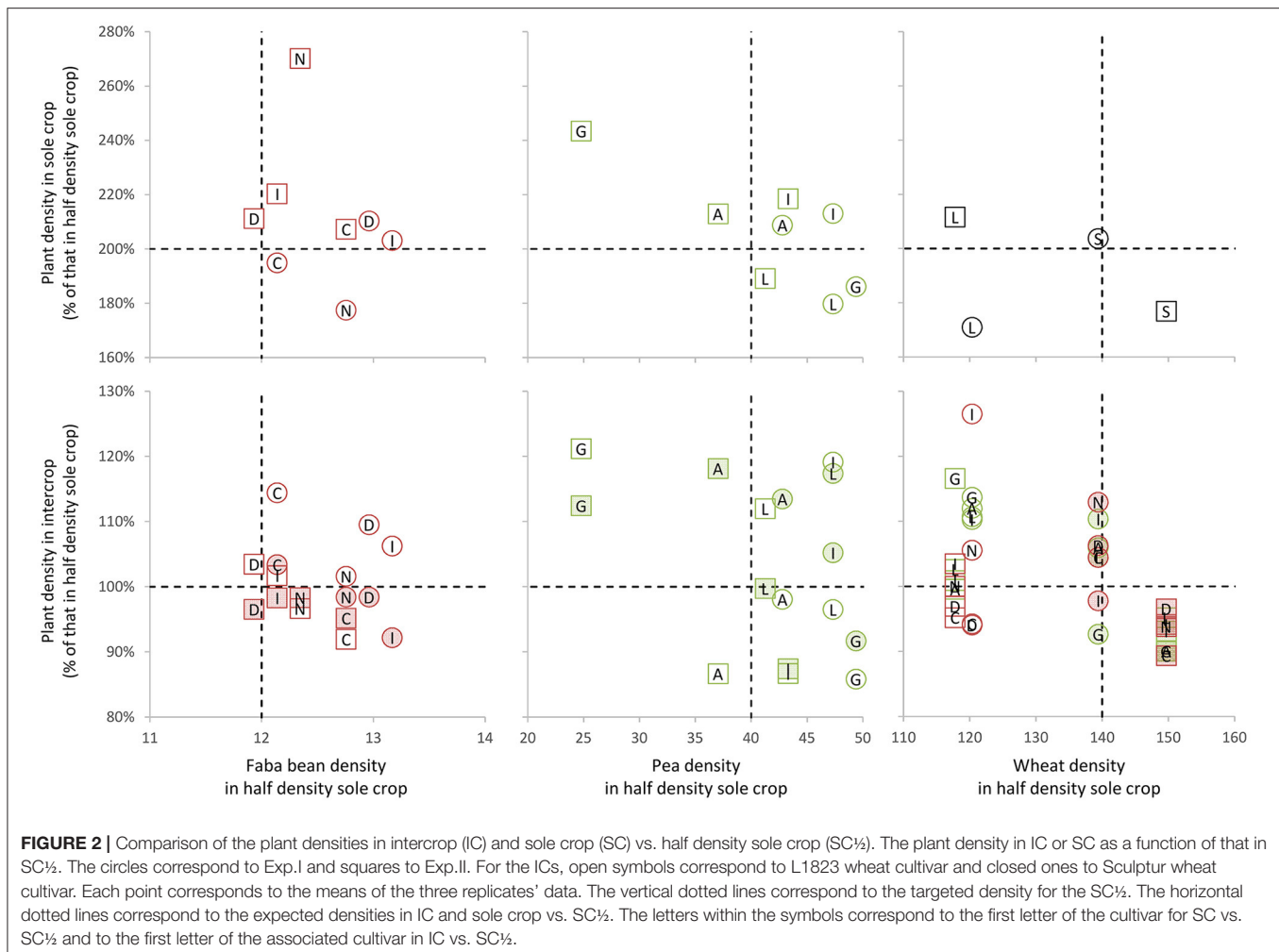
Emergence and Plant Densities

On average for all the treatments and the two experiments, the plant density was close to the objective with observed plant

density representing 107% of the expected values for faba bean (105% in IC, 110% in SC, and 104% in SC½), 106% for pea (107% in IC, 106% in SC, and 104% in SC½), and 93% for wheat (96% in IC, 90% in SC, and 94% in SC½).

As illustrated in **Figure 2**, the plant densities in IC were very similar to those in SC½ (103 ± 13, 100 ± 6, 102 ± 9%, for pea, faba bean, and wheat, respectively). The plant densities in SC were nearly two times higher than those in the SC½ (206 ± 19, 212 ± 25, 191 ± 17%, for pea, faba bean, and wheat, respectively).

No difference was found between the two experiments except for pea with lower plant density in Exp.II than in Exp.I (94 and 117% of the expected density, respectively). A significant difference ($p < 0.01$) was found between the wheat cultivars for both experiments (85 and 101% of the expected density for L1823 and Sculptur, respectively). No difference was found between the faba bean cultivars (104–108% of the expected density) and between the pea cultivars except for Geronimo



that had significantly ($p < 0.001$) lower density in Exp.II than the other three cultivars (70 and 102% of the expected density, respectively).

Species Yields in Durum Wheat–Grain Legume Intercrop Depend Partially on Their Sole Crop Yields

Legume Yield in Durum Wheat–Grain Legume Intercrop and Sole Crop

The legumes grain yield achieved in IC (1.8 Mg ha^{-1}) is always significantly lower ($p < 0.10$) than the corresponding SC yield (3.5 Mg ha^{-1}), due to both the response to density and interspecific competition, and slightly correlated to it (Figure 3A). Legume grain yield achieved in IC is always significantly lower ($p < 0.10$) than the corresponding $\text{SC}\frac{1}{2}$ grain yield (3.4 Mg ha^{-1} ; Figure 3B) due to interspecific competition only.

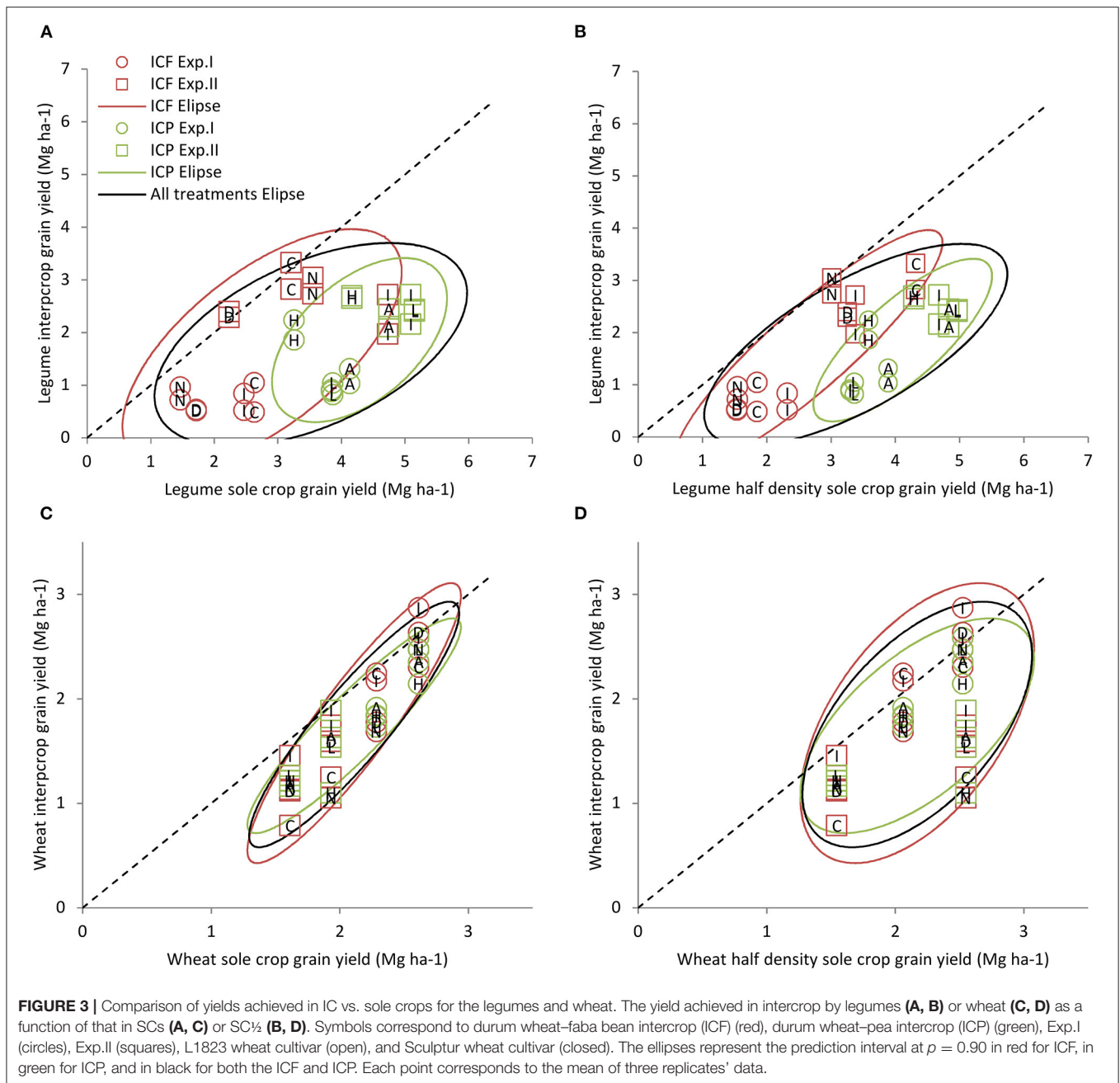
For both legumes, the SC and $\text{SC}\frac{1}{2}$ grain yields were similar, underlining the ability of the legumes to compensate for lower densities. In addition, Figures 3A,B show that the behaviours of the two legumes are different as illustrated by distinguishable

ellipses. More precisely, pea produced a higher yield than faba bean in both SC (4.3 vs. 2.7 Mg ha^{-1} , respectively) and $\text{SC}\frac{1}{2}$ (4.1 vs. 2.7 Mg ha^{-1} , respectively) while they yielded similarly in the IC (1.9 and 1.7 Mg ha^{-1} , respectively). Therefore, the grain yield loss between $\text{SC}\frac{1}{2}$ and IC was higher for pea than for faba bean, suggesting that pea is more sensitive than faba bean to wheat competition.

Wheat Yield in Durum Wheat–Grain Legume IC and Sole Crop

For the wheat, yield achieved in IC was on average slightly lower than that in $\text{SC}\frac{1}{2}$ (1.8 vs. 2.2 Mg ha^{-1} , respectively) indicating a limited competition by the legume in IC (Figure 3D). Additionally, the wheat yield achieved in IC did not vary on average with the associated species [1.8 and 1.7 Mg ha^{-1} for durum wheat–faba bean intercrop (ICF) and durum wheat–pea intercrop (ICP), respectively] but depended on the associated cultivar (Figures 3C,D).

The wheat yield in SC (2.1 Mg ha^{-1}) was similar to that in $\text{SC}\frac{1}{2}$ underlining the well-known ability of wheat to compensate for low density. The correlation between IC and $\text{SC}\frac{1}{2}$ wheat grain yield, which reveals only the response to interspecific



competition, was worse (**Figure 3D**) compared with that between IC and SC (**Figure 3C**) corresponding to the response to both the density and interspecific competition.

These results confirm that the production of a given species in IC cannot be easily predicted neither from its SC or SC½ yields due to the species responses to density and interspecific competition. Therefore, the best varieties for sole cropping are not necessarily the best ones for intercropping, in line with the results obtained by, e.g., Francis et al. (1978) or Smith and Zobel (1991). These results also revealed the limits of the land equivalent ratio (LER; Willey and Osiru, 1972) defined

as the relative land area required when growing sole crops to produce, the yield achieved in an IC with the same species proportion. The land equivalent ratio is used in about 11% of the articles on intercropping published between 2000 and 2010 (Bedoussac et al., 2015) and is a relevant indicator to quantify mixture productivity per unit of soil surface for yield as compared with the sole crops. The land equivalent ratio has a didactic virtue to assess the IC performance due to the final balance of competition, complementarity, cooperation, and compensation between the species as named “the 4C approach” by Justes et al. (2021). Nevertheless, the LER cannot identify the intraspecific

and interspecific interactions because it is dependent on the sole crop reference and reveals the species responses to both the intraspecific and interspecific competition (Jolliffe, 2000; Bedoussac and Justes, 2011).

In Durum Wheat–Grain Legume IC, the Yield of a Species Depends Negatively on That of the Associated Species

Figures 4A,B show that the higher the wheat yield in the mixture the lower that of the legume and conversely. Exp.I is characterised by a high wheat yield in the mixture representing 76 and 63% of the total IC grain yield in ICF and ICP, respectively. The converse was observed in Exp.II with the wheat representing only 32 and 36% of the total IC grain yield in ICF and ICP, respectively. Because of the balance between the two associated crops, the total durum wheat–grain legume ICs grain yield remained statistically stable at $p = 0.05$ with ICF and ICP total grain yield varying from ± 14 and $\pm 6\%$, respectively when compared with the average yield over the two experiments.

As the main soil characteristics, such as mineral nitrogen availability, was very similar between the two experiments, the differences in the SC, SC $\frac{1}{2}$, and IC yields were very probably explained by the climatic conditions. Indeed, they were drastically different and could explain the inversion of the yield proportions between the two experiments though only partially.

Climatic Conditions Partially Explain the Inversion of the Yield Proportions

The legumes grain yields (**Figures 3A,B**) were significantly lower ($p < 0.01$) in Exp.I compared with Exp.II (respectively, 1.0 vs. 2.6 Mg ha $^{-1}$ for IC, 2.9 vs. 4.1 Mg ha $^{-1}$ for SC, and 2.7 vs. 4.1 Mg ha $^{-1}$ for SC $\frac{1}{2}$). Conversely, the wheat grain yields (**Figures 3C,D**) were significantly higher ($p < 0.01$) in Exp.I than in Exp.II (respectively, 2.2 vs. 1.3 Mg ha $^{-1}$ for IC, 2.4 vs. 1.8 Mg ha $^{-1}$ for SC, and 2.3 vs. 2.0 Mg ha $^{-1}$ for SC $\frac{1}{2}$). Our results show that the pea yields varied less than those of the faba bean (± 19 vs. $\pm 40\%$, respectively compared with the average yield over the two experiments). This tends to indicate that pea was less sensitive than faba bean to our contrasting climatic conditions.

The Exp.I was indeed characterised by a very cold winter that affected the legume growth more than the wheat growth. In particular, the frost damage symptoms in Exp.I were obvious on both the legumes but especially severe on the faba bean shoots and upper tap roots, resulting in lethality on some plants and several weeks delay before growth resumption. Conversely, the climatic conditions of Exp.II have been favourable to both legumes growth due to the wet spring while it negatively affected the wheat grain yield.

In Exp.I, the wheat proportion in IC is higher than that which would be directly anticipated from the SC yields. This shows that the cereal was more competitive than the legume, in line with a number of reports concluding on the higher competitive ability of the cereal (Jensen, 1996; Hauggaard-Nielsen et al., 2001). However, this dominance potential was probably restricted by the low mineral nitrogen availability which is known as a less profitable situation for the cereal, high nitrogen demanding crop.

In Exp.II, the wheat proportion in IC is similar to that which would be directly anticipated from the SC yields. Thus, even if the cereal was potentially more competitive than the legume, the climatic conditions in Exp.II with less favourable conditions to the cereal than to the legume has probably influenced the competitive balance between the crops. These results confirmed that the species production in IC cannot be predicted from the SC yields only, because of complex genotype $\times$ cropping mode interactions and species responses to interspecific competition in IC.

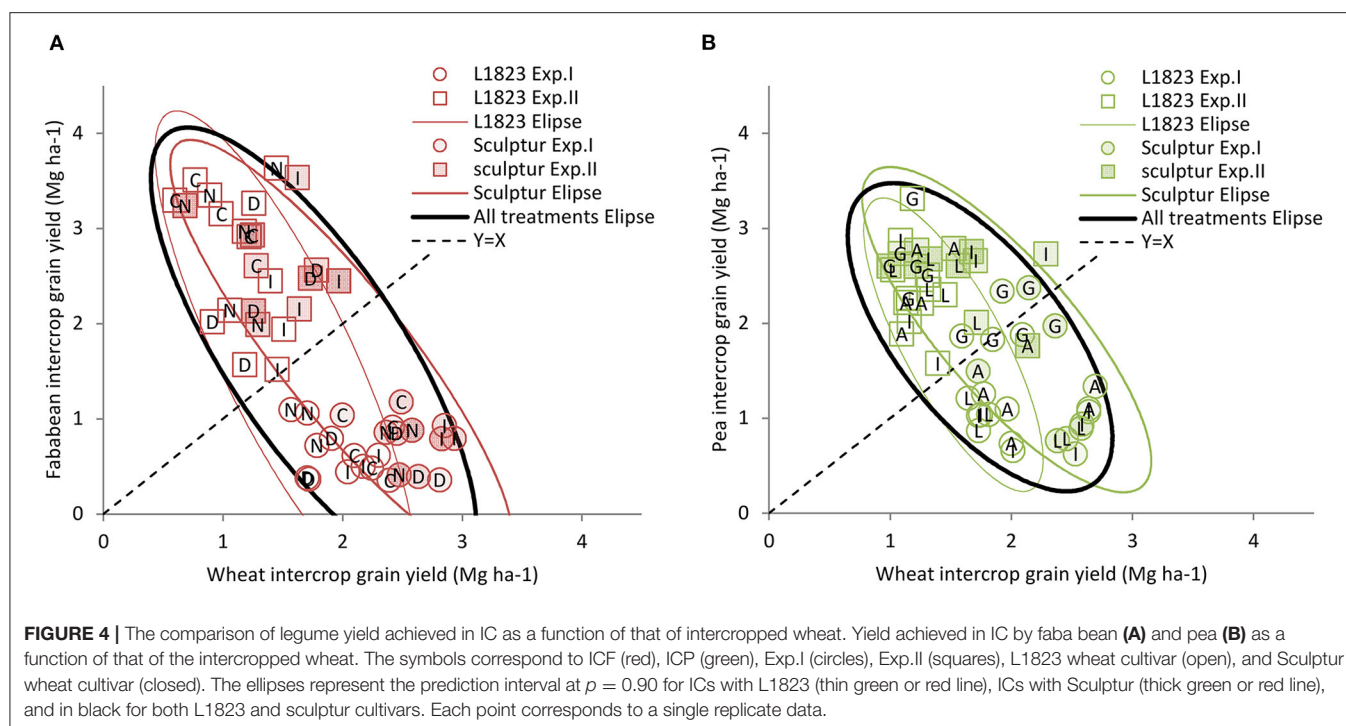
Species Production in Durum Wheat–Grain Legume IC Depends Also on Genotype $\times$ Genotype Interactions

Considering durum wheat–grain legume IC yields of the various cultivars of each species, the wheat L1823 had a lower yield compared with Sculptur (**Figure 4**; 1.5 and 2.0 Mg ha $^{-1}$, respectively on average for ICP and ICF and the two experiments). Surprisingly, the grain legume yield was similar with L1823 and Sculptur (**Figure 4**; 1.7 and 1.8 Mg ha $^{-1}$, respectively on average over ICP and ICF and the two experiments). Consequently, the whole durum wheat–grain legume IC grain yield was lower ($p < 0.01$) with L1823 than with Sculptur (**Figure 4**; 3.2 and 3.8 Mg ha $^{-1}$, respectively on average over ICF and ICP and the two experiments).

No significant difference ($p > 0.10$) was observed between the faba bean or pea cultivars for their effect on the total IC grain yield on average over the two experiments (values ranging from 3.2 to 3.6 Mg ha $^{-1}$ for faba bean cultivars and from 3.4 to 3.9 Mg ha $^{-1}$ for pea cultivars; **Figure 4**). However, the legumes cultivars showed distinct behaviours in their productivity in IC (**Figure 4**) with: (i) for faba bean cultivars Diver and Irena producing lower yields (1.4 and 1.5 Mg ha $^{-1}$, respectively) than Castel and Nordica (2.0 and 1.9 Mg ha $^{-1}$, respectively) and (ii) for pea cultivars Lucy, Isard, and AOPH10 producing lower yields (1.7 Mg ha $^{-1}$) than Geronimo (2.4 Mg ha $^{-1}$, respectively). Consequently, the wheat grain yield was significantly ($p < 0.01$) higher (**Figure 4**) when intercropped with Diver and Irena (1.8 and 2.1 Mg ha $^{-1}$, respectively) than with Castel and Nordica (1.6 Mg ha $^{-1}$ for both). Conversely, the wheat grain yield in IC was not affected by the pea cultivars (values ranging from 1.6 to 1.9 Mg ha $^{-1}$; **Figure 4**).

These results indicate that the total durum wheat–grain legume IC yield and its composition is not only determined by the choice of the two intercropped species but also by each species cultivars, in line with other studies (Davis and Woolley, 1993; Nelson and Robichaux, 1997; Hauggaard-Nielsen and Jensen, 2001). In fact, the cultivars within a species display diverse characteristics which contribute to determining the level of complementarity or competition with the second species in IC, leading to genotype $\times$ genotype interactions with the effect of cultivars which can be higher than that of species.

These elements can be illustrated by the high complementarity obtained with the photoperiod-sensitive cultivar Geronimo. This could be the consequence of a delayed and later vegetative growth combined with its more active stem branching



(Weller et al., 1997; Lejeune-Hénaut et al., 2008). Indeed, this possibly results in the different times in peak requirements for resources such as nitrogen and light and thus characterising an over-time complementarity situation (Bedoussac and Justes, 2010b). Thus, these results confirm that the species production in IC depends on genotype $\times$ genotype interactions which must be taken into account for modelling approaches to estimate the yield achieved in IC from the SC data.

Interspecific Interaction Index Value Depends on the Associated Species Yield

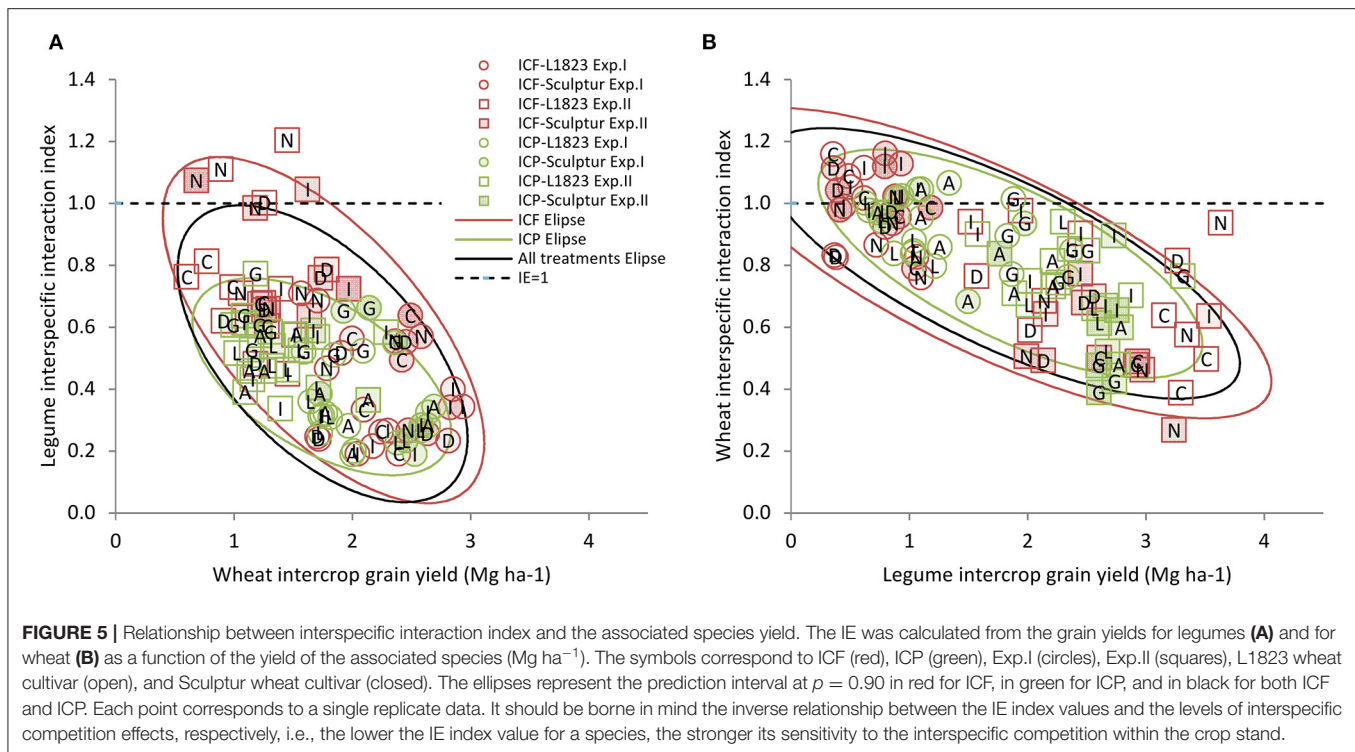
Interspecific interactions can only be relevantly analysed by comparing the ICs with sole crops sown at half-density and not directly with sole crops sown at normal density as for the land equivalent ratio (Bedoussac and Justes, 2011). The IE index is an indicator that compares the production of one component species in IC with its production in SC $\frac{1}{2}$. The IE index thus reflects the intensity of the interspecific competition effect on a species. For the three species studied, the IE values were almost always lower than 1 (**Figure 5**) indicating a lower yield achieved in IC than in SC $\frac{1}{2}$ due to interspecific competition. The IE values were also negatively and significantly correlated ($p < 0.001$) with the yield of the associated species (**Figure 5**). This clearly indicates that the greater the associated species yield, the stronger the interspecific competition effect on the first species, in line, for example, with, Bedoussac and Justes (2010b).

On average for the two experiments, the slopes of ellipses are significantly steeper ($p = 0.01$) for the legumes (**Figure 5A**) than for the wheat (**Figure 5B**). This result indicates that the legumes are more affected than the wheat by the increase of the associated species yield. More precisely, for a similar associated species yield,

the legume yield loss proportionally to SC $\frac{1}{2}$ is higher than for the wheat ($IE_{\text{Wheat}} > IE_{\text{Legumes}}$) revealing that the legume is more sensitive to the interspecific interactions than the wheat. No difference was found between the slopes of the ellipses for the two legumes (**Figure 5A**). This signifies that an increase of, e.g., 1 Mg ha $^{-1}$ of wheat yield leads to the same reduction of IE value for both pea and faba bean. However, on average for the two experiments, the IE values of pea were lower than those of faba bean (0.44 vs. 0.59, respectively) for a similar wheat grain yield (1.7 vs. 1.8 Mg ha $^{-1}$, respectively). This indicates that for a similar wheat yield, the pea yield loss in IC compared to SC $\frac{1}{2}$ is higher than that of the faba bean ($IE_{\text{Pea}} < IE_{\text{Fababean}}$), i.e., the pea is more sensitive to the interspecific interactions than the faba bean.

Finally, the response of wheat to interspecific competition was similar irrespective of the legume species as illustrated by the same ellipses slopes values (**Figure 5B**) and, on average for the two experiments, the same IE_{Wheat} values (0.81 for both ICF and ICP) for a similar legume grain yield (1.9 and 1.7 for both ICF and ICP, respectively).

Considering a simple linear regression between IE and grain yield of the associated species should not mask that the response to the associated species yield increase is certainly not linear. Indeed, for the low yield values, the interspecific competitions are almost null. In such a case and except if facilitation occurs, IC can be considered as an SC $\frac{1}{2}$ leading to the IE values close to 1. Such a situation was observed (**Figure 5B**) in Exp.I with the high IE_{Wheat} values (0.8–1.1) associated with low legume yield (mostly below 1 Mg ha $^{-1}$) while at the same time (**Figure 5A**) low IE_{Legume} values (0.2–0.6) correspond to the great wheat yield (2–3 Mg ha $^{-1}$) reflecting the strong disequilibrium between the two species. Conversely, Exp.II leads to a situation in which both



the wheat and legume had intermediate IE values compared to Exp.I (Figures 5A,B).

In conclusion, an analysis of the relationship between IE and the associated species yield in a variety of situations is an informative approach to determine and compare the competitive abilities and tolerance to the competition of various cultivars within and among durum wheat–grain legume intercropped species. This leads us to formulate the hypothesis that the mean of IE values over all ICs and over the two experiments calculated for a given genotype can be considered as an indicator characterising its global tolerance response to the interspecific competition.

Estimation of Cultivar Yields in Durum Wheat–Grain Legume ICs From Both the Sole Crop Yields and Average IE Indices Modelling IC Grain Yield

We showed that, under a given set of pedo-climatic conditions, the behaviour of each cultivar in durum wheat–grain legume IC is related to: (i) its growth potential in a pure stand (Figure 3), (ii) its response to the density when that of the pure stand reference is different (Figure 3), and (iii) its response pattern to the interspecific competition (Figures 4, 5) which is related to the growth potential in the pure stand of the associated cultivar (Figure 3).

Therefore, we here formulate the hypothesis that it should be possible to estimate the durum wheat–grain legume IC yield of each intercropped cultivar based on the SC and SC½ yielding of each of the two cultivars and their IE mean values over all durum wheat–grain legume ICs and experiments ($\overline{IE}$) as an indicator of their response pattern to interspecific competition. Note that

using the $\overline{IE}$ values avoid a direct and circular mathematical link with the IC grain yield, conversely to the use of IE values calculated as the mean of the three replicates of a given treatment.

An analysis of covariance (ANCOVA) procedure was first applied to test the relationships between the cultivar IC grain yield and the six explanatory variables mentioned above. We added the type of species (legume or wheat) and the legume species (i.e., faba bean or pea) as co-variables to determine if these relations were different among the groups. The ANCOVA showed significant ($p < 0.01$) effects of SC and SC½ grain yields altogether with the $\overline{IE}$ mean values on the grain yield achieved by a cultivar in durum wheat–grain legume IC. Testing species as a co-variable indicated that these relations were different between the wheat and legumes and between the pea and faba bean leading to the following structure of statistical linear “complete” models:

- Durum wheat–pea complete model (ICP_{Complete} model):

$$\begin{aligned} \text{Yield}_{P-ICP} &= a \times \text{Yield}_{SCW} + b \times \text{Yield}_{SC1/2W} + c \times \overline{IE}_W \\ &\quad + d \times \text{Yield}_{SCP} + e \times \text{Yield}_{SC1/2P} + f \times \overline{IE}_P + g \\ \text{Yield}_{W-ICP} &= a' \times \text{Yield}_{SCW} + b' \times \text{Yield}_{SC1/2W} + c' \times \overline{IE}_W \\ &\quad + d' \times \text{Yield}_{SCP} + e' \times \text{Yield}_{SC1/2P} + f' \times \overline{IE}_P + g' \end{aligned}$$

- Durum wheat–faba bean complete model (ICF_{Complete} model):

$$\begin{aligned} \text{Yield}_{F-ICF} &= a \times \text{Yield}_{SCW} + b \times \text{Yield}_{SC1/2W} + c \times \overline{IE}_W \\ &\quad + d \times \text{Yield}_{SCF} + e \times \text{Yield}_{SC1/2F} + f \times \overline{IE}_F + g \end{aligned}$$

TABLE 2 | Parameters values and adjustment quality for the complete and simplified models.

	Complete model				Simplified model			
	ICF Model		ICP Model		ICF Model		ICP Model	
	Wheat	Faba bean	Wheat	Pea	Wheat	Faba bean	Wheat	Pea
Yield _{SCW}	1.50	−1.54	1.26	−1.73	1.26	−0.50	1.28	0.02
	$p = 0.02$	$p = 0.04$	$p < 0.0001$	$p < 0.001$	$p < 0.01$	$p = 0.25$	$p < 0.0001$	$p = 0.95$
Yield _{SC1/2W}	−0.55	−0.31	−0.41	0.19	−	−	−	−
	$p = 0.26$	$p = 0.59$	$p = 0.25$	$p = 0.58$				
IE _{Wheat}	−5.71	−12.84	−5.30	−9.13	−	−	−	−
	$p = 0.33$	$p = 0.09$	$p = 0.18$	$p = 0.03$				
Yield _{SCLeg}	0.13	−0.01	0.05	−0.66	−	−	−	−
	$p = 0.18$	$p = 0.93$	$p = 0.88$	$p = 0.07$				
Yield _{SC1/2Legume}	−0.18	0.51	−0.09	0.29	−0.10	0.89	0.10	0.98
	$p = 0.33$	$p = 0.04$	$p = 0.74$	$p = 0.32$	$p = 0.44$	$p < 0.001$	$p = 0.45$	$p < 0.001$
IE _{Legume}	−1.37	2.32	−1.00	1.12	−1.39	3.16	−0.99	4.33
	$p = 0.11$	$p = 0.04$	$p = 0.39$	$p = 0.32$	$p = 0.08$	$p < 0.01$	$p = 0.08$	$p < 0.001$
Constant	5.17	12.93	4.72	13.35	0.19	−1.48	−0.94	−4.15
	$p = 0.39$	$p = 0.10$	$p = 0.20$	$p < 0.0001$	$p = 0.87$	$p = 0.37$	$p = 0.38$	$p = 0.02$
RMSE	0.19	0.23	0.14	0.13	0.22	0.30	0.15	0.22
BIAS	$7.1 \cdot 10^{-16}$	$-5.3 \cdot 10^{-16}$	$1.6 \cdot 10^{-15}$	$-1.3 \cdot 10^{-15}$	$-2.8 \cdot 10^{-17}$	$-4.6 \cdot 10^{-16}$	$1.4 \cdot 10^{-16}$	$-9.5 \cdot 10^{-16}$
EF	0.89	0.95	0.91	0.96	0.84	0.90	0.88	0.89

Yield units are Mg ha^{−1}. The level of statistical significance of the associated variable in the models corresponds to that revealed by the multiple regressions.

$$Yield_{W-ICF} = a' \times Yield_{SCW} + b' \times Yield_{SC1/2W} + c' \times \overline{IE_W} + d' \times Yield_{SCF} + e' \times Yield_{SC1/2F} + f' \times \overline{IE_F} + g'$$

However, such an elevated number of variables precludes practical use. Subsequently, to simplify the model, a multiple regression procedure was applied for the cultivars of wheat, pea, and faba bean separately and considering only three variables (Yield_{SCW}, Yield_{SC1/2P} and $\overline{IE_P}$ for durum wheat–pea; Yield_{SCW}, Yield_{SC1/2F}, and $\overline{IE_F}$ for durum wheat–faba bean) resulting in the following linear “simplified” models:

- Durum wheat–pea simplified model (ICP_{Simplified Model}):

$$Yield_{P-ICP} = a \times Yield_{SCW} + b \times Yield_{SC1/2P} + c \times \overline{IE_P} + d$$

$$Yield_{W-ICP} = a' \times Yield_{SCW} + b' \times Yield_{SC1/2P} + c' \times \overline{IE_P} + d'$$

- Durum wheat–faba bean simplified model (ICF_{Simplified Model}):

$$Yield_{F-ICF} = a \times Yield_{SCW} + b \times Yield_{SC1/2F} + c \times \overline{IE_F} + d$$

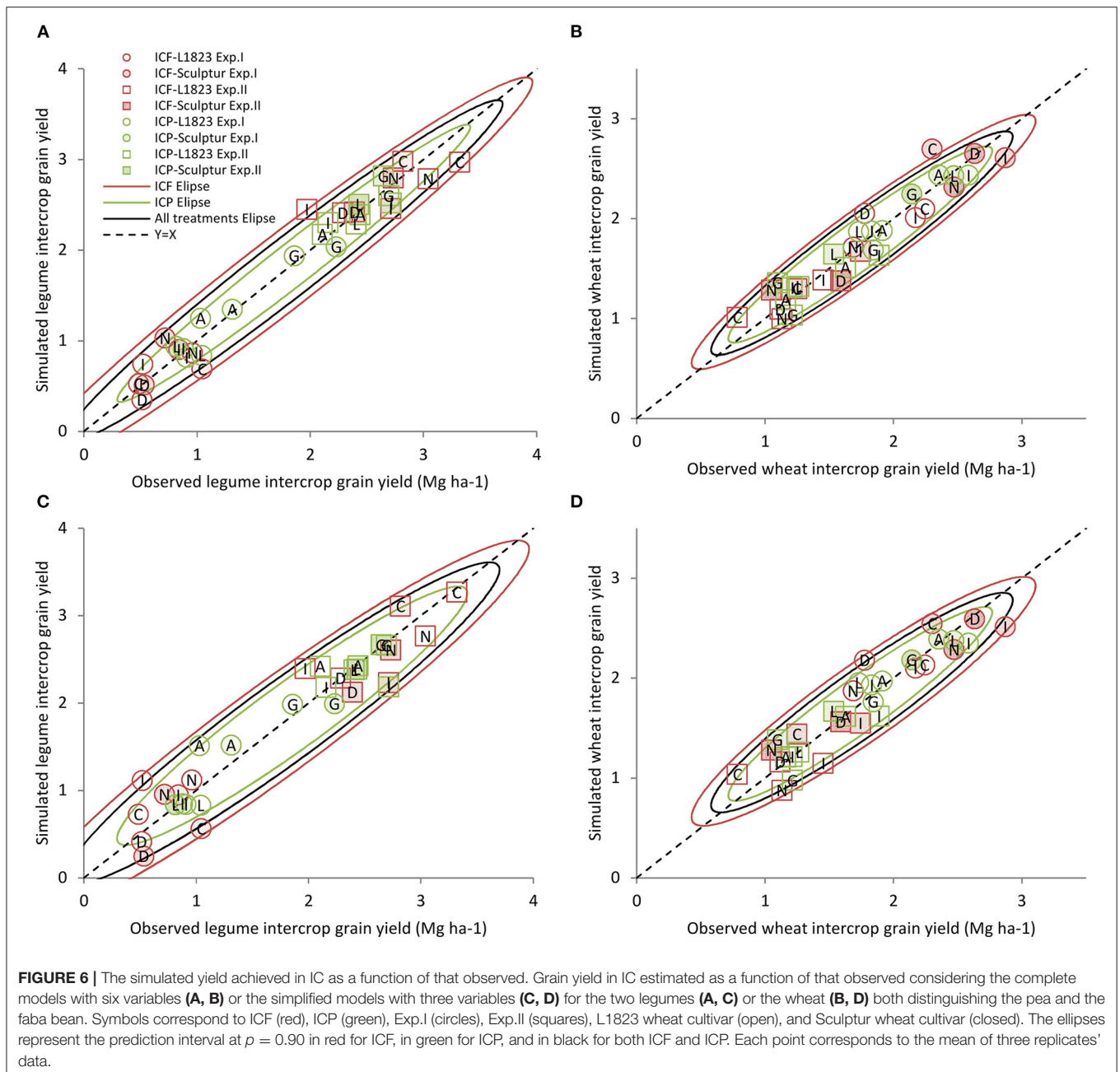
$$Yield_{W-ICF} = a' \times Yield_{SCW} + b' \times Yield_{SC1/2F} + c' \times \overline{IE_F} + d'$$

Simplified vs. Complete Model

Considering only three explanatory variables in the simplified model makes the cultivar IC yield fitting more robust and

functional with only a slightly lower quality of adjustment than the complete model (RMSE ranging from 0.15 to 0.30 vs. 0.13 to 0.23; **Table 2**). This confirms that the model fitting quality does not always depend upon its complexity or number of variables. In both the complete and simplified models, wheat IC yield is positively related to that in SC while the IC legume yield is positively correlated to the SC_{1/2} yield (**Table 2**). This is consistent with results described in **Figure 3** revealing contrasted responses between the species to plant density. More precisely, it underlines that the legumes are less prone to compensate for a low density than the wheat, which is able to produce more tillers upon favourable pedo-climatic conditions thus leading to quite similar yields in SC and SC_{1/2}. Because of the balance between the two species in IC, the wheat IC yield is negatively related to the legume SC_{1/2} yield and the IC legume yield is negatively correlated to the wheat SC yield.

The $\overline{IE_P}$ and $\overline{IE_F}$ variables can be considered as an indicator of the tolerance to interspecific competition in IC of the pea and the faba bean, respectively. They are positively correlated to the IC legume yield because the higher the $\overline{IE_P}$ and $\overline{IE_F}$ values the lower the loss between SC_{1/2} and IC legume yields. Oppositely, $\overline{IE_P}$ and $\overline{IE_F}$ are negatively correlated with the IC wheat yield as shown in **Figure 6**. Indeed, the high $\overline{IE_P}$ and $\overline{IE_F}$ values correspond to low IC wheat yield which mostly indicates a strong competitive effect of the legume, the converse being true for the low $\overline{IE_P}$ and $\overline{IE_F}$ values. This statement is reinforced by the fact that, in the equation for wheat IC yield, the $\overline{IE_F}$ parameter was lower than



that of $\overline{IE_P}$ (-1.39 vs. -0.99 , respectively) altogether with the higher $\overline{IE_F}$ value than $\overline{IE_P}$ value (0.59 vs. 0.44 , respectively). This reflects that the effect of faba bean cultivars on IC wheat grain yield was more significant than that of the pea cultivars.

In our situation, the quality of the simplified model is satisfactory to identify the most important variables explaining both the wheat and legume IC yields for the two experiments with contrasting climatic conditions. However, the potential yield as expressed by the SC and the SC $\frac{1}{2}$ yields, for wheat

and legumes, respectively, are greatly dependent on the pedoclimatic conditions. Moreover, the $\overline{IE_P}$ and $\overline{IE_F}$ values are strongly dependent on the experiments and particularly on the diversity of genotype $\times$ genotype combinations used, making the predictive quality of the model still questionable. For these reasons, there is now a need to understand in a dynamic fashion the link between the $\overline{IE_P}$ and $\overline{IE_F}$ values and the plant characteristics to be able to define relevant phenotypic indicators of the competitive ability of a cultivar.

CONCLUSIONS

Durum wheat–grain legume IC yield and its composition are greatly influenced by species and cultivar choice, and we observed a significant wheat cultivar $\times$ grain legume cultivar interaction. This work makes the proof of concept that a simple statistical model could allow predicting the yield of each durum wheat–grain legume IC component from only three simple and easy to measure and calculate variables: (i) the sole crop yields of wheat cultivars, (ii) SC $\frac{1}{2}$ yields of legume cultivars, and (iii) an indicator of legume cultivar tolerance to interspecific competition. However, the predictive quality of the model is probably limited and further studies on more diverse genotypes and growing conditions should be conducted to enlarge this finding. The applicability of the model could thus be extended to a variety of typical species $\times$ climate $\times$ management combinations. Moreover, further mechanistic understanding is required to better evaluate the links between the tolerance to interspecific interactions and the plant phenotype characteristics (traits). Such links will be useful for specific breeding programs of cultivars for intercropping as already pointed out by several authors (e.g., Nelson and Robichaux, 1997; Hauggaard-Nielsen and Jensen, 2001; Annicchiarico et al., 2019) to reveal the plant characters, such as height, leaf area, or root architecture to optimise complementarity between the species.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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AUTHOR CONTRIBUTIONS

EJ: funding acquisition. BK: data collection and formatting. BK and LB: data analysis. BK, E-PJ, and LB: Writing original draft. BK, E-PJ, EJ, and LB: writing, review and editing. All authors contributed to the article and approved the submitted version.

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Breeding Beyond Monoculture: Putting the “Intercrop” Into Crops

Peter M. Bourke^{1*}, Jochem B. Evers², Piter Bijma³, Dirk F. van Apeldoorn^{4,5}, Marinus J. M. Smulders¹, Thomas W. Kuyper⁶, Liesje Mommer⁷ and Guusje Bonnema^{1*}

¹Plant Breeding, Wageningen University & Research, Wageningen, Netherlands, ²Centre for Crops Systems Analysis, Wageningen University & Research, Wageningen, Netherlands, ³Animal Breeding and Genomics, Wageningen University & Research, Wageningen, Netherlands, ⁴Farming Systems Ecology Group, Wageningen University & Research, Wageningen, Netherlands, ⁵Field Crops, Wageningen University & Research, Lelystad, Netherlands, ⁶Soil Biology, Wageningen University & Research, Wageningen, Netherlands, ⁷Plant Ecology and Nature Conservation, Wageningen University & Research, Wageningen, Netherlands

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Jérôme Enjalbert,
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Reviewed by:

Nobuhito Sekiya,
Mie University, Japan
Radu E. Sestras,
University of Agricultural Sciences
and Veterinary Medicine of Cluj-
Napoca, Romania
Jean-Paul Sampoux,
Institut National de Recherche pour
l'Agriculture, l'Alimentation et
l'Environnement (INRAE), France

*Correspondence:

Guusje Bonnema
guusje.bonnema@wur.nl
Peter M. Bourke
peter.bourke@wur.nl

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Intercropping is both a well-established and yet novel agricultural practice, depending on one's perspective. Such perspectives are principally governed by geographic location and whether monocultural practices predominate. Given the negative environmental effects of monoculture agriculture (loss of biodiversity, reliance on non-renewable inputs, soil degradation, etc.), there has been a renewed interest in cropping systems that can reduce the impact of modern agriculture while maintaining (or even increasing) yields. Intercropping is one of the most promising practices in this regard, yet faces a multitude of challenges if it is to compete with and ultimately replace the prevailing monocultural norm. These challenges include the necessity for more complex agricultural designs in space and time, bespoke machinery, and adapted crop cultivars. Plant breeding for monocultures has focused on maximizing yield in single-species stands, leading to highly productive yet specialized genotypes. However, indications suggest that these genotypes are not the best adapted to intercropping systems. Re-designing breeding programs to accommodate inter-specific interactions and compatibilities, with potentially multiple different intercropping partners, is certainly challenging, but recent technological advances offer novel solutions. We identify a number of such technology-driven directions, either ideotype-driven (i.e., “trait-based” breeding) or quantitative genetics-driven (i.e., “product-based” breeding). For ideotype breeding, plant growth modeling can help predict plant traits that affect both inter- and intraspecific interactions and their influence on crop performance. Quantitative breeding approaches, on the other hand, estimate breeding values of component crops without necessarily understanding the underlying mechanisms. We argue that a combined approach, for example, integrating plant growth modeling with genomic-assisted selection and indirect genetic effects, may offer the best chance to bridge the gap between current monoculture breeding programs and the more integrated and diverse breeding programs of the future.

Keywords: intercropping, plant breeding, functional-structural plant modeling, indirect genetic effects, plant-plant interactions, mycorrhiza, plasticity

INTRODUCTION

Agriculture is at a crossroads. On the one hand, industrialized agricultural systems have delivered high yields of staple crops, achieved through a combination of chemical inputs, improved varieties, mechanization and large-scale agribusiness farms (Tilman et al., 2002). Despite its successes, modern agriculture is a system that is clearly out of balance and one that has led to widespread problems for soil, water, biodiversity, climate, and health (Steffen et al., 2015). One of the solutions proposed is to re-align our agricultural system with natural processes and cycles through the re-diversification of our cropping systems (Vandermeer, 1992; Brooker et al., 2015). Such diversified cropping systems (alternatively referred to as intercropping, mixed cropping, or polyculture) are already widely deployed in smaller-scale farming operations in many parts of the world [particularly in Latin America, Africa but also China (Brooker et al., 2015)].

The simultaneous cultivation of more than a single crop, including a diversity of genotypes of a single-crop species (Smithson and Lenné, 1996; Chateil et al., 2013), can lead to higher yields and increased yield stability and food security (Raseduzzaman and Jensen, 2017) of critical importance in low-input, often small-scale agricultural systems. There is an urgent need to investigate how more diverse cropping systems can be applied on larger spatial scales (Feike et al., 2012; Bybee-Finley and Ryan, 2018), particularly in the context of the current set of Sustainable Development Goals and the sustainable intensification needed to achieve them (Struik and Kuiper, 2017). Up-scaling of crop mixtures will require a re-designing of the technology currently employed in large-scale agricultural systems.

Among these technical means, one of the key components is modern improved varieties, as these have significantly contributed to the increase in yield and other important agronomic and economical traits. One example is the high-yielding modern dwarf varieties of wheat and rice that were first deployed during the Green Revolution of the 1950s and 1960s (Evenson and Gollin, 2003). Most modern varieties are bred specifically for monoculture, where a single genotype is grown (spatial monoculture). In our definition, this is irrespective of what crop was grown in preceding or subsequent seasons. However, modern varieties bred for monoculture are unlikely to be the best adapted genotypes for diverse cropping systems (Hamblin and Zimmermann, 1986; Hill, 1990; O'Leary and Smith, 1999; Brooker et al., 2015; Annicchiarico et al., 2019). Current breeding strategies focusing on the selection of the best performing genotypes in pure stands have overlooked the benefits of positive inter- and intraspecific interactions between crops or genotypes. Breeding practices and protocols are geared toward breeding for pure stands, ignoring the potential impact of trait variation of a companion crop on a plant's performance.

Biodiversity is one of the key factors underpinning ecosystem functioning (Tilman et al., 2014; Weisser et al., 2017; Leclère et al., 2020) and is a priority within the United Nation's sustainable development goal 15 (sdgs.un.org). Biodiversity is the combination of ecosystem diversity, species diversity, and

genetic diversity within species. While most ecological studies have focused on the importance of species diversity for ecosystem functioning, the erosion of crop genetic diversity is often seen as a more critical issue (Hajjar et al., 2008) in agriculture. Examples include genetic bottlenecks arising from breeding activities (Louwaars, 2018) or the replacement of farmers' landrace varieties with modern cultivars (F.A.O., 2019). Over the last half century, there has been a general trend toward reduced diversity in cropping systems both across and within species, with a concentration of agricultural production from an increasingly small number of key or staple species (Dawson et al., 2019). Intercropping provides an opportunity to re-diversify agricultural systems on many levels: increased diversity of crop species within land parcels, increased diversity within a crop species across cropping systems, and increased non-crop diversity within the agricultural landscape of wild species (Koricheva and Hayes, 2018; Beillouin et al., 2019).

Although there are clearly many reasons why plant breeding programs *should* accommodate diversity (Østergård et al., 2009; Lammerts Van Bueren et al., 2018), in practice many modern plant breeding programs are commercial operations that make breeding decisions based on economic justifications. If plant breeding companies are to begin to breed for more diversified agricultural systems, they will do so only when a number of economic justifications are already satisfied. These could include (1) forecasts on which crop combinations will primarily be used by farmers and growers in the future and at what scale this will occur, (2) the market potential for an adapted cultivar for intercropping over a standard cultivar, and (3) the relative efficiency versus costs of breeding under mixed stand conditions compared with pure stands. There is an urgent need to explore these questions together with breeders, some of whom already recognize the benefits of diversification but do not yet consider there to be a need to actively begin breeding for such systems (Dawson et al., 2019). The reasons for this could be economic as listed above, but could also be practical as there is currently little guidance or expertise on how breeding for intercropping should be performed.

Other authors have highlighted the issue of breeder engagement and suggest that farmers should be involved in the process of breeding for intercropping through participatory plant breeding programs (Annicchiarico et al., 2019). For now, we assume that breeders are ready and willing to take up the challenge. We therefore focus primarily on the challenges faced by breeders in developing new variety combinations and the potential of modern computational methods for use in more diverse breeding programs. We identify a number of breeding directions for intercrop performance. We firstly explore the idea of "trait-based breeding," taking inspiration from the results of plant growth modeling and ecological theory to provide specific trait-based breeding targets to define a crop ideotype. A complementary breeding approach is what might be termed "product-based" breeding, in which a statistical black-box approach is used to optimize the system rather than breeding by design toward an ideotype. Quantitative breeding approaches are already widely used in animal and plant breeding programs, for example, in the use of genomic

prediction models (Meuwissen et al., 2001). In intercrop breeding, genomic prediction could reduce the need for extensive phenotyping (Annicchiarico et al., 2019) while potentially achieving greater genetic gains than traditional phenotypic selection programs (Bančić et al., 2021). We propose that an integrated framework that combines information from both approaches could lead to both continual genetic improvement and the prediction of breakthrough trait combinations.

To be able to discuss the integration of these mechanisms into breeding for intercropping, we first review our current understanding of the biological mechanisms that can lead to improved performance of crop mixtures over pure stands.

BIOLOGICAL MECHANISMS IN INTERCROPPING

Eco-Physiological Mechanisms Underlying Crop Mixture Performance

Growing mixtures of species or genotypes is often more productive than pure stands (Bedoussac et al., 2015; Brooker et al., 2015), demonstrating higher nutrient efficiencies and increased biocontrol, leading to more sustainable agricultural systems (Boudreau, 2013; Li et al., 2014). However, relatively little work has been done to explore the potential of crop mixtures for modern agriculture, even though this potential has been shown for mixtures of species (Yu et al., 2015; Fletcher et al., 2016; Juventia et al., 2021) and genotypes (Tooker and Frank, 2012; Sapoukhina et al., 2013; Ditzler et al., 2021). Here, we consider a crop mixture to include both mixtures of genotypes of a single species, or mixtures of different species, encompassing a range of possible spatial and temporal arrangements (Brooker et al., 2015).

Recent research has started to focus on the mechanisms that explain the increased performance and efficiency of mixed-species systems (Stomph et al., 2020). One of the reasons crop mixtures show these benefits can be traced back to the way plants of different species compete for resources. Relaxation of competition between species due to spatial or temporal complementarity in resource uptake is a strong determinant of mixture performance and efficiency (Yu et al., 2015; Li et al., 2020). For instance, differences in root growth or root architectural characteristics between species growing together may lead to complementary uptake of water or nutrients, when the root systems are (partly) spatially or temporally separated (Henry et al., 2010; Postma and Lynch, 2012). Similarly, differences in shoot architecture and photosynthetic efficiency can result in complementarity in light capture and light use efficiency (Stomph et al., 2020), especially when the component species are not sown or harvested simultaneously (Yu et al., 2015).

Further mechanisms underlying high performance and efficiency of mixtures relate to a reduction in the prevalence of weeds and diseases in mixed systems. In theory, high weed suppression by one of the component species in a mixture may lead to improved performance of the other, leading to more productive and resource-efficient crop systems.

Ideally, weed suppression should occur without incurring negative competitive effects on the component crop species, replacing weed biomass with crop biomass. Enhanced weed suppression in crop mixtures does occur (Stomph et al., 2020), while ecological studies have also demonstrated that invading species such as weeds have less opportunity to invade diverse plant communities compared to monocultures (Van Ruijven et al., 2003). Disease incidence can be reduced drastically in crop mixtures (Stukenbrock and McDonald, 2008; Boudreau, 2013; Wuest et al., 2021) for both leaf and soil-borne diseases. Disease suppression in mixtures has been attributed to host dilution, allelopathy, and microclimate effects, and depending on the design of the mixture, also physical barrier effects (Ampt et al., 2019).

Importantly, plant traits that may provide benefits in one type of mixed-crop system may not be relevant for high performance in another. Mixed-crop systems come at many different levels of temporal and spatial species segregation (Ditzler et al., 2021). For example in fully mixed designs, the component species are fully mixed within the crop rows. There are also a range of strip cropping systems (Van Oort et al., 2020). Narrow strip systems maximize interspecific interaction but rule out mechanical management (strips of one or two rows per species alternating). Alternatively, wide-strip systems show very little interspecific interaction but provide other benefits, such as complementary insect populations that improve pollination and herbivore reduction, or beneficial microclimate (alley cropping). It is therefore likely that crop genotypes with a particular set of traits may only show the typical mixed-crop benefits for a subset of mixture designs.

Deciphering Interactions in Intercropping

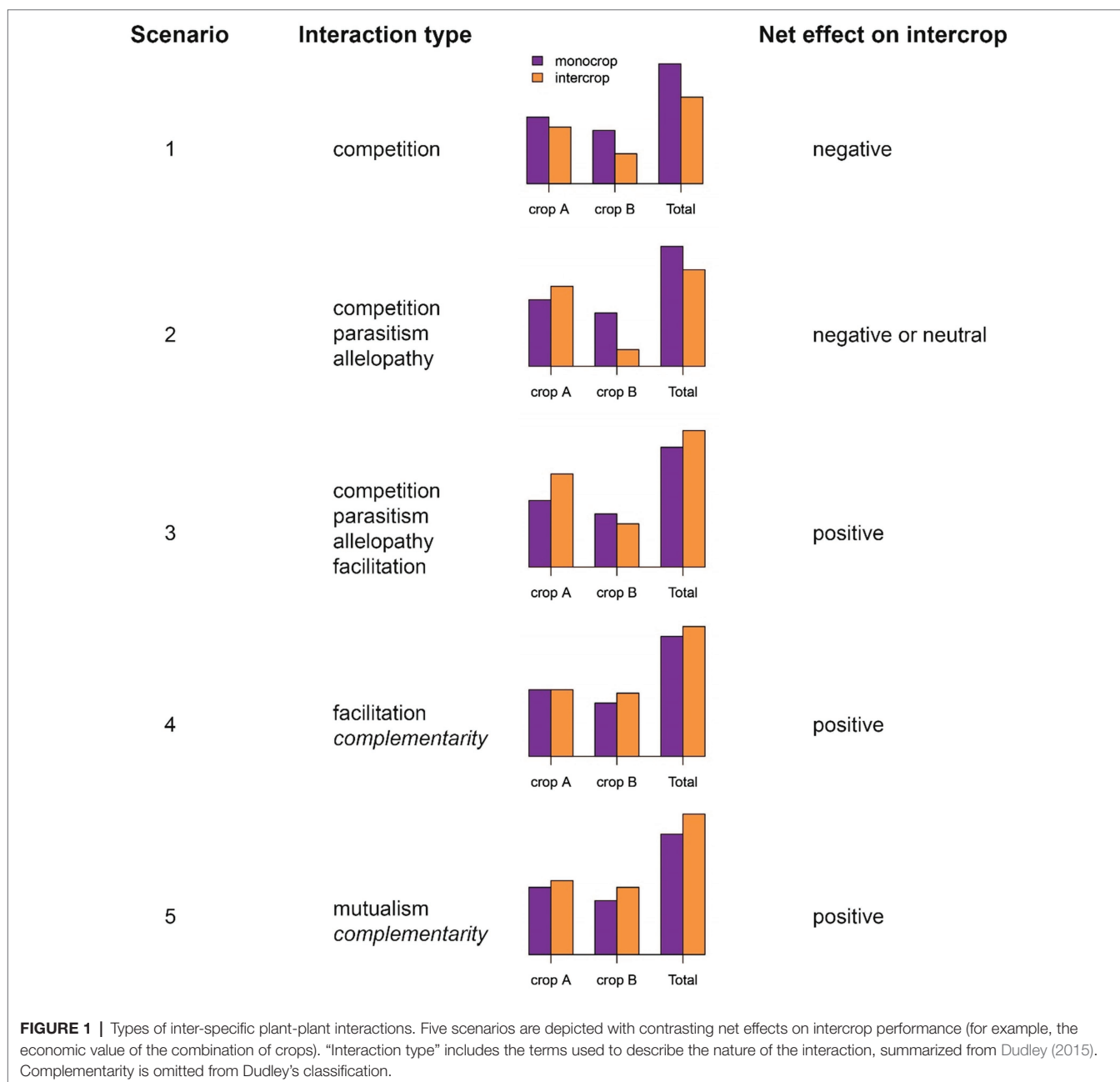
Central to the topic of intercropping is the extent to which interactions between plants will affect overall intercrop performance. A better understanding of these interactions can lead to insights into how best to design an intercrop system and may also provide leads for breeding. However, the literature on such interactions often contains discipline-specific terminology and classifications and requires some “deciphering” for the non-specialist reader.

In much of the general agronomic literature on intercropping, three types of plant–plant interactions are mentioned: competition, complementarity, and facilitation (Li et al., 2013; Bedoussac et al., 2015). Competition is generally framed as an undesirable interaction, leading to a negative impact on the performance of one or both species. Complementarity and facilitation, on the other hand, generate positive effects on intercrop performances (Li et al., 2014; Barry et al., 2019). However, competition effects in intercrops may also provide benefits, at least temporarily. For example, the combination of a cereal and a legume can benefit from the direct competition for soil inorganic N between the species, forcing the legume, as weaker competitor, to invest more in its rhizobial symbionts to supply its nitrogen needs (Jensen, 1996). This results in an emergent behavior of the mixed-cropping system to become more N-efficient (i.e., increased production per unit of N input),

an effect also known as N-sparing (Giller, 2001). Therefore, in this situation, competition ultimately leads to facilitation and complementarity.

More nuanced classifications of plant–plant interactions identify both costs and benefits to each component species and distinguish between inter- and intraspecific interactions (Dudley, 2015; Subrahmaniam et al., 2018). Identifying costs and benefits provides a sound classification framework. But from an agronomic and breeding perspective, one is most interested to know whether the net effect of a specific intercrop (effect = benefit – cost) is positive, neutral or negative to the overall performance metric to be maximized or improved. To illustrate this, we present five possible scenarios that demonstrate

different cost/benefit relationships between a pair of intercrop partners in **Figure 1**. In the first scenario, competition proves detrimental to both parties, with a negative net effect. Alternatively, one crop may benefit at the other's expense, resulting in either a neutral or negative net effect, depending on the magnitude of competition and the relative value of the component crops (scenario 2; **Figure 1**). In such circumstances, parasitism or allelopathy may be involved in the interaction, although they suggest particular life-cycle strategies or mechanisms that go beyond simple “competition.” In the third scenario, the benefit enjoyed by one crop exceeds the cost paid by the second crop, resulting in a positive net effect. Although competition still occurs (at least from the perspective



of the crop paying the price), such an interaction could also be termed “facilitation,” enabling a superior overall performance in combination (e.g., in a legume-cereal combination). Facilitation may also occur at no cost to the enabling partner (scenario 4; **Figure 1**). In the most ideal scenario, facilitation may be reciprocal, i.e., in a “mutualistic” interaction (scenario 5; **Figure 1**). However, in order to quantify costs and benefits, one needs information on pure stand performance. This is certainly of scientific interest, but it is unlikely that future intercrop breeding will involve calculations of costs and benefits (unless perhaps through *in silico* simulation). On the other hand, it is straightforward to assign economic weights to component crops in a joint crop analysis, an approach presented in more detail below (*cf.* section “The Direction of Selection”).

In the ecological literature, the concept of “niche differentiation” is generally used to describe the process whereby species have evolved to avoid each other’s specific niches (Zuppingier-Dingley et al., 2014; Meilhac et al., 2020). Niche differentiation leads to the avoidance of direct competition by expanding the range of microniches and ultimately leads to greater overall productivity of the assemblage (Gathumbi et al., 2002; Li et al., 2007; Ndufa et al., 2009; Mueller et al., 2013). Ecologists also frequently partition biodiversity effects into *complementarity* versus *selection* effects (Loreau and Hector, 2001). Positive selection effects in a mixture occur when highly productive species in monoculture also dominate in the mixture, and positive complementarity effects occur when species’ yields in the mixture are on average greater than expected from their yields in monoculture, weighted by their relative abundance in the mixture (Loreau and Hector, 2001). The complementarity effect is the difference between the net biodiversity effect (observed yield of the mixture minus the yield of the mixture expected without selection and complementarity effects) and the selection effect. It covers a range of plant–plant interactions including niche partitioning and facilitation (Barry et al., 2019). Thus, while complementarity is often presented as being distinct from facilitation in the general intercropping literature, the terms are not considered exclusive in the ecological additive-partitioning of biodiversity effects. The interested reader is directed to the review of Barry et al. (2019) that highlights this confusion and suggests how the study of complementarity might be better approached in future research (Barry et al., 2019).

Plant Plasticity

Plasticity in plant traits (the ability of a plant to morphologically adapt its phenotype to a particular environment) can help maintain a balance between intercrop partners through niche differentiation, which may ultimately lead to over-yielding. However, it potentially complicates the definition of an ideotype in a more variable growing environment such as an intercrop. Many types of intercrops typically have some degree of spatial heterogeneity due to differences between conditions experienced by individual plants. If plants are plastic, they can tailor their growth and development to the resources locally available (e.g., Zhu et al., 2016). Plants in mixed-cropping systems

encounter different local environments above and below ground due to their diverse neighboring plants. As a consequence, a plant phenotype is the result of these variable local phenotypic responses, maximizing resource uptake and potentially leading to higher overall performance. On the other hand, plasticity comes at a cost. Plants with limited resources may expend unnecessary resources in trying to acquire more resources, e.g., by stem elongation that may be detrimental to overall crop performance. In monoculture cultivation, such plastic responses (e.g., unwanted side-shoot development) are partly controlled through planting density. In an intercrop, that means of control may no longer be effective. Plasticity may also lead to certain non-uniformity in a crop that can be detrimental to marketable yield. Plasticity may thus help to improve intercrop performance, but may also reduce it. Plastic responses to acquire extra available resources are beneficial, but plastic responses to escape adverse conditions or those that reduce yields may ultimately be detrimental for whole crop performance. Breeding may therefore be needed to increase plasticity for some traits (e.g., those involved in competition) but not for others (e.g., those involved in marketable yields).

Using Functional–Structural Plant Modeling for Intercrop Breeding

Most studies on crop mixtures rely on field experiments and occasionally detailed pot experiments to understand and/or predict performance of intercrops. Such experiments provide very useful information on plant behavior in mixed systems and its consequences for overall crop performance. However, they are limited in the extent to which plant traits can be changed or manipulated, in the number of scenarios that can be tested and in the level of detail in the data they can generate. Simulation modeling has been a useful tool in complementing experimental work on species mixtures as well as in informing it (Gaudio et al., 2019).

An approach to capture mixture behavior in simulation is to adapt crop models that have been developed to simulate pure crops and modify them to represent crop mixtures (Corre-Hellou et al., 2009; Chimonyo et al., 2016). This approach is useful when representing full mixtures with little or no spatial heterogeneity. However, species mixtures with a distinct spatial arrangement, such as strip intercrops, cannot be represented satisfactorily in such models. This has led to the development of models that capture strip arrangements as combinations of small pure stands, still using traditional crop modeling approaches (Gou et al., 2017; Van Oort et al., 2020). Approaches relying on traditional crop modeling concepts do not allow the exploration of combinations of species phenotypes (development, physiology, architecture) for crop design optimization (e.g., varying the level of plant clustering in strips, population densities, amount of temporal overlap). This is because (1) the phenotype of the species used in simulation is captured in a relatively small set of parameters in such models and (2) the degree to which plant arrangement can be altered is limited.

The functional-structural plant (FSP) modeling approach (Godin and Sinoquet, 2005; Evers et al., 2018) does not have these drawbacks. In FSP models, plant development, growth and architecture are simulated in 3D over time and are governed by the effects of competition for capture of resources such as light, water, and nutrients (**Figure 2**). Originally developed to represent plant development realistically (Prusinkiewicz and Lindenmayer, 2012) and not to predict crop performance, plant traits such as leaf size and angle, stem length, and root branching are explicitly captured in FSP models. This makes FSP modeling ideally suited to explore the relationships between plant traits, plant arrangement, and performance. This has been done successfully for leaf traits in tomato pure stands (Sarlikioti et al., 2011) and wheat-pea mixtures (Barillot et al., 2014) as well as for root traits in single bean plants (Rangarajan et al., 2018).

These examples demonstrate the possibilities of FSP modeling to help breeding for diversity. However, to truly arrive at an optimized combination of species phenotypes and plant arrangement, FSP models should capture both above- and belowground processes in sufficient detail (Faverjon et al., 2019; De Vries et al., 2021). Plant growth in any crop type is restricted by the most limiting resource, and thus, the capture and use of those resources needs to be well represented to model plant growth (Evers et al., 2019). Ultimately, FSP models can be applied to explore the interaction between species traits and intercrop plant arrangement. Architectural ideotypes, complementary in resource use, could thus be determined as optimized phenotypes for mixture designs. A clear description of these architectural ideotypes could inform breeding programs while alleviating the challenge of having to test large numbers of genotypes and crop designs.

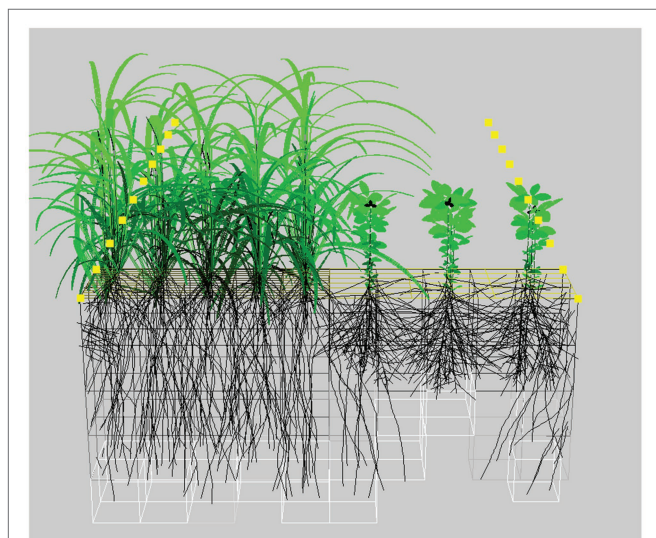


FIGURE 2 | Example of an FSP simulation of a relay cereal-legume system at 52 days after sowing. The brightness of the leaves corresponds to the leaf light capture. The boxes surrounding the roots represent the soil explored by the roots. Image created using the GroIMP modeling platform (Hemmerling et al., 2008).

Interactions In the Soil Involving Mycorrhiza

In breeding, most attention is focused on above-ground plant traits (with the obvious exception of root crops). In contrast to above-ground traits, our understanding of variation in root traits has lagged behind (Faget et al., 2013; Weemstra et al., 2016). However, our view of roots has recently been transformed (Bergmann et al., 2020; Laughlin et al., 2021), with a large proportion of root trait variation being explained by the propensity of a plant to form a symbiotic association with mycorrhizal fungi (beneficial associations between certain root-inhabiting fungi and plant roots). Among crop species, almost all crop plants form such mycorrhizas, contributing to enhanced uptake of nutrients of limited mobility (especially phosphate, also zinc and copper) and water, and increasing resistance or tolerance to biotic and abiotic stresses. Crop species differ in the extent to which they depend on and benefit from this mycorrhizal symbiosis. Variation in plant response to mycorrhiza has also been reported for many crops (Kuyper et al., 2021).

It is likely that modern agronomic practices (continuous monocropping, high fertilizer use, fungicide use, tillage, bare fallow in the winter season) have selected against mycorrhizal fungi with larger benefits to plants (Verbruggen and Kiers, 2010). It has also been hypothesized that plant breeding under these conditions has resulted in plants with lower benefits from the mycorrhizal symbiosis (Hetrick et al., 1992). Intercropping systems are often characterized by lower fertilizer and fungicide levels, less soil disturbance, and higher plant diversity and cover. Intercropping should therefore shift the current selection of mycorrhizal fungal species with ruderal life styles and limited plant benefit toward species that form more beneficial associations.

It is widely accepted that mycorrhizal fungi show little or no selectivity with regard to the plant species with which they associate. Consequently, the mycorrhizal mycelium in soil consists of a network through which plants are connected, known as common mycorrhizal networks (CMN). These CMNs have been shown to underlie the overyielding of plant species mixtures (Walder et al., 2012) or variety mixtures (Wang et al., 2020). Although the extent of plant and fungal control over movement of carbon and nutrients through such CMNs is poorly known, it is possible that such CMNs reduce rather than amplify competition, resulting in a negative selection effect and a positive complementarity effect. If this is a general pattern, it could imply that plant breeding for intercropping should ensure plants be sufficiently promiscuous, associating with a diversity of mycorrhizal fungi to promote the development of CMN and their resulting overyielding benefits.

The issue of breeding for mycorrhizal promiscuity or selectivity has received no attention to date, contrary to nitrogen-fixing bacteria (rhizobia) in soya bean (*Glycine max*). Soya bean cultivars have been bred in Africa that were able to associate with indigenous rhizobia, thereby foregoing the need for inoculation. Alternatively, soya bean cultivars have been bred in the United States that very specifically associate with a limited number of rhizobial strains (Giller, 2001, p. 155–157). This points to a genetic

basis for symbiont selectivity, and the merits and disadvantages of breeding to modify symbiont selectivity should be further investigated.

BREEDING DIRECTIONS FOR INTERCROP PERFORMANCE

Ideotype Breeding

In plant and animal breeding, ideotypes have been used to describe a conceptual direction toward which a breeding program can aim. An ideotype describes an ideal or hypothetical phenotype that is expected to maximize performance (usually yield) under a particular set of growing conditions. Particularly in plant breeding, individual performance of plants grown together is of secondary importance to their collective performance (Weiner, 2019). When originally proposed, the ideotype concept was used to describe an ideal wheat plant (*Triticum aestivum*): weakly competing and tolerant of both high planting densities and high soil fertility (Donald, 1968). Donald's wheat ideotype most likely benefitted from hindsight: dwarf rice and wheat varieties providing the inspiration for the broader concept of ideotype breeding (Rasmusson, 1987). The idea of breeding for an idealized individual, one that may demonstrate poor individual fitness under natural selection but leads to superior collective performance, has remained a powerful concept, particularly for plant breeders. In some crops like rice, there is evidence to suggest that following an ideotype breeding approach has led to higher genetic gains for yield than would have been expected under selection for yield alone (Peng et al., 2008).

Ideotype breeding focuses primarily on defining breeding targets for traits which are thought to contribute to higher crop performance, in a real or hypothetical environment (Donald, 1981). Most ideotypes assume a monoculture cropping system, where a plant experiences a neighborhood of identical genotypes. However, the definition of ideotype does not preclude cropping systems that involve non-kin neighbors. Indeed, the term “ideomix” has been coined to extend the ideotype concept to plant mixtures (Litrice and Violle, 2015).

An intercrop ideotype would ideally include a range of positive interaction effects that optimize collective performance. While a single wheat genotype can be selected to poorly compete with its conspecifics in a monoculture stand, it is less clear what sort of interactions should be selected for among intercrop partners, particularly given the dynamic and inter-dependent nature of these interactions. An increasingly detailed description of favorable interaction effects is being compiled, although it remains context-, crop-, and experiment-specific in many cases (Brooker et al., 2021). Efforts to generate *in silico* ideotypes are providing novel insights (Louarn et al., 2020), but still require confirmation of their ability to predict as-yet unidentified traits with significant agronomic impact. As we develop greater insight into the mechanisms involved in intercrop performance, it is likely that more detailed crop combination-specific intercrop ideotypes will emerge.

A Quantitative Genetic Approach to Breeding for Intercropping

Many relevant traits in plant production are quantitative and affected by many genes. This is particularly the case for yield. For such traits, quantitative genetics provides a powerful and mathematically explicit framework for genetic improvement. Developments in genomic prediction (Daetwyler et al., 2013) and indirect genetic effect (IGE; Bijma, 2014) make this approach very suitable for intercropping.

In most cases, the choice of a production system will precede the genetic improvement for that system, and the desired direction of genetic improvement follows from the properties of the production system. Hence, to discuss genetic improvement in the context of intercropping, we will assume here that the crops that are grown together have already been chosen. Thus, we will focus on genetic improvement in an existing intercropping production system, where the two (or more) species are a given. Though the focus is on a system of two species, the concept generalizes to more than two. Moreover, we will focus on recurrent selection, for example, to improve the *per se* value of populations in outbreeding species or to ultimately deliver hybrid cultivars, such as reciprocal selection or topcross selection.

Genetic improvement for intercropping differs from breeding for monoculture only when the two species grown together impact each other and when this impact shows genetic variation. Without such impact, the optimum breeding direction will be the same as for monoculture, while the absence of genetic variation makes breeding futile. For this reason, IGEs that act between the two crops grown together are the key element that differentiate genetic improvement for intercropping from breeding for monoculture. The importance of IGEs for intercropping has also been recognized by other authors, most notably in the contribution of Wright (1985) although there the term “associate effect” was used. We describe here the steps of incorporating IGEs in a prediction model for an intercrop to provide a bridging link in the literature and offer a fresh perspective on Wright's approach.

An IGE is a genetic effect of one individual on the trait values of another individual. Neighboring plants may, for example, impact each other's growth rate and this impact may have a genetic basis. Traditionally, IGEs have been defined for individuals of the same population and thus species (Griffing, 1967; Moore et al., 1997; Muir, 2005). However, there is no conceptual difficulty to extend the IGE concept to interactions between species. When breeding for monoculture production, within-population IGEs are implicitly accounted for when selection occurs at the level of plots of a single genotype or of a family (Griffing, 1976). With intercropping, however, also between-population IGEs matter, and those require specific attention.

The Direction of Selection

In an intercropping production system, interest is typically in the performance of the entire system, which may include the

performance of both crops, say M and F , indicating maize (*Zea mays*) and faba bean (*Vicia faba*) which we use as an example. Because the relevant importance may differ between the two crops, we may specify a quantitative breeding objective, say H , which is a weighted (w) sum of the relevant phenotypes of each of the two crops,

$$H = w_M y_M + w_F y_F$$

where for the sake of example, y_M represents the yield of maize and y_F the yield of faba bean. More generally, y_M and y_F could be a combination of multiple traits of each of the two crops. When the goal is to increase profit of the entire system, the weights w_M and w_F would be partial derivatives of profit with respect to yield of maize and yield of faba bean, respectively, following basic principles of selection index theory (e.g., Smith et al., 1986). When interest is in only one of the two crops, for example, when the second crop is a rhizobial symbiont grown to increase yield of the first crop, one can simply set w_2 to zero.

Maize yield will depend on the genes of maize (direct genetic effect, DGE), but may also be affected, *via* competition or facilitation, by genes of the faba bean. The latter represents a between-species IGE. The same applies to the yield of faba bean. Hence, in total we need to consider four quantitative genetic main effects: DGE of maize on yield of maize ($G_{M,D}$), DGE of faba bean on yield of faba bean ($G_{F,D}$), IGE of maize on yield of faba bean ($G_{M,I}$), and IGE of faba bean on yield of maize ($G_{F,I}$). There are also two inter-specific interaction effects, namely $G_{M,D} * G_{F,I}$ and $G_{F,D} * G_{M,I}$ (Sampoux et al., 2020). The previous equation for H can therefore be expressed more fully as

$$H = w_M (\mu_M + G_{M,D} + G_{F,I} + G_{M,D} * G_{F,I}) + w_F (\mu_F + G_{F,D} + G_{M,I} + G_{F,D} * G_{M,I})$$

where μ_M and μ_F are the mean contributions to H of maize and faba bean, respectively.

In a recurrent selection cycle where genotypes are randomly assembled, only the genetically additive parts of direct and indirect effects are inherited. Denoting the heritable component of H by H_{ADD} , we have

$$H_{ADD} = w_M (A_{M,D} + A_{F,I}) + w_F (A_{F,D} + A_{M,I})$$

where $A_{M,D}$, $A_{F,I}$, $A_{F,D}$, and $A_{M,I}$ are the additive genetic values inherited in the next generation by offspring of candidates to selection for $G_{M,D}$, $G_{F,I}$, $G_{F,D}$, and $G_{M,I}$, respectively. Note that the genetic effects are indexed by the crop from which they originate, because this crop is the gene pool relevant for the improvement of the genetic main effect. For example, $G_{F,I}$ is the IGE due to faba bean on the yield of maize; improvement of $G_{F,I}$ requires breeding in faba bean, but will benefit maize yield.

A relatively larger variance of the interaction terms indicates a smaller narrow sense heritability. Hence, the magnitude of the interaction variance is relevant for the choice between a recurrent selection scheme vs. a general mixing ability scheme (e.g., Sampoux et al., 2020). Furthermore, when the aim is to

develop a specific two-genotype combination (a “tandem” variety pair), the interaction between genotypes is of interest. Focusing on the additive part of the model, selection in maize would be for the selection index

$$H_{ADD,M} = w_M A_{M,D} + w_F A_{M,I}$$

while selection in faba bean would be for the index

$$H_{ADD,F} = w_F A_{F,D} + w_M A_{F,I}$$

Note that $H_{ADD,M}$ could be considered as a weighted general mixture ability of maize (respectively, $H_{ADD,F}$ for faba bean). If $w_M = w_F = 1$, then $H_{ADD,M}$ is the general mixture ability of maize (Sampoux et al., 2020). The direct component of $H_{ADD,M}$ and $H_{ADD,F}$ will be expressed in the crop itself, while the indirect component will be expressed in the partner crop.

The total genetic variation that breeders can use for improvement of the intercropping system by recurrent selection is equal to the variance of H_{ADD} (Wright, 1985; Bijma, 2011; Sampoux et al., 2020). For maize, this equals

$$\text{var}(H_{ADD,M}) = w_M^2 \text{var}(A_{M,D}) + w_F^2 \text{var}(A_{M,I}) + 2w_M w_F \text{cov}(A_{M,D}, A_{M,I})$$

and for faba bean

$$\text{var}(H_{ADD,F}) = w_F^2 \text{var}(A_{F,D}) + w_M^2 \text{var}(A_{F,I}) + 2w_M w_F \text{cov}(A_{F,D}, A_{F,I})$$

A trade-off due to competition will reduce this genetic variation. For example, if selection of maize for higher yield reduces yield of faba bean due to competition, then this will surface as a negative covariance between $A_{M,D}$ and $A_{M,I}$. The absolute magnitude of this competition is measured by the size of this (negative) covariance. The deviations of the corresponding correlations from a value of -1 indicate the degree to which this trade-off can be circumvented by selection. Since genetic correlations are rarely equal to -1 , the presence of a trade-off does not imply that simultaneous improvement of the yield of both crops is impossible; it merely means a slower rate of improvement. Moreover, these two correlations indicate that the total trade-off may originate from two different gene pools, and the strength of the trade-off may differ between the two gene pools. In other words, maize competing with faba bean does not imply faba bean competing with maize. In the absence of competition, positive covariances between DGE and IGE are possible, implying genetic variability for facilitation.

The relationship between direct and IGEs was also recently used to qualitatively classify the nature of interaction effects between intercrop partners (Haug et al., 2021). The authors developed an elegant classification system based on nine different potential combinations (either $-/0/+$ for the direct and indirect genetic effects) in a binary mixture, with terminology reminiscent of other plant–plant interaction classification systems previously mentioned (Dudley, 2015; Subrahmaniam et al., 2018). These provide a more intuitive understanding for breeders of the types of interaction effects that ultimately should be aimed at in breeding programs.

Estimating Direct and Indirect Genetic Effects

Genetic improvement of the overall performance of an intercropping system requires estimates of the direct and indirect genetic components of $H_{ADD,M}$ and $H_{ADD,F}$ to select the parents of the next generation. In monoculture, direct and indirect genetic effects for yield can be estimated from a combination of yield records on plants, data on their position in the field (so that their neighbors are known) and pedigree or genome wide marker data (Muir, 2005; Cappa and Cantet, 2008; Silva et al., 2013). In this approach, knowledge of the mechanisms or traits underlying the competitive effects is not needed; instead, the full competitive effects for the traits of interest are estimated directly from the resulting phenotypes together with the genetic relationships between individuals in the population, using statistical mixed-model technology.

Extension of this statistical approach to intercropping is straightforward in principle. It merely requires extending the mixed model with an additional indirect genetic random effect due to the identities of the neighbors of the other crop. However, optimization of the design with respect to the spatial organization of families of each crop in relation to their neighbors will require careful consideration, to avoid confounding and to maximize precision of the resulting estimates of G_D and G_I of each of the two crops. The availability of genome-wide marker data should considerably increase the precision of these estimates, because it provides precise information on genetic relatedness between all individuals in the data. In cases where such data is not available, factorial designs are needed, in which each genotype of a species is tested in several mixtures with different genotypes of the other species.

Analogy With Breeding for Hybrid Combining Ability

The analogy between hybrid breeding and intercrop breeding has already been drawn many times, in that both seek optimal combinations of genotypes. In hybrid breeding, the aim is to identify parental lines which, together, exhibit a good combining ability leading to heterosis in the F_1 generation. In order to identify such parents, test crosses with a single or small number of tester lines are often performed (alternative approaches include a poly-cross or diallel; Acquaah, 2020). For intercrop breeding, using a single tester line of crop B when trialling crop A reduces the complexity to a level similar to that of a single-crop breeding program, providing a simple method to screen for “general mixing ability” or “general ecological combining ability” (Harper, 1964, 1967; Hill, 1990). However, such an approach would not yield sufficiently accurate information on the IGEs of the focal crop on the tester crop. Furthermore, specific interactions with the tester genotype would be included in the estimated genetic merit of individuals of the target crop (present in both direct and IGEs), which may bias breeding value estimates. As an alternative, a small set of tester lines selected for their contrasting phenotypes could be assembled or mixed to represent the range of possible cropping partners (Holland and Brummer, 1999). This could be a pragmatic and cost-effective strategy to begin with, although the specific choice

of tester genotypes could potentially have a large influence on results. A highly competitive or dominant tester line may suppress genotypic differences in the target crop, while a weak tester may not provide sufficient inter-specific interaction (Hill, 1996). A recurrent selection scheme for the simultaneous improvement of two species was already proposed over 35 years ago (Wright, 1985) and has been recently included in a simulation study that compared different selection strategies for intercrop performance (Sampoux et al., 2020). In this study, the bulked progenies of candidate lines from crop B were used as a tester for crop A and vice versa (Sampoux et al., 2020).

F_1 hybrid breeding also distinguishes between general-combining and specific-combining abilities, with much focus on accurately estimating these parameters using phenotypic, pedigree, and genomic information. General mixing ability is the sum of the direct and IGEs, while specific mixing ability is the sum of the interaction terms between specific genotypes of both species (Forst et al., 2019; Haug et al., 2021). However, as pointed out earlier, in the context of a recurrent selection program for polygenic traits, specific combining effects are not inherited from one selection cycle to the next one.

Randomization

Randomization is one of the central tenets of good experimental design. It helps guard against unwanted confounding between effects and non-experimental variables and underpins the assumption of independence of errors from ordinary linear models. However, intercrop trials can obstruct the process of randomization, since the regular patterns between alternating rows or strips are often by necessity non-random. In trials where the neighbor crop is one of the experimental factors, this factor cannot be randomly applied to the experimental units (e.g., sub-plots within strips). One possible solution is the use of spatial models, which attempt to correct for spatial trends in the analysis rather than at the design stage. When applied to data for a series of intercropping experiments looking at border effects, spatial models were found to improve the model fit in some but not all tested datasets (Knörzer et al., 2010). For plant breeding programs with relatively “simple” breeding objects – for example, finding the best genotype combination of maize and bean, a regular planting design need not overly bias the results if each recorded plot experiences a similar interaction environment; here, neighboring species is not an experimental factor of interest. However, the introduction of systematic biases (e.g., light interception patterns due to strip orientation) is often unavoidable, and therefore, careful planning of experiments is needed. If breeding is being performed to select a specific genotype that performs well with a wide range of other companion crops (in the broadest sense, as a target crop and good neighbor), then randomization issues become extremely pertinent. Simple designs are not always best in such situations (Connolly et al., 2001).

Evolutionary Breeding

The idea of allowing natural selection to play a part in how a heterogeneous population develops has been termed “evolutionary

breeding” (Suneson, 1956) and, although usually applied to intraspecific diversity, does fall under the wider topic of breeding for more genetically diverse systems. The possible benefits of such diverse populations are well documented, particularly under lower input conditions (Phillips and Wolfe, 2005; Dawson and Goldringer, 2012), providing a level of buffering against environmental variability. They also offer the possibility of developing local strains or farmer varieties through on-farm seed saving. Composite cross-populations, generated from a diverse panel of founder genotypes, provide a starting point for evolutionary breeding and have also featured in experiments aimed at developing varieties for intercropping (Allard and Adams, 1969; Hill, 1990). The potential of evolutionary breeding as a tool for intercrop breeding has been again recently highlighted (Annicchiarico et al., 2019), allowing component crop species to co-evolve over a number of generations. The authors did caution about its applicability for inbred crops, which may have limited evolutionary scope to improve complementarity traits (Annicchiarico et al., 2019). Many self-fertilizing species naturally have a low level of outcrossing, but a refinement to the original evolutionary breeding strategy was to introduce a certain proportion of male sterility in the population, promoting outcrossing and leading to hybrid seed production over multiple generations (Suneson, 1951, 1956; Phillips and Wolfe, 2005). Assuming that sufficient out-crossing occurs to produce a representative quantity of seed on male sterile plants, this would allow evolutionary progress (as opposed to dominance of a single genotype) to take place over practical time-scales.

For evolutionary biologists, competition effects in communities play a central role in so-called tragedies of the commons, where co-operation among a group of individuals is continually vulnerable to invasion from selfish individuals (Hardin, 1968; Gersani et al., 2001). In an agricultural context, the superior individual performance of dominant highly competitive individuals is often not reflected in the collective performance of such individuals when placed together in a field or in a pen (Weiner et al., 2017). Indeed, the process of domestication and artificial selection

has often run contrary to natural evolutionary processes to avoid or circumvent such tragedies of the commons (Denison, 2012; Anten and Vermeulen, 2016; Montazeaud et al., 2020). On the one hand, evolutionary breeding may be vulnerable to potential tragedies of the commons. However, it could provide a complementary avenue to develop diverse and robust plant populations, particularly in the context of on-farm seed saving and farmer-engaged breeding efforts.

Genetic Resources for Intercrop Breeding

Breeding is the exploitation of genetic variation for humankind's benefit. It is an effort to both increase and decrease variation within the context of a single species (Louwaars, 2018; Schouten et al., 2019). Therefore, the issue of whether the genetic resources for improved intercrop performance are present in existing modern cultivars is of primary importance to intercrop breeding.

It is worth first examining whether existing genetic diversity within a crop species has demonstrated any functional purpose in crop mixtures. In natural systems, within-species genetic diversity is likely to play an important role in productivity (Hughes et al., 2008). In grassland systems, intraspecific diversity has been shown to result in positive biodiversity effects, for example in increased yield stability (Prieto et al., 2015). Fewer experiments have been performed in crop species, although meta-analyses of cereal performance (with a focus on wheat) have reported over-yielding to occur in crop mixtures (Kjær et al., 2009; Borg et al., 2018; Reiss and Drinkwater, 2018). Recent evidence suggests that there is a significant genotypic component in the ability of plant mixtures to over-yield (in this case, its domestication status: either wild or cultivated), tested over a range of important crop species (Chacón-Labela et al., 2019).

Chacón-Labela et al. (2019) also found that biodiversity effects may have been reduced in the process of domestication. This suggests possible increases in intercrop performance could be achieved by re-diversifying the genetic basis of agricultural crops (Figure 3), although the performance gap between modern

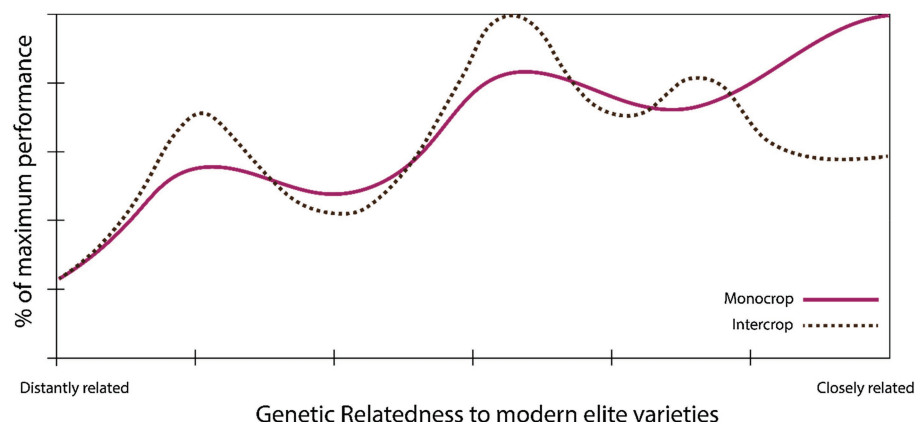


FIGURE 3 | Theoretical performance landscape of modern varieties for intercropping. Modern elite varieties may not be optimal for intercropping, due, for example, to reduced partitioning of assimilates to seeds or reduced biodiversity effects when grown in mixtures (Chacón-Labela et al., 2019; Chen et al., 2021). Breeding efforts may have to break through local optima (dashed line) by accessing wider pools of genetic diversity in order to re-equip crops with features suited to intercropping.

varieties, landraces, and crop wild relatives would require serious breeding attention.

Although not the only metric to judge relative performance, yield remains a central target of most breeding efforts, whether for monoculture or intercropping. It appears that modern cultivars may reduce the proportion of assimilates allocated to seed production when grown in mixtures, despite showing overall higher yields in both vegetative and reproductive plant parts in mixtures when compared to monoculture (Chen et al., 2021). As we do not know what theoretical limits exist regarding resource partitioning of crops grown in mixtures to seeds or other edible parts, it is too early to say whether breeding efforts could increase yield gains further, but the implication, particularly in the light of this recent evidence, is that it should be possible.

Studies have also shown differences in the root microbiome between wild ancestors and the cultivated progenitors, but again with a mixed pattern. For example, in barley (*Hordeum vulgare*), below-ground microbe communities were altered in small but significant ways depending on whether a modern cultivar, landrace, or wild accession was grown (Bulgarelli et al., 2015; Alegria Terrazas et al., 2020). For soya bean (Liu et al., 2019) and wheat (Valente et al., 2020), a more diverse microbiome was reported for wild ancestors than for crops, but for lettuce, domestication increased rhizobiome diversity (Cardinale et al., 2015). For maize, a history of 80 years of breeding did not leave an imprint on the microbiome (Emmett et al., 2018). While these studies showed differences in species composition and diversity, linking such differences in microbiome functioning has still turned out to be elusive in most cases. Only Liu et al. (2019) showed that, despite taxonomic divergence in the microbiome of the wild ancestor and modern soya bean, there was functional convergence between both microbiome communities.

Overall, there is a need to assess whether the genetic resources currently available contain sufficient heritable variation for intercrop performance, and, if not, whether this could be increased by accessing wider gene-pools beyond that of modern elite germplasm. Without a systematic assessment of this, we risk making only marginal improvements in intercrop performance at great effort.

DISCUSSION

A Powerful Troika: IGE, Plant Growth Models, and Genomic Prediction

In this paper, we have described two complementary breeding strategies for intercrop breeding: trait-based versus product-based. This dichotomy has also previously been recognized, where “trait-based” and “trait-blind” breeding approaches were identified (Gaba et al., 2015; Barot et al., 2017). Barot et al. (2017) proposed that these approaches be combined, using information on trait complementarity to perform an initial selection, after which a trait-blind strategy would select superior combinations. Here, we take a closer look at how these different strategies can complement each other.

Indirect genetic effect models come in two types: variance component models and trait-based models (McGlothlin and Brodie, 2009; Bijma, 2014). Variance component models do not model the IGE as a linear function of traits of the companion species, but instead partition the total genetic variance in the focal trait into a direct effect attributable to the focal individual and IGEs attributable to its social partners using linear models (e.g., Muir, 2005). They are empirically very powerful, but do not specify the causal traits and thus provide no knowledge of the underlying mechanisms. Trait-based models, in contrast, represent a functional approach that specify the IGE on an individual as a function of specific traits of its neighbors (Moore et al., 1997). Trait-based IGE models are a powerful approach when good prior information or a hypothesis on the traits underlying the IGE is available, particularly when phenotypes for these traits can be recorded precisely, but become statistically less tractable when multiple traits and reciprocal interactions are involved (Bijma, 2014).

To illustrate the two models, we compare the trait-based model of Moore et al. (1997) to the corresponding variance component model. Following Moore et al. (1997), considering interaction between two individuals, the value z_i for trait i of the focal individual may be expressed as the sum of an additive genetic component of the focal individual, a_i , a general (i.e., non-social) environmental component, e_{gi} , and a component due to the values z_j of each of $j=1$ to n traits of the partner,

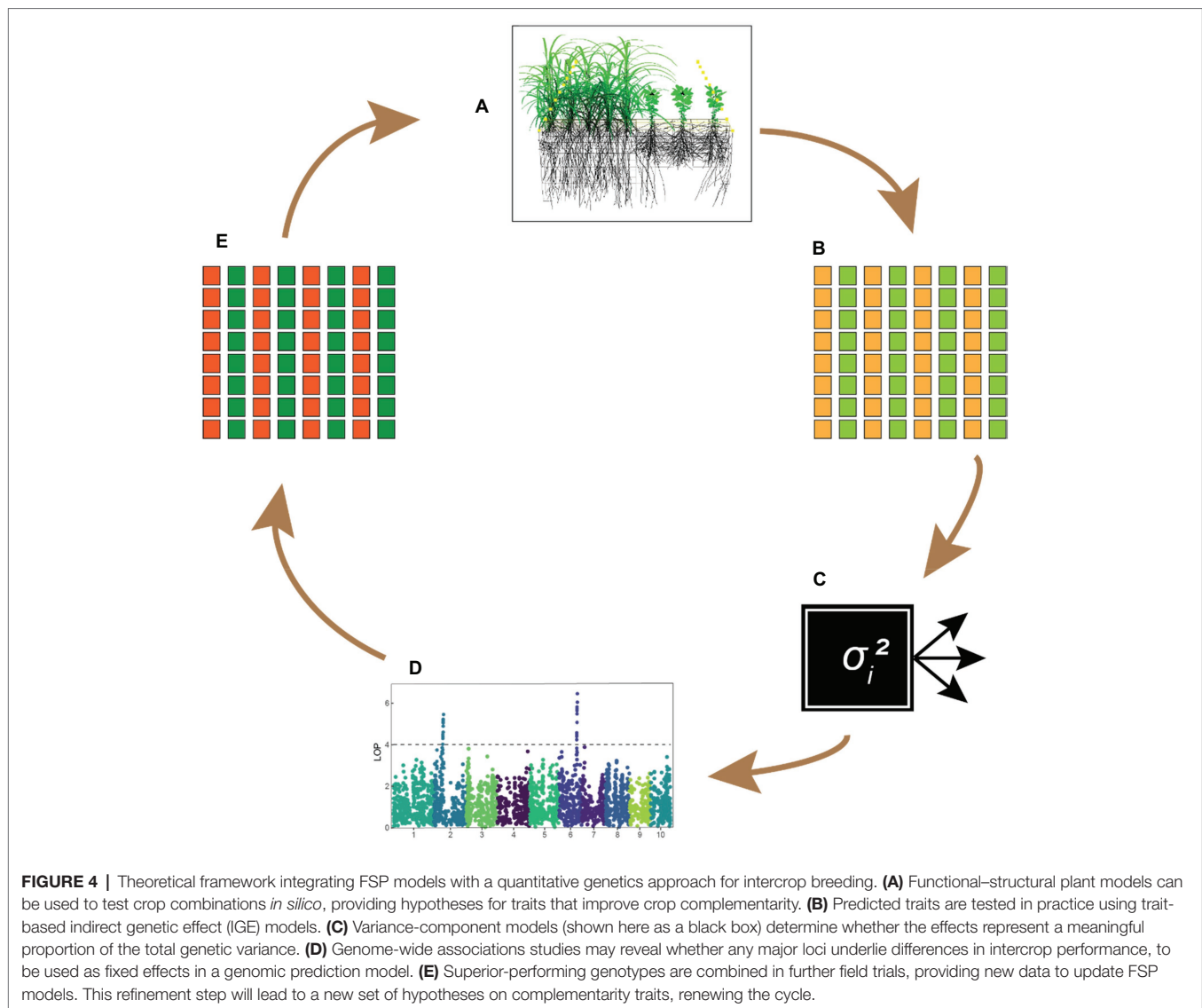
$$z_i = a_i + e_{gi} + \sum_{j=1}^n \Psi_{ij} z'_j$$

where the ' indicates the social partner. Here, the Ψ_{ij} is a path coefficient from trait j of the partner to trait i of the focal individual and the product $\Psi_{ij} z'_j$ specifies the impact of trait j in the partner on the value of trait i in the focal individual. Hence, this model attributes indirect effects to specific traits (j) of the social partner. The corresponding variance component model is given by

$$z_i = A_{D,i,\text{focal}} + A_{I,i,\text{partner}} + e_i$$

where $A_{D,i,\text{focal}}$ is the (direct) genetic effect of the focal individual on its own value for trait i and $A_{I,i,\text{partner}}$ represents the full IGE of the partner on the value of trait i in the focal individual, without making reference to specific causal traits in the partner. Trait-based IGE models represent a functional approach with a focus on between-plant interactions and could therefore be complemented by FSP models. While variance component models disregard the functional traits underlying plant-plant interactions, such knowledge could considerably advance the precision of the phenotypes and thus the accuracy of selection. For example, FSP modeling coupled with information on phenotypic correlations could be used to determine which trait combinations optimize intercrop performance and whether such combinations are feasible (Picheny et al., 2017; van Eeuwijk et al., 2019).

Moreover, statistical and functional models could be used as complementary approaches to identify the phenotypic traits functionally underlying the interactions (Figure 4). On the



one hand, predictions based on functional models could be compared to empirical data to see whether their predictions match observed effects, in particular whether predictions from functional models agree with estimated genetic regression coefficients, and potentially also to identify new traits not (yet) present in the current gene pool (**Figures 4A–C**). On the other hand, variance component models can be used as a black-box tool to select populations for lower competitiveness (**Figure 4C**). Subsequently, the observed changes in functional traits provide information on which phenotypic traits underlie the competitive interactions, which may be used to improve FSP models (**Figures 4D,E**). In this approach, breeders let “the plants figure it out.” This approach may also lead to the identification of new traits that play an important role in plant–plant interactions and thus also has an exploratory function. Furthermore, the ability of plant growth models to simulate an extensive range of phenotypes without the normal constraints has the potential to predict novel phenotypes or

phenotype combinations not yet encountered by breeders. Such traits could potentially provide breakthrough advances in intercrop breeding programs that might not have been otherwise achieved.

These traits may be included explicitly as predictors in a selection program using precision phenotyping (e.g., measured by sensors carried by unmanned arial vehicles or field robots, or using lab analyses). Recording many phenotypes at high precision is relatively costly, and breeders should utilize such information to the best possible extent. This is where genomic prediction (GP) can play a key role. With classical breeding for polygenic traits, the value of an individual phenotype is restricted to the individual itself and its close relatives. Hence, in a classical setting, precision phenotyping would need to be performed on “all” candidates for selection. With genomic prediction, however, information collected on a limited set of individuals can be utilized for a much broader set of candidates for selection that may be somewhat distantly related to the

phenotyped individuals. In this way, GP could considerably increase the value of precision phenotypes, while removing the dependency on having complete phenotypic information before selection decisions can be made. Moreover, multivariate application of GP would give insight into the (genetic) relationships between the different traits involved in plant–plant interactions and could therefore inform FSP models with stochastic elements.

Intercrop Breeding Without the Intercrop

Most breeding activities are currently performed in single-crop settings, reflecting the predominant monoculture agricultural paradigm. Although we have been considering specific breeding approaches for intercropping, we are assuming that IGEs are an important component in an intercrop system, not just in their magnitude but also in their potential correlation to direct genetic effects. However, this has yet to be firmly established for many important crop combinations and represents an important start for further research in this direction. This echoes the call to prioritize research into the linkage or correlation between “agronomic traits” and “interaction traits” (Litrico and Violle, 2015).

A high-input pure stand that discards data from border rows arguably provides a much more uniform environment than even a well-designed intercropping trial. In plant breeding, particularly in early-stage trials, the unit of selection is usually a single row or a small plot, which only loosely approximates the growing conditions of a large monoculture field. At later stages of a breeding program, plot sizes may increase as the number of genotypes to test decreases, but at this point many of the crucial early selections have already occurred. It is interesting to speculate that the necessity of selection procedures based on small plot performance (e.g., small seed lots, many genotypes to test, and limited space) may have inadvertently facilitated selection for intercrop performance or at least, to non-uniform competition effects from neighbors. However, these neighbors are usually of the same species as the target genotype. The literature on this topic tends to view such non-uniform effects as nuisance (Rebetzke et al., 2014) rather than as potentially beneficial for the long-term prospects of breeding for diversity.

If IGEs can be ignored, it would be preferable to continue to select in a more uniform pure stand than in an intercrop. A recent study on the application of genomic prediction for intercropping modeled a genetic correlation between monocrop and intercrop yield (Bančić et al., 2021) as the main parameter controlling the shared heritable information between a pure stand and mixed stand. Through simulation, it was found that the magnitude of this genetic correlation influences the optimum breeding strategy to apply (i.e., whether to include information from monocrop trials or not in a prediction model). The authors went on to argue that genotype $\times$ genotype interactions (which we understand to be another term for IGEs) will be minimized through the use of continuous complementary recurrent selection schemes (Hill, 1996; Bančić et al., 2021). However, it is not clear why heritable variation for $G \times G$ should tend to zero before that of direct genetic effects, nor whether

this is a desirable strategy in the context of long-term genetic gain (Gorjanc et al., 2018; Vanavermaete et al., 2020).

Another approach to the question “do we need to breed for intercropping in an intercrop” has been to compare selection efficiencies between pure stands and mixed stands. Selection efficiency has previously been defined as $S = \frac{Y - A}{X - A} \%$, where X is the number of genotypes selected in the pure stand, Y is the number of pure-stand selected genotypes that were also selected in the intercrop, and A is the random expectation for Y , sampled from a binomial distribution (Hamblin and Zimmermann, 1986). This parameter has some advantages in that it says something about the reality of a running breeding program and the selective pressure being applied in a specific situation. However, it says nothing about the IGEs of the focal crop on its neighbors. Framing the issue as a genotype $\times$ cropping system interaction has also been used to test whether selection efforts for intercropping should be done in an intercrop system or not, depending on the level of significance of the interaction term (Gebeyehu et al., 2006). Again, this approach remains limited unless both direct and indirect genetic effects are considered. In many studies, there has been evidence of weak correlations between traits across genotypes evaluated in intercrop and monocrop systems (Zimmermann, 1996; Holland and Brummer, 1999), which at least provides a motivation to breeders to start testing their varieties under intercrop conditions. Indeed, some traits are simply not expressed in pure stands (in particular, the effect a plant has on its neighbors and vice versa) and cannot be evaluated without a mixed-crop setting.

CONCLUDING REMARKS

We began this piece with agriculture at a crossroads. Diversified agriculture points a clear route toward more sustainable and productive systems. Although breeding for intercropping is by no means simple, it offers the possibility to re-align our crops with the cropping systems of the future, both above and below the ground. It is clear that breeding for intercropping will not become widespread without sufficient economic justification. Currently, research is underway to determine which crop combinations perform well together (not just in terms of yield, but also positive effects on bird and insect populations for example). There are also many well-established crop combinations that are used for intercropping worldwide (e.g., maize and bean) that provide well-tested models upon which to build. Once compatible cropping partners are known, the approaches described here can be used to estimate the magnitude of genetic variation for intercrop performance. Such knowledge, coupled with an increased uptake of diversified agriculture by farmers (perhaps incentivized for its positive impacts on biodiversity), will provide the breeding sector with a clear direction and justification. We are already witnessing a renewal of interest in the topic of intercrop breeding (not just in academia but in the wider breeding community) and anticipate further significant developments in this area in the coming years, in both theory and practice.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Application of Crop Growth Models to Assist Breeding for Intercropping: Opportunities and Challenges

Martin Weih^{1*}, Eveline Adam², Giulia Vico¹ and Diego Rubiales³

¹Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, ²Saatzucht Gleisdorf GmbH, Gleisdorf, Austria, ³Institute for Sustainable Agriculture, Consejo Superior de Investigaciones Científicas (CSIC), Córdoba, Spain

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*Correspondence:

Martin Weih
martin.weih@slu.se

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Intercropping of two or more species on the same piece of land can enhance biodiversity and resource use efficiency in agriculture. Traditionally, intercropping systems have been developed and improved by empirical methods within a specific local context. To support the development of promising intercropping systems, the individual species that are part of an intercrop can be subjected to breeding. Breeding for intercropping aims at resource foraging traits of the admixed species to maximize niche complementarity, niche facilitation, and intercrop performance. The breeding process can be facilitated by modeling tools that simulate the outcome of the combination of different species' (or genotypes') traits for growth and yield development, reducing the need of extensive field testing. Here, we revisit the challenges associated with breeding for intercropping, and give an outlook on applying crop growth models to assist breeding for intercropping. We conclude that crop growth models can assist breeding for intercropping, provided that (i) they incorporate the relevant plant features and mechanisms driving interspecific plant–plant interactions; (ii) they are based on model parameters that are closely linked to the traits that breeders would select for; and (iii) model calibration and validation is done with field data measured in intercrops. Minimalist crop growth models are more likely to incorporate the above elements than comprehensive but parameter-intensive crop growth models. Their lower complexity and reduced parameter requirement facilitate the exploration of mechanisms at play and fulfil the model requirements for calibration of the appropriate crop growth models.

Keywords: APSIM, biodiversity, complementary resource use, plant breeding, process-based models, STICS, mixed cropping

INTRODUCTION

Intercropping is the simultaneous cultivation of at least two crops in the same field (Willey, 1979), although without necessarily sowing or harvesting them at the same time. Intercropping has been a common agricultural practice over ages; however, the intensified agriculture of the last decades is based on uniform crops relying on mechanization and heavy use of synthetic fertilizers and pesticides, which has reduced intercropping (Hauggaard-Nielsen and Jensen, 2001). The negative side impacts of intensive agriculture on soil, water, and air quality and on biodiversity conservation are calling for a renewed interest on intercropping, among other practices (Malezieux et al., 2009).

Intercrops often use available resources more efficiently than the corresponding sole crops, as intercropped species can utilize resources in a complementary way and take advantage of other mechanisms such as niche facilitation. However, the outcome and success of intercrops depends on the competitive hierarchies and the role of asymmetric competition among the admixed species, as well as their individual performances (Andersen et al., 2007). For example, in cereal-legume intercrops, the cereal component is often a better competitor for soil inorganic nitrogen (N) than the legume component especially in early growth stages, due to rapid and deep root growth; while the legume component can exploit fixed N mainly in later crop growth stages when the soil N availability increasingly limits crop growth (Bedoussac et al., 2015). The mechanisms underlying competitive hierarchies and positive interactions can include the complementary use of less mobile soil resources such as phosphorus (Hinsinger et al., 2011), with several legume species being able to facilitate the acquisition of phosphorus by associated cereals (Li et al., 2014).

Traditionally, intercropping systems have been developed and improved by empirical methods within a specific local context, e.g., by combining species and varieties with anticipated complementary resource use and niche differentiation in a certain region. To support the development of promising intercropping systems, the individual component species of mixtures can be subject of breeding and genotypes with contrasting resource foraging characteristics selected to maximize mixture complementarity, reduce negative competitive interactions, and improve the production of each component species (Litrico and Violle, 2015). Such a process can be facilitated by modeling tools simulating, in a system approach, plant functioning and expected outcomes of the combination of different species' (or genotypes') traits for growth and yield development over time. As such, models can support the exploration of a wide range of plant properties and growth conditions without the need of extensive and time-consuming field testing; and even before actually breeding for these plant properties. Crop models have already been used successfully to assist plant breeding (Rötter et al., 2015). Yet, the focus of the previous approaches was on the design of cultivars grown as sole crops. Modeling intercrops involves additional challenges, mainly because often complex plant-plant interactions need to be considered; although, the underlying mechanisms in many of them still are poorly known. We discuss current advances and future directions for a more effective use of models in support of breeding for intercropping. We do this by (i) summarizing the specific challenges associated with breeding for intercropping; (ii) providing an update on existing crop growth models that can simulate intercrops; and (iii) evaluating their application to assist breeding for intercropping.

CHALLENGES IN BREEDING FOR INTERCROPPING

Breeding would be a straightforward task if the desired traits were clear, there was genetic variation in the traits to be selected for, and accurate but fast and economic screening protocols were available for the massive screenings needed. Breeding success

can be accelerated by adoption of valuable emerging technologies for phenotyping and genotyping. However, proper identification, prioritization of the traits and their combinations to target among the many possible ones remains a major challenge. Their definition is needed before they can be selected individually or in combinations in large segregating populations following different selection strategies (Annicchiarico et al., 2019; Bancic et al., 2021; Wolfe et al., 2021). In the absence of such knowledge on traits and trait combinations, breeding is still possible but needs to rely on heavy experimentation using proper designs (Barot et al., 2017; Haug et al., 2021). A better understanding of the mechanisms underlying intercropping benefits would facilitate the search of the existing variation for the traits of interest and enhance the breeding success. To date, the bottleneck remains our understanding of the most relevant traits to breed for in an intercrop.

In general, trait selection and crop breeding can be performed either on sole crops or intercrops. Yet, selection efficiency for intercropping adaptation under sole cropping conditions is generally moderate or low (Annicchiarico et al., 2019), and elite cultivars selected for sole cropping systems might not be the optimal ones for intercropping. This is because, in a sole crop, desirable traits are often those increasing resource acquisition, whereas, when the same crop is grown in an intercrop, traits that optimize complementarity or facilitation can be more relevant (Costanzo and Barberi, 2014) but require considering complex above- and below-ground interspecific interactions. Also, traits are plastic and likely differ when plants are grown in sole crops or intercrops; and the often observed significant genotype $\times$ cropping system interactions indicate that specific breeding for intercropping is needed to exploit the genetic variability of the traits of interest in an intercrop context (Nelson and Robichaux, 2006).

Particularly important, in an intercrop context, are traits related to competitive ability and compatibility, which can be selected for by incorporating the relevant traits into selection indices (Annicchiarico, 2003; Annicchiarico and Filippi, 2007). Still, the relevant adaptive traits can vary with the intercropping systems and over time, reinforcing the need for careful considerations of appropriate trait combinations (Jensen et al., 2015). For example, in general, leaf area, leaf area development, and plant height are all expected to enhance competitive ability. In a specific case, pea competitive ability was affected mainly by leaf area in early growth stages and plant height later on (Barillot et al., 2014), which needs to be considered when this species is to be grown in an intercrop. While leafless pea types are desired in sole crops to improve standing ability, leafy types might be preferred in intercrops due to a higher growth rate and competitive ability (Semere and Froud-Williams, 2001). Thus, breeding for intercrops requires setting specific objectives for each of the admixed species in relation to the other(s). For example, in cereal-legume mixtures the legume component is often less competitive due to a lower relative growth rate, so we could admix less competitive cereals or try to improve the competitiveness of the legume. This can be achieved by selecting for (i) higher relative growth rate and plant height in the legume or lower in the cereal or both; (ii) greater

plasticity of both, with implications for plant competition for light, including higher light absorption capacity under shading (Wang et al., 2006); and (iii) early establishment of rhizobium symbiosis in legumes (Hauggaard-Nielsen and Jensen, 2001).

The challenge is to discern the most relevant traits contributing to the possible intercropping benefits, and to prioritize them according to their predicted breeding value. Modeling could help to understand the net outcome of the complex interactions among the components' traits affecting complementarity and facilitation in intercrops; and to define how specific traits should be changed to take maximal advantage of complementarity and other intercropping benefits. In principle, there are two types of models, i.e., process-based and empirical models. Process-based models simulate detailed physical or biological processes inherent a system, while empirical models rely on correlative relationships in line with mechanistic understanding, but without fully describing the inherent processes. In reality, most models use a hybrid approach and combine process-based and empirical elements. Empirical approaches involve great uncertainty and bias especially when correlative relationships are extrapolated beyond observed variability. Simulation of the processes behind plant–plant and plant–environment interactions in intercrops usually involves the extrapolation of relationships beyond observed variability, because most of the available data sets are from sole crops and represent the relevant relationships under past conditions which not necessarily are the same in future conditions. The best suited models to address the complex interactions in intercrops are therefore those that explicitly describe the processes behind plant–plant and plant–environment interactions, as reviewed next.

PROCESS-BASED MODELS TO SIMULATE INTERCROPS

Mathematical process-based crop growth models integrate plant properties and environmental conditions in a system approach. They simulate plant functioning based on the individual plant properties of crop species or cultivars and the environmental and management (e.g., intercropping) conditions at the target location. Crop growth models quantify the final outcome of these interacting aspects on, e.g., crop yields, without depending on lengthy field test campaigns. As such, they can assist plant breeding, by highlighting which plant properties are sensitive to the model simulation conditions, how the outcome of these properties would respond to the anticipated changes in growth conditions, and ultimately where significant performance gains can be made by breeding.

Despite their promises, hitherto only few crop models have been developed and applied to simulate intercrops. Simple models have been developed to evaluate how plant–plant interactions in terms of competition and facilitation can affect plant growth and seed yield (Tilman et al., 1997; Klimek-Kopyra et al., 2013; Evers et al., 2019), but these models cannot be used to predict the net outcome of species mixtures in agriculture. Among the models designed for agronomic applications, the most frequently used for intercrops are APSIM (Agricultural Production Systems sIMulator) (Keating et al., 2003; Knörzer et al., 2011a; Chimonyo et al., 2016; Berghuijs et al., 2021) and STICS (Simulateur

multIdisciplinaire pour les Cultures Standard, or multidisciplinary simulator for standard crops) (Brisson et al., 2003, 2004; Corre-Hellou et al., 2009). Other crop growth models able to simulate intercrops are Daisy (Manevski et al., 2015) and DSSAT-CERES (Knörzer et al., 2011b; for reviews of model applications to intercrops, see Knörzer et al., 2010 and Chimonyo et al., 2015). Simulating intercrops with these models is often challenging, because they use many parameters, for which measured values from field experiments are required as inputs. Given the limited set of parameters typically available from most field experiments using intercrops, the uncertainties in the estimates of these parameters are large, and consequently the resulting simulation results are uncertain. An alternative approach is that of minimalist crop growth models. These models rely on fewer parameters, thus reducing the uncertainties in parameter estimations. These models also facilitate model adjustment to various species or variety combinations grown in an intercrop under different conditions (Van der Werf et al., 2007). Minimalist crop models have been recently developed for strip intercrops of wheat and maize (Gou et al., 2017; Liu et al., 2017; Tan et al., 2020) or wheat and faba bean grown under nitrogen-limited conditions (Berghuijs et al., 2020).

Modeling an intercrop requires capturing the extent of interspecific competition for limiting resources and how that is determined by the properties of the admixed species. In this context, it is interesting to note that process-based growth models often depart from conditions of unlimited plant growth. The simulated potential plant growth is then reduced by the environmental factors considered relevant, including neighbors of different species competing for the same resources. For example, the competitive ability of the species involved was affected by differences in canopy structure and crop height (Keating and Carberry, 1993; Pronk et al., 2003; Gou et al., 2017), root system architecture (Ozier-Lafontaine et al., 1998; Corre-Hellou et al., 2007), and nutrient uptake capacity (Corre-Hellou et al., 2006). The APSIM model considers these effects *via* the following crop-related model parameters: phenology stage (usually defined in degree days), leaf development and biomass growth rates, radiation use efficiency (RUE) in g biomass per unit of light, and water and/or nitrogen demand and deficit functions (Chimonyo et al., 2016; Berghuijs et al., 2021). The STICS model provides additional examples for crop related parameters that can account for the competitive ability and how it changes in intercrops: minimum and maximum root and biomass growth rates and species specific nitrogen dilution functions derived from theoretical optimum nitrogen contents in the admixed target species (Brisson et al., 2003; Corre-Hellou et al., 2009). Finally, in minimalist models, the following crop related parameters describing inter-specific competition have been used: minimum and maximum plant heights, relative growth rate, specific leaf area, RUE, nitrogen demand and dilution functions (Gou et al., 2017; Berghuijs et al., 2020; Tan et al., 2020).

DISCUSSION

Crop growth models use plant parameters to simulate growth and development of a crop for the given environmental

conditions. These models have been used previously to assist plant breeding, especially ideotype breeding of crops to be grown in sole culture (Martre et al., 2015; Rötter et al., 2015). In contrast to the many applications for growth models simulating sole crops, few crop growth models have been developed, calibrated, and validated for intercrops, to accommodate the specific mechanisms of plant–plant interactions that are important in intercrop performance (Knörzer et al., 2010; Chimonyo et al., 2015).

A remaining challenge for modeling intercrops is including the most important mechanisms of plant–plant interactions, such as different kinds of cues for neighbor detection: light quality (initiating, e.g., shading adaptation), root chemicals, and volatile organic compounds (Biedrzycki et al., 2010; Gruntman et al., 2017; Ninkovic et al., 2019). Most existing models applicable to intercrops lack several of these mechanisms. For example, APSIM does not simulate shading adaptation of the shorter species in the intercrop, and therefore systematically overestimates the growth of the taller species and underestimates the performance of the shorter species (Berghuijs et al., 2021). However, the addition of plant characteristics and mechanisms driving interspecific competition to existing crop growth models, such as APSIM and STICS, would make these already complex models even more so. There would be a further increase in the number of plant and environment parameters, which then would need to be assessed several times during a single growing season for model calibration. Among them, some are not commonly or easily monitored in the field trials targeted to plant breeding.

Yet, the most important limitation in using crop growth models in support of breeding for intercrops is the difficulty to link model parameters to breeding traits. While the parameter lists for many crop growth models include some “true traits”—i.e., plant characteristics that breeders could select for—many of the parameters included in these models cannot be easily translated into breeding traits. Hence, in spite of the strength of crop growth models in identifying highly influential plant properties, many of these properties are likely to be driven by the expression of several underlying traits and are, therefore, challenging to link to breeding traits. The dependence on environments adds a further level of complexity. At the same time, basing crop growth models only on breeding traits and their combinations and describing within the model how these traits are altered by the environment is generally unfeasible. Even if all the mechanisms involving these traits and their response to growing conditions were well understood and amenable to inclusion in a model, the latter would have large parameter requirements. While these parameters would be better linked to “true traits,” the large amount of field measurements needed for a proper model parameterization would diminish its wide applicability.

A case in point is the RUE. The RUE is a central parameter in most crop growth models, but needs to be decomposed into its component traits that breeders can select for. The component traits behind RUE include the leaf photosynthetic capacity and the spatial distribution of this photosynthetic

capacity over the canopy (Rodriguez et al., 1999). The latter is in turn affected by breeding traits such as leaf angle, leaf phenology, and the carbohydrate source-sink balance during the grain filling of cereals (Reynolds et al., 2000)—some of which could be altered by the plant–plant interactions that are important in intercrops. Although automated phenotyping facilities will enable monitoring the component traits behind RUE in the future (Furbank et al., 2019), their potential use in crop growth models to assist breeding can only be realized if the corresponding field assessments are performed in real intercrops to accommodate the physiological and biochemical mechanisms that are specific for the beneficial effects of intercrops. Keeping in mind the high degree of complexity in the existing comprehensive crop growth models such as APSIM and STICS, incorporating both some additional component traits behind RUE and the interspecific plant–plant interaction mechanisms required to truly simulate intercrops for breeding purposes, is perhaps unfeasible.

A more promising approach is developing dedicated minimalist crop growth models incorporating the plant characteristics and traits that are particularly important for the outcome of the intercrop. While even in these models some parameters cannot be immediately linked to traits for breeding, most of these minimalist crop growth models include parameters that either are true breeding traits important in an intercrop context, or could be easily linked to them; e.g., specific leaf area and plant height (Gou et al., 2017; Berghuijs et al., 2020; Tan et al., 2020). These are good candidates to be included in models to assist breeders. For example, plant height has been selected by breeders during several decades of cereal improvement and could be justified as a trait of interest also in the modeling.

A further aspect to consider when assessing the potential of crop growth models in intercropping is the ultimate goal of the intercrop, and how much such performance is affected by specific crop traits. For example, if maximizing the total intercrop community (seed) yield is the most important goal, then crop height of the component species might be less important. But if instead the individual yields of the component species matter most, then the crop height of the individual intercrop components is an important trait to consider (Berghuijs et al., 2020).

In summary, for models to be effective in assisting breeding of intercrops, they need to be designed so that they can be used to predict the best trait combinations for the specific end-use of the intercrop. To this end, models need (i) to incorporate the relevant plant features and mechanisms driving interspecific plant–plant interaction in the model; (ii) rely on parameters that are closely linked to the traits that breeders would select for; and (iii) be calibrated and validated with field data that are assessed in intercrops, if possible using advanced field phenotyping technologies to fulfil the parameter requirements of the common crop growth models. Due to their lower complexity and much reduced parameter requirement, minimalist crop growth models are more likely to incorporate the above elements than comprehensive and parameter-rich crop growth models such as APSIM and STICS.

AUTHOR CONTRIBUTIONS

MW: conceptualization. MW and DR: writing—original draft preparation. DR, EA, and GV: writing—review and editing. DR, EA, GV, and MW: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Supply Chain Perspectives on Breeding for Legume–Cereal Intercrops

Lars P. Kiær^{1*†}, Odette D. Weedon^{2†}, Laurent Bedoussac³, Charlotte Bickler⁴, Maria R. Finckh⁵, Benedikt Haug^{5,6}, Pietro P. M. Iannetta⁷, Grietje Raaphorst-Travaille⁸, Martin Weih⁹ and Alison J. Karley^{7†}

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*Correspondence:

Lars P. Kiær
lpk@plen.ku.dk

[†]These authors have contributed
equally to this work and share first
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¹Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark, ²Department of Ecological Plant Protection, University of Kassel, Kassel, Germany, ³AGIR, Univ Toulouse, ENSFEA, INRAE, Toulouse, France, ⁴Organic Research Centre, Cirencester, United Kingdom, ⁵Department of Crop Sciences, Research Institute of Organic Agriculture FiBL, Frick, Switzerland, ⁶Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE – Le Moulon, Paris, France, ⁷Ecological Sciences, The James Hutton Institute, Dundee, United Kingdom, ⁸Nordic Maize Breeding, Doniaga, Netherlands, ⁹Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Compared to sole crops, intercropping—especially of legumes and cereals—has great potential to improve crop yield and resource use efficiency, and can provide many other ecosystem services. However, the beneficial effects of intercrops are often greatly dependent on the end use as well as the specific species and genotypes being co-cultivated. In addition, intercropping imposes added complexity at different levels of the supply chain. While the need for developing crop genotypes for intercropping has long been recognized, most cultivars on the market are optimized for sole cropping and may not necessarily perform well in intercrops. This paper aims to place breeding targets for intercrop-adapted genotypes in a supply chain perspective. Three case studies of legumes and cereals intercropped for human consumption are used to identify desirable intercrop traits for actors across the supply chains, many of which are not targeted by traditional breeding for sole crops, including certain seed attributes, and some of which do not fit traditional breeding schemes, such as breeding for synchronized maturity and species synergies. Incorporating these traits into intercrop breeding could significantly reduce complexity along the supply chain. It is concluded that the widespread adoption and integration of intercrops will only be successful through the inclusion and collaboration of all supply chain actors, the application of breeding approaches that take into account the complexity of intercrop supply chains, and the implementation of diversification strategies in every process from field to fork.

Keywords: breeding strategies, crop mixtures, intercrop-adapted genotypes, legume–cereal intercropping, participatory breeding, species synergy, supply chain actors

INTRODUCTION

The practice of intercropping legumes and cereals is predicted to drive the sustainable intensification of food supply chains (Finckh, 2008; Finckh et al., 2021; Li et al., 2021a,b). Compared with sole crops, intercrops have great potential to improve yields and enhance land use efficiency (Yang et al., 2019; Li et al., 2020, 2021a,b; Weih et al., 2021). Additionally, legume–cereal intercrops can provide ecosystem services, such as (i) improved resource use efficiency (Li et al., 2021b; Zhang et al., 2021), particularly for nitrogen (Jensen, 1996; Bedoussac and Justes, 2010a; Naudin et al., 2010), (ii) greater biodiversity, including beneficial insects (Brandmeier et al., 2021); (iii) pest and pathogen regulation (Finckh and Wolfe, 2015; Zhang et al., 2019; Finckh et al., 2021); (iv) enhanced soil health (Yang et al., 2019; Uwase et al., 2021; Zhang et al., 2021); and (v) healthy and nutritious food products (Dwivedi et al., 2017). Although legume-based intercrops are not practiced widely in modern farming systems, they can contribute toward national and EU policy targets for reducing pesticide use, minimizing fertilizer losses, reversing biodiversity declines, and delivering secure and resilient food systems (Iannetta et al., 2021).

Each legume–cereal intercrop is part of a dedicated supply and value addition chain (referred to here as supply chains) with different end uses and actors requiring different outcomes and breeding targets. The major functions of legume–cereal intercrops from industrialized agriculture are animal feed in the form of grain, whole-crop forage, or silage. Their use for wholegrain and processed food products is currently small scale, although this is changing rapidly (Hamann et al., 2019), and legume species in addition to pea and faba bean are expected to become increasingly popular (Magrini et al., 2019; Mamine and Farès, 2020).

The benefits of intercrops for crop yields and other outcomes are often dependent on the specific genotypes used (Ajal et al., 2021), emphasizing the importance of breeding for mixtures. Cultivars that contribute specifically to optimizing intercrop benefits represent an emerging market opportunity for breeders and seed producers. However, while the need for developing intercrop-adapted genotypes has long been recognized (e.g., Finlay, 1976; Finckh, 2008; Lamichhane et al., 2018), and even occurred historically before pure-line breeding became popular (e.g., pea cultivars were selected and bred in species mixtures until the end of the 19th century; Zohary and Hopf, 1973), most cultivars on the market are optimized for sole cropping and might not perform well in intercrops (Kammoun et al., 2021). Recently, a few innovative breeders have initiated small-scale breeding programs for intercrop-adapted genotypes with specifically selected traits and characteristics (Hoppe, 2016; Adams, 2018; Starke, 2018; KWS, 2019; Raaphorst-Travaille, 2019). The lack of optimized cultivars is, however, one of several bottlenecks limiting a wider use of intercropping (Rosa-Schleich et al., 2019; Bonke and Mußhoff, 2020; Trivett et al., 2021).

Intercropping currently imposes added complexity at different levels of the supply chain (Tippin et al., 2019; Mamine and Farès, 2020), which is a key reason for the low demand for intercrop-adapted genotypes. The many challenges associated with

diversification strategies, such as intercropping, could be overcome through the integration of all actors within the supply chain from plant breeders to consumers (Lammerts van Bueren et al., 2018; Wolfe et al., 2021). The breeding of intraspecific mixtures for disease control is an example where close collaboration along the supply chain has been successful (Finckh and Wolfe, 2015). In rare cases, breeders might engage with mixture breeding to promote their own cultivars for novel uses (Labarthe et al., 2021). However, a key actor for trait selection is farmers, whose choice of intercrop traits depends on many factors, including pedoclimatic, end use, market quality requirements, crop rotation considerations, and availability of farm equipment (Verret et al., 2020). Where intercrop products require downstream processing, aggregators and processors are likely to focus on traits affecting mixed seed separation (e.g., seed size), product purity, and nutritional quality (including anti-nutritional factors), and/or other physico-chemical properties that affect processing efficiency (e.g., for milling, fermentation, extrusion). Growing societal expectations and consumer demands for agriculture to support biodiversity, environmental sustainability, and more nutritious products (e.g., Lienhardt et al., 2019a; Mamine and Farès, 2020; Marette, 2021) will influence trait selection by breeders and actors along the supply chain.

Here, three case studies of intercrop supply chains were used to: (i) determine challenges at each level of the supply chain, (ii) identify relevant trait categories to help overcome these challenges, and (iii) suggest potential breeding targets for “intercrop-adapted” genotypes. Finally, approaches and methods with potential to improve breeding for intercropping and increase supply chain acceptance are discussed.

DESIRABLE BREEDING TRAITS IN INTERCROP SUPPLY CHAINS

The three case studies draw on input from relevant stakeholder groups, including breeders, crop scientists, farmers, and processors. This was compiled from authors' experience, exchange with relevant stakeholders in Germany, France, Scotland, and Denmark, and a workshop held at the first European Conference on Crop Diversification (Budapest, Hungary, September 2019). Cases were selected among several candidates based on the criteria that they (i) be currently relevant legume-based intercrops for human consumption in an author's country, (ii) represent different types of supply chains, and (iii) reveal some experience with supply chains actors.

Case study 1: Winter wheat intercropped with pea in Germany. While traditionally grown for fodder, this combination is gaining attention for its potential to improve wheat baking quality. Many farmers are currently reluctant to grow this intercrop due to lack of expert advice and experience within farmer networks, as well as suitable pea cultivars for mixing with winter wheat.

Case study 2: Pea–barley intercropping in Scotland, using barley for distilling and pea protein by-products as a food ingredient. Barley is grown on over 60% of the arable land in Scotland and is used for brewing and distilling and animal feed, which are critically important to Scotland's economy and culture. Pea intercropping creates an opportunity to diversify the arable system.

TABLE 1 | Compiled breeding targets that were assessed as important for each actor in the supply chain in each of the three intercrop case studies.

Actor	Case study 1 Winter wheat—Pea (food)	Case study 2 Barley—Pea (alcohol and food protein)	Case study 3 Lentil—Cereal (food)
Plant breeders	All of the below	All of the below	All of the below
Seed multipliers and merchants	Abiotic and biotic stress tolerance/resistance Seed quality	Abiotic and biotic stress tolerance/resistance Seed quality	Abiotic and biotic stress tolerance/resistance Seed quality
Farmers	Yield Resource use efficiency Abiotic and biotic stress tolerance/resistance Lodging resistance Increased root vigor Wheat baking quality Resistance to pod shattering in legume* Resistance to seed splitting in legume* Seed size and color differentiation* Synchronization of ripening times* Species synergy*	Yield Resource use efficiency Abiotic and biotic stress tolerance/resistance Synchronization of ripening times* Species synergy*	Yield Resource use efficiency Abiotic and biotic stress tolerance/resistance Lodging resistance Increased root vigor Resistance to pod shattering in legume* Increased lentil pod harvest height* Seed size and color differentiation* Synchronized crop ripening times* Species synergy*
Aggregators	Seed size and color differentiation* Resistance to seed splitting in legume*	Seed size and color differentiation* Resistance to seed splitting in legume*	Seed size and color differentiation* Resistance to seed splitting in legume*
Processors	Wheat baking quality Less anti-nutritional factors	Resistance to seed splitting in legume* Ease-of-use of protein-rich co-product* Low N content in barley grain Starch-rich pea	
Wholesalers and Retailers	Quality, nutrition and sensory characteristics [§]		Quality, nutrition and sensory characteristics [§]
Consumers	Quality, nutrition and sensory characteristics [§]	Sensory characteristics [§]	Quality, nutrition and sensory characteristics [§]

*Traits only relevant for intercrop breeding are in bold, while the rest are general agronomic traits relevant for both sole crop and intercrop breeding (see section “General Agronomic Traits”). Traits related to synergistic plant–plant interactions are marked with “+” (see section “Species Synergy Traits”). Traits important for technical issues related to cultivation and post-harvest handling of intercrops are marked with “**” (see section “Traits Related to Technological Challenges”). Traits related to seed quality, nutrition, and sensory characteristics are marked with “§” (see section “Quality, Nutritional, and Sensory Characteristics”).*

Case study 3: Lentil intercropped with cereals for human consumption based in France, Denmark, and Germany. Lentil is a high-value food crop and intercropping with cereals in organic systems provides weed suppression and structural support resulting in increased lentil height and more efficient harvest.

Crop traits desirable to each supply chain actor were compiled for each case study (Table 1), revealing four overall trait categories. While breeders are an essential part of the supply chain our initial focus is on the other supply chain actors, who create the primary demand for specific intercrop traits and properties.

General Agronomic Traits

Several of the breeding traits identified as relevant within the supply chain for these intercrops (Table 1) are equally important for sole crops, including yield, stress tolerance/resistance, pest and disease resistance, weed suppressiveness, lodging resistance, root vigor, winter hardiness and quality traits, such as low levels of anti-nutritional factors (Gupta, 1987).

Selection of these traits for intercrops is particularly challenging due to the added complexity of managing crop species interactions (Brooker et al., 2015; Litrico and Violle, 2015), and their responses to crop agronomy, soil conditions, and climate (Allard, 1999;

Lithourgidis et al., 2011; Saxena et al., 2018). For example, selection for yield in an intercrop should aim to maximize complementary resource use and minimize asymmetrical competition, as the overall yield of intercrops often depends on the yield of the less-competitive component (Harper, 1977; Kammoun et al., 2021). This stresses the importance of selecting for competitive ability of less-competitive crop partners (Annicchiarico et al., 2021), particularly when the less-competitive species is also more economically valuable, which is often the case for legumes (Hamann et al., 2020).

Species Synergy Traits

The main advantage of intercrops is often described as being the result of the “4C effects” (Justes et al., 2021) corresponding to three positive interactions (complementarity, cooperation, and compensation) and one negative interaction (competition) occurring simultaneously and dynamically between species over the whole cropping cycle. Positive legume–cereal interactions are underpinned by mechanisms of niche differentiation, such as for soil mineral nitrogen vs. biological nitrogen fixation (Bedoussac et al., 2015; Cowden et al., 2021), and facilitation, such as soil phosphate release by

legume root exudates (Homulle et al., 2021) and physical support to prevent lodging. One of the main challenges for improving intercrops is characterizing the trait combinations that maximize these positive interactions while minimizing negative interactions (Brooker et al., 2021; Homulle et al., 2021; Justes et al., 2021). The intercrop ideotypes that optimize these processes will vary with the intended outcome, whether to increase fertilizer use efficiency, minimize lodging, improve weed control, or promote biodiversity (Brooker et al., 2015; Gu et al., 2021; Homulle et al., 2021). Identifying clear goals to be achieved by intercropping is crucial when choosing candidate germplasm in the selection process, as different goals may necessitate separate breeding programs or simply the correct selection of existing genotypes.

Selection for “species synergy” (Table 1), that is, traits and trait combinations that optimize complementary interactions above and belowground, is expected to “force the positive relation between diversity and yield” (Litrice and Violle, 2015). Aboveground traits include plant morphology, physiology, phenology, and developmental trajectories (Lithourgidis et al., 2011; Isaacs et al., 2016; Saxena et al., 2018; Bourke et al., 2021; Nelson et al., 2021). Belowground traits (summarized in Homulle et al., 2021) include rooting patterns and architecture (Lithourgidis et al., 2011; Streit et al., 2019; Bourke et al., 2021; Timaeus et al., 2021), nutrient-releasing or pathogen-suppressive root exudates, and associations with beneficial microbes including common mycorrhizal networks (Barto et al., 2012; Brooker et al., 2015; Bourke et al., 2021). A positive effect of increased plant diversity on soil communities and plant microbiomes (Strecker et al., 2015; Tiemann et al., 2015; Saleem et al., 2020) can act as a driver for the diversity–productivity relationship (Raynaud et al., 2021).

Traits Related to Technological Challenges

Many of the breeding targets identified within legume–cereal supply chains (Table 1) have implications for technical issues and the additional costs associated with intercropping. Improving these traits could increase the efficiency of mechanical and technological processes embedded within intercrop supply chains, aided by precision technologies for crop agronomy and harvesting (Banfield-Zanin et al., 2021).

Resistance to seed splitting in legumes, for example, is important for both sole crop and intercrop production (Endres et al., 2016), but the separation of split legume grains from cereal grains of similar color and size is particularly challenging (Tippin et al., 2019). Selection for crop differences in seed size and color may increase seed sorting efficiency, reduce the number of seed separation cycles and improve final product purity and quality, while reducing costs (Viguier et al., 2018; Bonke and Mußhoff, 2020). Conversely, differences in seed size might be an undesirable feature during sowing, leading to seed segregation in the drill hopper, which interferes with sowing both species simultaneously as a blend.

Synchronization of ripening times between species and the reduction of pod shattering and seed splitting in legumes are important not only to improve harvesting and seed sorting

efficiency, but also to reduce additional post-harvest handling, such as drying (Tippin et al., 2019; Trivett et al., 2021).

Selection for increased lentil canopy height would improve mechanical pod harvesting efficiency (Viguier et al., 2018), while also raising the combine header off the ground and reducing abrasion damage from stones. This has the added benefit of reducing soil and stone contamination of the grain, improving product quality and purity, and thereby increasing marketable yields and gross margins (Viguier et al., 2018).

Quality, Nutritional, and Sensory Characteristics

While quality, nutritional, and sensory characteristics are breeding targets that are important in sole crops as well as intercrops, intercropping will often influence quality parameters. Cereal grain protein can be improved through intercropping with legumes (Hauggaard-Nielsen et al., 2001; Bedoussac and Justes, 2010b; Bedoussac et al., 2015), although desirable protein levels depend on the end use (Black et al., 2021) and could be mitigated by higher grain starch contents of barley or the intercrop (Lienhardt et al., 2019a,b). Conversely, quality characteristics might be negatively affected by intercropping. For example, differential ripening and inefficiencies in sorting and drying intercrop grains can lead to higher grain moisture content and favor mycotoxin production (Daou et al., 2021), which could be addressed by improving traits related to technological challenges. Product purity will also be a key consideration for removing allergens related to favism and gluten allergy.

Although the benefits of diversified diets are well known (Dwivedi et al., 2017), highlighting the need for diversified crop products for human consumption, the improvement of nutritional and sensory characteristics for intercrops are not well explored. The development of heterogeneous cereal populations with unique sensory characteristics (Vindras-Fouillet et al., 2014, 2021) demonstrates potential opportunities for creating novel and innovative food products using intercrops.

DISCUSSION

Our assessment has identified several desirable intercrop traits for actors across supply chains, including several seed attributes not targeted by modern breeding for sole crops, including synchronized maturity and species synergies. Dedicated crop improvement strategies and collaborations are evidently needed for intercropping to support the sustainable intensification of food and feed supply chains.

Crop breeding priorities are often set by the dominant industry demands for characteristics, such as disease resistance, ease of harvest, or processing quality. Intercropping requires consideration of additional traits; while this might add to breeding complexity, it presents opportunities to reduce complexity for other supply chain actors and encourage intercrop innovations in desirable traits and end products, while contributing to agricultural sustainability.

Taking advantage of potential intercrop innovations will require the involvement and empowerment of all supply chain actors, including regulators who incentivize farmers to adopt legume–cereal intercrops (e.g., by limiting nitrogen inputs or by direct payments for crop diversification). Processors might be more willing to challenge purity requirements when there is a close working relationship with producers (Tippin et al., 2019). Smaller-scale artisan processors often possess the skills to adapt to changes in product composition, although investment is needed to rebuild lost artisanal and short supply chain capacities (Form, 1987; Fitzgerald, 1993; Iannetta et al., 2021). Changes in regulatory procedures could encourage intercrop seed production and certification and facilitate intercrop placement within field-to-fork contexts (Hamann et al., 2018). Additionally, existing infrastructure will need to be redesigned to ensure the efficient processing and storage of intercrop mixtures and their components on regional and national levels (Tippin et al., 2019; Mamane and Farès, 2020). The increasing environmental and food literacy of consumers, combined with policy targets for reduced agrochemical use, net zero carbon, and reversing biodiversity declines, create potential drivers for practices, such as intercropping (Vasconcelos et al., 2020; Balázs et al., 2021a,b).

From the breeder's perspective, the intercrop traits presented above have differing levels of complexity, implying differences in genetic background, variable importance of trade-offs between traits, and a need for different breeding schemes. We identified three categories of traits: (i) general agronomic traits, such as disease resistance and grain yield; (ii) specific traits for intercropping success due to their role in technical, quality, and other downstream processes, including ripening time and seed color; and (iii) complementary traits related to species synergy during the growth period, for example, “mixing ability” and “species compatibility,” which are more complex, not yet clearly defined and undoubtedly involve more genes than the other categories.

These different breeding targets present novel opportunities and challenges for existing breeding programs, especially when considering plant–plant and plant–environment interactions (Gaba et al., 2015). Breeding for Category 1 traits fits readily into existing breeding programs, although selection in sole crops does not necessarily produce genotypes best suited to intercrops (Litrice and Violle, 2015; Bourke et al., 2021). Category 2 breeding traits are also likely to be identified within existing breeding programs. However, lack of supply chain integration means that relevant traits might not be considered important, especially as modern breeding has become driven by scientists and breeders (Tveitereid Westengen and Winge, 2019). Breeding for Category 3 traits related to overall species synergy is currently not pursued within mainstream breeding programs. The “breeding gaps” for these three trait categories present an opportunity for novel “systems-level” breeding approaches that involve selection within mixtures.

Recent advances in breeding tools and approaches highlight the growing interest in their potential use for intercrop breeding. Application of Function-Structural Plant Models (FSPM) and process-based minimalistic models could

significantly reduce the complexity of intercrop breeding, and minimize the need for experimental evaluation of multiple crop genotype combinations and spatial designs (Berghuijs et al., 2020; Blanc et al., 2021; Bourke et al., 2021). While simulation has shown the utility of genomic selection for intercrop breeding (Bančič et al., 2021), using both phenotypic and genomic selection tools is strongly recommended (Annicchiarico et al., 2021; Wolfe et al., 2021). Additionally, methods for estimating both general and specific mixing ability correlated with simple-to-measure indicator traits could provide a cost-efficient and effective methodological framework for intercrop breeding (Haug et al., 2021). The use of additional selection indices, such as cultivar competitive response, can significantly improve genotype selection for intercropping (Kammoun et al., 2021). Furthermore, relevant traits can be pooled into a selection index for indirect selection for intercrop performance under sole crop conditions (Annicchiarico et al., 2019). Studies of genotype-by-cropping system interactions could reveal within-species variation, allowing selection of genotypes most suited to intercropping (e.g., Moutier et al., 2021).

Participatory breeding has been successful at increasing yields of several crops (Ceccarelli et al., 2001; Sperling et al., 2001; Desclaux et al., 2012; van Frank et al., 2018) and this presents an excellent opportunity to engage farmers and other supply chain actors in breeding for intercropping, while simultaneously encouraging its adoption. Heterogeneous populations have also indicated great potential for intercropping (Khan, 1973; Annicchiarico et al., 2019), and evolutionary breeding in mixtures (Suneson, 1956) represents another valuable approach to on-farm breeding for intercrops, especially as breeding for climate resilience becomes more important.

While the diversification of agroecosystems through intercropping is gaining attention, the widespread adoption and integration of intercrops will only be successful through the inclusion and collaboration of all supply chain actors, and the application of different breeding approaches. This requires challenging the “predominant monoculture agricultural paradigm” prevalent in breeding programs (Bourke et al., 2021), and implementing diversification strategies in every process from field to fork.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, and further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LK and AK conceived the idea. LK, OW, LB, and PI collated case study information. LK, OW, and AK wrote the manuscript, with input from all authors. All authors contributed to the article and approved the submitted version.

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A Comprehensive Approach to Evaluate Durum Wheat–Faba Bean Mixed Crop Performance

Stefano Tavoletti^{*†} and Ariele Merletti[†]

Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Ancona, Italy

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Jérôme Enjalbert,
Institut National de Recherche pour
l'Agriculture, l'Alimentation et
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Reviewed by:

Raphael Paut,
Institut National de Recherche pour
l'Agriculture, l'Alimentation et
l'Environnement (INRAE), France
Hans-Peter Kaul,
University of Natural Resources
and Life Sciences Vienna, Austria

*Correspondence:

Stefano Tavoletti
s.tavoletti@staff.univpm.it

[†] These authors have contributed
equally to this work

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Plant breeding for intercropping is lagging because most varieties currently available in the market are selected for sole cropping systems. The present study analyzed the response of durum wheat (12 varieties) and faba bean (3 varieties) in pure and mixed cropping. Field trials were conducted in 2019 and 2020. The performance of each variety in mixed and pure cropping was evaluated using both univariate and multivariate analyses of the grain yield and land equivalent ratio (LER). For durum wheat, grain protein content was also evaluated. Durum wheat varieties were characterized by good performance in both years, whereas faba bean varieties were more affected by the growing season, suggesting that much breeding effort is warranted to improve the latter as a pure and mixed crop. Moreover, the relative performance of all varieties was affected by their combination in mixed cropping, as evaluated based on the ratio (LERratio) between LER for wheat (LERw) and LER for faba bean (LERfb). To further evaluate the overall performance of wheat and faba bean in mixed cropping, total yield, LERtotal (LERw + LERfb), and ln(LERratio) were subjected to principal component and cluster analyses. The first principal component combined the total yield and LERtotal in a single index of the overall performance of each mixed crop combination. The second principal component, based on ln(LERratio), highlighted the relative performance of varieties in each mixed crop combination. The proposed multivariate approach can be applied in the breeding programs for intercropping to identify variety combinations based on crop performance and the relative importance of the proportion of cereal and legume grains in the total harvest.

Keywords: durum wheat, *Vicia faba* minor, breeding for intercropping, principal component analysis, land equivalent ratio

INTRODUCTION

Intercropping is the cultivation of different crops in the same field at the same time and it has been recognized as an alternative to pure crops for the development of more sustainable agricultural systems (Malézieux et al., 2009; Lithourgidis et al., 2011; Costanzo and Bàrberi, 2014; Brooker et al., 2015; Martin-Guay et al., 2018; Li et al., 2020; Maitra et al., 2021). Much attention has been paid to cereal–grain legume intercropping, including both cool- and warm-season crops (Hauggaard-Nielsen et al., 2008; Bedoussac et al., 2015). Among the cool-season crops, research has mainly

focused on bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* ssp. *durum* Desf. Husn.), and barley (*Hordeum vulgare* L.) as cereals intercropped with faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) as grain legumes (Bedoussac and Justes, 2010a,b; Sahota and Malhi, 2012; Abdel-Wahab and El Manzlawy, 2016; Galanopoulou et al., 2019; Kammoun et al., 2021; Nelson et al., 2021). In pure cropping, these crops are grown in dense stands (Hauggaard-Nielsen and Jensen, 2001), with a higher plant density for cereals (300–400 plants m^{-2}) than for grain legumes (on average, 35–45 plants m^{-2} for faba bean and 80–90 plants m^{-2} for pea). Typically, in intercropping, the cereal–legume combinations are grown as mixed crops, with the plants of the two crops planted as a mixture in the field without a specific row arrangement (Aziz et al., 2015; Layek et al., 2018). Under such growing conditions, strong interspecific interactions occur at both the aerial and root levels (Bedoussac and Justes, 2011; Pivato et al., 2021). Thus, morphophysiological traits characterizing each component warrant attention because of their importance to the overall performance of the mixed crops.

Increased biodiversity due to intercropping is advantageous for soil health (Wahbi et al., 2016) as well as nitrogen (N) and P bioavailability (Fustec et al., 2010; Li et al., 2016; Kaci et al., 2018; Ingraffia et al., 2019). In particular, the complementary use of N resources, that is, mineral N for cereals and atmospheric N_2 for legumes, entails lower N fertilization, which reduces carbon dioxide (CO_2) emissions and N losses, ultimately lowering the inputs in more sustainable agricultural systems (Ghaley et al., 2005; Pelzer et al., 2012; Jensen et al., 2020). Simultaneously, compared with legume pure crops, cereal–grain legume mixed cropping enhances the competitive ability of crops against weeds through the allelopathic effects of cereals, reducing the use of herbicides, offering further opportunities to increase grain legume production through low-input conventional and organic farming systems (Wu et al., 2001; Agegnehu et al., 2008; Corre-Hellou et al., 2011; Zander et al., 2016; Bybee-Finley and Ryan, 2018). Moreover, mixed cropping can reduce the damage caused by diseases and parasitic plants (*Orobanche* spp.), which represent the major hurdles to increase grain legume cultivation, particularly in the Mediterranean countries (Fernández-Aparicio et al., 2011, 2016; Karkanis et al., 2018). Overall, intercropping is a reliable alternative to intensive agricultural systems, which rely upon pure crops, for lowering the environmental impacts of agriculture through reduced use of agrochemicals, increased biodiversity within cultivated fields, and enhanced ecosystem services while increasing the crop yield and ensuring stable production (Malézieux et al., 2009; Bedoussac et al., 2015; Raseduzzaman and Jensen, 2017; Hawes et al., 2019; Weih et al., 2021).

In the process of transition toward more sustainable agricultural systems, plant breeding may play a vital role in facilitating the transition from pure cropping to intercropping (Lulie, 2017; Raseduzzaman and Jensen, 2017; Fung et al., 2019). However, selection for sole cropping cannot produce the best genotypes for intercropping, and alternative breeding schemes must be established for intercropping (Lithourgidis et al., 2011; Gaba et al., 2015; Litrico and Violle, 2015; Haug et al., 2021).

Recurrent selection (Sampoux et al., 2020), incomplete factorial designs (Haug et al., 2021), and genomic selection (Bančić et al., 2021) have recently been proposed as the strategies for mixed crop breeding. Moreover, models to identify traits that are the most closely linked to mixed cropping performance have been developed (Berghuijs et al., 2020; Louarn et al., 2020).

In the present study, we explored the mixed cropping of durum wheat and faba beans (*Vicia faba* L. var *minor* Beck). Durum wheat is mainly cultivated for human consumption, whereas small-seed faba beans are primarily used as a protein concentrate in feedstock (Mariotti et al., 2018). While there has been marked progress in terms of plant breeding for durum wheat (Royo et al., 2009; Beres et al., 2020; Xynias et al., 2020), genetic selection for faba bean, although applied effectively (Maalouf et al., 2019; Carrillo-Perdomo et al., 2020), has been limited. These discrepancies are reflected in the performance of cereal and grain legumes, particularly yield stability; as such, faba bean yield is much more variable across years than durum wheat yield (Annicchiarico et al., 2019).

In two successive years, mixed crop combinations of durum wheat and faba bean varieties, which are commonly cultivated in central Italy, were evaluated to (1) compare the performance of mixed crop combinations with that of sole crops, (2) compare the effects of mixed and sole cropping on durum wheat grain protein content, and (3) develop an approach based on multivariate analyses (principal component and cluster analyses) for the characterization of the overall performance of durum wheat–faba bean mixed crop combinations. Although based on commercial varieties, the results of the present study provide information that could be applied to gather a comprehensive evaluation of durum wheat–faba bean combinations in breeding programs for intercropping.

MATERIALS AND METHODS

Field Trials

Two field trials were set at the experimental station of the Università Politecnica delle Marche (Italy) on December 10, 2018 (trial 1: 43°31′54.41″N and 13°22′00.93″E) and January 22, 2020 (trial 2: 43°32′41.09″N, 13°21′34.13″E). Regarding crop rotation, the preceding crops were sunflower and barley for trials 1 and 2, respectively. The high level of precipitation (**Supplementary Figure 1**) registered from September to December 2019 delayed the sowing time for trial 2. However, delayed sowing is rather common in this area of central Italy and the two sowing times in the present field trials represent a normal trend in the local agricultural systems.

Both field trials were conducted in silty–clayey soils, with the relative sand, silt, and clay content of 11.7, 42.4, and 45.9% in trials 1 and 17.4, 43.3, and 39.3% in trial 2, respectively. Soils in trials 1 and 2 were characterized by similarly high pH (8.13 and 8.14), very high (130 $g\ kg^{-1}$), and high (55 $g\ kg^{-1}$) active calcium carbonate content, moderate (11.9 $g\ kg^{-1}$) and low (9.5 $g\ kg^{-1}$) available P, high available K (305 and 295 $g\ kg^{-1}$), low (16.9 $g\ kg^{-1}$), and moderate (20.6 $g\ kg^{-1}$) organic matter content, and moderate total N content (1.20 and 1.15 $g\ kg^{-1}$), respectively.

Mixed crops were sown with intermixed cereal and faba bean seeds in a single step. Therefore, a sowing depth of 3 cm was used, which was a compromise between the sowing depths of 2–3 cm for wheat and 3–5 cm for small-seeded faba beans suggested for our pedo-climatic conditions.

Different levels of N fertilization were applied to durum wheat pure (180 kg N·ha⁻¹) and mixed crops (90 kg N·ha⁻¹) because the amount of N fertilizer (urea, 46%) was set based on durum wheat seed density. Pure faba bean crops were not fertilized, according to the local farming practices.

For both trials, a randomized complete block design with four replicates was applied and each plot comprised eight rows (length, 5 m) spaced 15 cm apart.

Durum Wheat and Faba Bean Varieties

A total of 12 durum wheat and three faba bean varieties, cultivated as pure crops in central Italy and representing a wide range of varieties in terms of grain yield and quality, were included in the field trials to assess their responses to mixed cropping (**Supplementary Table 1**). A total of 11 durum wheat varieties were chosen because of their relatively good performance across years, whereas Aureo was included because of its very high protein content, despite its lower yield than that of the other varieties available on the market. Three faba bean varieties (Chiaro di Torrelama, Prothabat69, and Rumbo) were included in 2019 (**Supplementary Table 1**), whereas Rumbo was not included in 2020 because the seeds of this variety were not available in that growing season. Therefore, results involving Rumbo in 2019 are summarized in **Supplementary Material**.

Since a preliminary trial performed in 2017 suggested that the 50:50 replacement ratio was suboptimal, mixed crops were sown at a seed density ratio of 50 and 65% for durum wheat and faba bean, respectively (additive design, seed density expressed as a percentage of the respective pure crops). A factorial design was applied to evaluate 36 and 24 mixed crop combinations in 2019 and 2020, respectively. In July 2019 and 2020, each plot was harvested using a Wintersteiger Delta combine harvester for field experimental trials.

Traits Evaluated

The total grain yield (Mg ha⁻¹) of pure and mixed crops was measured. Wheat and faba bean grains from mixed cropping were separated *via* sieving to determine the yield (Mg ha⁻¹) of each crop. Moreover, durum wheat grain protein content (%) was evaluated.

For each mixed crop combination, the land equivalent ratio (LER) of each crop and total LER were calculated as follows (Vandermeer, 1989; Bedoussac et al., 2015):

LER for wheat (LER_w) = Durum wheat yield as a mixed crop/durum wheat yield as a pure crop

LER for faba bean (LER_{fb}) = Faba bean yield as a mixed crop/faba bean yield as a pure crop

Total LER (LER_{total}) = LER_w + LER_{fb}

Intercropping is considered to present better land-use efficiency than sole cropping when LER_{total} exceeds one. Since the partial LER value (LER_w and LER_{fb}) represents the relative performance of a specific variety in terms of its performance

as a pure crop, the LER_{ratio} = LER_w/LER_{fb} was used as an index of the relationship between the relative performances of the two crops in each mixed crop combination. The LER_{ratio} recalls the competitive ratio (CR) proposed by Willey and Rao (1980), but the ratio LER_w/LER_{fb} was not corrected for the proportions in which the crops were initially sown, as applied for the CR coefficient.

The rationale behind the use of LER_{ratio} was as follows: LER_{ratio} = 1 indicates that LER_w = LER_{fb}, therefore both crops show equal relative performance in mixed cropping. In contrast, LER_{ratio} > 1, that is LER_w > LER_{fb}, indicates that durum wheat performed better than faba bean in mixed cropping, or *vice versa* if LER_{ratio} < 1, that is LER_w < LER_{fb}.

For data analysis, the raw data of LER_{ratio} were log-transformed (natural log transformation, ln) to obtain ln(LER_{ratio}). The ln-transformation was needed to overcome the heteroscedasticity of residual errors due to the different ranges of variation of raw LER_{ratio} data when LER_{ratio} > 1 or when LER_{ratio} < 1 (0 < LER_{ratio} < 1). Moreover, after ln-transformation, complementary situations of cereal and legume performance in mixed cropping share the same absolute ln(LER_{ratio}) but with the opposite sign. For instance, if the performance of durum wheat is two times higher than that of faba bean, LER_{ratio} = 2/1 = 2; if the performance of faba bean is two times higher than that of durum wheat, LER_{ratio} = 1/2 = 0.5. Following ln-transformation, the ln(LER_{ratio}) values are ln(2) = +0.693 and ln(0.5) = -0.693. After ANOVA and mean comparisons based on ln-transformed data, the ln(LER_{ratio}) means were subsequently transformed to LER_{ratio} ratios using the exponential (exp) function.

Univariate Data Analyses

Analysis of variance (ANOVA) was performed on the data of only Chiaro di Torrelama and Prothabat69 as faba bean varieties since they were included in both years. Different ANOVA fixed models were applied based on the results of the Shapiro-Wilk and Bartlett's tests, applied to assess the normal distribution of residuals and homogeneity of variances, respectively.

Pure crop yield (Mg ha⁻¹), including both durum wheat and faba bean varieties, was analyzed using the following ANOVA model:

$$y_{ijk} = \mu + \alpha_i + \rho_{j(i)} + \beta_k + \alpha\beta_{ik} + \varepsilon_{ijk} \quad (\text{Model1})$$

where y_{ijk} = pure crop yield; μ = overall mean; α_i = year effect ($i = 1, 2$); $\rho_{j(i)}$ = blocks ($j = 1, \dots, 4$) nested within the year; β_k = pure crop effect ($k = 1, \dots, 14$; 12 wheat + 2 faba bean varieties); $\alpha\beta_{ik}$ = year × pure crop interaction; and ε_{ijk} = residual error.

For mixed crops, the durum wheat yield (Mg ha⁻¹), total yield (Mg ha⁻¹), and ln(LER_{ratio}) were analyzed using the following ANOVA model, including year as the main effect and its first- and second-order interactions:

$$y_{ijkl} = \mu + \alpha_i + \rho_{j(i)} + \beta_k + \gamma_l + \beta\gamma_{kl} + \alpha\beta_{ik} + \alpha\gamma_{il} + \alpha\beta\gamma_{ikl} + \varepsilon_{ijkl} \quad (\text{Model2})$$

where y_{ijkl} = measured variable; μ = overall mean; α_i = year effect ($i = 1, 2$); $\rho_{j(i)}$ = blocks ($j = 1, \dots, 4$) nested within the year; β_k = wheat ($k = 1, \dots, 12$); γ_l = faba bean ($l = 1, 2$); $\beta\gamma_{kl}$ = wheat $\times$ faba bean interaction; $\alpha\beta_{ik}$ = year $\times$ wheat interaction; $\alpha\gamma_{il}$ = year $\times$ faba bean interaction; $\alpha\beta\gamma_{ikl}$ = year $\times$ wheat $\times$ faba bean interaction; and ε_{ijkl} = residual error.

For the faba bean yield in mixed cropping, the 2019 and 2020 data were separately analyzed using the following ANOVA model:

$$y_{ijk} = \mu + \rho_i + \alpha_j + \beta_k + \alpha\beta_{jk} + \varepsilon_{ijk} \quad (\text{Model3})$$

where y_{ijk} = faba bean grain yield; μ = overall mean; ρ_i = block effect ($i = 1, \dots, 4$); α_j = wheat ($j = 1, \dots, 12$); β_k = faba bean ($k = 1, 2$); $\alpha\beta_{jk}$ = wheat $\times$ faba bean interaction; and ε_{ijk} = residual error.

For durum wheat grain protein content, the data from 2019 and 2020 were separately analyzed because of the highly significant heteroscedasticity of residual errors. The cropping system, including pure and mixed crop combinations with the two faba bean varieties, was included as the main effect in the following ANOVA model:

$$y_{ijk} = \mu + \rho_i + \alpha_j + \beta_k + \alpha\beta_{jk} + \varepsilon_{ijk} \quad (\text{Model4})$$

where y_{ijk} = wheat grain protein content; μ = overall mean; ρ_i = block effect ($i = 1, \dots, 4$); α_j = wheat ($j = 1, \dots, 12$); β_k = cropping system ($k = 1, 2, 3$: wheat pure cropping and mixed cropping with two faba bean varieties); $\alpha\beta_{jk}$ = wheat $\times$ cropping system interaction; and ε_{ijk} = residual error.

Tukey's honestly significant difference (HSD) test was applied for multiple comparisons among means for the main effects, and pairwise comparisons (with Bonferroni correction) were used for interactions. Moreover, confidence intervals were calculated to test significant differences from one of the mean LER_{total} values.

Multivariate Analysis

To obtain a comprehensive picture of the effectiveness of mixed cropping, a multivariate approach was applied based on a combined analysis of the most representative variables describing the overall performance of mixed crop combinations: total yield, LER_{total} , and $\ln(LER_{ratio})$. Specifically, principal component analysis (PCA) was performed using Pearson's correlation matrix (Rencher, 2002), followed by cluster analysis (CA; Euclidean distance and UPGMA clustering), to identify the possible patterns of mixed crop combinations on the PCA scatterplot. For multivariate analysis, data from both years were combined in a single data file, with each combination of mixed crop and year considered as operational taxonomic units (OTUs) (Sneath and Sokal, 1973).

Faba Bean Variety Rumbo

Data of the variety Rumbo were evaluated only in 2019, and the results of univariate analysis and PCA are summarized in **Supplementary Material**.

RESULTS

Pure Cropping

All sources of variation (ANOVA, Model 1) were significant. As expected, mean grain yield across years was significantly higher for most durum wheat varieties than for faba bean varieties (**Table 1A**), with a highly significant positive correlation between years ($r = 0.84$, $P < 0.01$). Claudio was the best performing durum wheat variety, and its grain yield was significantly higher than that of most other varieties; Aureo was the lowest yielding variety. Pairwise contrasts (**Table 1B**) showed that the significant pure crop $\times$ year interaction was mainly due to the lower yield of both faba bean varieties in 2020 than in 2019 ($P < 0.001$). Only Nazareno and Aureo produced significantly ($P < 0.05$) higher mean yield in 2020 than in 2019. Therefore, in pure cropping, faba bean varieties were more influenced by the growing season than durum wheat varieties, reflecting the well-known yield instability of faba bean (Flores et al., 1996).

Mixed Cropping

Durum Wheat Grain Yield

The year main effect ($P = 0.13$) and the wheat $\times$ faba bean interaction ($P = 0.07$) were not significant (ANOVA, Model 2). All the remaining sources of variation were highly significant ($P < 0.001$). Therefore, particular attention was paid to the second-order interaction (wheat $\times$ faba bean $\times$ year) and **Figure 1** summarizes the performance of durum wheat varieties in mixed cropping with the two faba bean varieties.

TABLE 1 | Pure crop yields (Mg ha^{-1}).

Varieties	(A) Pure crop ¹	(B) Pure crop × Year		
		2019	2020	p ²
Durum wheat				
Claudio	6.77 ^a	6.60	6.93	
Antalis	6.20 ^{ab}	6.18	6.21	
Marco Aurelio	6.03 ^{ab}	5.77	6.29	
Nazareno	6.00 ^{ab}	5.43	6.58	*
Achille	6.00 ^{ab}	6.15	5.85	
Odisseo	5.68 ^b	5.72	5.65	
Natur	5.39 ^{bc}	4.94	5.83	
Rangodur	5.35 ^{bc}	4.94	5.75	
Tirex	5.32 ^{bc}	4.88	5.76	
Svevo	5.31 ^{bc}	5.15	5.46	
SanCarlo	4.61 ^{cd}	4.38	4.84	
Aureo	3.99 ^d	3.39	4.58	*
Faba bean				
Chiaro di Torrelama	4.27 ^d	5.05	3.50	***
Prothabat69	3.93 ^d	4.52	3.33	***

(A) Pure crop main effect: multiple comparisons of mean yield across years (HSD test). (B) Pure crop $\times$ Year interaction: contrasts (with Bonferroni correction) performed separately for each variety between 2019 and 2020.

¹Difference in means followed by different letters are statistically significant ($P < 0.05$).

²P-value: contrasts between means followed by * or *** are significant at $P < 0.05$ or $P < 0.001$, respectively.

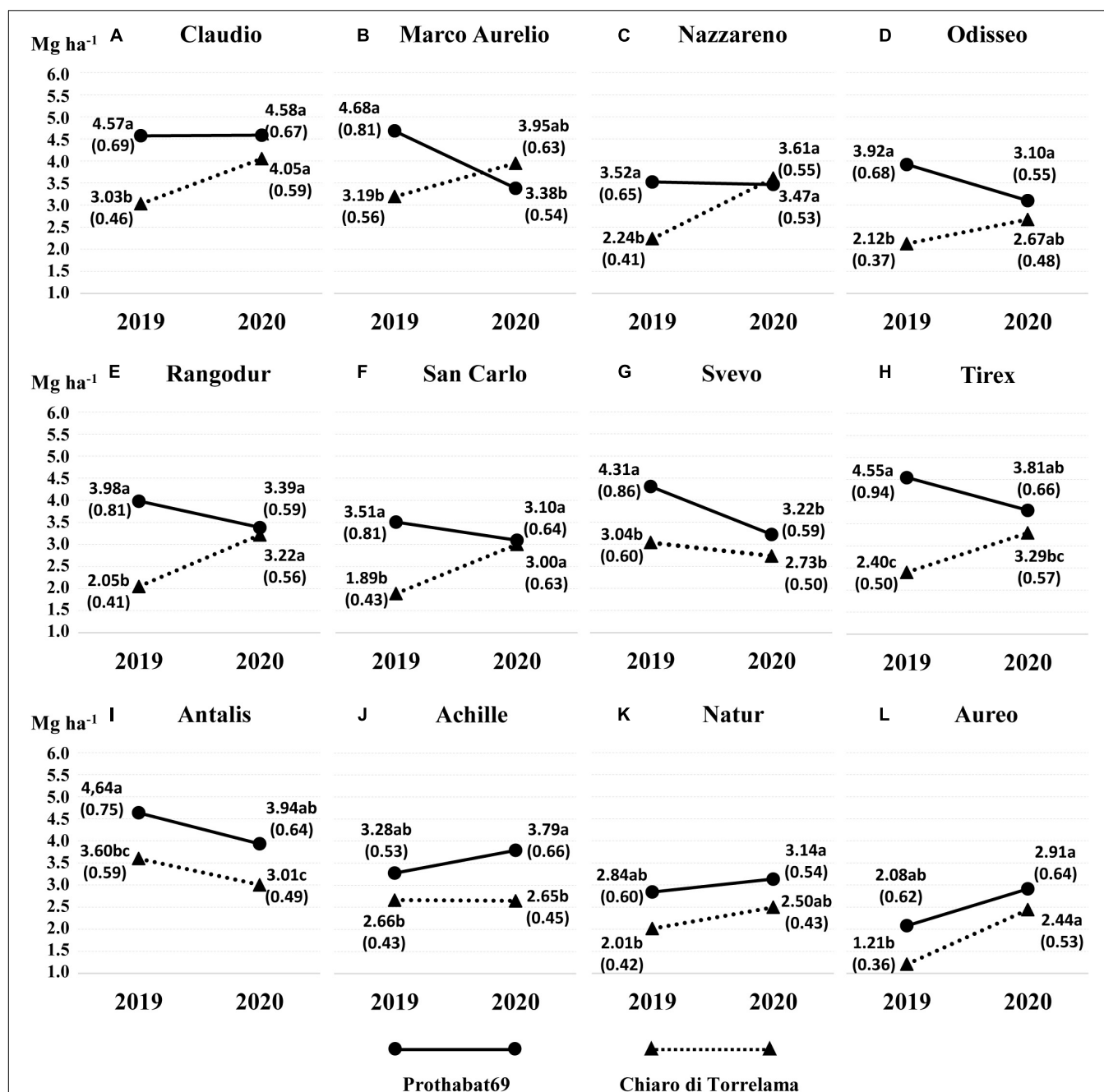


FIGURE 1 | Grain yield in 2019 and 2020 of each durum wheat variety (A–L) in mixed cropping with two faba bean varieties (Chiaro di Torrelama and Prothabat69). Means followed by different letters are significantly different ($P < 0.05$). In parenthesis, the land equivalent ratio (LER) of wheat (LER_w) values are reported.

Eight durum wheat varieties (Figures 1A–H) were characterized by a significantly higher yield with Prothabat69 than with Chiaro di Torrelama in 2019, whereas no significant differences were observed in 2020. Therefore, in 2019, these varieties highlighted a significantly lower performance in mixed cropping with Chiaro di Torrelama than with Prothabat69, as also reflected by the respective LER_w values. Significantly higher yields in combination with Prothabat69 than Chiaro di Torrelama were recorded for Antalis in both years (Figure 1I)

and Achille in 2020 (Figure 1J), while the performance of Natur was relatively stable across years (Figure 1K). Aureo and Nazzeno were characterized by a significant increase in yield from 2019 to 2020 in pure cropping (Table 1); in mixed cropping, however, this trend was detected only in the combination with Chiaro di Torrelama (Figures 1C,L). Moreover, pure crop yield was not correlated with LER_w in both years.

Overall, the results of grain yield and LER_w highlighted a wide range of responses of the 12 durum wheat varieties in

mixed cropping with the two varieties of faba bean. Performance as pure and mixed crops was also differently affected by the year of cultivation.

Faba Bean Yield

The statistical analysis of faba bean yield in mixed cropping was performed separately for each year because of the highly significant heteroscedasticity of residual errors. ANOVA (model 3) revealed significant main effects, but it was only in 2020 that the wheat $\times$ faba bean interaction was significant.

The mean yield of Chiaro di Torrelama was significantly higher than that of Prothabat69 both in 2019 (3.38 vs. 2.14 Mg ha⁻¹) and 2020 (1.58 vs. 1.38 Mg ha⁻¹), suggesting different general mixing abilities of the two faba bean varieties. Moreover, the performance of both faba bean varieties was poorer in 2020 than in 2019, reflecting the same trends observed in pure cropping. Regarding the main effect of durum wheat varieties on faba bean yield, in 2019, mixed cropping with four varieties, namely Nazareno, Natur, Achille, and Odisseo, showed significantly higher faba bean yields than most other combinations (Table 2A).

In 2019, there was a highly significant and negative correlation ($r = -0.72$, $P < 0.01$) between durum wheat and faba bean yield, suggesting that the average performance of faba bean significantly varied according to the durum wheat variety included as a combination crop. Therefore, the average yield of faba bean has increased as the yield of wheat decreased, suggesting some level of balance due to the interaction between the two crops in mixed cropping. In contrast, in 2020, no correlation between durum wheat and faba bean yield was detected, as a consequence of the lower average performance of faba bean. Interestingly, the mean faba bean yield in mixed cropping with Natur was the highest in both years, suggesting that Natur could be considered as the least competitive variety for faba bean in the set of durum wheat varieties evaluated.

Although in 2020 the wheat $\times$ faba bean interaction was significant, Chiaro di Torrelama performed better than Prothabat69 only in mixed cropping combination with Achille and Aureo (Table 2B).

Overall, faba bean was more affected by the different growing seasons than durum wheat, and the response of both faba bean and durum wheat was closely related to the companion variety included in mixed cropping.

Total Yield and LER_{total}

The main effects of durum wheat and faba bean, as well as the durum wheat $\times$ faba bean $\times$ year interaction, were significant. Total yields of mixed crops including Claudio, Marco Aurelio, and Antalis, averaged across faba bean varieties and years, were significantly higher than those from most other combinations, whereas the mean total yield of mixed crops including Aureo was the lowest (Table 3A). Therefore, high variability due to the average effect of durum wheat varieties on the total yield of mixed crops was detected.

The mean total yield of mixed crops including Prothabat69 (5.41 Mg ha⁻¹) was significantly higher than that of mixed crops including Chiaro di Torrelama (5.25 Mg ha⁻¹). Therefore,

TABLE 2 | Faba bean yield (Mg ha⁻¹).

Durum wheat varieties	(A) Faba bean yield (Mg ha ⁻¹) in 2019			
	Main factor ¹	Wheat $\times$ Faba bean		
		ChTL ²	Pr69 ²	P ³
Nazareno	3.28 ^a	3.95 (0.78)	2.62 (0.58)	***
Natur	3.24 ^a	4.01 (0.79)	2.47 (0.55)	***
Achille	3.09 ^a	3.56 (0.71)	2.61 (0.58)	**
Odisseo	3.06 ^a	3.54 (0.70)	2.59 (0.57)	**
Rangodur	2.94 ^{ab}	3.72 (0.74)	2.16 (0.48)	***
Aureo	2.89 ^{abc}	3.52 (0.70)	2.25 (0.50)	***
SanCarlo	2.79 ^{abc}	3.58 (0.71)	2.01 (0.44)	***
Marco Aurelio	2.43 ^{bc}	3.11 (0.62)	1.75 (0.39)	***
Tirex	2.40 ^{bc}	2.97 (0.59)	1.83 (0.40)	***
Antalis	2.35 ^{bc}	2.77 (0.55)	1.93 (0.42)	*
Claudio	2.35 ^{bc}	3.07 (0.61)	1.63 (0.36)	***
Svevo	2.27 ^c	2.74 (0.54)	1.80 (0.40)	**
Durum wheat varieties	(B) Faba bean yield (Mg ha ⁻¹) in 2020			
	Main factor ¹	Wheat $\times$ Faba bean		
		ChTL ²	Pr69 ²	P ³
Natur	1.74 ^a	1.78 (0.51)	1.71 (0.51)	
Achille	1.64 ^{ab}	1.90 (0.55)	1.37 (0.41)	***
Antalis	1.55 ^{abc}	1.71 (0.49)	1.40 (0.42)	
Rangodur	1.51 ^{abc}	1.56 (0.45)	1.47 (0.44)	
Claudio	1.50 ^{abc}	1.54 (0.44)	1.46 (0.44)	
Marco Aurelio	1.50 ^{abc}	1.49 (0.43)	1.51 (0.45)	
Nazareno	1.44 ^{bc}	1.50 (0.43)	1.37 (0.41)	
Odisseo	1.43 ^{bc}	1.41 (0.41)	1.44 (0.44)	
Svevo	1.39 ^{bc}	1.54 (0.44)	1.25 (0.37)	
SanCarlo	1.38 ^{bc}	1.33 (0.38)	1.43 (0.43)	
Aureo	1.36 ^c	1.80 (0.52)	0.92 (0.28)	***
Tirex	1.31 ^c	1.40 (0.40)	1.21 (0.36)	

Results of 2019 (A) and 2020 (B) field trials, including multiple comparisons (HSD test) among the mean yield of faba bean varieties (wheat as the main factor) and contrasts between mean yields of the two faba bean varieties within each mixed crop combination (wheat $\times$ faba bean interaction). Land equivalent ratio (LER) for faba bean (LER_{fb}) values are shown in parenthesis.

¹Means followed by different letters are significantly different ($P < 0.05$).

²Variety name abbreviations: ChTL, Chiaro di Torrelama; Pr69, Prothabat69.

³P-value: contrasts between means followed by *, **, or *** are statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

including the faba bean variety with the lowest performance in mixed cropping resulted in a higher mean total yield because of the increased performance of durum wheat.

Regarding the wheat $\times$ faba bean $\times$ year interaction, pairwise contrasts (Table 3B) revealed that the total yields of mixed crops including Claudio and Aureo were the most stable across faba bean varieties and years, whereas for mixed cropping including Antalis, Nazareno, Odisseo, and Svevo the total yield was significantly higher in 2019 than in 2020 for combinations with both faba bean varieties. In contrast, mixed cropping including Marco Aurelio, Rangodur, and Tirex produced a significantly lower total yield in 2020 only in combination with

Prothabat69, whereas Achille, Natur, and San Carlo produced lower yields in 2020 in combination with Chiaro di Torrelama. Interestingly, comparisons within the year highlighted no significant differences in total yield among the varieties, except for Tirez in 2019.

Moreover, in 2019, LER_{total} values were significantly higher than one for almost all mixed crop combinations, except for Claudio (Table 3C). Meanwhile, in 2020, the LER_{total} values for almost all mixed crop combinations were not significantly different from one, except the Claudio–Prothabat69 and Odisseo–Chiaro di Torrelama combinations, which showed LER_{total} values significantly higher and lower than one, respectively.

Overall, a trend toward convergence to similar overall performance in terms of total yield and LER_{total} was noted for the mixed crop combinations of each durum wheat variety, together with different responses between years. However, the total yield and LER_{total} did not provide information on the relationship between the relative performances of the varieties of the two crops, as expressed by LER_w and LER_{fb} , the values of each mixed crop combination. For this purpose, the LER_{ratio} was analyzed following the $\ln$ -transformation.

$\ln(LER_{ratio})$

The ANOVA (model 2) revealed highly significant wheat and faba bean main effects and significant wheat $\times$ faba bean $\times$ year interactions ($P < 0.001$). Regarding the average effects of the two faba bean varieties, the mean $\ln(LER_{ratio})$ values were positive (0.410) and negative (−0.112) for Prothabat69 and Chiaro di Torrelama, respectively, and the difference was highly significant. Therefore, the average relative performance of the durum wheat was significantly better than faba bean in mixed cropping with Prothabat69, whereas Chiaro di Torrelama showed, on average, a better performance than durum wheat in mixed cropping.

The results of multiple comparisons among overall mean $\ln(LER_{ratio})$ values of the durum wheat varieties are presented in Table 4A. There were significant differences between two groups of varieties, characterized by positive (from 0.262 to 0.411) and negative (from −0.183 to −0.006) $\ln(LER_{ratio})$ values. Three varieties, namely San Carlo, Rangodur, and Aureo, were ranked as intermediate, while Tirez (the highest) and Natur (the lowest) showed significantly different extreme means.

Regarding the two-way interaction (Table 4B), in 2019, the mean $\ln(LER_{ratio})$ values were negative and positive for almost all mixed crop combinations including Chiaro di Torrelama and Prothabat69, respectively. The negative $\ln(LER_{ratio})$ values for Chiaro di Torrelama indicate that in 2019, LER_{fb} was higher than LER_w , whereas a contrasting trend was noted for Prothabat69. Therefore, in 2019, Chiaro di Torrelama showed a better performance than Prothabat69 in mixed cropping with most durum wheat varieties. However, the performance of Chiaro di Torrelama in 2020 was much poorer than that in 2019. Consequently, the mean $\ln(LER_{ratio})$ values were positive for most mixed crop combinations with both faba bean varieties (Table 4B). Only Achille and Natur showed negative $\ln(LER_{ratio})$ values with Chiaro di Torrelama in 2020, suggesting that in less favorable growing seasons for faba bean, these two durum wheat

varieties were characterized by the lowest competitive ability against the best performing faba bean variety.

Overall, the analysis of $\ln(LER_{ratio})$ values confirmed that the relative performance of cereal and legume crops is an important parameter when assessing the effectiveness of durum wheat–faba bean mixed cropping.

Durum Wheat Grain Protein Content

In both years, durum wheat grain protein content showed highly significant variances for cropping system and durum wheat $\times$ cropping system interaction (ANOVA, model 4). Multiple comparisons among cropping systems revealed that in both years, on average, mixed cropping increased the protein content of durum wheat (Table 5A), and the two faba bean varieties significantly differed in terms of their overall effect on this durum wheat quality trait. Regarding the wheat $\times$ cropping system interaction (Table 5B), most durum wheat varieties showed a significant increase in grain protein content in mixed cropping with Chiaro di Torrelama in both years, whereas little differences between mixed cropping with Prothabat69 and pure crops were detected in 2019.

Comprehensive Mixed Crop Performance

To further analyze the information obtained through univariate analyses, a more comprehensive approach based on PCA, followed by CA, was applied. For this analysis, the most important variables, summarizing different features of mixed cropping performance, were considered: total yield, LER_{total} , and $\ln(LER_{ratio})$. The combined results of the PCA and CA, including eigenvalues and eigenvectors, are presented in Figure 2. The dendrogram obtained through CA is shown in Supplementary Figure 2.

The first two principal components (PC1 and PC2) were highly significant (Bartlett test, $P < 0.001$) and explained 93.6% of the total variation. PC1 was related to the total yield and LER_{total} , and both variables showed positive PC1 eigenvector coefficients. Therefore, the higher the PC1 score, the higher the overall mixed crop performance as a combination of the total yield and LER_{total} . Moreover, PC1 effectively highlighted the different performances of mixed crops in the 2 years; the PC1 scores were mainly positive and negative for the mixed crops evaluated in 2019 and 2020, respectively.

The $\ln(LER_{ratio})$ was not correlated with either total yield ($r = -0.06$, ns) or LER_{total} ($r = 0.003$, ns), but it was important for PC2, explaining 33.35% of the total variance. For PC2, high positive, intermediate, and low negative scores were related to mixed crops with a better performance of durum wheat than of faba bean, a similar performance of the two crops, and better performance of faba bean than of durum wheat, respectively.

The effectiveness of $\ln(LER_{ratio})$ in the characterization of mixed crop combinations was further confirmed using CA, which identified main clusters of mixed crops within each year, with only a few exceptions (Figure 2 and Supplementary Figure 2). In 2019, three main clusters (A, B, and C) with positive PC1 scores but different PC2 scores were detected. Only Claudio–Prothabat69, evaluated in 2020, was included in cluster A. Clusters A, B, and C were primarily discriminated against based

TABLE 3 | Total yield and LER_{total}.

Durum wheat varieties		Total yield (Mg ha ⁻¹) ^{1,2}							
	(A) Overall Mean	(B) Durum wheat × Faba bean × Year				(C) LER _{total} ³			
		2019		2020		2019		2020	
		ChTL ⁴	Pr69 ⁴	ChTL ⁴	Pr69 ⁴	ChTL ⁴	Pr69 ⁴	ChTL ⁴	Pr69 ⁴
Claudio	5.98 ^a	6.09 ^a	6.20 ^a	5.59 ^a	6.04 ^a	1.07	1.05	1.03	1.11*
Marco Aurelio	5.76 ^{ab}	6.30 ^a	6.43 ^a	5.43 ^{ab}	4.88 ^b	1.18**	1.20**	1.05	0.99
Antalis	5.74 ^{ab}	6.37 ^a	6.56 ^a	4.71 ^b	5.33 ^b	1.14**	1.18**	0.98	1.06
Nazareno	5.57 ^{abc}	6.18 ^a	6.14 ^a	5.11 ^b	4.84 ^b	1.20**	1.24**	0.98	0.94
Achille	5.46 ^{bc}	6.23 ^a	5.89 ^{ab}	4.55 ^c	5.15 ^{bc}	1.15**	1.11*	1.00	1.06
Rangodur	5.38 ^{bcd}	5.76 ^{ab}	6.14 ^a	4.77 ^b	4.85 ^b	1.15**	1.29**	1.01	1.03
Tirex	5.36 ^{bcd}	5.37 ^b	6.38 ^a	4.69 ^b	5.01 ^b	1.09	1.34**	0.98	1.02
Odisseo	5.20 ^{cd}	5.67 ^a	6.51 ^a	4.09 ^b	4.54 ^b	1.07	1.26**	0.88*	0.98
Svevo	5.16 ^{cd}	5.78 ^a	6.11 ^a	4.27 ^b	4.47 ^b	1.14**	1.26**	0.94	0.97
Natur	5.11 ^{cd}	6.02 ^a	5.30 ^{ab}	4.27 ^c	4.84 ^{bc}	1.22**	1.14**	0.94	1.05
San Carlo	4.96 ^d	5.47 ^a	5.52 ^a	4.33 ^b	4.53 ^{ab}	1.14**	1.25**	1.01	1.07
Aureo	4.28 ^e	4.73 ^a	4.33 ^a	4.24 ^a	3.83 ^a	1.06	1.11*	1.05	0.92

(A) Multiple comparison (HSD test) of the mean total yields of mixed crops, averaged across faba bean varieties and years. (B) Second-order interaction: pairwise contrasts between mean total yields of mixed crop combinations within each durum wheat variety. (C) LER_{total} values.

¹Overall mean yield: differences in means followed by different letters are significant ($P < 0.05$).

²Pairwise contrasts (by rows) of mixed crop combinations for each durum wheat variety: differences in means followed by different letters are significant ($P < 0.05$).

³LER_{total} values are significantly higher or lower than one at $P < 0.05$ (*) or $P < 0.01$ (**).

⁴Variety name abbreviations: ChTL, Chiaro di Torrelama; Pr69, Prothabat69.

TABLE 4 | ln(LER_{ratio}).

Durum wheat varieties		(B) Durum wheat × faba bean × year interaction						
	(A) Main factor		ln(LER _{ratio}) in 2019				ln(LER _{ratio}) in 2020	
	Durum wheat		ChTL ²			p ³	PR69 ²	
	Mean ¹	Ratio	ChTL ²	PR69 ²	p ³		ChTL ²	PR69 ²
Tirex	0.411 ^a	(1.51:1)	−0.168 (1:1.18)	0.851 (2.34:1)	***		0.358 (1.43:1)	0.602 (1.83:1)
Svevo	0.354 ^{ab}	(1.42:1)	0.094 (1.10:1)	0.748 (2.11:1)	**		0.121 (1.13:1)	0.454 (1.57:1)
Marco Aurelio	0.301 ^{ab}	(1.35:1)	−0.099 (1:1.10)	0.746 (2.11:1)	***		0.382 (1.47:1)	0.177 (1.19:1)
Claudio	0.277 ^{ab}	(1.32:1)	−0.270 (1:1.31)	0.679 (1.97:1)	***		0.287 (1.33:1)	0.412 (1.51:1)
Antalis	0.262 ^{ab}	(1.30:1)	0.063 (1.06:1)	0.579 (1.78:1)			−0.010 (1:1.01)	0.418 (1.52:1)
San Carlo	0.255 ^{abc}	(1.29:1)	−0.501 (1:1.65)	0.624 (1.86:1)	***		0.495 (1.64:1)	0.402 (1.50:1)
Rangodur	0.114 ^{bcd}	(1.12:1)	−0.583 (1:1.79)	0.525 (1.69:1)	***		0.224 (1.25:1)	0.289 (1.36:1)
Aureo	0.104 ^{bcd}	(1.11:1)	−0.686 (1:1.99)	0.234 (1.26:1)	***		0.038 (1.04:1)	0.829 (2.29:1)
Nazareno	−0.006 ^{cde}	(1:1.01)	−0.635 (1:1.89)	0.121 (1.12:1)	***		0.233 (1.26:1)	0.259 (1.30:1)
Odisseo	−0.022 ^{cde}	(1:1.02)	−0.648 (1:1.91)	0.174 (1.19:1)	***		0.156 (1.17:1)	0.228 (1.26:1)
Achille	−0.076 ^{de}	(1:1.08)	−0.498 (1:1.65)	−0.085 (1:1.09)			−0.184 (1:1.20)	0.464 (1.59:2)
Natur	−0.183 ^e	(1:1.20)	−0.678 (1:1.97)	0.071 (1.07:1)	***		−0.172 (1:1.19)	0.046 (1.05:1)

(A) Multiple comparisons (HSD test, $P < 0.05$) of overall means across faba bean varieties and years. (B) Second-order interaction: pairwise contrasts (with Bonferroni correction) performed within the year between mixed crops of each durum wheat variety. For each ln(LER_{ratio}) mean, the corresponding LER_w/LER_{fb} is shown in parenthesis; negative values are boldfaced.

¹Means followed by different letters are significantly different ($P < 0.05$).

²Variety name abbreviations: ChTL, Chiaro di Torrelama; Pr69, Prothabat69.

³Differences in means are significant at ** $P < 0.01$ or *** $P < 0.001$.

on PC2 scores because the range of variation in PC1 scores was rather similar among the three clusters.

Cluster A included mixed crop combinations characterized by having only Prothabat69 as the faba bean companion, and ln(LER_{ratio}) values were always positive, ranging between 0.41

(Claudio–Prothabat69 in 2019, LER_w = 1.51 × LER_{fb}) and 0.85 (Tirex–Prothabat69; LER_w = 2.34 × LER_{fb}). These results indicate that in the mixed crop combinations of cluster A, durum wheat varieties overcame Prothabat69 in relative performance, and all mixed crop combinations, except the one including Claudio

TABLE 5 | Durum wheat grain protein content (%) in 2019 and 2020.

	Grain protein (%) ³					
	2019			2020		
	Pure	ChTL ³	Pr69 ³	Pure	ChTL ³	Pr69 ³
(A) CS¹	16.0 ^c	17.2 ^a	16.6 ^b	13.8 ^c	14.8 ^a	14.5 ^b
(B) Variety²						
Achille	14.9	15.5	14.6	13.0	13.7	13.2
Antalis	14.4	15.1	14.9	12.8	13.7**	13.7**
Aureo	19.6	20.6*	20.4	15.5	17.5***	16.5***
Claudio	15.7	16.4	15.6	13.8	14.5*	14.4
Marco Aurelio	16.5	17.8**	17.4	14.7	15.6**	15.2
Natur	16.15	16.7	16.4	13.2	14.4***	14.4***
Nazareno	15.8	16.9**	16.2	14.4	14.8	14.8
Odisseo	14.9	17.1***	15.4	13.5	14.3**	14.1
Rangodur	16.1	16.7	16.8	13.1	14.3***	13.9**
SanCarlo	16.5	18.2***	17.1	14.1	15.4***	15.4***
Svevo	16.8	18.1***	17.6	14.2	15.0**	14.8
Tirex	15.1	17.6***	16.6***	12.9	14.2***	14.2***

(A) Cropping system (CS) as the main factor: multiple comparisons among overall means of 12 durum wheat varieties in pure and mixed cropping systems within each year. (B) Wheat × Cropping system interaction: pairwise contrasts, between the mean of pure crop and each mixed crop combination for each durum wheat variety.

¹Within a year, means followed by different letters are significantly different (HSD test, $P < 0.05$).

²Within a year, the mean of each mixed crop is significantly different from that of the respective pure crop at * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

³Faba bean variety name abbreviations: ChTL, Chiaro di Torrelama; Pr69, Prothabab69.

in 2019 ($LER_{total} = 1.05$), showed LER_{total} values significantly higher than one. Interestingly, based on CA, the mixed crop combinations of Claudio–Prothabab69 evaluated in 2019 and 2020 formed a sub-cluster within cluster A (Supplementary Figure 2), with the lowest PC1 scores.

Cluster B showed intermediate PC2 scores. The lowest and highest PC2 scores were recorded for Claudio–Chiaro di Torrelama and Odisseo–Prothabab69, with $\ln(LER_{ratio})$ values of -0.27 ($LER_w = 0.76 \times LER_{fb}$) and 0.17 ($LER_w = 1.18 \times LER_{fb}$), respectively. Therefore, cluster B included mixed crop combinations that showed a more balanced LER_w/LER_{fb} ratio in 2019.

Cluster C was characterized by mixed crop combinations, including only Chiaro di Torrelama, and showed low negative PC2 scores because the relative performance of the faba bean variety was higher than the durum wheat varieties. The $\ln(LER_{ratio})$ values ranged between -0.68 (Natur–Chiaro di Torrelama; $LER_w = 0.51 \times LER_{fb}$) and -0.50 (San Carlo–Chiaro di Torrelama; $LER_w = 0.61 \times LER_{fb}$).

The variability for PC2 scores was lower in 2020 than in 2019, as shown by the range of PC2 scores in the bi-dimensional scatterplot, and three main clusters (D, E, and F) were identified by CA (Figure 2). The PC1 scores in 2020 were lower than those in 2019 and the narrower range of variation in the PC2 scores reflected the lower performance of faba bean varieties in 2020 than in 2019.

The main difference between clusters D and E was related to the total yield, which was mostly higher and lower than 5 Mg ha^{-1} , respectively, and their LER_{total} values were not significantly different from 1. Interestingly, cluster F showed negative PC2 scores and, similar to cluster C, included mixed crops combinations with only Chiaro di Torrelama as the faba bean companion crop.

Finally, the durum wheat variety Aureo warrants specific attention. In fact, PCA revealed that this variety acted as an outgroup in 2019 when cultivated in combination with Chiaro di Torrelama as well as in 2020 when cultivated in combination with Prothabab69. Aureo is a well-known low-yielding but high-quality variety. As a mixed crop, Aureo showed the poorest performance with both faba bean varieties in 2019 and with Prothabab69 in 2020. Its PC1 scores were low and, based on the PC2 score, this variety showed a better competitive ability against Prothabab69 than against Chiaro di Torrelama in both years. These results suggest that Aureo should be selected as a mixed crop with caution, as its performance is more closely linked to the companion faba bean variety and growing season than the other durum wheat varieties.

Results Including the Variety Rumbo

The faba bean variety Rumbo was included in 2019 alone and results regarding this variety are summarized as Supplementary Table 2 and Supplementary Figure 3 (principal component analysis with Rumbo). Mixed crops including Rumbo showed a wider range of PC1 scores and intermediate PC2 scores when compared to 2019 results of the other two faba bean varieties. Therefore, although limited to one trial, these results confirm that an interesting variability in mixed cropping characterized the set of faba bean varieties evaluated.

DISCUSSION

The present study examined the durum wheat–faba bean mixed cropping system using a set of varieties that are commonly cultivated in central Italy as pure crops. Two years of field trials provided information that could be usefully extended to future breeding for mixed cropping.

Durum wheat is a very important cereal in Italy and the Mediterranean Basin, this crop has been subjected to intense breeding, a wide range of varieties is currently available for farmers (De Vita et al., 2007), and as a pure crop, it ensures high and stable yields across environmental conditions. However, mixed cropping improves land-use efficiency, reduces the use of herbicides and nitrogen fertilizers, leading to more sustainable crop management than pure cropping. The results of the present study showed that by reducing N fertilization by 50%, mixed cropping resulted in a higher grain protein content.

Due to the good performance as a pure crop, farmers could not positively look at the replacement of pure durum wheat with mixed crops. Therefore, a proper and effective strategy, aimed at the valorization in the final products of the advantages related to agroecological and grain quality aspects, should be applied to make farmers choose to intercrop as an effective alternative

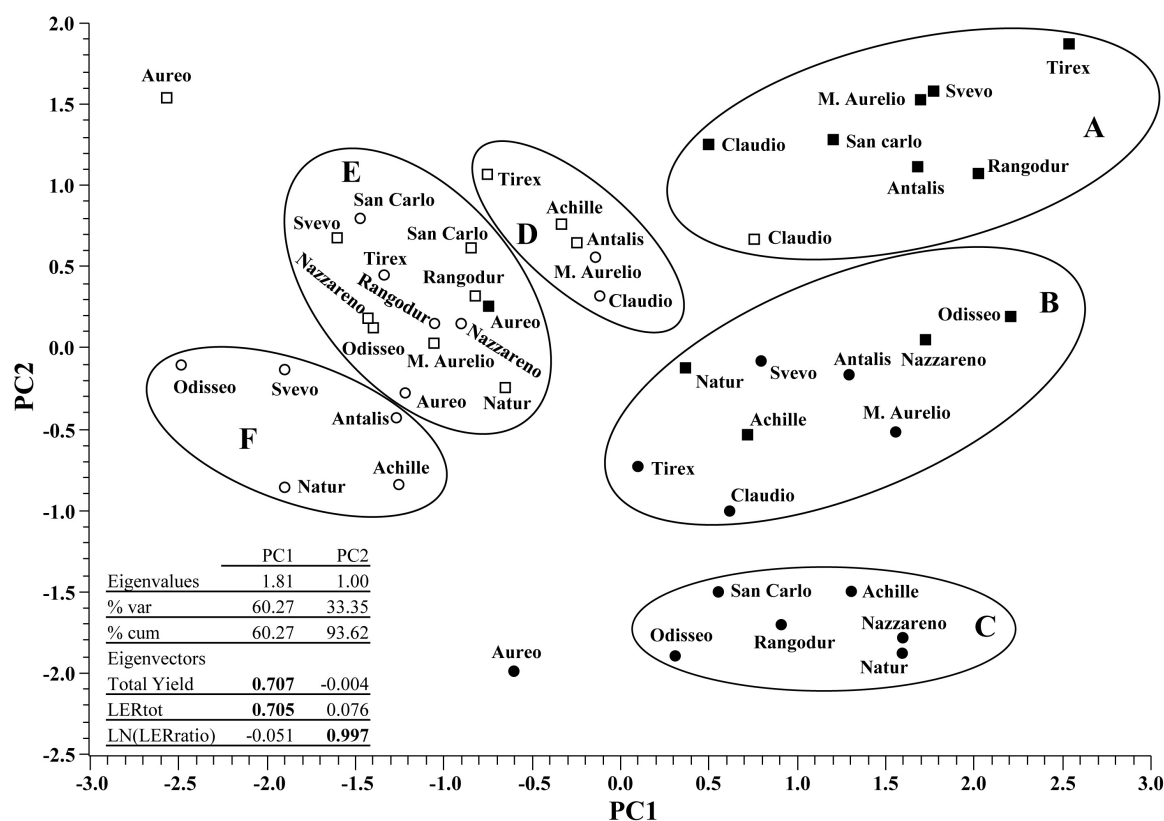


FIGURE 2 | Scatterplot of principal component analysis (PCA). Circles and squares represent mixed crop combinations with Chiaro di Torrelama and Prothabat69, respectively. Mixed crops grown in 2019 and 2020 are indicated in black and white, respectively. Capital letters (A–F) indicate groups identified in cluster analysis (as shown in **Supplementary Figure 2**).

practice to durum wheat pure crop. However, further studies are necessary on the effects of intercropping on other important durum wheat quality traits, such as yellowness and gluten index (Borrelli et al., 1999; De Vita et al., 2007; Magallanes-López et al., 2017).

In both years a highly significant correlation ($P < 0.01$) was found between durum wheat yield as pure and in mixed cropping, but pure crop yield was not correlated with LER_w . Therefore, for the set of varieties evaluated here, the performance in pure crop could not be considered as an index of durum wheat performance in mixed cropping. For example, the variety Claudio ranked among the highest yielding ones in 2019 and 2020 both as pure and in mixed cropping. However, in 2019 the LER_{total} values of mixed crops including Claudio were the lowest. These results were due to its low LER_w value ($LER_w = 0.46$) in combination with Chiaro di Torre Lama and its high LER_w value ($LER_w = 0.69$) with Prothabat69 that was followed by very low performance of this faba bean variety ($LER_b = 0.36$). Differently, in 2020, both combinations including Claudio were the best-performing ones in terms of the LER_{total} , because the high LER_w value compensated for the low general performance of faba bean. These results suggested that a specific relationship between the durum wheat and the faba bean variety, as also affected by environmental conditions, determined the overall performance of each mixed crop combination. This trend was

further confirmed by the negative correlation found in 2019, followed by no correlation in 2020, between durum wheat and faba bean yields in mixed cropping.

Regarding faba bean, this crop is mainly used as feedstuff and plant breeding programs have been less extensively applied for this grain legume than for durum wheat, although it was traditionally a very important legume crop for animal feeding. Therefore, the range of faba bean varieties commercialized in Italy is restricted as compared to durum wheat, and the reintroduction of faba bean at the large scale is currently impeded by the instability of its performance over years, together with many socio-economic aspects, as highlighted by Brooker et al. (2015), Magrini et al. (2016), and Mamme and Farès (2020). Nevertheless, the differences in mixed cropping performance observed among the three faba bean varieties, including Rumbo that was evaluated only in 2019, suggested that genetic variability is available for breeders to select better faba bean genotypes for mixed cropping with durum wheat. The yield fluctuation of faba bean over years is also a very important constraint that hampered the spread of this grain legume in more rational crop rotations. Therefore, breeding to improve faba bean yield stability is of utmost importance to obtain high legume performance both in favorable and unfavorable growing seasons.

Interestingly, our results showed that the total yield of mixed crops was much higher than that of faba bean as a pure crop in

both years, and farmers could also exploit the advantage of mixed cropping for better control of weeds as compared to faba bean pure cropping, due to the allelopathic effects of the cereal. For these reasons, farmers could, at present, look at mixed cropping as a valuable alternative to faba bean pure crops, especially because in unfavorable years for the legume crops, the yield of wheat would balance the lowered yield of the grain legume in the harvested mixed grain. Hopefully, plant breeding will identify new faba bean genotypes that will avoid the differences in the overall performance of durum wheat-faba bean mixed cropping observed comparing the results obtained in 2019 and 2020.

However, given the lack of genotypes selected for mixed cropping, at present, the implementation of durum wheat-faba bean mixed cropping in the transition toward more sustainable agricultural systems must rely on varieties available in the market and selected for sole cropping. Therefore, at present, mixed cropping could be considered a valid alternative to faba bean pure crops, whereas breeding efforts are requested to make mixed cropping more competitive than durum wheat pure crops for farmers.

Moreover, the LER_{ratio} highlighted that the relative performance of the cereal and the legume varied across different mixed crop combinations and over years. Therefore, in inbreeding programs, the LER_{ratio} could be a valuable parameter, together with selection for better stability across years, for the characterization of mixed crop combinations. As a matter of fact, in 2019 durum wheat performance was influenced by the faba bean variety included as a companion crop, because most durum wheat varieties were characterized by a significantly higher yield in combination with Prothabat69 than with Chiaro di Torrelama, and mixed crops including Rumbo showed an intermediate behavior. In 2020, most durum wheat varieties did not show a significant difference in grain yield between combinations with the two faba bean varieties, only Antalis and Achille, retaining a significantly higher yield with Prothabat69 than Chiaro di Torrelama. Therefore, in both years, the durum wheat-faba bean combination significantly affected the overall performance of the mixed crop as a whole, and the LER_{ratio} showed a high discriminant ability among the mixed crop combinations, reflecting an important aspect that would have otherwise been missed by considering only the total yield and LER_{total} .

Overall, the univariate (ANOVA) and multivariate (PCA and CA) analyses provided complementary information for interpreting the performance of durum wheat-faba bean mixed cropping. The average performance of each variety in mixed cropping could provide information on the general mixing ability, but the negative correlation between durum wheat and faba bean yield in 2019 and the overall clustering of mixed crop combinations over years suggested that specific mixing ability should not be disregarded. Therefore, the multivariate analysis of grain yield, LER, and LER_{ratio} allowed the characterization of all mixed crop combinations through a comprehensive evaluation of their overall performance. Indeed, in 2019 all mixed crops of durum wheat varieties grouped in cluster A (high positive PC2 score) shifted to cluster B or C when Chiaro di Torrelama replaced Prothabat69 as a companion crop. Therefore, the relative performance of these durum wheat varieties decreased

in combination with Chiaro di Torrelama. The same result was observed for durum wheat varieties that, in combination with Prothabat69, were included in cluster B but shifted to cluster C when Chiaro di Torrelama was the companion crop. Interestingly, this trend was retained also in 2020, although the range of variation of PC2 score was lower than that detected in 2019.

The multivariate approach highlighted the clear differentiation between mixed crops based on the combinations of durum wheat and the faba bean varieties. Therefore, the principal component scores could be applied as indices of selection within breeding programs aimed to simultaneously improve both cereal and legume performance. This approach could allow the identification of the best combinations that could be considered as an alternative to durum wheat pure crop, faba bean pure crop, or both. Of course, the interpretation of PC scores could vary based on the features of the genotypes of durum wheat and faba bean under evaluation.

CONCLUSION

The present study involved durum wheat, which is probably the most important cereal in the Mediterranean area, and it is involved in a market that asks the farmers to combine high yield with high-quality parameters. All varieties included in the present study not only reflected these needs but also represented a good sample to test their mixing ability with the aim of gathering information on genetic variability that could be available for future breeding programs. The evaluation of this representative set of durum wheat varieties highlighted that efforts are needed to select new durum wheat genotypes because selection for pure crops did not reflect their performance in mixed cropping.

Moreover, although a restricted number of faba bean varieties was included, results of PCA and CA suggested that different combinations of durum wheat and faba bean varieties could result in different relative performances of the cereal and the legume crops. The faba bean choice deeply influenced the performance of durum wheat and, consequently, of the whole mixed crop combination, as better highlighted by the multivariate rather than univariate analysis. Therefore, the analysis at the mixed crop combination level is an important feature to be considered in further breeding programs for durum wheat-faba bean mixed cropping. However, breeding efforts for faba bean in mixed cropping must also be addressed to reduce the instability of performance over years. For this purpose, a wider range of faba bean varieties available in the EU Common Catalog of Plant Varieties, together with further germplasm accessions, could be evaluated and included in specifically targeted breeding programs for mixed cropping.

In conclusion, consideration is necessary about climate change. In the last years, agriculture has been facing the effects of strong year-to-year variation in environmental conditions that deeply influenced crop performance. The 2 years involved in the present study reflected the variability of crop performance under very contrasting growing seasons, confirming,

based on the set of varieties available in the market, the much higher resilience of durum wheat than faba bean. Therefore, our results highlighted the need for a much more intense breeding work for faba bean than for durum wheat to let mixed cropping be more positively evaluated by farmers as an alternative to durum wheat pure crops. *Vice versa*, durum wheat-faba bean mixed cropping is a real opportunity as an alternative to faba bean pure cropping, especially in low input conventional or organic farming. However, the more constant performance of faba bean varieties in mixed cropping is requested, and therefore breeding programs for this grain legume should also involve multiyear trials together with selection carried out under mixed cropping with durum wheat genotypes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Harnessing the Potential of Wheat-Pea Species Mixtures: Evaluation of Multifunctional Performance and Wheat Diversity

Johannes Timaeus, Odette Denise Weedon and Maria Renate Finckh*

Department of Ecological Plant Protection, Faculty of Organic Agricultural Sciences, University of Kassel, Witzenhausen, Germany

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Hamid Khazaei,
World Vegetable Center, Taiwan

*Correspondence:

Maria Renate Finckh
mfinckh@uni-kassel.de

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Species mixtures and heterogeneous crop populations are two promising approaches for diversified ecological cropping systems with increased resilience and reduced dependency on external inputs. Inter- and intraspecific diversity were evaluated in combination using 15 wheat (*Triticum aestivum*) entries, including line cultivars and heterogeneous populations (HPs), from central Europe and Hungary and one winter pea cultivar under organic conditions. Monocultures and wheat mixtures were evaluated multi-functionally for yield, quality, land use efficiency, crop protection, and wheat entry traits. Mixtures increased cereal grain quality, weed suppression, resource use efficiency, yield gain, and reduced lodging. Effects were stronger in 2018/19, which were characterized by dry and nutrient-poor conditions than in 2019/20 when nutrient levels were higher. Wheat entries varied considerably in protein content and yield in both mixtures and monocultures. Under higher nutrient availability, entry-based variation was reduced in both systems, and peas were suppressed. Because of low disease pressure, the wheat entries varied little in terms of disease protection services, and mixture effects on the disease were low. The multi-criteria framework identified stability of yield, yield gains, and quality under high environmental variability of mixtures as clear agronomic advantages with HPs being considerably more stable than line cultivars. Some line cultivars outperformed the HPs in either protein content or yield across environments but not both simultaneously. Trait analysis revealed a possible link between harvest index and reduced competition in mixtures, which can increase yield performance in specific line cultivars. System cultivar interactions were generally very low and highly dependent on environmental conditions. We conclude that while cultivar breeding for mixtures can be successful in monocultures, high environmental variation highlights the necessity of evaluating cultivars in mixtures. In addition, use of intraspecific diversity within interspecific mixed cropping systems can be a valuable addition to further improve mixture performance and its stability under increasing environmental stresses due to climate change.

Keywords: species mixtures, intercropping, diversification, heterogeneous population, multifunctional agriculture, yield gain, composite cross

INTRODUCTION

High-input single species cropping systems are very vulnerable to unpredictable climatic conditions that are the result of climate change (IPCC, 2021, Chapt. 11). Additionally, they rely on fossil fuel-based nitrogen fertilizers and plant protection chemicals for reliable productivity. Nitrogen fertilizer production requires 65–100 MJ per kg with associated emissions of 2.1–5.5 kg CO₂ equivalents per kg (Jensen et al., 2020). There is an urgent need for highly resilient and resource-efficient cropping systems that contribute simultaneously to climate change adaptation and mitigation. Intra- and interspecific diversification of cropping systems has been identified as one of the important building blocks of such agricultural systems (Østergård et al., 2009; Finckh et al., 2021).

Intraspecific diversity enhances resilience against biotic and abiotic stress and can be achieved through evolutionary breeding approaches resulting in HPs (Suneson, 1956; Finckh, 2008; Döring et al., 2011). HPs are highly adaptive to environmental stress and provide higher yield stability than genetically homogenous line cultivars in wheat (Brumlop et al., 2017, 2019; Weedon and Finckh, 2019, 2021). In recognition of the valuable contribution of intraspecific diversity to crop resilience, the new EU Organic Regulation 2018/848 that will come into force in 2022 will provide a legal framework for HPs, enabling further mainstreaming.¹

Intraspecific diversity in cropping systems can be further enhanced by adding interspecific diversity. A key lever to harness interspecific diversity in agriculture is to use the complementarity in nitrogen acquisition strategies of cereals and legumes (Bedoussac et al., 2015). A recent global study estimated that intercropping cereals and legumes could decrease the required fertilizer globally by 26% compared to sole crops (Jensen et al., 2020). In addition, cereal-legume species mixtures in arable cropping systems provide services, such as improved crop quality, weed suppression, land-use efficiency (Bedoussac et al., 2015), crop health (Finckh et al., 2000; Boudreau, 2013; Finckh and Wolfe, 2015), and lodging resistance (Kontturi et al., 2011; Podgórska-Lesiak and Sobkowicz, 2013). Species mixtures also provide ecosystem services, such as soil (Stefan et al., 2021) and water conservation (Yin et al., 2020).

A major effect of plant species diversity on natural ecosystems and cultivated grasslands is increase in ecosystem multifunctionality (Hector and Bagchi, 2007; Gamfeldt et al., 2008; Isbell et al., 2011, 2017; Suter et al., 2021). Consequently, taking a multifunctional perspective on arable cropping systems is needed to fully appraise cropping system diversity (Huang et al., 2015; Schmidtke, 2021). Studies that assess genotype effects of species mixtures on yield, quality, resource efficiency, and crop protection from a multifunctional perspective are missing, and many studies are only focused on some aspects of cropping system performance.

Species mixtures of wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) are touted as a model species mixture (Pelzer et al., 2012; Mamane and Farès, 2020), as these two species are complementary for many needs and, in combination, may help in mitigation of climate change-related challenges.

Wheat protein content and baking quality strongly depend on timely plant-available soil nitrogen (Wieser and Seilmeier, 1998; Xue et al., 2016). However, high-input fertilization practices to improve wheat quality provide nutrients throughout the season, which are only partially utilized by the crop, often resulting in nutrient leaching into the environment (Häußermann et al., 2019). Nitrogen (N) uptake by winter wheat in the fall is minimal; however, sufficient availability of N during grain filling is critical to achieve good baking quality (Xue et al., 2016). Multiple mechanisms can contribute to improved wheat grain quality in species mixtures, and the most often cited is reduced competition for nitrogen in mixtures compared to wheat monocultures (Bedoussac et al., 2015; Stomph et al., 2020), but other mechanisms, such as transfer of nitrogen from legumes to non-legumes, have also been discussed for pasture ecosystems (Thilakarathna et al., 2016). Winter peas with determined growth, flower and mature earlier than most winter wheat cultivars (Bioland, 2021). Biological nitrogen fixation of legumes ceases after flowering, and N is released from nodules. If this coincides with N requirement during grain filling of wheat, grain protein content should be improved. This may interact with water use efficiency in mixtures that can be higher than in monocultures because of several mechanisms, such as change in evapotranspiration, hydraulic lift, and Spatio-temporal differentiation of water use. For example, Daryanto et al. (2020) found that moisture in deeper soil levels can be reduced while increased in shallower soil levels in mixtures compared to monocultures. Peas can shade the ground in between wheat plants, which, besides suppressing weeds, help reduce soil temperature and evaporation, potentially mitigating soil drought conditions that hamper soil mineralization processes, and reduce nitrogen availability. On the other hand, pea monocropping systems face a range of challenges, such as lodging, pests, and diseases, and high weed pressure, causing strong yield fluctuations (Watson et al., 2017), which can potentially be mitigated by mixed cropping. In contrast, in single-species cropping systems, these challenges can only be controlled by increased external inputs hindering climate change mitigation. Most empirical research studies on cultivar effects in legume-cereal mixtures so far have focused on legume cultivars, such as pea cultivars in mixtures with cereals (Hauggaard-Nielsen and Jensen, 2001; Annicchiarico et al., 2017, 2019; Baxevanos et al., 2017; Haug et al., 2021). Broader and systematic evaluation of cereal cultivar effects is missing so far. Considering the value of intraspecific diversity, it is of interest to assess both homogeneous line cultivars and HPs in this context, as suggested by Saxena et al. (2018) and Annicchiarico et al. (2019) for legumes. Addition of HPs can contribute to improve species mixture performance and integrate biological diversity at multiple levels of cropping systems, including diversity at the inter- and intraspecific levels.

This study addresses two main research goals. First, an overall multifunctional performance evaluation of wheat-pea species mixtures is conducted by comparing them to pea and wheat monocultures for yield, crop quality, resource efficiency, and crop protection services. Second, wheat cultivars, including line cultivars and HPs, are evaluated addressing three

secondary aims: (a) study the magnitude of cultivar effects and system-cultivar interactions to evaluate the need to specifically breed for species mixtures, (b) compare the performance and stability of line cultivars and HPs, (c) identify candidate traits, such as phenological, and yield traits, such as harvest index, that explain system-cultivar interactions and performance in mixtures. The secondary aims are crucial to improve breeding for species mixtures.

In the experiments presented here, sowing densities were partially additive (70% of wheat pure stands and 50% of pea pure stands), with an explicit aim to enhance wheat performance especially with respect to baking quality. Total density (pea + wheat) in mixtures was therefore 83% (290 seeds/m²) of wheat pure stand (350 seeds/m²) and 322% of pea pure stand (90 seeds/m²). Additive designs make it difficult to distinguish density from mixture effects, in contrast to replacement designs where total densities are held constant (Harper, 1977; Bybee-Finley and Ryan, 2018). However, additive designs are used more often in practice than replacement designs (FiBL, 2017). Therefore, our main research question addresses mixture effects under realistic farming conditions and wheat cultivar effects on mixture performance.

MATERIALS AND METHODS

Study Site and Design

Experiments were conducted in 2017/18, 2018/19, and 2019/20 at the University of Kassel Research Station in Neu-Eichenberg (51°22′24.7″ N and 9°54′12.5″ E, 247 m asl). The soil is classified as Haplic Luvisol with 76 soil points according to the German soil classification system (0–100). Mean annual temperature from 2000 to 2020 was 9.1°C, and the mean annual precipitation was 626 mm. The site has been managed organically since 1982 without the addition of synthetic fertilizers or pesticides. In 2019/20, 20 t ha⁻¹ vetch-rye silage was added to the field as a routine nutrient amendment. Weeds were controlled by harrowing and hoeing at tillering. A split-plot design with four replicate blocks with mixtures/monocultures as main plots and randomized cultivar plots nested within the main plot for each block was used. Plot size was 13 × 1.5 m² with five rows at 28 cm distance. Wheat and pea monocultures were sown with 350 and 90 seeds m⁻², respectively. Sowing rates in mixtures were 70% for wheat and 50% for pea to increase protein values in wheat. Weather data were recorded by the weather station located in the experimental site.

Soil N levels were 17, 25, and 42 kg N ha⁻¹ at a depth of 0–60 cm in February 2018, 2019, and 2020, respectively. In June 2019 and 2020 (BBCH of wheat 70–80), nitrogen levels were 11 and 22 kg, respectively. Data for 2018 could not be taken because of extreme drought.

The mean temperature in the 2017/18 season was 10.5°C; the total precipitation was 471 mm and deviated considerably from the long-term annual mean. Black frost, in February 2018 killed nearly all winter peas, was followed by extremely dry conditions throughout critical developmental phases of wheat. These dry conditions persisted until late November 2018, also

affecting the second season at sowing (**Figure 1**). In 2018/19 and 2019/20, mean temperatures were 10.4°C with warm winter but less extreme summer. The total annual precipitation of 583 mm in 2018/19 did not compensate for the 2017/18 drought, but it was adequate for wheat growth. In May 2019, torrential rain of 70 mm in 3 h resulted in heavy lodging of pea monocultures. In 2019/20, total precipitation was similar to the long-term average of 671 mm.

Plant Material

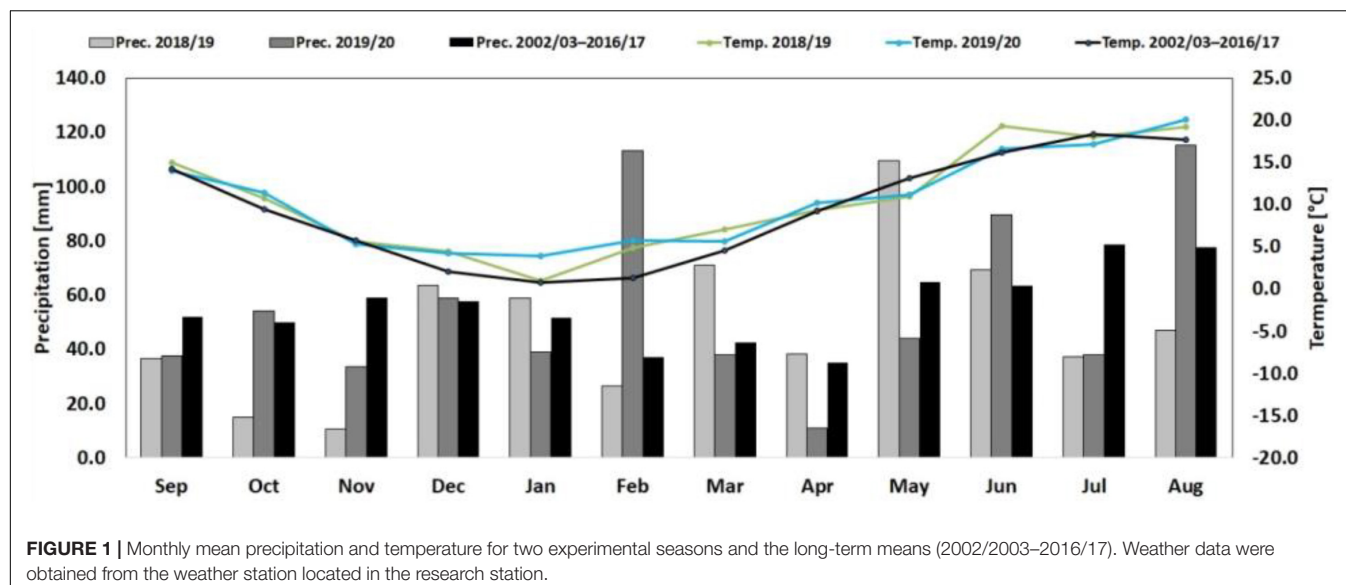
In 2017/18, the winter pea cultivar “Dexter” (white flowers, *determinate* short stature) was sown but killed in February 2018 by black frost. Therefore, in 2018/19 and 2019/20, the more frost-tolerant winter pea cultivar “Fresnel” was chosen. “Fresnel” is a French winter pea cultivar with *determinate* growth, short stature, and early maturation suited for mixtures with barley (Petersen, 2021).

The wheat entries consisted of line cultivars and HPs. The year 2018 was extremely hot and dry. Therefore, an HP (H-HP) and four cultivars from Hungary (H-lines) were included in 2018/19 and 2019/20 to include more adapted materials to summer heat and increase the trait variation of wheat (**Table 1**). The line cultivars “Butaro” and “Wiwa” were bred organically by Dottenfelderhof and Getreidezüchtung Peter Kunz, respectively (Bioverita, 2020). “Achat” and “Capo,” both bred by Probstdorfer Saatzeit, Austria, are baking cultivars popular in organic farming and have relatively good foliar health (Naturland, 2018). The Hungarian line cultivars “Nemere,” “Toborzo,” “Kolompos,” and “Karizma” were bred by the Agricultural Research Center of the Hungarian Academy of Science in Martonvásár.

The HPs from the University of Kassel (K-HP, **Table 1**) evolved from HPs created in 2001 by the Organic Research Centre and the John Innes Institute in the United Kingdom (Döring et al., 2015). “OYQI” and “OYQII” are the result of crossing 8 high-yielding (Y) × 11 baking quality (Q) parents, plus all 19 parents crossed with the cultivar “Bezostaya,” while “OQI” and “OQII” resulted from a half-diallel cross of 12 baking quality cultivars. Since 2005 (F₅), these HPs have been maintained under organic (O) conditions as parallel non-mixing (I, II) populations at the University of Kassel without conscious selection, apart from the removal of plants taller than 130 cm during the first 3 years (Brumlop et al., 2019). “OYQII” is registered in Germany under the name “EQuality” (OSS, 2020). In F₈, a pooled seed of “OYQI” and “OYQII” was sown broadcast and maintained without mechanical weed control in two non-mixing populations since F₉ (“BSFI,” “BSFII”) (Döring et al., 2015; Brumlop et al., 2017, 2019). “Brandex” and “Liocharls” are recently released German organic baking quality HPs bred by Dottenfelderhof (D-HP; Spieß and Vollenweider, 2016), and “Elit CCP” is a Hungarian HP (H-HP) made of 7 high-yielding and high-quality Hungarian cultivars for which F₄ was available (Costanzo and Barberi, 2016).

Data Collection

All assessment dates are summarized in **Supplementary Table 1**. Plant emergence was determined along three row sections of 0.5 m in three inner rows of each plot to avoid edge effects



(BBCH 10). BBCH growth stages (Lancashire et al., 1991) were assessed at each field visit and every second day starting at the end of booting to determine heading dates. Weed cover (%) was estimated visually before booting (BBCH 20–25) six times per plot in an area of 0.1 m² using a metal sampling frame. Lodging was estimated as percentage of the plot area (BBCH 70–80). At maturity, three 0.5-m rows (0.42 m²) were cut, and wheat and pea plants were separated. Total dry biomass, grain weight, and thousand-grain weight (TGW) were measured, and the number of ear-bearing tillers was counted.

Non-green leaf area (NGLA, in %) due to disease and senescence was assessed visually twice per season starting when diseases became relevant in early/mid-June, and from beginning/end of flowering to end of milk stage (BBCH 50–70). The two most important causes of NGLA were recorded. Foot diseases were assessed in year two and in three of 8 entries in early July (BBCH 70–80) (“Achat,” “Butaro,” “Capo,” “Kolompos,” “Nemere,” “Toborzo,” “Elit CCP” and “OYQII”). Per plot, a total of 30 tillers were pulled out with the root crown from five to six places within the plot. The outer stem sheaths were removed and symptoms of *Fusarium* spp., *Oculimacula yallundae*, and *Ceratosporium cereale* were identified on the stem base using a pictorial key (Bayer, 2013) and scored on a 0–3 scale (Bockmann,

1963), where 0 indicates a healthy stem, 1 (< 50%) and 2 (> 50%) of the stem diameter show symptoms, and 3 indicates broken stems (*O. yallundae* only).

Grain yield was determined by combining harvesting and subsequent separation of pea and wheat grains and yields and adjusted to 14% moisture. A NIRS-based analysis of wheat grains for protein, gluten, and water content, sedimentation, and hectoliter weight was conducted with Foss Infratec 1,241 Grain Analyzer. NIRS results were used to categorize entries into wheat quality groups based on thresholds given for protein, where a protein content of < 10% is classified as fodder, 10–11% as second-class baking quality, and > 11% as first-class baking quality (Drangmeister, 2011).

Data Processing and Statistical Analysis

The area under the curve for non-green leaf area (AUNGLA) was calculated as described by Shaner and Finney (1977) for AUDPC based on NGLA. Foot disease index (DIA) was calculated according to Bockmann (1963) for all three foot pathogens individually and combined as described in detail by Weedon and Finckh (2021). Harvest index (HI) was calculated as the ratio of grain to total biomass. Days to heading (DTHs) were calculated from the sowing date.

To assess cropping system performance, yield gain (YG) and land equivalent ratio (LER) were calculated. Yield gain (YG) quantifies differences in the yield of mixtures (Y_{mix_t}) compared to the expected mixture yield (Y_{mix_e}) calculated from pure stands adjusted by sowing densities (Li et al., 2020):

$$YG = Y_{mix_t} - Y_{mix_e} \quad (1)$$

$$Y_{mix_e} = (Y_{1mon} * D_1 + Y_{2mon} * D_2) \quad (2)$$

where D_n is the relative density of species in mixture compared to monoculture.

LER quantifies the land area required in mixture relative to the area that would be required to obtain the same yield in pure

TABLE 1 | Wheat entries used, grouped by type (HP, heterogeneous population; Line, line cultivar) and origin (C, central Europe; H, Hungary, K, Kassel University; D, Dottenfelder Hof).

Group	Entries (origin)
C-Lines	Achat (DE), Butaro (DE), Capo (AU), Wiwa (DE),
H-Lines	Karizma, Kolompos, Nemere, Toborzo, (HU)
K-HPs ²	OYQII, OQII, BSFI, BSFI (2001 in United Kingdom, since F ₅ at Univ. of Kassel, used generations ≥ F ₁₆)
D-HPs	Brandex, Liocharls, (DE)
H-HPs	Elit CCP (HU)

stand, and is defined as the sum of yield ratios of both crop species in mixtures and pure stands (Mead and Willey, 1980):

$$LER = \frac{Y_{1\text{ mix}}}{Y_{1\text{ mon}}} + \frac{Y_{2\text{ mix}}}{Y_{2\text{ mon}}} \quad (3)$$

where $Y_{n\text{ mon}}$ and $Y_{n\text{ mix}}$ are the yields of species n in monoculture and mixture, respectively.

Relative mixture effects (RMEs) were calculated based on response ratios (Hedges et al., 1999) for each individual plot:

$$RME = \left(\frac{R_{\text{mix}}}{R_{\text{mon}}} - 1 \right) \times 100 \quad (4)$$

where R_n refers to the response variable (yield, protein) in the respective system.

A mixed model approach was taken into account for the nested structure of the split-plot experiment (Piepho and Edmondson, 2018). Additionally, because of significant interactions between year and system for wheat and pea for experimental seasons 2018/19 and 2019/20 (**Supplementary Table 4**), experimental years were analyzed separately. For the response variable (R) for yield, protein, and diseases, two factorial models were built with system, entry, and replicate as fixed effects and main plot nested in replicate as a random effect:

$$R \sim \text{system} * \text{entry} + \text{replicate} + 1 | \text{replicate} : \text{mainplot}$$

For YG and LER, models were constructed with wheat entry as fixed and replicate as a random effect:

$$R \sim \text{entry} + 1 | \text{replicate}$$

If confidence intervals did not cross zero for yield gain and did not cross 1 for LER, mixture effects were judged as robust. For lodging data with many zero values, a glm with a Poisson family distribution was fitted.

The response ratio model for estimating relative mixture effect (RME) means and confidence intervals was specified as:

$$RME \sim R + 1 | \text{replicate}$$

RMEs are present if estimated confidence intervals do not cross zero. Mixture effects could not be calculated for lodging data, as this would result in a denominator of zero. Response ratios were divided among four classes indicating multifunctionality: yield (pea and wheat, total yield), resource use efficiency (yield gain), crop protection (weeds, disease, lodging), and wheat grain quality (water, protein, and gluten content, hectoliter weight, and sedimentation).

The linear mixed models were complemented by a genotype main effect plus genotype-by-environment interaction analysis and biplots as a visual tool for analysis (GGE, Yan et al., 2007; Yan, 2014). GGE is a principal component analysis optimized for analysis of genotype suitability across environments and focuses on entry and entry-environment interaction effects. First, GGE was applied to assess entry association with cropping system and seasons defined as environments. In a further genotype by trait (GT) analysis, relationships between performance indicators

(LER, protein, YG) and traits (HI, kernels per ear, tillers m^{-2} , TGW, DTH) were analyzed to identify trait profiles of the entries.

All statistics were calculated using R (R. Core Team, 2020). Dplyr (Wickham et al., 2020b) was used for data aggregation and handling, and ggplot2 (Wickham et al., 2020a) and ggpubr (Kassambara, 2020) for plotting. Normality was assessed with histograms, and variance heteroscedasticity was tested by Levene's test for model residuals and visual methods. The package lme4 (Bates et al., 2020) was used for mixed-effects models for absolute performance data, and nlme (Pinheiro et al., 2020) for mixed effect models with weighted variances to account for heteroscedasticity of response ratios. Estimated marginal means and confidence intervals (CIs) were calculated with the emmeans package (Lenth et al., 2020) followed by a *post-hoc* test with pairwise comparison and Holm correction. GLMs were constructed with base R. GGE and GT analyses were performed using the metan package (Olivoto and Lúcio, 2020).

RESULTS

In 2018/19, the mean pea emergence rate was 100% in the mixtures and 97% in the monocultures. Pea winter survival was 55 and 107 seedlings m^{-2} in the mixtures and monocultures, respectively. Mean emergence rates of wheat were 74 and 75% in the mixtures and monocultures, respectively. In 2019/20, mean pea emergence rates were 82% in the mixtures and 72% in the monocultures. Respective survival rates were 33 and 51 seedlings m^{-2} in the mixtures and monocultures. Mean emergence rates of wheat were 86 and 80% in the mixtures and monocultures, respectively.

Multifunctional Evaluation of Relative Mixture Effects

Due to failure of the peas in the 2017/18 season, relative mixture effects could only be analyzed for 2018/19 and 2019/20 (for statistical summary, see **Supplementary Tables 2, 3**). Based on sowing frequencies, expected wheat yields were 70% and pea yields 50% of the respective pure stands. Yields of wheat compared to pure stands at full sowing density were 75.7% in 2018/19 and 92.4% in 2019/20, and for peas 77.2% in 2018/19 and 18.9% in 2019/20. The RMEs of total yields compared to wheat in the monocultures were close to 100% in both years while compared to peas in the monocultures they were 139 and 120% in 2018/19 and 2019/20, respectively (**Figure 2A**). Relative yield gain was 20.8% in 2018/19 and 8.1% in 2019/20 (**Figure 2B** and **Supplementary Table 3**). Sedimentation and protein and gluten content of wheat were considerably higher in the mixtures in both years and had greater effects in 2018/19 (**Figure 2C** and **Supplementary Table 3**). RMEs for weed cover were especially high compared to pea in the monocultures but agronomically irrelevant with respect to wheat as weed levels in wheat were very low (**Figure 2D**). RMEs for AUNGLA and DIA in wheat were moderate to low in both years except for a 19% reduction in AUNGLA in 2019/20.

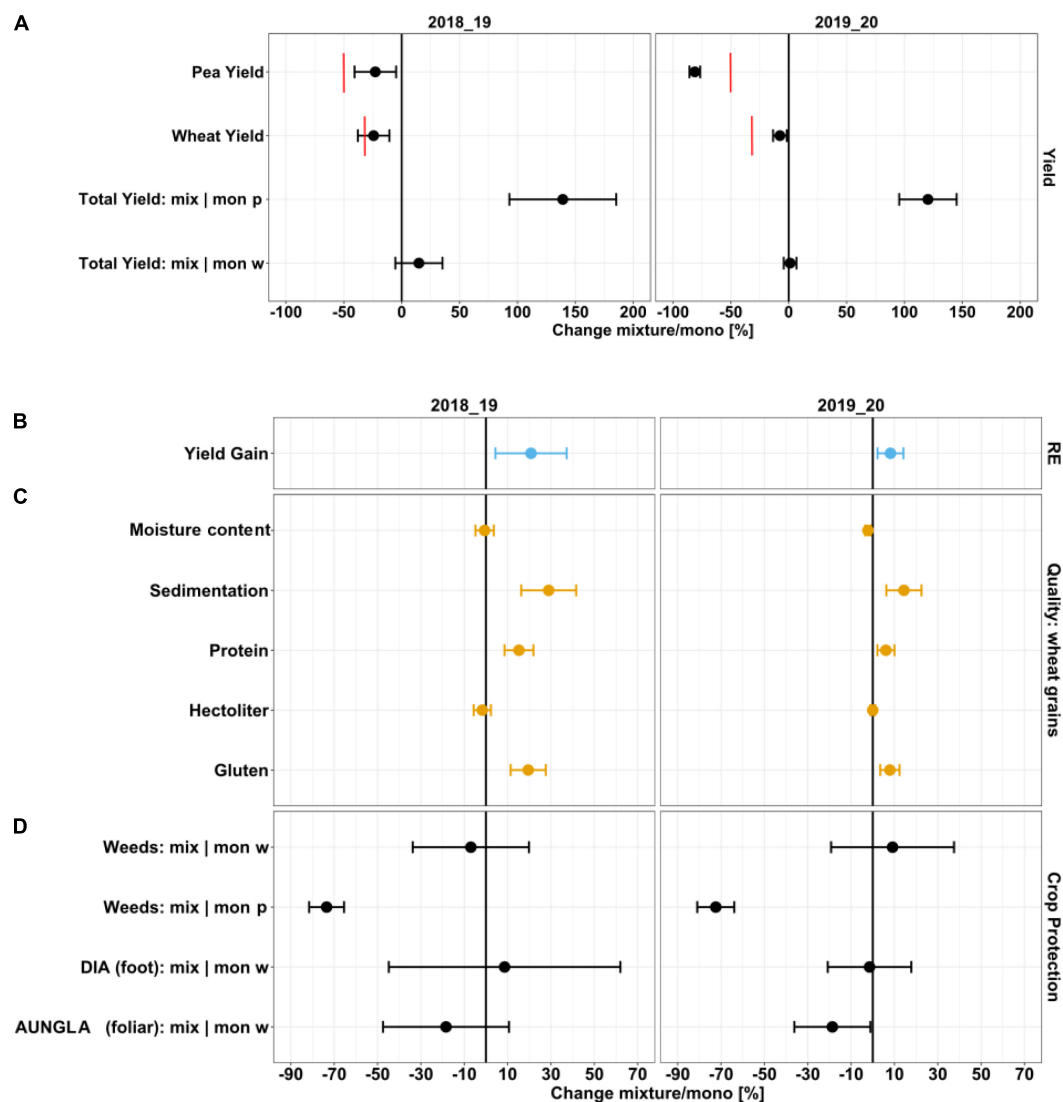


FIGURE 2 | Mixture effects on (A) yield, (B) yield gain as a measure of resource efficiency (RE), (C) quality parameters of wheat, and (D) crop protection. Red bars indicate expected yield levels of wheat and pea-based on sowing densities in mixtures compared to pure stands. Estimated marginal means and 0.95 confidence intervals were derived from nlmes. Total yield and weeds are referred to either relative to wheat monocultures (mix|mon w) or relative to pea monocultures (mix|mon p).

Wheat Entry and Interaction Effects

Lodging in the wheat monocultures and mixtures was also low in both seasons (max 3.8% in the “Liocharls” monoculture 2020). In the pea monocultures, however, lodging was significantly higher than in all the mixtures in both years (75 vs. 3% in 2018/19 and 29 vs. 1.6% in 2019/20).

Growing system (mixed vs. monoculture) and wheat entry indicated significant system $\times$ entry interaction effects for yield, total mixture yield, wheat protein content, AUNGLA, and weeds in at least one of two seasons (2018/19 or 2019/20). However, the main effects were usually more than one order of magnitude greater than the interaction effects. Relatively strong system $\times$ entry interactions occurred for total yield in 2018/19 with insignificant system effects. Similarly, the system $\times$ entry

interaction was significant for weed cover in wheat in 2019/20 (Supplementary Table 5).

Crop Protection

Weed pressure in wheat was generally low in 2018/19 and 2019/20, with 1.8 and 2.3% weed cover in the mixtures and 1.9 and 2.5% in the pure stands. While the wheat entry $\times$ system interacted, at such low weed pressure this is biologically irrelevant. In the pea monocultures, weed cover was also relatively low in both years but significantly higher than in wheat and in comparison, to all mixtures with 7.3 and 9.2% ($P < 0.01$) in the two respective seasons (Data not shown).

In 2018/19, mean NGLA on the first assessment date was 4.2% in the monocultures and 3.6% in the mixtures. On the second

assessment date, the mean NGLA was 17.7% in the monocultures and 13.2% in the mixtures. In 2019/20, mean NGLA on the first assessment date was 7.4% in the monocultures and 6.5% in the mixtures. On the second assessment date, mean NGLA was 37.3% in the monocultures and 29.6% in the mixtures. For both years and assessment dates, NGLA was mainly caused by senescence and *Drechsleratritici-repentis* (DTR), with respective incidences of nearly 100%. In very few cases, (2–3% in 2019 and 13% on the first assessment in 2020), stripe rust (*Puccinia striiformis*) was more prevalent than DTR. Mean AUNGLA for wheat in the mixtures was 23 and 19% lower than in the monocultures in 2019 and 2020, respectively (Figure 3). Wheat entry interacted significantly with system in 2018/19 [$F_{(14, 87)} = 4,996$, $P \geq 0.01$] but not in 2019/20. In both years, the main effects of entry and system were highly significant ($P < 0.01$) and in 2019 considerably larger than interaction (Supplementary Table 6), warranting a closer look at the main effects. However, there was no discernable pattern among or within entry groups within or across years (Figure 3).

Overall, cereal foot disease pressure was similar in both seasons, but no discernable mixture effect or lodging could be associated with it. Incidence of *O. yallundae* was highest at 0.77 and 0.82 in 2019 and 2020, respectively, while incidence of *Fusarium* spp. (0.27 and 0.11, respectively) and *C. cereale* (0.1 and 0, respectively) were rather low. Joint disease indices in 2018/19 were 47.6 in the monocultures and 47.3 in the mixtures, indicating moderate severity. In 2019/20, they were 46.7 for the monocultures and 44.8 for the mixtures. No mixture effects were detected.

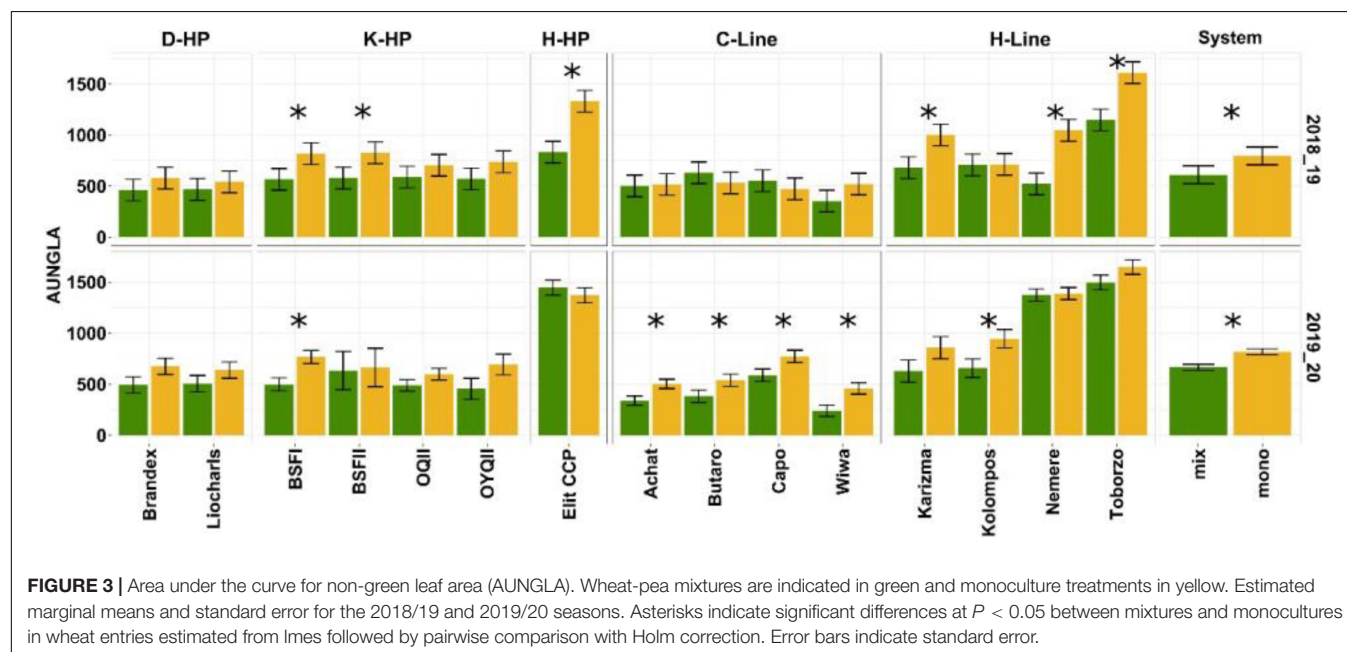
Grain Yield and Quality

Overall mean wheat yield in the monocultures and mixtures was 4.2 and 3.2 t ha⁻¹ in 2018/19 and 5.8 and 5.3 t ha⁻¹ in 2019/20, respectively. In both seasons, the mean wheat yield in

the pure stands was significantly higher than in the mixtures that had been sown at 70% seed density (Figure 4). The respective mean pea yield in the monoculture and mixtures was 2 and 1.5 t ha⁻¹ in 2018/19 and 2.7 and 0.5 t ha⁻¹ in 2019/20. While significant system $\times$ entry interactions were found, the main effects were considerably greater than the interactions (Supplementary Table 7).

Despite the lower monoculture yield of peas in 2018/19, the contribution of peas to total mixture yield was considerable in that year (1.2–2 t ha⁻¹), with no significant reductions in pea yield through mixing in 10 out of 15 cases. In response, almost all wheat cultivars except “Kolompos” indicated significantly lower yield in the mixtures than in the monocultures (Figure 4). Wheat yield in the monocultures ranged from 3.6 (“Toborzo”) to 4.9 t ha⁻¹ (“Achat”) and in the mixtures from 2.3 (“Toborzo”) to 4.6 t ha⁻¹ (“Kolompos,” Figure 4). Ranks in the monocultures for total yield were different to the ranks for wheat yield in the mixtures, with the lowest total yield of 3.6 t ha⁻¹ in the “Butaro” pea mix and highest in the “Kolompos” pea mix (6 t ha⁻¹). Mixtures with “Elit CCP,” “Kolompos,” and “Nemere” had significantly higher total yield than the wheat monocultures (Figure 4).

In 2019/20, wheat yield in the monocultures ranged from 4.6 (“Toborzo”) to 7.3 t ha⁻¹ (“Kolompos”) and in the mixtures from 4.4 (“Wiwa”) to 6.9 t ha⁻¹ (“Kolompos”). While pea monoculture yield was higher than in 2018/19, the contribution of peas to total mixture yield was very low in 2019/20 (0.3–0.9 t ha⁻¹), and peas yielded significantly less in all the mixtures than in the monocultures (Figure 4). “Achat,” “Elit CCP,” “Karizma,” and “Wiwa” yielded significantly lower in the mixtures than in the monocultures (Figure 4), resulting in rank changes between systems and, thus, interaction effects. Total yield (wheat + pea) was significantly affected by wheat entry [$F_{(14, 84)} = 22.4$, $P < 0.01$] and ranged from 5 (“Toborzo,” pea) to 7.1 t ha⁻¹ (“Kolompos,” pea). With one exception of “Liucharls” in the



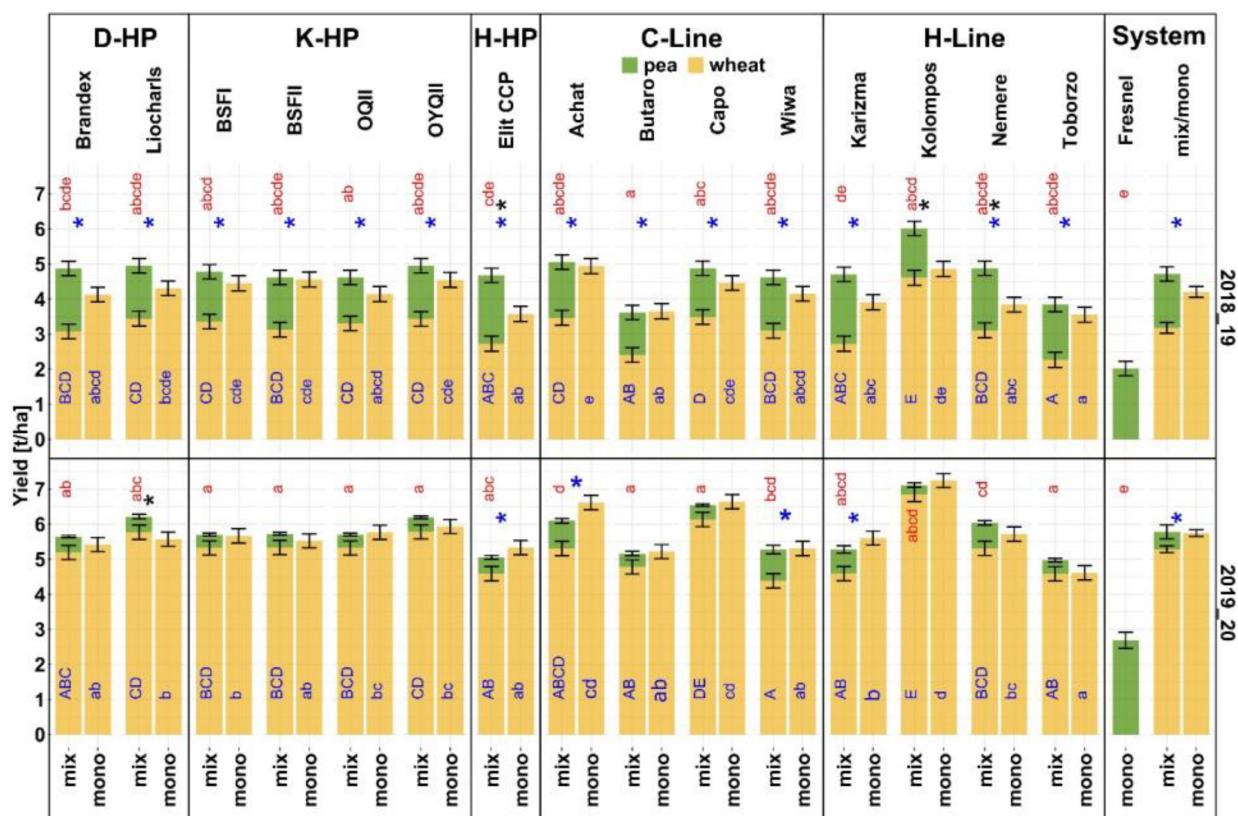


FIGURE 4 | Yield in the mixtures and monocultures (2018/19 and 2019/20). Estimated marginal means and standard error from linear mixed effect models are plotted. Significant differences at $P < 0.05$ estimated from mixed models with pairwise comparison and Holm correction are shown. Small blue letters indicate significant differences among wheat entries in the monocultures and blue capital letters among the wheat cultivar mixtures. Red letters indicate significant differences in pea yield. Asterisks indicate significant differences in wheat yield (blue) and total yield (wheat + pea) (black) between systems.

mixtures, total pea-wheat yield did not differ from wheat monoculture yield (Figure 4).

In 2018/19, mean protein content in the mixtures was 12.3% (range: 10.8, “Capo”; 14.3%, “Toborzo”) and 10.8% in monocultures (range: 9.4, “Achat”; 12.6%, “Toborzo”). All the wheat entries significantly increased their protein content in the mixtures compared to monocultures, and apart from entries that had already reached the baking quality class in monoculture (“Wiwa,” “Karizma,” “Nemere,” and “Toborzo”), the remaining wheat entries increased their protein ranking class from fodder to baking wheat or from intermediate baking to top baking quality (Figure 5). Although a significant but weak interaction occurred between system and wheat entry [$F_{(14, 84)} = 2$, $P = 0.025$], main effects of entry and growing system were much greater (Supplementary Table 8). In 2019/20, wheat grain protein content in the mixtures was 13.7% and in the monocultures 12.9%, ranging in the mixtures from 12 (“Kolompos”) to 15.8% (“Wiwa”) and in the monocultures from 11.7 (“Kolompos”) to 14.3% (“Butaro”). As all the entries were classified as top baking quality in the monocultures, no improvement in baking quality classification was found between the two growing systems.

In the genotype main effect plus genotype-by-environment interaction analysis (GGE), the GGE explained 95% of wheat

yield variation and 96% of variation in wheat grain protein (Figures 6A,B). First, the GGE was applied to assess entry association with cropping system and seasons defined as environments. For yield, the HPs (except for “Elit CCP”) clustered close to the biplot origin, indicating less yield variability across all environments (Figure 6A). “Kolompos” showed the strongest association with all environments, while Achathad a strong yield advantage in both monoculture environments and “Capo” and “OYQII” in the mixtures. The yield performance of “Wiwa,” “Elit CCP,” “Toborzo,” “Butaro,” and “Brandex” was not associated with any specific environment.

The mean vs. stability plots (Figures 6B,D) allowed for the assessment of genotype performance and stability ranking across environments. The green horizontal axis is called the average environment axis (AEA) and ranks the genotypes by their yield performance. The average environment coordination (AEC) axis is the second green axis that runs through the biplot origin and perpendicular to the AEA; the farther the distance from the origin of the biplot along this line, the greater the instability of the genotype, which means that the longer the length of the line or vector connecting the genotype to the AEA, the higher the GE interaction and the greater the instability of the genotype in all environments. Overall, it is conspicuous that almost all the HPs,

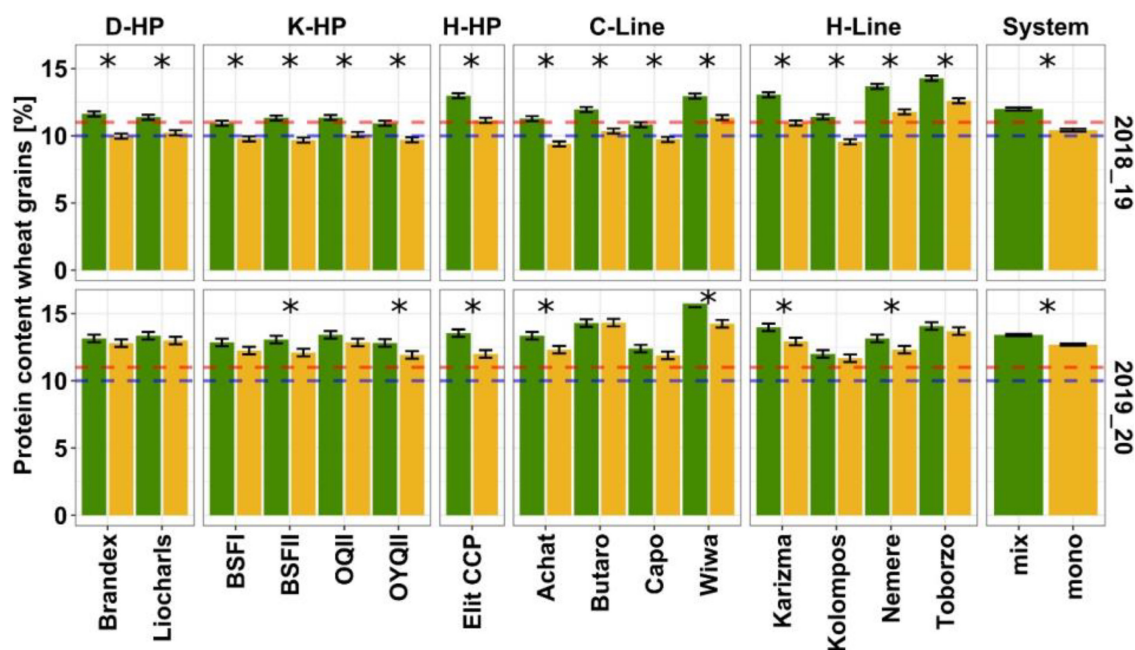


FIGURE 5 | Wheat grain protein content (%) in the mixtures (green) and monoculture (yellow). Estimated marginal means and standard error for from linear mixed effect models are plotted. Asterisks show significant differences at $P < 0.05$ estimated from mixed models with pairwise comparison and Holm correction between the mixtures and monocultures in wheat entries. Dashed lines indicate quality thresholds for protein given by Drangmeister (2011). Red dashed line marks 10% and the blue dashed line 11% protein content. Quality classes are as follows: fodder wheat < 10%, baking wheat = 10–11%, and high-quality baking wheat > 11%.

with the exception of “Liocharls,” display greater yield stability than the majority of the line cultivars (Figure 6B).

In the protein-GGE, the experimental seasons were not associated with each other; however, both cropping systems in each experimental season were similar for protein. Additionally, different entries were strongly associated with the two experimental seasons for protein content (Figure 6C). The entry pattern for protein GGE is opposite to that of yield analysis. Here, “Butaro” and “Wiwa” are associated with both systems in 2019/20, while “Toborzo,” “Nemere,” “Elit CCP,” and “Karizma” are associated with both systems in 2018/19. With the exception of “Elit CCP,” the HPs displayed highest stability in protein content with some of the line cultivars indicating strong association to specific environments (Figures 6C,D).

Yield Gain and Land Equivalent Ratio

In 2018/19, yield gain (YG) was 0.8 t ha^{-1} , equaling a relative yield increase of 19% compared to the monocultures, with significant wheat entry effects [$F_{(14, 42)} = 5.7, P = 0$, Supplementary Table 9]. YG ranged from 0.1 t ha^{-1} (2.4%, “Butaro”) to 1.6 t ha^{-1} (38%, “Kolompos”; Figure 7A), and it differed significantly from zero for “Kolompos” (1.6 t ha^{-1}), “Nemere” (1.2 t ha^{-1}), and “Elit CCP” (1.2 t ha^{-1}), all from Hungary.

In 2019/20, the mean YG across entries was only 0.4 t ha^{-1} (7% yield increase) with no significant differences between entries or from zero (Figure 7A). Relative yield gain ranged from 0.3 (“Elit CCP”) to 16.7% (“Liocharls”). In contrast to 2018/19, when the Hungarian entries dominated the top ranks, no specific group dominated in 2019/20. Conspicuously, the D-HP and

K-HP-based mixtures resulted in similar YG in both years, which was not the case for H-HP or the Hungarian cultivar mixtures. For example, “Elit CCP” over-yielded in 2018/19 by 1.2 t ha^{-1} but not in 2019/20. Thus, the range of over-yielding in these two contrasting seasons was 1.2, i.e., twice as high as the mean YG (0.6 t ha^{-1}). In contrast, mixtures with “OYQII” over-yielded by 0.75 and 0.7 t ha^{-1} in the 2 years; thus, over-yielding varied by only 0.05 t ha^{-1} , albeit with different fractions of wheat and peas in the different years (Figure 4).

Although all the LER values were > 1 in both years, the effects were considerably greater in 2018/19 (Figure 7B), and entry effects were significant [$F_{(14, 42)} = 6.5, P = 0$, Supplementary Table 9], with almost the same ranking as for the YG. In 2019/20, LER ranged from 1 (“Elit CCP”) to 1.2 (“Liocharls”) (mean 1.1), with no significant entry effects explaining the apparent dissimilarity in the ranks of YG and LER in that year. Most conspicuously, while YG in mixtures with “Kolompos” was second highest, the LER of these mixtures was second-lowest (Figure 7B). Nevertheless, LER and YG were highly correlated in both 2018/19 ($r = 0.9, P < 0.01$) and 2019/20 ($r = 0.86, P < 0.01$).

Effects of wheat on peas and peas on wheat were visualized by plotting the partial LERs per species against each other (Figure 7C). For peas at sowing density of 50%, $pLER > 0.5$ indicates over-yielding, while for wheat (sowing density 70%), $pLER > 0.7$ indicates over-yielding. In both seasons, the wheat entries significantly varied for their $pLER$ and their effects on the $pLER$ of peas (Supplementary Table 9). However, while wheat and pea $pLER$ s in 2018/19 were greater than the expected $pLER$, although with few exceptions, in 2019/20, the $pLER$ s of wheat

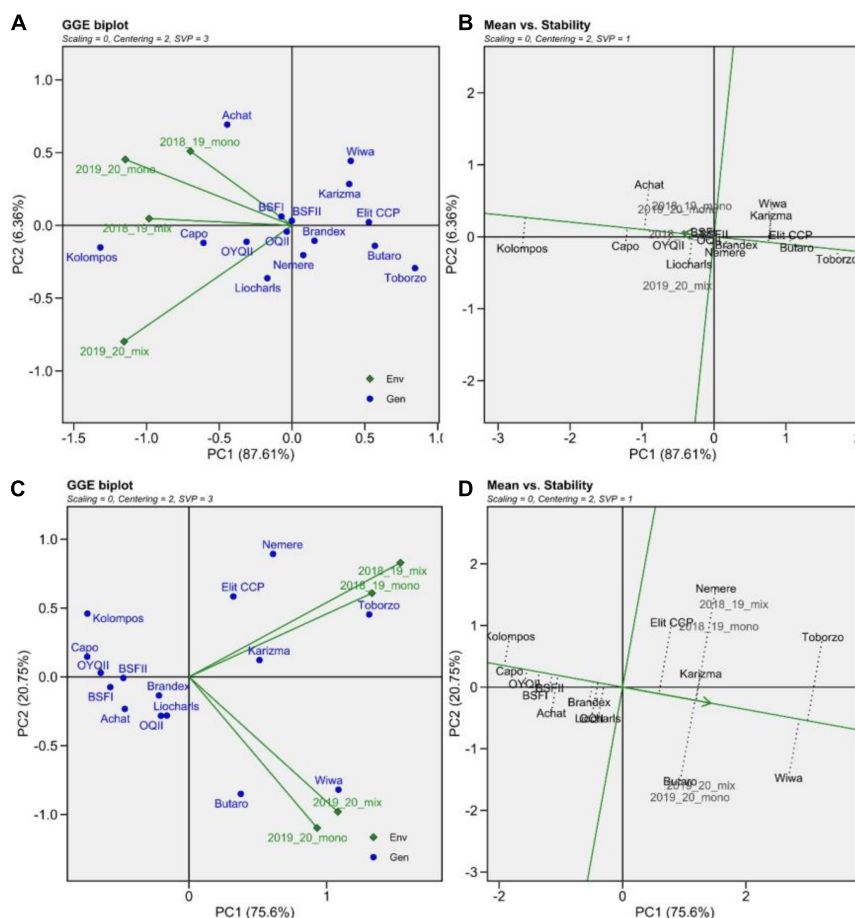


FIGURE 6 | GGE biplots for wheat (A) yield and (B) stability and protein (C) content and (D) stability across seasons 2018/19 and 2019/20 seasons. For the symmetrical (SVP = 3) GGE biplot entries are indicated in blue and environments in green. For the mean vs. stability biplot, entries are black and environments are gray.

were greater and those of peas were lower than the expected values (Figure 7C).

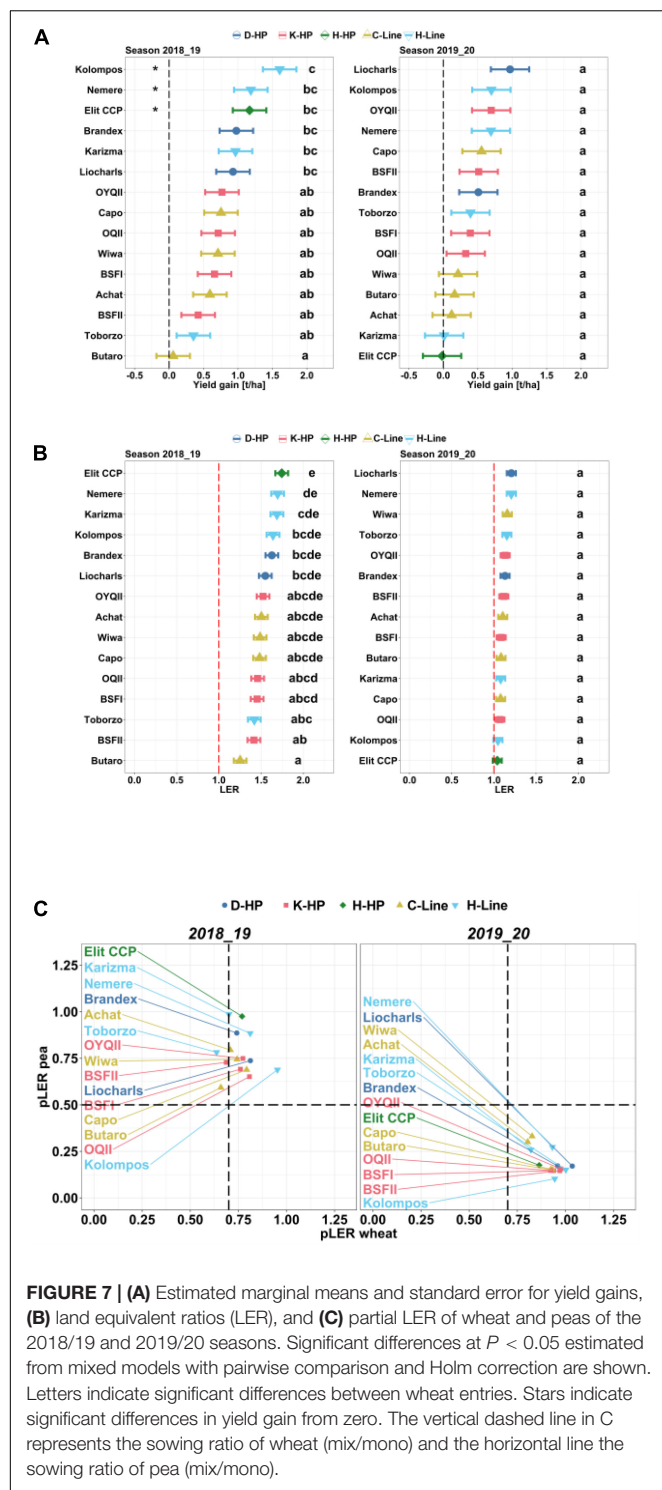
Wheat Traits

In the set of evaluated entries, there was a considerable variation in days to heading (DTH, BBCH 50) due to the inclusion of Hungarian and central European entries. Mean DTH in the 2018/19 season was 224 and 208 days in 2019/20, corresponding to the fact that the experiment was sown 2 weeks earlier in 2018/19. Entry had by far the strongest effect on DTH in both years. A significant systems effect was found in 2019/20, while in 2018/19 the system $\times$ entry interaction was small. The system $\times$ entry interaction effects were one or two orders of magnitude smaller than the entry effects (Supplementary Table 11). The relative ranking of the entries was consistent across both years (Supplementary Table 12). The Hungarian entries were the earliest, and the organic line cultivars and “Achat” were the latest. The K-HP entries and “Capo” constituted a group of mid-early entries relative to the entire entry set. “Kolompos” was the latest Hungarian entry and grouped with the mid-early entries in 2018/19.

Changes in HI were generally small and mostly insignificant. Reactions of the wheat entries were very variable and sometimes contrasting in the two seasons. In the monocultures, the ranking of the entries differed between years, e.g., “Nemere” and “Kolompos” were intermediate in 2018/19 but highest in 2019/20. In mixture with pea, the HI of “Nemere” was significantly lower in 2018/19 (mix: 0.33, mono: 0.38) and 2019/20 (mix: 0.39, mono: 0.44), while that of “Kolompos” was significantly higher in 2018/19 (mix: 0.44, mono: 0.38) but was reduced in 2019/20 (mix: 0.41, mono: 0.43). For “Wiwa” and “OQII,” HI showed a similar pattern to “Kolompos” but in contrast to “Elit CCP” (Figure 8A).

In mixture with pea, the different wheat entries indicated high variability for kernel number per ear depending on the experimental year. Entry responses indicated a similar pattern to HI with the exception of “Wiwa,” which, despite a significant reduction in HI in the mixtures in 2019/20, slightly increased its number of kernels per ear in that year. “Kolompos” indicated an increase in kernel number per ear in the mixtures in 2018/19 (Figure 8B and Supplementary Table 13).

The GT biplot (genotype by trait) explained 68% of the total trait variation in 2018/19 and 61% in 2019/20 (Figure 9). The



interpretation is similar to that of the GGE biplot of yield and protein (Figure 6), in that the cosine of the angle between two traits approximates the correlation between them. Additionally, the length of the vectors indicates closeness of association with other traits. Traits with shorter vectors tend to have weaker associations with other traits. The same is true for genotypes and

traits in terms of cosine of the angle between the genotypes and the traits. Genotypes found closely located ($< 90^\circ$) to specific traits indicate high propensity to a trait or traits, referred to as genotype trait profile. If trait profiles between genotypes are different, they represent contrasting trait profiles.

Of interest is the question of how the measured traits (HI, kernels per ear, ears m^{-2} , and DTH) interact and how they affect the yield and quality performance of the entries in mixtures (LER, YG, TGW, and protein). The number of kernels per ear and HI had vectors of similar length in both years, and the cosine angle indicates that they were correlated in 2018/19 but not in 2019/20. DTH was correlated with both kernels per ear and HI in 2018/19; however, ears m^{-2} was not associated with these traits. In 2019/20, DTH was still correlated with kernels per ear. In addition, HI and ears m^{-2} were positively correlated; however, these two traits were negatively correlated with the former two (Figure 9).

With respect to yield performance, it is not surprising that wheat pLER, YG, and LER are all closely associated. However, while pea pLER grouped with the aforementioned traits in 2018/19, it was weakly correlated with LER and negatively with YG in 2019/20, the year when more nitrogen was available in the soil. Nevertheless, with respect to quality, protein content was always closely associated with pea pLER. In contrast, HI was always negatively related with protein content, while it was correlated strongly with wheat pLER in 2019/20. This indicates that HI directly enhanced wheat competitiveness. The negative effect on pea pLER is the logical consequence. In contrast to HI, kernels per ear and days to heading were negatively associated with protein content in the first but positively associated in the second year. In 2019/20, both TGW and ears m^{-2} correlated with wheat pLER.

As depicted by the longer blue vectors, entry trait associations were stronger in 2019/20 than in 2018/19. Three Hungarian pure line cultivars and “Butaro” indicated stronger associations to plant traits in the first year. In 2019/20, most pure line cultivars, the H-HP “Elit CCP,” and the D-HP “Liocharls” indicated stronger associations to specific traits. The K-HPs, in general, tended toward the GT biplot origin in both years (Figure 9).

DISCUSSION

The mixture effects found in this study are confirmed by previous reports on cereal-legume mixtures with respect to improved cereal grain quality, weed suppression, resource use efficiency (Bedoussac et al., 2015), yield gain (Li et al., 2020), and lodging resistance (Kontturi et al., 2011; Podgórska-Lesiak and Sobkowicz, 2013). The two cropping seasons, 2018/19 and 2019/20, when the pea-wheat mixtures could be realized were relatively similar with respect to moderate to low water availability and temperature conditions but differed mostly in nitrogen availability. In 2018/19, when nitrogen was deficient, wheat baking quality could be improved to a high-quality baking standard. In 2019/20, when nitrogen provision was more adequate, standard high baking quality could be already achieved in the pure stands, while in the mixture’s protein

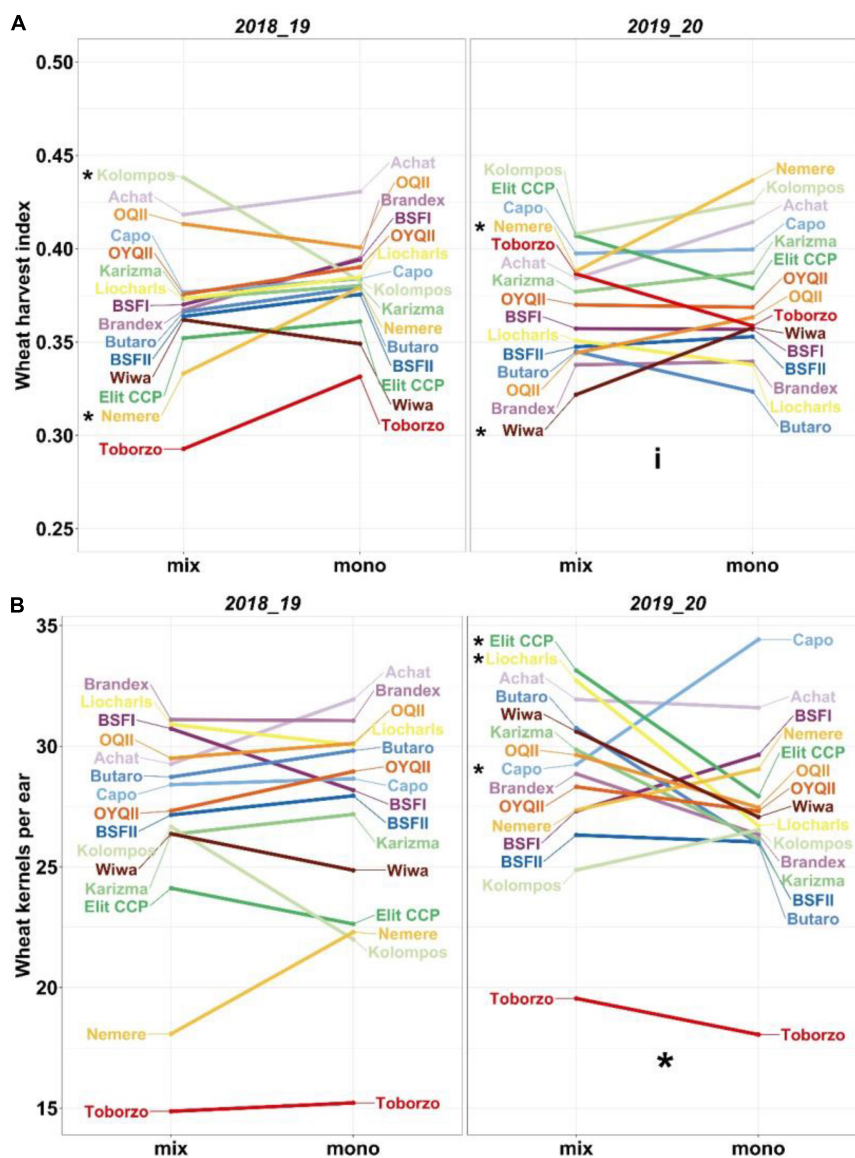


FIGURE 8 | Interaction plots for **(A)** harvest index and **(B)** kernels per ear. Estimated marginal means from linear mixed effect models are plotted. Small stars indicate significant differences in entries between systems and large stars between systems across entries, and “i” indicates significant interactions at $P < 0.05$ estimated from mixed models with pairwise comparison and Holm correction.

content was increased even further. Weeds and foliar diseases were generally reduced, albeit only moderately, because of low weed and disease pressure and no specific resistance-related differences to foliar pathogens became evident in wheat under these conditions. The fact that senescence, overall, was delayed in the mixtures, reducing the area under the non-green leaf area curve (AUNGLA) points to beneficial interactions due to changes in wheat density and the addition of peas with respect to resource use dynamics over time. No effects on foot diseases were found, as it is often the case when studying mixtures (Finckh and Wolfe, 2015).

In reaction to the differing nitrogen supply, wheat entries displayed a broad range of reactions to growing in pure stands

and wheat-pea mixtures with respect to yield and quality on the one hand. For example, in 2018/19, earliness could be one explanation for high YG in some Hungarian entries, except for “Toborzo” that seemed to be too early, while “Butaro,” the latest entry, had the lowest YG in this season. Earliness was strongly associated with kernels per ear and HI in 2018/19; however, in 2019/20, HI was affected negatively by earliness. Depending on the year, different traits, such as harvest index (HI), days to heading (DTH), and ears m^{-2} , were correlated with the performance traits of wheat and peas, such as LER or partial LER, yield gain (YG), and wheat grain protein content. Nevertheless, in both experimental years, the HI of wheat appeared to be a robust measure for predicting effects on pea

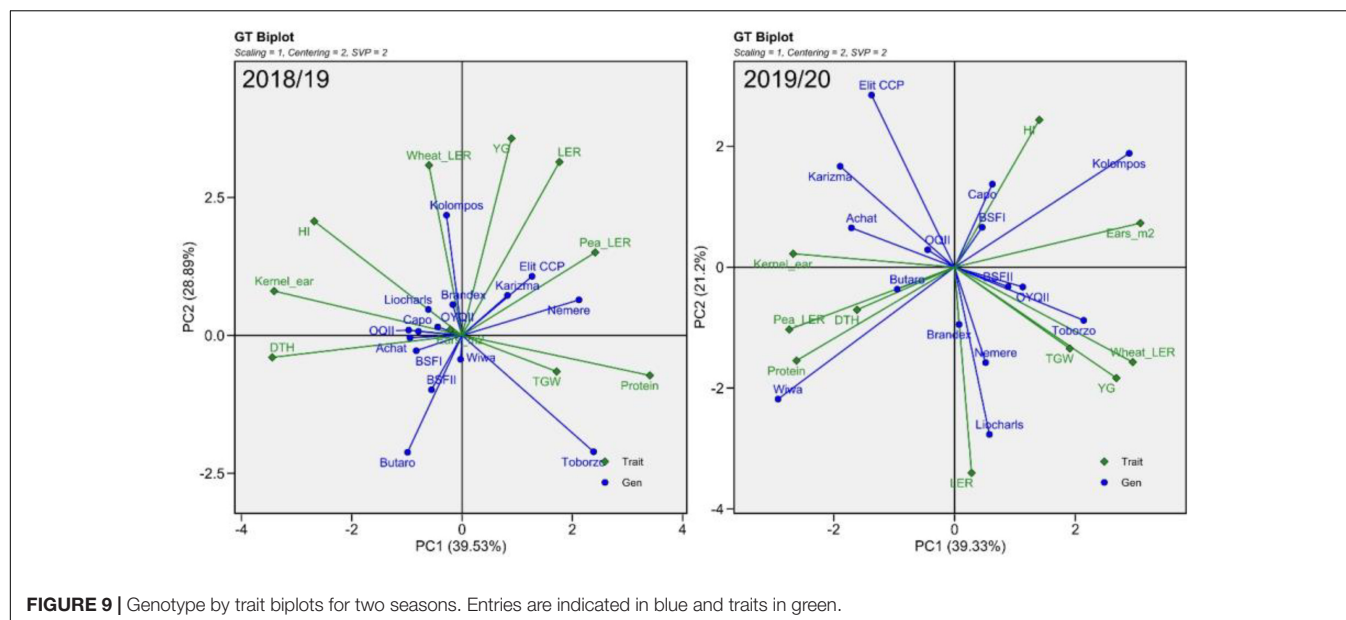


FIGURE 9 | Genotype by trait biplots for two seasons. Entries are indicated in blue and traits in green.

performance (pea pLER) and, subsequently, on wheat quality in mixtures, as protein content was negatively correlated with HI and positively with pea pLER.

With the exception of the Hungarian “Elit CCP” independent of the growing season, the HPs displayed highest stability with respect to yield and quality and no specific trait profiles. This indicates that while selection of specific traits might be of use when selecting pure line cultivars for mixtures, this avenue might not be the best when dealing with HPs. The high stability of the HPs was at an overall intermediate level for yield and protein values. Some of the Hungarian line cultivars achieved either high yield or protein content in the two experimental years. This was due to the fact that weather conditions, overall, were very warm and similar to more continental summers. Increased environmental variability and weather extremes challenge future agricultural production and need to be mitigated. Therefore, HPs can add an additional level of environmental stress-buffering capacity to wheat-pea species mixtures.

The multicriteria evaluation confirms the relevance of inter- and intra-specific diversity for multifunctionality as was previously reported for multifunctional grasslands (Isbell et al., 2017, 2011). Such a multifunctional perspective in agriculture is by no means established in practice. Rather, the focus, even in organic agriculture, is on yield of single crop species. This is, in large part, due to lack of established value chains based on mixed cropping (Kjær et al., 2022, this volume).

Entry Effects and System × Entry Interactions of Performance

Although system × entry interactions were mostly weak compared to the main effects of the wheat entries, they were generally greater in 2018/19 than in 2019/20. Water availability was likely somewhat lower in 2018/19 than in 2019/20 because of the extreme drought in 2017/18. Complementarity, with respect

to water use between wheat and pea, likely played a role. Thus, differences in YG and LER among the wheat entries were only significant in 2018/19.

The complementarity of legumes and cereals with respect to nitrogen pools accessible by cereals (soil) and legumes (soil and atmospheric nitrogen), and different competitive abilities with respect to soil nitrogen of cereals (high) and legumes (low) depend on these resources being limited (Bedoussac et al., 2015), which was most likely the case in the 2018/19 season. Under the relatively low nutrient levels in 2018/19, peas were more competitive and contributed with 33% more than expected to total yields in mixture compared to monoculture. In contrast, in 2019/20, total yield was 20% higher than in the previous year, but peas were suppressed by wheat, and their contribution to total yield was only 9%, with strong effects of the wheat entries on pea performance. The fact that growing system and entry had similarly strong effects in 2018/19, but entry effects were stronger than system effects in 2019/20 seem to confirm other studies (Moutier et al., 2021) that competitive interactions among peas and wheat were strongly driven by nitrogen availability. The spatial and temporal dimensions of niche differentiation, such as different rooting patterns and plant phenology, are, here, of particular relevance (see next section). It should be noted that even the higher N-levels in the second year were relatively low compared to conventional farming systems. The K-HPs that were bred for and selected in organic systems often outperform line cultivars under organic but not under conventional conditions (Weedon and Finckh, 2019, 2021). Nevertheless, wheat yield is more predictable under high nutrient conditions. LER seems more sensitive to cultivar variation; however, the overall picture is similar to YG. Leveling of cultivar-based performance differences also makes it challenging to make robust statements with respect to different entry groups and relationships between traits and performance.

The GGE analysis revealed that the K-HPs and “Brandex” had a lower variation for grain yield and protein than “Elit CCP,” C-lines, and H-Lines. With respect to yield performance in mixtures with the pea cultivar “Fresnel” and the monocultures “Kolompos,” “Achat,” and “Capo” (the former two are conventional relatively short cultivars compared to the other entries evaluated) performed best in the monocultures. In the mixtures, they also performed well; however, “Achat” did so less. The other line cultivars varied greatly among years in their relative performance. In contrast, with the exception of the Hungarian “Elit CCP” in 2018/19, the HPs were only outperformed in the pure stands by “Achat” and in the mixtures by “Kolompos.” In 2019/20, the HPs were mostly outperformed by “Achat,” “Capo,” and “Kolompos” in the pure stands while in the mixtures only “Kolompos” out-yielded all the HPs. Thus, while most of the HPs, with the exception of “Elit CCP” were well suited for mixtures, among the pure line cultivars, “Kolompos” and “Capo” appeared particularly well suited with respect to yield.

Using the 4C approach (Justes et al., 2021), the pLERs allowed us to draw conclusions with respect to competition, complementarity, compensation, and cooperation. In 2018/19, all the wheat entries except for “Toborzo,” “BSFII” and “Butaro” fall in the top right section of the pLER plot, indicating that complementarity and cooperation were stronger than the competition. In 2019/20, competition was stronger than cooperation and complementarity, and wheat suppressed the peas. Still, in 2018/19, pea pLER was highest in combination with four of the five best-performing entries (“Karizma,” “Elit CCP,” “Nemere” and “Brandex”). Pea pLER was somewhat reduced with “Kolompos,” but the latter had the highest wheat pLER. All the other wheat entries indicated greater reduction in yield in relation to pea. This points to overall asymmetric competitive interactions depending on wheat entry (Weiner, 1990). In parallel to the pLERs, HI varied considerably. In 2018/19, most of the entries did not differ significantly in HI between systems, indicating similar competition in the mixtures and monocultures, while in 2019/20 some interactions occurred. In contrast, the HI of “Kolompos” increased in the mixtures compared to the monocultures in 2018/19 but not in 2019/20, while the HI of “Nemere” decreased in the mixtures in both years. All the other entries had no significant changes in their HI. This confirms a differential competitive response of wheat entries with respect to resource allocation and depending on environment, indicating different routes to high LER or YG values that ideally should be exploited.

In contrast to yield, the most important factor affecting protein content of wheat within the year was growing system (i.e., mixed cropping), but the increase in protein content in mixture with pea was almost twice as high under low nitrogen levels (+1.5%) compared to higher nitrogen levels (+0.7%), and significant system $\times$ entry interactions only occurred in 2018/19. The line cultivars “Wiwa” and “Toborzo” outperformed the HPs in terms of protein content but not yield across environments. The reversed pattern of yield and protein GGEs confirm the well-known trade-off between yield and quality in wheat (Michel et al., 2019) and the fact that the effect of

cereal-legume mixtures on wheat quality is almost entirely due to nitrogen (Bedoussac et al., 2015). Nevertheless, the increase in protein content in both years, particularly 2019/20, was greater in the pure line cultivars than in the HP entries, except for “Elit CCP.” The fact that HP entries are heterogeneous results in higher variation in protein potential. The German HPs bred for good baking quality (“Brandex,” “Liocharls,” and: “OQII”) had higher protein content in the pure stands under high N-input in the second year than the more diverse HPs, “OYQII,” and the two BSF-HPs. However, in the mixtures, these differences were no longer evident, suggesting greater plasticity of the HPs for that trait. Thus, it appears that under high nitrogen input levels, the relative yield performance of wheat when mixed with a determinate pea cultivar is quite predictable from the wheat pure stands. In contrast, selection for improved mixture performance and improved protein levels of wheat in the mixtures with peas may only be useful under low nitrogen levels to identify entries highly efficient for nitrogen use.

While foliar disease levels were very low in our experiments, leaf senescence played a more prominent role and was the main factor contributing to the non-green leaf area. AUNGLA, as an indicator for leaf senescence, was reduced in the mixtures compared to the monocultures in 2018/19 and 2019/20. Delayed senescence could be a possible explanation for higher protein contents, since the onset of senescence explained up to 86% of the variation in nitrogen utilization efficiency in wheat (Gaju et al., 2011).

In contrast to yield and protein effects, with respect to weeds, system $\times$ entry interactions were high under higher nitrogen levels, even if absolute weed levels were low. Weed pressure is increased by high nutrient levels and is an important issue not only in organic growing systems, but particularly in species mixtures, as herbicides are usually incompatible with such mixtures. Thus, improved weed suppression and management through optimization of weed-suppressive crop mixtures and associated breeding programs should be highlighted as important aims to improve crop mixtures and their wider application.

Wheat Trait Effects on Mixture Performance

Increased competition through increased plant density or reduced resources in monocultures increases the allocation of resources to vegetative relative to reproductive plant organs, leading to lower HI in wild plants (Keddy, 2017, p. 128) and arable crops (Li et al., 2015). Chen et al. (2020) found a greater increase in total biomass than in grain yield in mixtures, because in mixtures reproductive effort is reduced (lower harvest index) relative to monocultures because of increased competition. Nevertheless, this does not necessarily indicate a trade-off, as is demonstrated with diversified wheat populations (Weedon and Finckh, 2021), and HI may, therefore, be a valuable indicator of reduced competition and increased complementarity in mixtures. Exploiting reduced competition and increased allocation to grains in mixtures may be a route for breeding to increase mixture performance in terms of grain yield (Chen et al., 2020).

The significantly increased harvest index of Kolompos in 2018/19 coincided with increased kernels per ear in the mixtures, and the reverse was true for Nemere. Even though these changes in kernels per ear were not statistically significant, the coherent contrasting pattern of both entries for the two variables suggests they might explain the changes in HI. It is possible that the reduced investment in the competition of Kolompos in the mixtures resulted in more kernels per ear, increasing its HI, while the presence of pea apparently played no significant role. Likely, the phenology of the mid-early Kolompos, relative to the set of evaluated entries, is complementary to the pea cultivar “Fresnel” contributing to (temporal) niche differentiation and, thus, reduced competition. Interestingly, “Kolompos” also had the longest seminal roots in a recent hydroponic study, even longer than those of wheat plants selected specifically for long roots (Timaues et al., 2021). As noted before, in our experiment, mixture effects are due to species mixing and density effects in an absolute sense, but comparing different entries in the same experimental setup still enables some insights. Temporal niche differentiation, as indicated by differences in maximum daily growth rates of component species, is an important driver of yield gain in mixtures of canola with soybean or maize (Dong et al., 2018) and mixtures of maize with small-grain cereals or legumes (Li et al., 2020). These studies compared different crop species combinations. Only few studies have at least, in part, attempted to investigate the effect of intraspecific variation of crop species phenology on mixture performance as pointed out by (Demie et al., 2022, this volume). To breed new line cultivars or HPs, we should systematically exploit the positive effects of temporal niche differentiation.

Experimental studies show that early vigor has a positive impact on nitrogen use efficiency (Liao et al., 2004) and, therefore, likely on protein content. As pointed out above, earliness was not always beneficial among the wheat entries. In contrast to Moutier et al. (2021) who found that wheat cultivar earliness is significantly correlated with wheat protein content increase, we did not detect a significant correlation between DTH and protein. Thus, in 2018/19, the very early cultivar “Toborzo” had, by far, the highest protein content, followed by “Nemere,” “Karizma,” and “Elit CCP.” The mid-early cultivar “Kolompos” had rather low protein content values, likely due to its high yield, while “Wiwa,” a late cultivar, had comparatively high protein values. The interactions between a range of different factors, such as DTH, speed of leaf senescence, yield level, and their effect on protein content, are not well explored.

Future Avenues for Breeding Research

The magnitude of stresses on cropping systems will have strong impacts on the relevance of cropping systems diversified at multiple levels. Increased frequencies of extreme weather events (IPCC, 2021) and additional socioeconomic factors, such as increasing market price and regulation for synthetic nitrogen fertilizers, could lift these systems to high relevance for farming practice. This would justify increased efforts in breeding programs and breeding research for diversified cropping systems.

Both wheat line cultivars bred for monocultures and HPs that evolved in single-species populations can be used to harness

the advantages of cereal-legume mixtures, as indicated by the multifunctional evaluation. HPs add additional performance stability to species mixtures under environmental stress but their genetic background and selection environment used for their evolution need to be taken into account (Weedon and Finckh, 2019, 2021). Interaction of diversity at the intra- and interspecific levels in cropping system performance needs further study, particularly in different environments. Research on species mixtures in multiple environments might profit from a stronger link to crop community ecology. Gliessman introduced the concept of crop communities for cropping systems and community ecology as a discipline to refocus agricultural management and research to harness emergent effects and properties of plant communities that can be used in agriculture (Gliessman, 1987, p. 161). Systematic empirical research on species mixtures combined with plant ecology might contribute to the development of crop community ecology as a basis for multifunctional cropping systems, as described by Litrico and Violle (2015).

Upscaling experimental research across environments and integration of new conceptual perspectives from ecology needs to be complemented by experiments that investigate diversification mechanisms and interactions related to plant traits in more detail, particularly those related to the 4C approach (Justes et al., 2021). Traits should not only be studied as targets for breeding but also as indicators of competition/complementarity and adaptation to species mixtures. For example, genotypes can systematically be screened for maintenance or even increased HI in mixtures indicating reduced susceptibility to competition. Some key results, such as differences in variation in the harvest index of “Kolompos” and “Nemere,” were detected by combining the results of *post-hoc* tests and visual interaction plots. Elucidating such variation in system comparisons can help to pinpoint mechanistic relationships between reduced competition between peas and specific wheat entries (increased HI in mixture), plant traits (phenology), and performance advantages under certain environmental conditions. Such experiments can support future breeding efforts to more systematically study traits and how they are influenced by the 4Cs to further reduce competition and increase complementarity in mixtures. Hitherto, intraspecific variation of phenology as a source of variation of niche differentiation between crop partners seems to be largely unexplored. Exploring variation in temporal niche differentiation could also increase resource efficiency and yield gains in high-input farming systems (Li et al., 2020).

Despite the many well-known advantages of mixed cropping, many obstacles for species mixtures currently preclude their adoption in general (Kjaer et al., 2022). With respect to wheat-pea mixtures for food products, market and processing opportunities are rare to nonexistent. Innovations in cropping system design may help to address practical challenges. In relay mixtures, for example, sowing of crop species and their harvest is done in a staggered fashion, avoiding the issue of grain separation prevalent in other mixture designs. This is especially relevant, since some specialized food processors require the highest grain purity because of consumer allergies. This highlights the importance of specific mixed cropping system designs and the need for their

systematic investigation and development while integrating plant ecology and agronomy (Brooker et al., 2021).

Harnessing the advantages of species mixtures in farming practice needs to go beyond the cropping system perspective. The multifunctional agronomic advantages identified here can only be harnessed in practice for food crops if major obstacles for food grain mixtures are addressed downstream of the food supply chain (Meynard et al., 2013, 2018). This includes the need for public support to establish sufficient grain sorting facilities, storage, and logistics in an emerging sector that needs to scale up. Lack of infrastructure may slow down or even impede the adoption of such farming practices (Mamine and Farès, 2020). Failing to account for these challenges in the food system will result in failure to harness mixture advantages as emphasized in the food-system turn in agroecology (Francis et al., 2003; Gliessman, 2015). For this reason, tailored research strategies that integrate plant ecology, agronomy, breeding science, and practical value chain aspects are urgently needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MF, OW, and JT conceptualized the study and contributed to methodology, validation, and writing, reviewing, and editing. JT and OW contributed to formal analysis. MF contributed

to resources, project administration, and funding acquisition. JT contributed to data curation and visualization. JT and MF wrote the original draft. MF and OW contributed to supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.846237/full#supplementary-material>

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Plant Breeding for Intercropping in Temperate Field Crop Systems: A Review

Virginia M. Moore^{1*}, Brandon Schlautman², Shui-zhang Fei³, Lucas M. Roberts⁴, Marnin Wolfe^{1,5}, Matthew R. Ryan⁶, Samantha Wells⁴ and Aaron J. Lorenz⁴

¹Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, United States, ²The Land Institute, Salina, KS, United States, ³Department of Horticulture, Iowa State University, Ames, IA, United States, ⁴Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN, United States, ⁵Department of Crop, Soil and Environmental Sciences, College of Agriculture, Auburn University, Auburn, AL, United States, ⁶Soil and Crop Sciences Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, United States

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Agricultural University of Athens,
Greece

*Correspondence:

Virginia M. Moore
vm377@cornell.edu

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Monoculture cropping systems currently dominate temperate agroecosystems. However, intercropping can provide valuable benefits, including greater yield stability, increased total productivity, and resilience in the face of pest and disease outbreaks. Plant breeding efforts in temperate field crops are largely focused on monoculture production, but as intercropping becomes more widespread, there is a need for cultivars adapted to these cropping systems. Cultivar development for intercropping systems requires a systems approach, from the decision to breed for intercropping systems through the final stages of variety testing and release. Design of a breeding scheme should include information about species variation for performance in intercropping, presence of genotype $\times$ management interaction, observation of key traits conferring success in intercropping systems, and the specificity of intercropping performance. Together this information can help to identify an optimal selection scheme. Agronomic and ecological knowledge are critical in the design of selection schemes in cropping systems with greater complexity, and interaction with other researchers and key stakeholders inform breeding decisions throughout the process. This review explores the above considerations through three case studies: (1) forage mixtures, (2) perennial groundcover systems (PGC), and (3) soybean-pennycress intercropping. We provide an overview of each cropping system, identify relevant considerations for plant breeding efforts, describe previous breeding focused on the cropping system, examine the extent to which proposed theoretical approaches have been implemented in breeding programs, and identify areas for future development.

Keywords: agroecology, ecosystem services, intercropping, plant breeding, polyculture, sustainable cropping systems

INTRODUCTION

Crop diversity provides an array of benefits (Altieri, 1999; Letourneau et al., 2011) and can appear at multiple spatial and temporal scales (e.g., across landscapes, seasons, farms, or fields). Intercropping represents within-field diversity, is defined as growing two or more crop species simultaneously in the same field, and encompasses a range of practices including mixed

intercropping (growing component crops simultaneously with no distinct row arrangement), row intercropping (growing component crops simultaneously in different rows), strip intercropping (growing component crops simultaneously in different strips), and relay intercropping (growing component crops with overlapping growth periods; Andrews and Kassam, 1976). Intercropping can provide valuable benefits, including increased yield (Trenbath, 1974; Hauggaard-Nielsen et al., 2001; Nyfeler et al., 2009; Finn et al., 2013; Martin-Guay et al., 2018; Li et al., 2020), yield stability (Rao and Willey, 1980; Raseduzzaman and Jensen, 2017), improved crop quality (Sleugh et al., 2000; Bélanger et al., 2014), reduced pest and disease impacts (Altieri, 1999; Boudreau, 2013; Gaba et al., 2015), improved weed management (Hauggaard-Nielsen et al., 2001; Finn et al., 2013; Johnson et al., 2017; Connolly et al., 2018; Hoerning et al., 2020), reduced input needs (Nyfeler et al., 2009; Gaba et al., 2015; Raskin et al., 2017), improved soil health (Cong et al., 2015; Li et al., 2021), support for a wide range of native pollinators (Eberle et al., 2015; Forcella et al., 2021), and a range of other ecosystem services, such as wildlife conservation, soil conservation, water quality improvements (Weyers et al., 2021), and carbon sequestration (Malézieux et al., 2009). Intraspecific diversity in the form of cultivar mixtures can provide benefits for productivity and resilience (Reiss and Drinkwater, 2018), but this review focuses on interspecific diversity through intercropping.

Intercropping is an ancient practice that has been used for thousands of years and remains an important practice in many parts of Africa, Asia, and Latin America. However, temperate regions have seen shifts away from intercropping and toward monoculture production, which is associated with greater mechanization, specialization, and input use (Anil et al., 1998; Altieri, 1999; Crews and Peoples, 2004). Intercropping systems are generally seen as labor-intensive and incompatible with mechanization and the need for standardized products (Brooker et al., 2015). However, with the array of environmental problems associated with modern agriculture (Foley et al., 2005; Pretty et al., 2018), there is interest among researchers and farmers in increasing diversity in cropping systems (Arbuckle and Roesch-McNally, 2015; Mortensen and Smith, 2020), and with new technological advances (e.g., new machinery, precision agriculture technology, and genomic tools), there are new possibilities of developing intercropping systems for modern agriculture in temperate field crops (Brooker et al., 2015). Modern plant breeding efforts in temperate field crops have primarily focused on monoculture production (Henkhaus et al., 2020). Still, as interest in intercropping for temperate agriculture increases, cultivars must be adapted to these cropping systems.

Experimental approaches and breeding schemes to improve germplasm for intercropping have been widely studied (Keller, 1946; Hamblin et al., 1976; Mead and Willey, 1980; Wright, 1985; Hill, 1990; Brooker et al., 2015), yet critical knowledge gaps exist that prevent greater utilization of intercropping. We briefly review the relevant literature in ecology, agronomy, and plant breeding and describe core experimental, breeding, and cropping system design approaches, and apply these core concepts to temperate field crops in three case studies of

intercropping systems at various stages of breeding research and development: (1) forage mixtures, (2) perennial groundcover (PGC) systems, and (3) intercropping with winter oilseeds. Case studies were selected to represent a range of temporal and spatial interactions, agronomic and ecosystem service goals, and maturity of breeding efforts. Through these case studies, we examine the extent to which proposed theoretical approaches have been implemented in breeding programs and identify areas for future development.

Defining the Problem and Solution Spaces

When a plant breeder considers whether to breed for intercropping systems, they first need to identify the cropping system goals, and whether major cropping system constraints can be addressed through plant breeding (**Figure 1**). The possible benefits of planting crops in an intercropping system are diverse and support agronomic goals and ecosystem services (Tamburini et al., 2020). The relative importance of agronomic and ecosystem service goals varies by cropping system, and likewise, the relative contribution of each crop component toward those goals will vary. Some intercrops are planted to maximize short-term profitability by increasing productivity or quality, while others are used for their regulating and supporting ecosystem services (e.g., cover crop mixtures).

In systems where all crop components are harvested as cash crops, and in which the components are of relatively similar value, the main goal is often to increase total productivity of the system. However, if regulating and supporting ecosystem services are goals of the intercropping system, the main goal may be to balance tradeoffs between short-term profitability and benefits that might only manifest over longer periods or under certain circumstances. In this scenario, the system goals will likely be focused on the productivity of the primary crop, as ecosystem services or yield provided by the secondary crop must be achieved without compromising the primary crop. Programs can focus on avoiding yield or quality reductions in the primary crop by breeding for differential resource-use relative to the secondary crop. Alternatively, breeding programs may focus on adapting a secondary crop to use resources not needed for the primary crop, better tolerate the stress imposed by the primary, or even to facilitate the primary crop. Depending on the specific goal of the system, selection may take place within one or more component species, and selection criteria may be based on the total productivity of combined crop components or based on maximizing the productivity of a single component species.

When developing intercropping systems for temperate field crops, breeding decisions, and overall cropping system design will depend on these goals and the role each component crop plays in their realization. Both plant breeders and agronomists work to design and improve intercropping systems, and as plant breeders identify potential breeding goals, particularly in novel and complex intercropping systems, it is critical they engage with both agronomists and end users to identify breeding needs. Because plant breeding is a resource-intensive endeavor, it is also prudent for plant breeders to

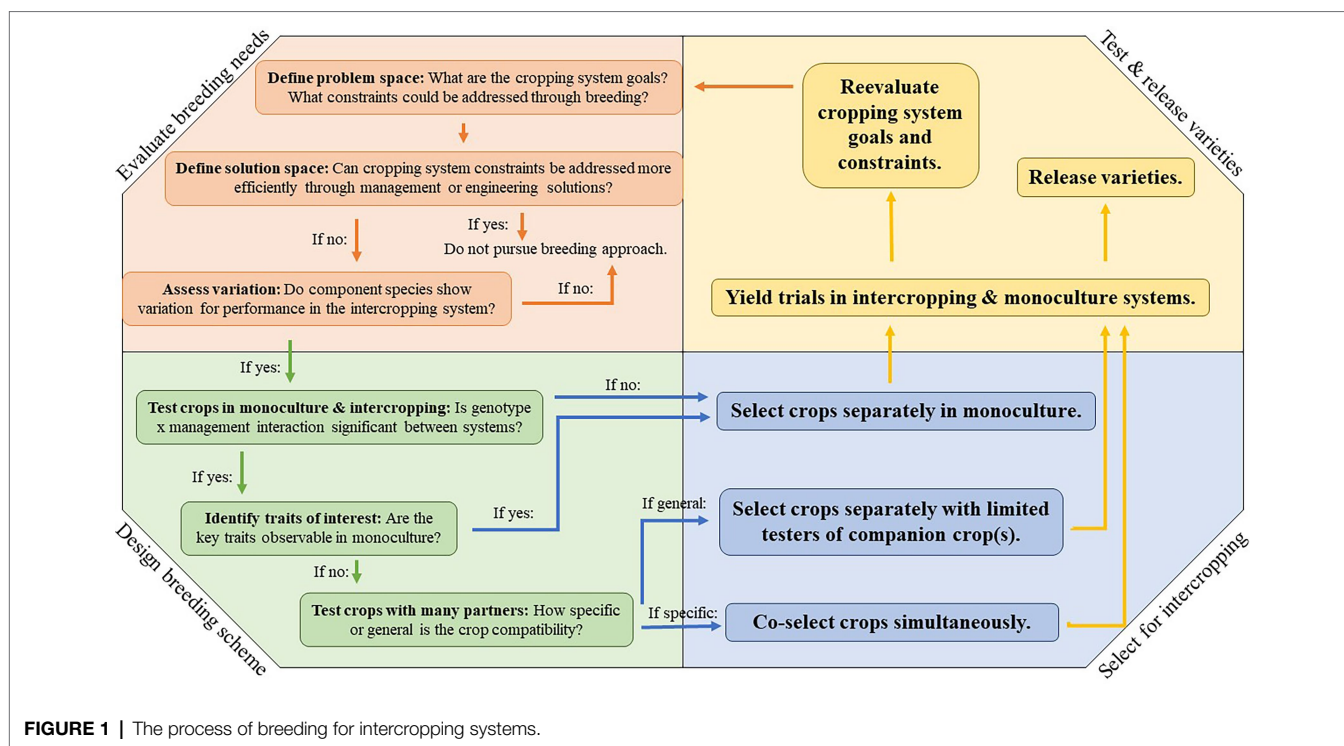


FIGURE 1 | The process of breeding for intercropping systems.

explore alternative approaches to improving intercropping system performance. This could include cropping system management (e.g., altering plant spacing, timing, or fertility) or engineering (e.g., adapting planting or harvesting equipment for multiple species) solutions. Agronomists, engineers, and other specialists should be engaged in this process of “defining the solution space” (Figure 1).

Assessing Variation and Genotype $\times$ Management Interaction

Identifying meaningful variation within the target species is a prerequisite for crop improvement efforts. Early studies often include screening diverse germplasm for performance in intercropping (Wright, 1985; Haug et al., 2021). In addition, to determine whether an intercropping-specific breeding program is merited, it is important to determine whether genotypes show differential performance in monoculture compared to intercropping systems. If genotype $\times$ management (G $\times$ M) interactions are not significant, then genotypes selected in monoculture can be used in intercropping systems (Annicchiarico et al., 2019). However, in the presence of significant rank changes, there is a need for intercropping-specific breeding efforts. To evaluate the significance of G $\times$ M interactions, diverse germplasm should be evaluated in both monoculture and intercropping systems (Brooker et al., 2015). Such comparative studies allow for the calculation of variance components, correlations, and heritabilities (Annicchiarico, 2003), and can inform breeder decisions about breeding methods and whether mixture-focused breeding efforts are required.

Performance in Intercropping: Competition and Overyielding

When designing a selection scheme for intercropping systems, a major question is which traits should be considered in the selection process? Ecological theory can provide insights to understand interspecies interactions and productivity in these systems (Li et al., 2014; Brooker et al., 2015; Litrico and Violle, 2015). While intercropping systems provide numerous environmental and agronomic benefits, competition between component species can reduce productivity. Competition between component species may come in the form of exploitation competition (competition for the same resources such as light, water, or nutrients) or interference competition (directly altering the resource acquisition behavior of another organism; Case and Gilpin, 1974; Schoener, 1983).

Intercropping systems are often challenged by asymmetric exploitation competition, such that one species has a competitive advantage over another, which can reduce productivity and overall benefits of the system (Connell, 1983; Thomas, 1992; Correhellou et al., 2006; Bybee-Finley et al., 2016). The competitive advantage may depend on growing conditions; for example, low N availability favors legumes over other plants, and moisture limitations in arid regions may favor one component species over another. Such genotype $\times$ environment (G $\times$ E) and genotype $\times$ environment $\times$ management (G $\times$ E $\times$ M) interactions will inform the regional focus and breeding approaches within intercropping breeding programs. Competition between partners may also change over time, depending on the phenology, stress tolerance, and persistence of component crops (Raskin et al., 2017; Ginakes et al., 2020). Temporal dynamics play a role in

both cropping system design and breeding. For example, understanding key growth periods during which competition will be more detrimental (e.g., through modeling yield loss due to competition) may help to select appropriate crop pairings and determine breeding objectives (e.g., early maturity; Gaudio et al., 2019; Cherié et al., 2020; Bourke et al., 2021; Schlautman et al., 2021).

Allelopathy, or chemical inhibition of one plant by another, is a common form of interference competition. Allelopathic ability has been a focus for breeding programs with an interest in weed suppression, and screening and selection for allelopathy have been conducted in cereals and other crop species, including rice, wheat, barley, oat, rye, cassava, sunflower, and sorghum (Worthington and Reberg-Horton, 2013). In the context of intercropping systems, the role of allelopathy may be important depending on the component species, and selection criteria may include reduced allelochemical production or reduced susceptibility to the allelochemicals produced by the partner species. In general, asymmetric competition may be more or less important depending on the goals of the system. For example, when one crop is planted primarily for ecosystem services, farmers may be less willing to compromise yield of its cash crop partner, whereas in intercropping systems involving two cash crops of comparable value, some yield reduction of each component may be acceptable.

Overyielding, or increased productivity in more diverse natural and agricultural ecosystems, often occurs in intercropping systems and can be explained through complementary interactions such as niche differentiation and facilitation. Niche differentiation is the process by which sympatric species avoid competitive interactions by differentially using resources; it can lead to greater productivity in natural and agricultural systems through increased resource acquisition and reduces interspecific competition (Hector et al., 1999; Fargione and Tilman, 2005; Hauggaard-Nielsen and Jensen, 2005; Li et al., 2007, 2014; Litrico and Violle, 2015). Previous research shows that, when designing and breeding for intercropping systems, an overarching goal should be to increase niche differentiation as a way to reduce competition between component crops and increase overall productivity of the system (**Figure 2**; Li et al., 2014; Brooker et al., 2015; Litrico and Violle, 2015; Annicchiarico et al., 2019). Niche differentiation may occur either spatially or temporally. For example, a focus on rooting depth or above-ground plant architecture could differentiate the resource space exploited by each component crop, whereas a focus on phenology could differentiate crops based on the period of maximum growth (Litrico and Violle, 2015). Increasing phenotypic plasticity could also contribute to species complementarity by increasing niche differentiation when planted in intercropping systems (Zhu et al., 2015).

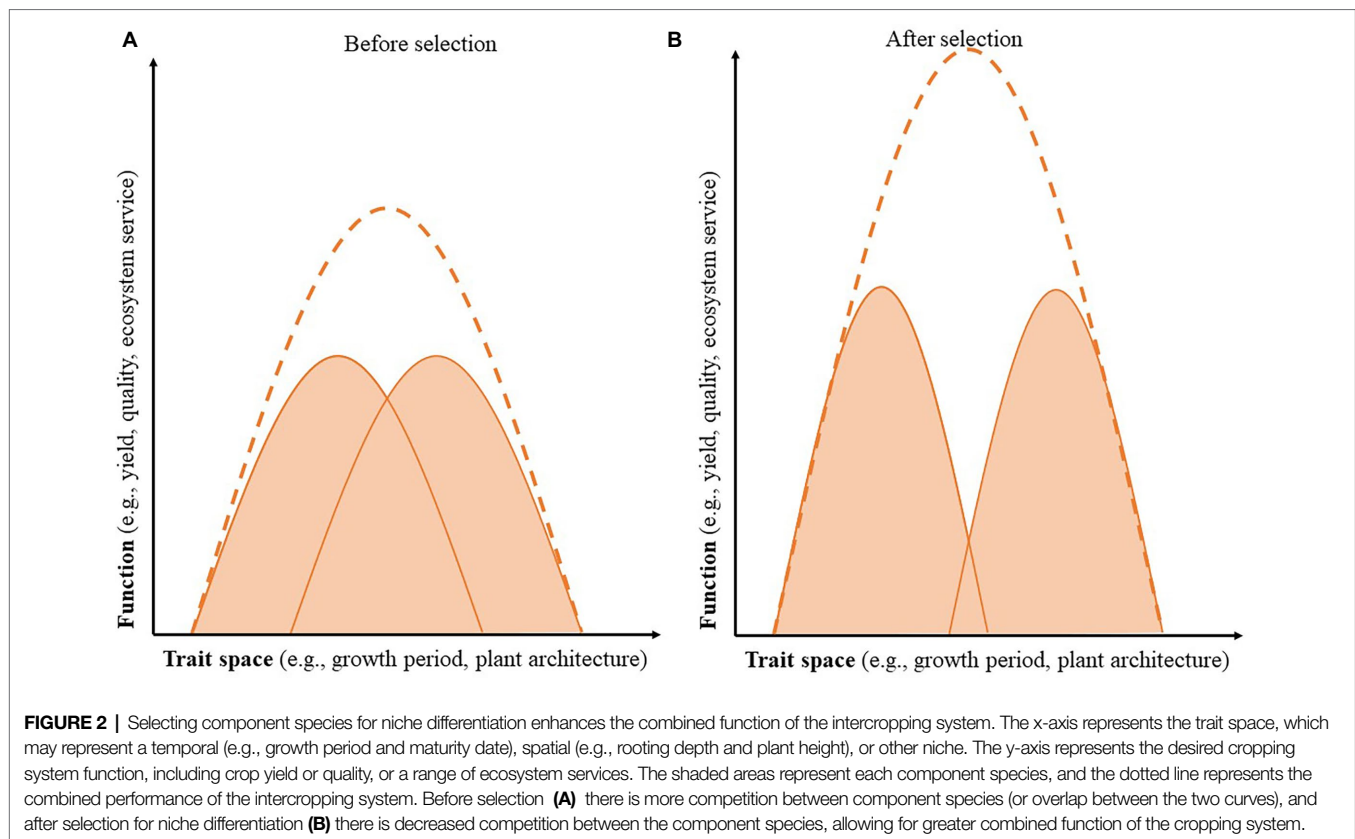
According to Callaway (1995), facilitation, or positive interactions among plants, can occur directly, for example, by reducing environmental stress or increasing resource availability. Facilitation can also occur indirectly through elimination of competitors (e.g., through allelopathic effects on susceptible weeds), promoting other beneficial organisms, or providing protection from herbivores. Both niche differentiation and

facilitation contribute to increased productivity in intercropping systems and can be difficult to distinguish in practice (Loreau and Hector, 2001). Facilitation has been observed in intercropping systems through mechanisms, such as improved nutrient availability (e.g., nitrogen fixation or mobilization of other nutrients), modification of root architecture, and suppression of pests and diseases (Hauggaard-Nielsen and Jensen, 2005; Li et al., 2014). Facilitation is more likely to occur with perennial intercrops compared to annual systems, since annuals have a more limited time to see beneficial effects from their partner species (Bybee-Finley and Ryan, 2018). Selecting for facilitation-related traits may therefore be more fruitful in perennials than annuals.

Designing the Breeding Scheme

One challenge, in practice, is to identify traits that are straightforward to phenotype and have high correlation with intercropping performance. If these traits are known, highly correlated and observable without intercropping, then it will be possible to select component crops in monoculture. This is known as a trait-informed approach. Otherwise, it may be more efficient to select directly in an intercropping system, otherwise known as a trait-blind approach (Barot et al., 2017). Preliminary experiments are required, first to determine the appropriateness of the trait-informed or trait-blind approach by evaluating candidate traits in both mono- and mixed cropping (Brooker et al., 2015; Annicchiarico et al., 2019). If traits can be identified that are observable under monoculture, which have a sufficiently strong genetic correlation, it may be most efficient to select indirectly for intercropping performance based on monoculture data (Atlin and Frey, 1989; Brummer, 2006). Indirect selection can be more effective than direct selection when the heritability of the trait is larger in an off-target environment (monoculture system) than in an on-target environment (intercropped system; Holland and Brummer, 1999; Bänziger and Cooper, 2001; Brummer, 2006). Selecting in monoculture may also be desirable when intercropping involves more complicated management including narrower windows of operation or greater precision for weed and nutrient management.

When breeding for systems with multiple species, the number of combinatorial interactions can quickly become impractical experimentally. The concepts of general mixing ability (GMA) and specific mixing ability (SMA) introduced by Wright (1985) (also referred to by Hill, 1990 as general and specific ecological combining ability) have been established to understand the necessity of recurrent selection and variety development for each component intercrop. The GMA and SMA are analogous to the classical concepts of general and specific combining ability (Sprague and Tatum, 1942; Griffing, 1956) and have also been applied to understand variety mixtures and multilines (Dawson and Goldringer, 2011). Genotypes with high GMA would perform well in a wide range of intercropping scenarios regardless of the identity of their partner, whereas high SMA and low GMA indicate good performance with another specific genotype but lack of general adaptation to many intercropping systems. In the ideal scenario, GMA would have a larger



contribution relative to SMA, allowing the breeding program to test material with a narrow set of entries (Annicchiarico et al., 2019). Temporal rotation and spatial intercropping systems might often be targets for focus on improving GMA, since breeders' products are expected to be paired in the field with varieties chosen by growers. However, breeders developing mixtures in contexts where they may control the varietal combinations employed by farmers will potentially be able to exploit SMA.

Despite the importance of determining the relative importance of GMA and SMA to optimize efficiency of intercropping-focused breeding programs, this question has been investigated in only a limited set of intercropping systems (Waldron et al., 2017). The GMA/SMA approach is feasible to integrate into the later stages of a breeding pipeline, but in order to make rapid progress, intercrop breeding needs to be applied recurrently and in earlier breeding stages (Wright, 1985; Hill, 1996; Sampoux et al., 2020). In early breeding stages, the massive number and diversity of possible intercrop combinations between any two species are intractable with the full-factorial designs necessitated by the GMA/SMA approach.

Genomic and phenomic technologies potentially make early stage and rapid recurrent intercrop selection more feasible by enabling strategic rather than complete sampling of intercrop combinations and the use of partial-factorial designs. The use of genomics and especially genome-wide marker data to enhance breeding decisions is becoming pervasive across crop and livestock breeding (Butruille et al., 2015; Bernardo, 2016; Hickey

et al., 2017; Georges et al., 2019; VanRaden, 2020). The process of choosing new parents or advancing new candidate cultivars on the basis of genomics-enabled predictions (GP) of their performance is known as genomic selection (GS; Meuwissen et al., 2001). GS has previously been suggested for improving mixture performance but has not yet been applied to do so (Annicchiarico et al., 2019; Bančič et al., 2021; Bourke et al., 2021; Wolfe et al., 2021).

In this special issue, two simulation studies (Bančič et al., 2021; Haug et al., 2021) and a perspective article (Wolfe et al., 2021) collectively highlight the advantage GS has to offer intercrop breeding. Haug et al. (2021) showed a clear advantage of partial-factorial designs even without using genomic information. Bančič et al. (2021) simulated several designs for using GS and sparse sampling in a two-species recurrent selection program all of which outperformed phenotypic selection. Bančič et al. (2021) modeled performance in pure vs. mixed stands as genetically correlated traits enabling breeding designs to be flexible and use a combination of mixed and pure plot trials, a feature likely to facilitate integrating intercrop breeding within established monoculture programs. Wolfe et al. (2021) point out that from a quantitative genetics perspective, the phenotype of any individual is the result of its response to an environment that is partially (or largely) determined by the other individuals present, both current and past.

The advantages of intercropping like yield stability and improved soil condition (i.e., ecosystem services) are observable primarily over multiple seasons and locations and occur because

of multiple interacting species. For this reason, joint-selection approaches enabled by genomic prediction and sparse designs are needed. Genomic prediction approaches that model GxE and genome-by-genome (GxG) interactions (Burgueño et al., 2012; Cuevas et al., 2016) should therefore be adaptable to enable intercrop-level selection in either a trait-blind or trait-informed way.

As in breeding programs focused on monoculture systems, breeding material developed for intercropping systems will go through the process of variety testing and release. Variety trials should be undertaken in intercropping systems, but monoculture trials may be useful as well, depending on the cropping system and breeding context. Variety release may have added complications when dealing with multiple species, especially if intercrop compatibility is highly variety-specific, and could necessitate unconventional variety release arrangements.

Optimal methods for breeding for intercropping have been described in the literature, and some studies have validated specific breeding methods for intercropping systems. However, there is a lack of literature bridging the scales between the conceptual and the specific to describe the design of breeding pipelines in the context of specific intercropping systems. Below, we describe three intercrop breeding systems in some detail. We hope readers will draw parallels between and see differences among these cases. Our nuanced and more specific understanding of each system will in turn inform design and implementation of future intercrop breeding programs.

FORAGE MIXTURES

Forages are frequently grown in grass-legume mixtures (Riday and Brummer, 2014). In alfalfa (*Medicago sativa*), the most widely grown forage crop, planting practices vary by region, with mixtures more common in the northeast and upper Midwestern United States (Undersander et al., 2011). White clover (*Trifolium repens*) is grown almost exclusively in mixed stands (Riday and Brummer, 2014). In general, legumes are weaker competitors compared to grasses, with the exception of alfalfa which often dominates mixtures when included as the legume component (Haynes, 1980; Zannone et al., 1986; Jones et al., 1989; Annicchiarico and Proietti, 2010; Brophy et al., 2017; Maamouri et al., 2017). Estimates of the optimum legume percentage for maximum dry matter, protein, and animal production range between 20 and 50% (Thomas, 1992). However, environmental conditions (e.g., temperature, moisture, soil pH, and fertility) and management (e.g., harvest height and timing) can affect the proportion of each component (Jungers et al., 2019).

Grass-legume forage mixtures can provide important production benefits. Numerous studies have found forage mixtures to provide increased yield relative to grass or legume monocultures, and also greater yield stability over the growth season and/or over a multi-year period (Zannone et al., 1986; Menchaca and Connolly, 1990; Annicchiarico and Piano, 1994; Sleugh et al., 2000; Malhi et al., 2002; Frankow-Lindberg et al., 2009; Picasso et al., 2011; McElroy et al., 2012;

Papadopoulos et al., 2012; Finn et al., 2013; Sanderson et al., 2013; Bélanger et al., 2014; Sturludóttir et al., 2014; Tracy et al., 2016). Additional documented benefits include improved forage quality (Sleugh et al., 2000; Malhi et al., 2002; Bélanger et al., 2014), reduced pest pressure (Lamp, 1991; Roda et al., 1996; Picasso et al., 2008; Frankow-Lindberg et al., 2009; Drenovsky and James, 2010; Sanderson et al., 2012, 2013; Finn et al., 2013; Bélanger et al., 2014; Sturludóttir et al., 2014), and reduced input needs (Zemenchik et al., 2001; Malhi et al., 2002; Nyfeler et al., 2009; Rasmussen et al., 2012; Frankow-Lindberg and Dahlin, 2013; Crème et al., 2016). Including grass species in forage mixtures have been shown to increase fiber digestibility, reduce bloating (Majak et al., 2003; Veira et al., 2010), and improve stand persistence (Sleugh et al., 2000) while the legume component fixes nitrogen (Thomas, 1992; Carlsson and Huss-Danell, 2003; Temperton et al., 2007; Nyfeler et al., 2011) and improves nutritive value (Barnett and Posler, 1983; Sleugh et al., 2000).

As mentioned above, forage mixtures often display asymmetrical competition, with the legume as the weaker competitor in most cases (Haynes, 1980; Annicchiarico and Proietti, 2010; Bybee-Finley et al., 2016; Brophy et al., 2017). This can be problematic if the legume proportion in the mixture drops to lower levels since the ecosystem services provided (e.g., N fixation) will be reduced as well (Thomas, 1992). The competitive dynamics between component species also change over time (Zannone et al., 1986; Chamblee and Collins, 1988; Marquard et al., 2009; Picasso et al., 2011; Baxter et al., 2017); in some respects, this is beneficial. For example, within a given season, temporal niche differentiation may allow one component to maximize its growth while the other is dormant (Zannone et al., 1986; Dong et al., 2018). Over a multi-year period, some species may experience reductions in plant populations and/or yield, while others show yield increase, e.g., due to compensation (Picasso et al., 2011). This can allow greater yield stability and persistence overall. However, forage quality parameters may be less stable and predictable compared to forages grown in monoculture (Grieder et al., 2021), which can bring added management complexity for producers.

Breeding forages for performance in mixtures has received more attention than many other temperate intercropping systems (Annicchiarico et al., 2019). Among forages, white clover-grass mixtures have a longer history of research and breeding, since white clover is grown predominantly in mixtures (Dijkstra and De Vos, 1972; Hill, 1990). Other forage legumes, including alfalfa, red clover (*Trifolium pratense*), and birdsfoot trefoil (*Lotus corniculatus*) have received less attention in terms of breeding specifically for mixture systems (Riday and Brummer, 2014).

The importance of breeding for forage mixtures has been established across multiple species by screening for variation in mixture performance and GxM interactions. Studies across multiple species have established variation for performance in mixtures (Atwood and Garber, 1942; Annicchiarico, 2003; Maamouri et al., 2015). The significance of GxM interactions among mixtures and pure stands varies across studies. When planting alfalfa with or without a grass companion, Zannone

et al. (1986) found the best mixtures were composed of the highest-yielding genotypes when planted in monoculture, indicating a lack of G×M interaction. However, other studies in white clover (Dijkstra and De Vos, 1972; Caradus et al., 1989; Annicchiarico, 2003), alfalfa (Maamouri et al., 2017) and orchardgrass (*Dactylis glomerata*; Xie et al., 2014) have found low correlation or significant G×M interaction between mixtures and monocultures. In general, less work has focused on genetic variation and G×M interaction in grasses than in legumes (Waldron et al., 2017). The significance of G×M also likely varies with the competitiveness of the companion species and the diversity of breeding material included in a given trial (Hill and Michaelson-Yeates, 1987; Annicchiarico and Piano, 1994; Grieder et al., 2021).

To implement a trait-informed breeding approach, studies have screened for candidate traits that impact performance in mixtures, and in some cases used these traits as selection criteria in breeding programs. Haynes (1980) describes a wide range of traits that impact competitive ability in grass-legume forage mixtures, including both physiological traits (e.g., symbiosis with microbes, growth rate, and phenology, light requirement, and water use) and morphological traits (e.g., growth habit, foliage architecture, and root morphology). Most traits specifically examined in an experimental setting fall into the latter category. Many studies have evaluated morphologically divergent material not selected in the same environment (e.g., Evans et al., 1985; Turkington, 1989; Elgersma and Schlegers, 1997), which limits conclusions that can be drawn due to confounding variables (Riday and Brummer, 2014). In white clover, traits including leaf size, stolon density, and petiole elongation and plasticity have been found to be associated with performance in mixtures (Atwood and Garber, 1942; Dijkstra and De Vos, 1972; Annicchiarico and Piano, 1994; Annicchiarico, 2003). Martin and Field (1984) found in a study of white clover and perennial ryegrass (*Lolium perenne*) that both shoot and root characteristics played a role in competitive dynamics but that their relative importance shifted over time. Zarroug et al. (1983) found that tall fescue (*Festuca arundinacea*) genotypes with low-density, high-yielding tillers allowed for greater contributions of birdsfoot trefoil in a mixed stand. Short and Carlson (1989) successfully improved orchardgrass compatibility with birdsfoot trefoil by selecting for traits including canopy height, tillering, and maturity. In alfalfa, Maamouri et al. (2017) identified internode length, shoot number, leaf size, and growth habit as key traits mediating competitive ability. Across species, most of the examined traits are related to competition and niche differentiation (e.g., access to light, nutrients, and other resources) rather than facilitation.

Trait-blind approaches have also been used when selecting forages for mixture systems. Forage breeding nurseries are frequently planted in a spaced-plant arrangement for efficient data collection, distinguishing among individual plant genotypes, and increasing environmental uniformity (Casler and van Santen, 2010). However, such arrangements also eliminate competition both within and among species, and alternate arrangements may be more appropriate when selecting for intercropping systems. Riday and Brummer (2014) selected birdsfoot trefoil

with and without a grass companion and found improved vigor and persistence among those selected with the grass. Forage legume breeding programs also commonly plant grasses for weed suppression purposes in space planted nurseries, with the additional benefit of selecting for performance in grass-legume mixtures (Riday and Brummer, 2014). Creeping red fescue (*Festuca rubra*) is often used since it is relatively prostrate and allows for easier viewing of space plants. However, this growth habit is quite different from that of forage grasses commonly planted with alfalfa and other legumes. The operating assumption is that creeping red fescue is an adequate proxy for other forage grasses (i.e., the effect of specific combining ability is small). Few studies have evaluated this assumption, but Grieder et al. (2021) tested red and tall fescues including both forage- and turf-types and found high phenotypic correlation between alfalfa cultivars planted across cultivation systems.

Although the efficiency of different selection schemes varies by system, previous research shows that direct selection in mixtures is most efficient when selecting forages for mixed systems. Rowe and Brink (1993) calculated predicted response to selection of white clover when planted in mixture and monoculture and found that planting in mixture would be 12–31% more effective when mixtures are the target cropping system. Where Annicchiarico (2003) compared a trait-informed approach (planted in pure stand) with two trait-blind approaches (direct selection in mixtures and selection in pure stand for biomass) in white clover and found that direct selection in a mixture was most efficient, followed by the trait-informed approach. Likewise, Waldron et al. (2017) compared selection of tall fescue in monoculture and in mixture and found direct selection in mixture to be more efficient.

Previous studies have found some specificity in the performance of forage mixtures depending on partner species. Rumbaugh and Pendery (1991) planted alfalfa clones with five associated forage species and found a significant genotype×species interaction, indicating the importance of SMA in this case. When comparing white clover performance in monoculture and in mixture with several grass species, Annicchiarico and Piano (1994) found variability of clover genotype performance to be driven by the competitiveness of the grass companion, indicating the possibility of identifying species groupings or companion “testers” based on vigor or other key traits. Given the large number of potential species pairings in forage mixtures, identification of tester species would be extremely valuable.

Over the long history of selecting forages for performance in mixtures, the bulk of breeding efforts for forage mixtures has been focused on improving the competitive ability of the less competitive species. Simultaneous selection of both grass and legume mixture components has been minimal, and we were also unable to identify programs using genomic selection as a tool in forage mixture programs. Developing forage breeding programs utilizing these approaches could increase efficiency and improve forage mixture yields.

Recognizing the ecosystem advantages of perennial forages and forage mixtures compared to annual grain production systems, efforts have been initiated to develop dual-purpose

perennial grain and forage crops that produce both human edible grain and valuable forage. Of these, intermediate wheatgrass (IWG), a perennial cool-season forage grass (Ogle et al., 2011), is one of the most promising. Efforts to domesticate IWG as a dual-purpose perennial grain-forage crop were initiated in the 1980s because it produces higher seed yields relative to most perennial grasses (Knowles, 1977; Lee et al., 2009) and has edible seeds, synchronous seed maturity, low shattering, and disease resistance (Wagoner, 1989, 1990). Continuous IWG breeding and domestication efforts have been underway since 2001 and it is now the nation's first commercially available perennial grain crop (i.e., Kernza®; DeHaan et al., 2018; Bajgain et al., 2019).

Grazing IWG in the late fall, winter or early spring, much like dual-purpose management of winter wheat is common in the High Plains (Lollato et al., 2017), could increase the profitability and early adoption of IWG for Kernza® perennial grain production (Jungers et al., 2017; Ryan et al., 2018; Pugliese et al., 2019). However, the majority of the IWG annual biomass production, which can exceed 10Mg ha⁻¹, is low quality straw (crude protein <60 g kg⁻¹) remaining after the grain is harvested (Favre et al., 2019). Intercropping IWG with alfalfa could improve the forage yield, quality, and seasonal distribution compared to IWG monocultures (Barnett and Posler, 1983; Sleugh et al., 2000; Aponte et al., 2019; Favre et al., 2019). Intercropping with alfalfa has also been identified as a potential strategy to meet IWG nitrogen (N) demands in perennial grain systems (Crews et al., 2016) and maintain stable IWG grain yields across years (Tautges et al., 2018; **Figure 3**).

Designing and breeding for IWG-alfalfa dual-purpose forage and grain production will likely be very different than for mixtures managed solely for forage. For example, in dual-purpose systems, IWG grain becomes the primary breeding target and forage yield is a secondary target, which has implications for selection decisions. IWG grain breeders are focused on improving seed size, seeds per head, and percent naked seed, rather than forage yield or quality-related traits (DeHaan et al., 2018). Breeding for IWG-alfalfa intercropping systems is in the initial stages of defining the problem space (cropping system goals) for the species and assessing variation in commercial alfalfa

varieties for impact on IWG grain yield and quality. Potential traits of interest include altered alfalfa growth habit (e.g., decumbent vs. prostrate growth), temporal distribution of growth (i.e., fall dormancy), or N-fixation potential to improve IWG-alfalfa complementarity, IWG-alfalfa forage yields and quality, and efficiency of IWG grain harvest in IWG-alfalfa dual-purpose systems. Regardless of breeding goals and trait targets, there is evidence that genotype×management interactions exist in this cropping system, at least for the IWG, that are better observed in sward than in spaced plant breeding nurseries (Mortenson et al., 2019).

PERENNIAL GROUNDCOVER SYSTEMS

Perennial groundcover systems are an emerging form of intercropping which pairs high-yielding row crops (e.g., maize, soy, cotton, and sorghum) with ecologically complementary PGCs (e.g., turfgrasses and clovers; **Figure 4**) to achieve productivity and natural resource conservation outcomes in the same field (Moore et al., 2019). The primary role of the groundcover is to provide continuous soil cover that radically reduces soil displacement from within crop fields and delivery to surface waters (Grabber and Jokela, 2013; Schlautman et al., 2021). It is critical that in this role, the PGC species do not interfere with the cash crop, whose primary role is maximum productivity and economic return (Flynn et al., 2013; Sanders et al., 2017).

Nearly all PGC research has been conducted using cash crop and PGC species and cultivars that were bred and adapted for other purposes and management practices (Moore et al., 2019). Researchers have found that creating spatial and temporal niche differentiation between the cash crop and PGC are critical to reduce interspecific competition and avoid reductions in cash-crop productivity (Bartel et al., 2020). Without access to PGC-adapted germplasm, adequate spatial and temporal differentiation have mainly been accomplished through management using mechanical (i.e., strip-tillage) and chemical (i.e., banded applications of contact herbicides) suppression of the PGC during key periods of the cropping season—at or

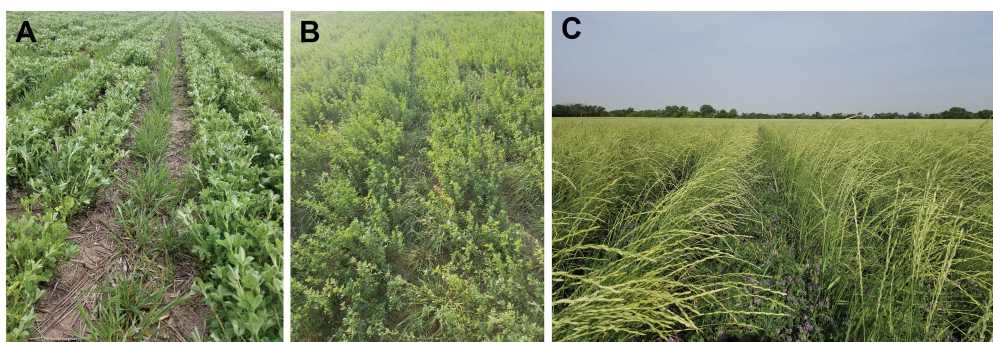


FIGURE 3 | Intermediate wheatgrass (IWG) intercropped with alfalfa for dual-purpose Kernza® perennial grain and forage production in a field near Canton, KS. The IWG is planted on 30-in rows with two rows of alfalfa (10 in apart) between each pair of IWG rows. Available spring (**A**, April 6, 2020) and fall (**B**, October 14, 2020) forage and ripening Kernza® perennial grain (**C**, June 24, 2020) are shown.



FIGURE 4 | Examples of maize intercropped with turfgrass perennial groundcovers (PGC). **(A)** Maize intercropped with strip-tilled but not chemically suppressed Kentucky bluegrass PGC. **(B)** Maize intercropped with a creeping red fescue PGC that has been chemically suppressed. Chemical suppression reduces the likelihood that the maize undergoes a shade avoidance response (SAR), which results in yield loss. **(C)** Maize intercropped with Kentucky bluegrass on August 2, 2020 in Ames, IA, United States.

just before planting and during the cash-crop critical weed-free period (Martin et al., 1999; Wiggans et al., 2012a; Bartel et al., 2017; Alexander et al., 2019). However, inter- and intraspecific variation in compatibility has been observed in screens of candidate PGCs, suggesting that spatial and temporal niche differentiation between component species in PGC systems can and should be improved through breeding (Flynn et al., 2013; Verret et al., 2017).

Because multiple cash crop species will be planted in rotation in PGC systems, PGCs generally must be compatible with multiple row-crop species and varieties (i.e., have high GMA) to fit within the desired crop rotation system. Examination of the more successful PGC candidate species reveals some shared common traits: low-growing growth habit, moderate to excellent shade tolerance, excellent winter hardiness, and shallow fibrous roots (Table 1). Together, these traits allow PGC to occupy spatial and temporal niches that do not overlap significantly with cash crops in corn-soybean rotations (Flynn et al., 2013; Bartel et al., 2017; Moore et al., 2019; Schlautman et al., 2021).

Cool-season grasses, and some cool-season legumes [e.g., kura clover (*Trifolium ambiguum* M. Bieb) and white clover (*T. repens* L.)] possess some or all of these desirable traits. The group of approximately 20 species cool-season grasses that possess culmless stems, making them mowing- or grazing-tolerant, commonly referred to as turfgrasses, appear especially well-suited as PGC in corn-soybean rotations (Hyder, 1972; Flynn et al., 2013). Turfgrasses have shallow fibrous root systems and they thrive in cool-moist climates (Beard, 1972). They have the C3 photosynthetic pathway, with an optimum growth temperature between 15.5 and 23.9°C (Beard, 1972), which is much cooler than the optimum growth temperatures for maize

and soybean: around 30°C for vegetative growth (Hesketh et al., 1973; Sánchez et al., 2014) and 26°C for anthesis (Boote et al., 2018). In studies using Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*F. rubra* L.) as PGC with maize, minimal or no reduction in grain yield was observed when the turfgrasses were chemically suppressed during maize establishment, and increases in soil water content were observed, potentially because the PGC functioned as a living mulch (Wiggans et al., 2012a,b).

Summer dormancy, which is strongly expressed in bulbous bluegrass (*Poa bulbosa* L.) and a few other *Poa* species, can further reduce the overlap period to be nearly non-existent. Induction and release of summer dormancy in *P. bulbosa* are controlled by photoperiod and to a lesser degree by temperature (Ofir and Kigel, 1999); therefore, its expression is strongly predictable (Figure 5). Summer dormancy can occur in Kentucky bluegrass but is likely an ecophysiological response to unfavorable environmental conditions, most likely low soil moisture, and is therefore not as reliable (Ervin and Koski, 1998; Suplick-Ploense and Qian, 2005). Summer dormancy is a key PGC trait because it has the potential to make chemical suppression of PGC unnecessary, reducing labor and cost and fitting well within both conventional and organic systems.

Perennial groundcover management undoubtedly affects the microclimate for maize or other cash crops compared to conventional management by altering the quality of the light, soil temperature, soil moisture, soil structure, and the biotic complexity of the agroecosystem (Wiggans et al., 2012b; Flynn et al., 2013; Banik et al., 2020). A few studies have demonstrated genetic variation in maize hybrid performance under PGC management (Ziyomo et al., 2013; Bowden, 2014); however, the relative importance of genotype × management (G × M)

TABLE 1 | Turfgrass and maize ideotypes in monoculture and intercropping systems.

	Crop and cropping system		
	Turfgrass		Maize
	Monoculture	Intercropped	Intercropped
Trait category			
Agronomic	Winter hardiness, dark green color, fine leaf texture, and high shoot density.	<i>Low input needs, winter hardiness.</i>	Early-season vigor, cold tolerance for non-tilled, lower temperature soils.
Phenology	Reduced summer dormancy to maintain year-round green color.	Early maturing, summer dormancy to reduce maize SAR.	
Architecture	Deep rooting, short stature, and reduced growth to minimize mowing.	Short, prostrate growth habit, and shallow, fibrous root systems for reduced above- and below-ground competition.	Deep rooting system that extends beyond the PGC root system.
Abiotic	Drought and heat tolerance for year-round persistence.	Shade tolerance to persist under the maize canopy, enhanced wheel traffic tolerance.	Drought tolerance, reduced SAR under altered red/far red light conditions.
Biotic	Ability to host fitness-enhancing endophytes.	Biological nitrification inhibitors to reduce N-loss, AMF.	

In each trait category, contrasting turfgrass breeding objectives are highlighted across the cropping systems. Characteristics of the intercropped maize ideotype are also highlighted.

interactions to maize hybrid performance remains unknown. If crossover GxM interactions (i.e., different maize hybrids are optimal under the two management conditions) exist, then establishing dedicated breeding programs for PGC-adapted maize is advisable. If not, then the elite, locally adapted germplasm from existing breeding programs can be utilized for PGC systems.

Many potential breeding targets exist for maize adaptation to PGC management including increased tolerance to shade competition and other abiotic stresses (e.g., cold soil temperatures) as seedlings, tolerance of drought conditions as mature plants, and perhaps tolerance to unknown pests for which the PGC may provide new habitats (Berti et al., 2021). Early indications suggest that minimizing the shade avoidance response (SAR) in maize will be critical to achieving yields under PGC management that are equivalent or better than those under conventional management (Moore et al., 2019). A green PGC, even if low-growing and minimally competitive, can alter the spectrum of reflected light received by maize leaves, causing the maize to perceive potential competitors and triggering a SAR (Rajcan et al., 2004), i.e., a cascade of physiological and morphological changes that can cause irreversible crop yield loss when it occurs during the crop's critical weed-free period (Bosnic and Swanton, 1997). While chemical suppression (which

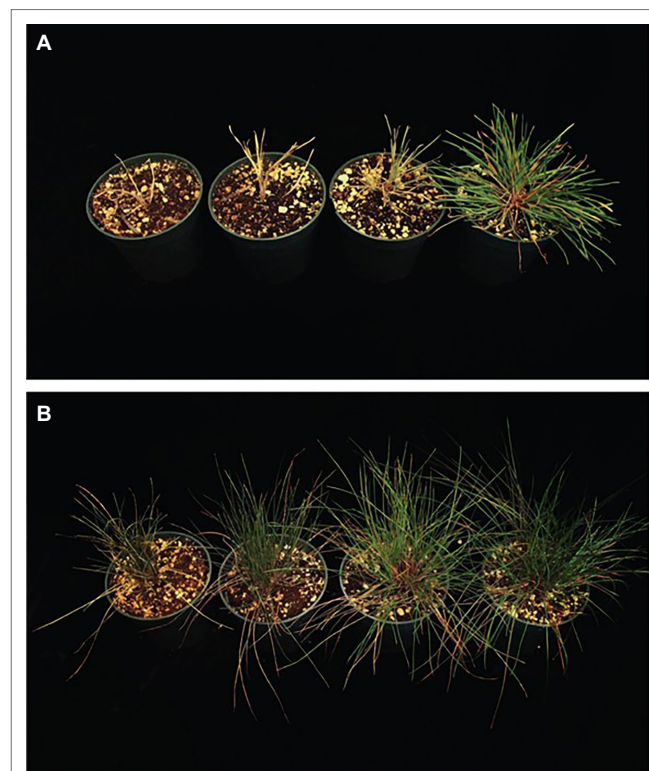


FIGURE 5 | (A) Summer dormancy response of sandberg bluegrass (*Poa secunda* J. Presl.) accession PI 232348 to various photothermal combinations representative of Ames, IA, United States. **(B)** Non-summer dormant red fescue (*Festuca rubra* L.), cv. "Audubon" under identical photothermal conditions. Summer dormant PGCs could reduce competition with cash-row crops (e.g., maize) during the growing season.

desiccates the PGC) or summer dormant PGC (whose leaves desiccate naturally) can reduce SAR, we expect that the maize SAR in PGC management could be mitigated through maize breeding. Population density insensitive maize hybrids provide evidence that maize can be, and indeed already has been, bred to tolerate intraspecific competition (Messina et al., 2021). Although still unknown, some of the same physiological mechanisms may allow maize to tolerate or fail to perceive interspecific competition in PGC management.

Cultivar development for PGC-based cropping systems is lacking. Cultivars and accessions that have been evaluated for their suitability as PGC are either wild collections or cultivars from turfgrass and forage grass breeding programs. Traits desirable for turfgrass are improved esthetic quality, which is a complex trait consisting of a number of component traits, such as shoot density, leaf color, and texture while traits desirable for forage grasses are high biomass yield and better nutritional quality. These traits are not inherently in conflict with traits for PGC except summer persistence, also a complex trait that is highly desirable for turfgrass cultivars and most forage grass cultivars but may be of less importance to PGC. Despite a relatively short history in turfgrass breeding, a large number of turfgrass cultivars have been released for major turf species including Kentucky bluegrass, Tall fescue (*F. arundinaceae*

Schreb.), perennial ryegrass (*L. perenne* L.), and red fescue (National Turfgrass Evaluation Program, <http://nstep.org>). There are also numerous wild collections for major cool-season grasses and legumes maintained at USDA GRIN,¹ most of which have not been well characterized and serve as an untapped resource for developing dedicated PGC germplasm. For species such as Kentucky bluegrass in which apomixis is the predominant reproductive mode or for species that reproduce by vivipary as in *P. bulbosa*, unimproved wild accessions may be directly deployed as PGC following field evaluation and seed increase. It is well known that abundant variation exists among cultivars of major cool-season grasses. For example, cultivars of Kentucky bluegrass vary so greatly that there are at least 16 groups of cultivars that each differs in morphology and development patterns from others (Honig et al., 2012). Screening of commercially available cultivars for their “mixing ability” with row crops is the most cost-effective strategy at this point to further refine the ideotype for PGC and facilitate trait-informed selection in the future.

The availability of compatible PGC cultivars that maintain adequate ground coverage without causing yield reduction to row crops is critically important to the success of PGC-based cropping systems. No dedicated PGC cultivars are currently available and the need for developing such cultivars is clearly present. Unlike selection and cultivar development for monoculture which deals with intraspecific interactions (typically among highly related plants within the same species), selection for PGC for intercropping has to consider the unique interspecific interactions. The inter-row space where PGC is grown is a unique microenvironment where air and soil temperatures, air and soil moisture, and light quality all differ from that of monoculture. It is therefore important that selection for superior genotypes is done in such an environment. While the near-term goal for breeding PGC is to identify or develop cultivars that provide adequate ground coverage without reducing yield of the row crop, future breeding needs to develop value-added traits such as the ability to inhibit nitrification (biological nitrification inhibition, BNI; Subbarao et al., 2007, 2009) or the enhanced capability of arbuscular mycorrhiza (AM) colonization. BNI can reduce N leaching and improve N use efficiency and has been reported in a number of grass species including perennial ryegrass (Moore and Waid, 1971; Subbarao et al., 2021). Symbiont AM can help plants capture nutrients such as phosphorus from soil (Deguchi et al., 2017). PGC cultivars with value-added traits should facilitate adoption of the PGC-Crop system.

INTERCROPPING SOYBEANS WITH WINTER OILSEEDS

Numerous legume-oilseed intercropping systems have been developed, and some have shown significant potential for commercial potential (e.g., canola-pea intercropping) and

advantages in terms of yield and nutrient-use efficiency (Dowling et al., 2021). In this case study, we focus on the development of novel intercropping systems including soybeans and winter oilseed crops. Winter oilseeds are being incorporated into existing cropping systems as an alternative to traditional winter annual cover crops. Like cover crops, they can provide environmental benefits (e.g., winter soil protection) and can also be harvested as a cash crop. Intercropping with winter oilseeds has become feasible with recent advances in domesticating cold hardy brassicas (Frels et al., 2019; Marks et al., 2021). In regions with harsh winter conditions, field pennycress (*Thlaspi arvense*) and winter camelina (*Camelina sativa*) offer suitable options (Cubins et al., 2019; Zhang and Auer, 2019). For regions that experience a milder winter, carinata (*Brassica carinata*) serves as a cool-season alternative (Gasol et al., 2007). Members of the Brassicaceae family are particularly suitable for this cropping system due to their cold tolerance (Warwick, 2011; Song et al., 2020). Furthermore, this adaptation for winter growth can capitalize on the observed increases in winter temperatures for the Northern Hemisphere (McCabe and Wolock, 2010).

In intercropping systems that include winter oilseeds, yield of both winter and summer crops are considered primary breeding goals. To a greater degree than in other systems previously discussed, these systems accomplish niche differentiation through temporal separation of the component crops (Brooker et al., 2015); there are extended periods in which a single species is growing in the field and narrower windows of overlap among the component crops. Winter annuals are established in the fall and harvested in early summer. In intercropping systems, the winter annuals are interplanted with summer annual row crops such as corn or soybean prior to seed formation (Figure 6). This strategy reduces the fallow period between crops, provides ecological benefits such as pollen for early-season pollinators and reduced leaching of nitrogen into groundwater sources (Weyers et al., 2019; Forcella et al., 2021), and produces harvestable yield from both cropping system components.

As most breeding programs for these winter annuals are less than a decade old, their focus has mainly been on key domestication traits (Chopra et al., 2020). Advances have also been made for heritable variations in plant morphology in the University of Minnesota's pennycress breeding program (Figure 7). Active breeding for intercropping systems has only recently been initiated, and there is a need to define an ideotype for intercropped winter oilseeds to facilitate the breeding of cultivars specifically adapted to intercropping to maximize yield. Some key differences in breeding objectives between monoculture and intercropping systems are likely to be important (Table 2).

Soybeans (*Glycine max*) offer a compatible and plastic option as a relay crop in a winter oilseed production system (Hussain et al., 2020). In the United States, soybeans are commonly double cropped with winter wheat, especially in parts of the Mid-South Region (Chan et al., 1980; Buehring et al., 1990; Wallace et al., 1992). Soybeans are cultivated on a global scale with greater than 120 million Ha harvested in 2019 [Food and Agriculture Organization (FAO), 2019]. With such a large

¹<https://npgsweb.ars-grin.gov/gringlobal/crop?id=110>

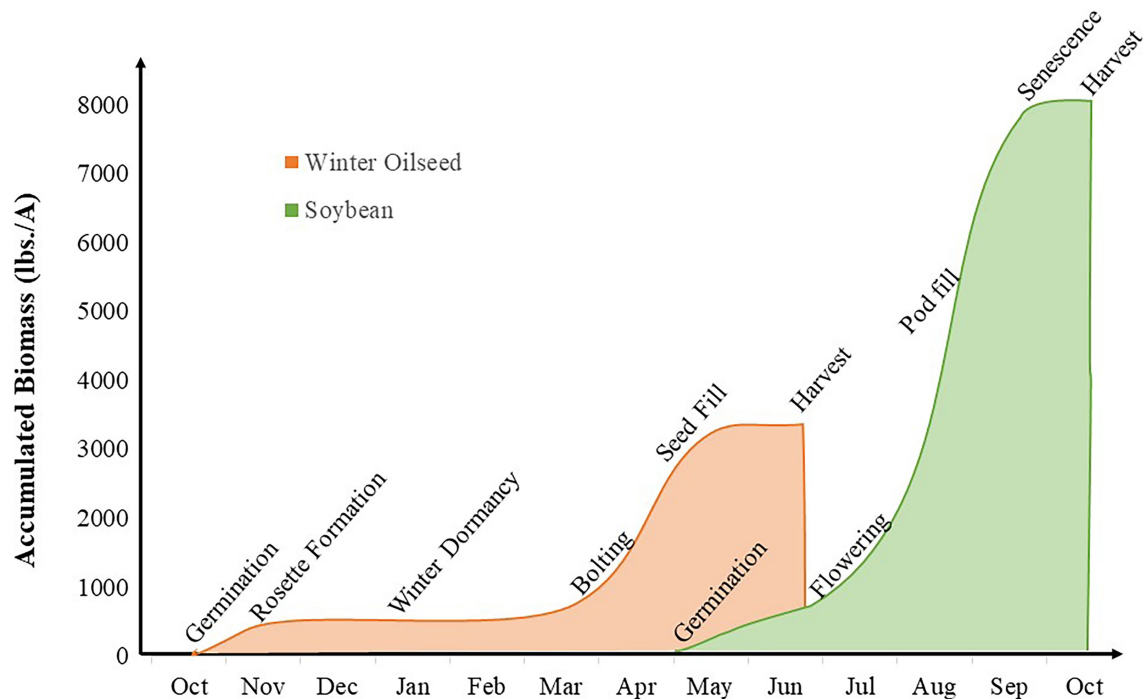


FIGURE 6 | Intercropping interaction window for winter oilseed intercropping systems. The orange curve represents biomass production and key growth stages in winter oilseed crop production and the green curve represents biomass production and key growth stages in soybean production.



FIGURE 7 | Trait variation in pennycress. (A) Wild type, (B) non-tillering, (C) Fasciated, (D) wild type, (E) dwarfing, (F) wild type, (G) glucosinolate null, and (H) wild type.

distribution, there are a plethora of intercropping studies between soybeans and various component species (sugarcane: Li et al., 2013; cassava: Tsay et al., 1988; sunflower: Saudy and El-Metwally, 2009; maize: Fan et al., 2020; and wheat: Li et al., 2001). In a winter annual oilseed relay system, soybean yield is highly dependent on environmental conditions with observed reductions in yield ranging from 0 to 47% (Hoerning et al., 2020).

Davis and Woolley (1993) also note that the genotype-by-cropping system interaction is more important for the understory crop (in this case the soybean). Negative effects on soybean yield may be due to a range of stressors including direct competition for resources, increased pest pressure, and allelopathy.

Direct competition in this system occurs on a shorter time frame than other intercropping systems, yet the effects on

TABLE 2 | Winter oilseed and soybean ideotypes in monoculture and intercropping systems.

Trait category	Crop and cropping system			
	Winter oilseed		Soybean	
	Monoculture	Intercropped	Monoculture	Intercropped
Agronomic	Reduced shattering to maximize harvestable yield.	<i>Reduced shattering to maximize harvestable yield and prevent late-season competition.</i>	Early-season vigor, cold tolerance.	<i>Early-season vigor, cold tolerance for non-tilled, lower temperature soils.</i>
Phenology	Late maturity to maximize seed yield.	Early maturity for early harvest to minimize competition window.	Early flowering and long reproductive period to maximize yield.	Late flowering so reproductive period does not overlap with intercropping competition.
Architecture	Reduced height for reduced lodging. Increased tillering to maximize yield.	Increased height of first silique for harvestability above soybean canopy. Reduced tillers to reduce competitive impact on soybean.	Rapid canopy closure through enhanced branching. Deep rooting.	<i>Rapid canopy closure through enhanced branching. Deep rooting.</i>
Abiotic	Nutrient-use efficiency.	<i>Nutrient-use efficiency for growth in competition with soybean.</i>	Drought tolerance.	<i>Drought tolerance, especially early-season in moisture-depleted soils. Shade tolerance.</i>
Biotic	High glucosinolates to suppress weed pressure.	Low glucosinolates to reduce allelopathic effects on soybean.		Tolerance to allelopathy.

In each trait category, contrasting breeding objectives are highlighted across the cropping systems. *Italic text indicates that the breeding objective is of greater importance in one cropping system than the other.*

juvenile soybean can have long lasting consequences for yield components, including height, biomass, and pod counts (Ott et al., 2019). One of the resources that become limited is light through the rapid canopy closure in pennycress and camelina stands where 40%–70% of available photosynthetically active radiation is blocked to the underlying soybeans (Ott et al., 2019). Current research is ongoing on allowing a greater amount of light to penetrate into the canopy through employing non-tillering pennycress lines (personal communication with Ratan Chopra), and it would also be beneficial to develop shade tolerance in soybeans. Planting winter oilseeds can deplete soil moisture levels, which can result in poor germination of the summer annual crop (Gesch and Johnson, 2015). One method to ameliorate reduced soil moisture may be to choose large-seeded soybeans which have been associated with early-season vigor (Smith and Camper, 1975).

Another biotic stressor that is the consequence of cultivating two species together is the potential for one to attract pests to the later crop. For example, field pennycress has shown to be an alternative host for soybean cyst nematodes (SCN; Johnson et al., 2008; Hoerning, 2019). Soybean cultivars with strong resistance to SCN are widely available, and longer rotation schedules are also a viable option. Sclerotinia has been shown to infect pennycress under controlled conditions (Boland and Hall, 1994), but there are currently no reports of infection of cultivated pennycress in the field. However, there have been reports of field infection of Camelina (Séguin-Swartz et al., 2009). In addition, the development stage of soybean plays a significant role in pathogen dynamics. Soybeans are susceptible once they begin to flower (Peltier et al., 2012), which typically would occur after winter oilseed harvest. Nevertheless, there could be a buildup of sclerotia in the soil, making major outbreaks

more likely. The pathogen dynamics in this intercropping system merit further study. Management tools that could help mitigate major outbreaks could include planting-resistant varieties, lowering planting density, using longer rotation schedules, and applying fungicides.

Allelopathy has long been a tool in cropping systems to reduce weed pressure (Putnam et al., 1983). Winter oilseeds have observed phytotoxic effects where weed biomass was suppressed up to 100% in pennycress and up to 87% in winter camelina (Hoerning et al., 2020). The only glucosinolate present in pennycress is in the form of sinigrin and the partial breakdown of sinigrin results in highly allelopathic isothiocyanates (Bialy et al., 1990; Chopra et al., 2019). Allelopathy tolerance is poorly characterized in soybeans but would be a necessity in developing varieties for a winter oilseed intercropping system.

Breeding for a winter oilseed relay system presents challenges not faced in other intercropping systems. Since winter oilseeds are still early on in the development pipeline and a market value is not yet established, it is unclear if importance should be given to maximizing the winter oilseed yield or reducing the yield penalties on soybeans. This differentiation is important in breeding for multiple traits because it has strong implications on selection indices, for example, one common approach is a base index selection where the weight of a trait is dependent on the market price (Kauffmann and Dudley, 1979). A number of breeding goals for monoculture and intercropping systems are mutually exclusive and signify the need for separate breeding pipelines. For example, earlier-flowering pennycress lines reduce the interaction window at the expense of pennycress yields, and reduced glucosinolate production corresponds to less allelopathic effects on soybeans at the expense of introducing insect and weed vulnerabilities to the pennycress (Table 2). Until the crops and markets are

more established, prioritizing these breeding goals may remain difficult. Despite these challenges, the development of a winter oilseed intercropping system has shown promise in terms of increased total yield, and additional markets may benefit producers by spreading risk (Gesch et al., 2014). These advantages, combined with ecosystem services such as reduced erosion and increased pollinator support, merit further efforts in breeding and management to help expand these systems into the future.

DISCUSSION

While all focused on breeding for intercropping, each of these case studies is distinct in its specific objectives. In forage mixture systems, both grass and legume components are harvested together as a single product, and the breeder seeks to maximize productivity of this mixture as a whole. In the unique case of perennial grain-forage systems, the grain becomes the primary target, with forage yield as a secondary goal. By contrast, in the PGC system, only the row crop is harvested, while the PGC is grown purely for ecosystem service purposes. The breeding focus will be primarily on improving the ability of the PGC to function within the row crop context (e.g., improving survival without impacting row crop yields). Some breeding may also be conducted in the row crop for improved compatibility with the PGC (e.g., to eliminate SAR), but such breeding efforts are likely to be fruitful only if they do not impact yield. In the winter oilseed intercropping systems, both summer and winter annual crops are harvested but as two separate crops. Yield of both crops are currently treated as primary breeding goals, but the cropping system is still developing, and as the production systems and markets mature, it will be critical to determine whether breeding programs should maximize yield of one component crop at the expense of the other.

The approaches used to minimize competition and maximize complementarity are also somewhat different in each case study. In the case of both PGC and winter oilseed intercropping systems, both temporal and spatial niche differentiation are important. In PGC systems, summer dormancy (temporal) and growth habit (spatial) have been important factors driving the choice of species to include in the system and could be enhanced through breeding (Table 1). In winter oilseed systems, maturity timing and growth habit are also important selection criteria for intercropping systems (Table 2). In both systems, there is also a need to select row crop components to reduce negative interactions associated with intercropping (eliminating SAR

in corn for PGC systems and reducing susceptibility to allelochemicals in soybean for winter oilseed systems). In contrast to other systems, breeding efforts for forage mixtures have seen a greater focus on spatial niche differentiation (e.g., selecting plants with compatible morphology), since component species are grown together for the entire cropping period, although facilitation (e.g., nitrogen fixation) and temporal traits (e.g., emergence and maturity timing) are also important.

Each of these cropping systems is also at a different stage in the development and implementation of a breeding pipeline for intercropping. Forage mixtures are certainly the most mature among the cropping systems discussed. Studies have been published addressing each major step along the pipeline (Figure 1) for at least some forage species and mixture combinations. Forage legume breeding also frequently takes place with a turf groundcover, mimicking a forage mixture. However, there is a need to deploy the methods described in this review in a more systematic way and to accelerate efforts to breed for mixture systems, e.g., through genomic selection and/or selection of both component species. Breeding for PGC and winter oilseed systems is comparatively much less developed. While there has been significant research focused on management of PGC systems, no breeding has taken place. Winter oilseed systems are at an early stage of development, with some species still being domesticated. In both cases, there is an opportunity to design a breeding pipeline that incorporates intercropping systems as one of its primary goals. Although nascent, breeding for intercropping systems holds great potential for improving intercropping systems and realizing the potential of this crop diversification strategy for addressing sustainability challenges.

AUTHOR CONTRIBUTIONS

VM contributed to conception and design of the manuscript. AL, BS, LR, MW, S-zF, and VM wrote sections of the manuscript. VM compiled and revised author contributions and wrote the first draft of the introduction and discussion. All authors contributed to the article and approved the submitted version.

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Mixture × Genotype Effects in Cereal/Legume Intercropping

Dereje T. Demie^{1*}, Thomas F. Döring², Maria R. Finckh^{3†}, Wopke van der Werf^{4†}, Jérôme Enjalbert^{5†} and Sabine J. Seidel¹

¹ Crop Science Group, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany,

² Agroecology and Organic Farming Group, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany, ³ Department of Ecological Plant Protection, Faculty of Organic Agricultural Sciences, University of Kassel, Witzenhausen, Germany, ⁴ Crop Systems Analysis Group, Wageningen University, Wageningen, Netherlands, ⁵ Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, Gif-sur-Yvette, France

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*Correspondence:

Dereje T. Demie
dereje.demie@uni-bonn.de

[†]These authors have contributed
equally to this work

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Cropping system diversification through annual intercropping provides a pathway for agricultural production with reduced inputs of fertilizer and pesticides. While several studies have shown that intercrop performance depends on the genotypes used, the available evidence has not been synthesized in an overarching analysis. Here, we review the effects of genotypes in cereal/legume intercropping systems, showing how genotype choice affects mixture performance. Furthermore, we discuss the mechanisms underlying the interactions between genotype and cropping system (i.e., sole cropping vs. intercropping). Data from 69 articles fulfilling inclusion criteria were analyzed, out of which 35 articles reported land equivalent ratio (LER), yielding 262 LER data points to be extracted. The mean and median LER were 1.26 and 1.24, respectively. The extracted genotype × cropping system interaction effects on yield were reported in 71% out of 69 publications. Out of this, genotype × cropping system interaction effects were significant in 75%, of the studies, whereas 25% reported non-significant interactions. The remaining studies did not report the effects of genotype × cropping system. Phenological and morphological traits, such as differences in days to maturity, plant height, or growth habit, explained variations in the performance of mixtures with different genotypes. However, the relevant genotype traits were not described sufficiently in most of the studies to allow for a detailed analysis. A tendency toward higher intercropping performance with short cereal genotypes was observed. The results show the importance of genotype selection for better in cereal/legume intercropping. This study highlights the hitherto unrevealed aspects of genotype evaluation for intercropping systems that need to be tackled. Future research on genotype effects in intercropping should consider phenology, root growth, and soil nutrient and water acquisition timing, as well as the effects of weeds and diseases, to improve our understanding of how genotype combination and breeding may help to optimize intercropping systems.

Keywords: cultivar combination, intercropping performance, mixture, mixing ability, trait combination

INTRODUCTION

In the past few decades, agricultural intensification has resulted in increased yields of pure line crops (Blomqvist et al., 2020); this has been accompanied by the simplification and homogenization of production systems and concentration on very few species as human diet staples (Khoury et al., 2014). Genetic uniformity and loss of diversity in the agricultural landscape (Hazell and Wood, 2008; Gregory and George, 2011) are characteristics of intensive agriculture, increasing vulnerability to climate change (Lin et al., 2008), and pathogen invasions (Anderson et al., 2004; Savary et al., 2019). Diversifying crop production systems is a promising pathway to tackle such vulnerabilities (Renard and Tilman, 2019; Hufnagel et al., 2020; Tscharncke et al., 2021). Diversification approaches can be classified into two categories: (1) integration of underutilized crops into the system; and (2) diversification of the production system through crop rotation, mixed cropping, and/or catch crops (Mustafa et al., 2019). More efficient utilization of resources with beneficial effects on the environment could also be gained by the integration of livestock with temporal and spatial crop diversification, such as forage legume intercropping with grain cereals (Danso-Abbeam et al., 2021). Crop diversification includes practices that significantly improve crop productivity, especially benefiting rural smallholders (Makate et al., 2016), and enhance overall ecosystem services without compromising crop yield (Tamburini et al., 2020; Beillouin et al., 2021; Ditzler et al., 2021). Annual intercropping is one form of cropping system diversification, which allows high productivity and reduction of fertilizer and pesticide input (Bedoussac et al., 2015; Li et al., 2020b) thereby substantially minimizing the negative environmental impacts of agriculture. Furthermore, crop diversification provides insurance against crop failure for farmers (Lithourgidis et al., 2011; Gaba et al., 2015).

Mixing crop species may be done with annual crops or perennial crops on a gradient of complexity from two to several species (Malézieux et al., 2009; Finckh and Wolfe, 2015). Cereal/legume intercropping systems are widely used across the world, particularly by smallholders, producing high-quality cereal and legume grains in an economically sustainable, environmentally friendly, and efficient way. Using legume crops in a mixture with cereals may significantly mitigate N₂O fluxes derived from fertilizer, hence providing an effective way to reduce greenhouse gas emissions from cropping systems (Senbayram et al., 2015). Furthermore, intercropping was found to produce higher cereal protein concentration (Bedoussac and Justes, 2010; Timaeus et al., 2021b), higher grain yields (Yu et al., 2016), higher yield stability (Raseduzzaman and Jensen, 2017), and better abiotic and biotic stress resistance (Bedoussac et al., 2015; Timaeus et al., 2021a) than sole crops.

Intercropping performance is often measured by the land equivalent ratio (LER), an index measuring the relative land area required to produce the same yields (or any other services, such as biomass) in sole crops as obtained from a unit area of intercrop. An LER greater than one indicates that intercropping uses the land more efficiently than pure stands to produce the desired outputs (Mead and Willey, 1980).

Several studies have shown that the general performance of intercropping systems depends on the genotypes used in the mixture (e.g., Hauggaard-Nielsen and Jensen, 2001) and that the performance in a mixed stand can be poorly correlated to performance in a pure stand (Annicchiarico et al., 2019). Different genotypes of legumes may have different responses in terms of phenology and morphology (Annicchiarico and Filippi, 2007) when compared in sole crops vs. mixtures. Hence, a specific selection of genotypes for intercropping is important (Giles et al., 2017), and intercrop yield advantage could be achieved by selecting specific traits of both species (Berghuijs et al., 2020). Therefore, it has been suggested that specific breeding of genotypes for intercropping is needed to improve complementarity of the intercropping partners (Annicchiarico et al., 2019; Haug et al., 2021).

Cereal/legume mixtures could include systems where both species have similar phenology but contrasting morphology, or, alternatively, contrasting phenology and morphology, resulting in temporal and/or spatial niche complementarity (Gaudio et al., 2019). The ecological niche separation concept posits that the different species involved may have different resource requirements at different times, as well as for different sources of nutrition (Malézieux et al., 2009). In addition to niche complementarity, intercrop performance can also be due to additional ecological mechanisms (Loreau and Hector, 2001). Facilitation effects may exist between mixed species, such as synergy in the use of phosphorus (Hinsinger et al., 2011; Li et al., 2020). The species complementarity effect, which measures the overall shift of relative yields in mixtures vs. sole crop, has a higher effect on yield gain than the selection effect, which defines how these shifts in relative yields are correlated to sole crop yields (Li et al., 2020a). Complementarity is a paramount feature in cereal/legume intercrops grown under low-nitrogen (N) conditions, in which biological N fixation by the legume and strong competition for soil-N by the cereal may synergize to enhance yield and grain quality.

Choosing plant genotypes for specific intercropping systems is, however, laborious and costly, if only because assessing intercropping performance also requires the inclusion of sole crops in field experiments for comparison and estimation of the benefits of mixing. Testing genotypes in mixtures easily results in a curse of dimensionality. For instance, with five genotypes of a cereal and five genotypes of a mixture, 25 mixtures should be tested along with 10 pure stands. Optimal species traits likely depend on the companion species, such that all possible combinations are preferably tested. Note that incomplete designs have been proposed to deal with this challenge of dimensionality (Hinsinger et al., 2011), and shown to be efficient to estimate mixing abilities (Haug et al., 2021). Testers and reciprocal breeding schemes have been proposed to co-breed species (Sampoux et al., 2020). Recent technologies, such as genomic selection strategies, could help select traits for breeding for intercropping accurately (Bančić et al., 2021). However, better knowledge on genotypes and their associated trait effects in intercropping is needed to make selection more targeted.

General and specific mixing ability of genotypes of single species has been studied to determine contrasting traits in sole cropping and in mixtures, and the theoretical background has been discussed with respect to species mixtures (Wright, 1985). Historically, multiple studies have evaluated different crop genotypes for complementarity in intercropping (Francis et al., 1976; Smith, 1985; Smith and Zobel, 1991; Davis and Woolley, 1993). Abundant research has been conducted, but the knowledge on genotype effects in intercropping is fragmented and has not been compiled to deliver necessary knowledge for designing optimized intercropping systems. Here, we aim to provide a current update by linking recent advances through a review. In particular, we address the knowledge gap concerning the mechanisms involved in genotype × cropping system interaction. This review is intended to answer the following questions: (i) How do different genotypes and/or traits of a species in cereal/legume intercropping systems affect the performance of the mixture? (ii) What are the mechanisms underlying the interaction of the genotypes in the intercropping system? and (iii) What are the current knowledge gaps in genotype evaluation for intercropping systems?

MATERIALS AND METHODS

Literature Search and Publication Screening

We conducted a systematic map, using the science databases Web of Science, Scopus, Science Direct, and Google Scholar. Keywords used for searching suitable publications were “genotype interaction in inter/mixed cropping system” OR “cultivars interaction in inter/mixed cropping system” OR “varieties interaction in inter/mixed cropping system” OR “cereals in inter/mixed cropping system” and scientific names (genus and species name) and common names of cereals species with intercropping and mixed cropping. The slash (/) was not used in a search; here, it is used for simplified expression of search terms (i.e., intercropping OR mixed cropping). A full list of the search terms is given in the **Supplementary Table 1**. In addition, secondary literature cited in selected articles were also looked up and included if relevant. The latest search was conducted on 12 April 2021.

To select the relevant articles, we used the following inclusion criteria: (i) studies from cereal/legume intercropping with both grain and forage legumes, (ii) studies evaluated at least two genotypes of at least one of the mixed species, (iii) peer-reviewed full-length articles published in English, (iv) studies reporting original research data, and (v) only field experiments, excluding greenhouse or pot experiments. No restriction was made against the type of mixture design, e.g., with respect to plant density, such as additive, replacement (substitution), or intermediate design. The information extracted from the original research articles was categorized in a digital database and analyzed following the Preferred Reporting Items for

Systematic Review and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Variables and Data Extraction

Data on genotype performance originated from different management and different zones, resulting in large differences in yield. Hence, an index was necessary to characterize the performance of genotypes in intercropping in relation to their respective pure stands (Mead and Willey, 1980). We used the LER (Equation 1) as a key metric to measure intercrop yield advantage (or disadvantage) by reference to the pure crop yields of mixed genotypes. We also retrieved the results of any ANOVA analyzing genotype and cropping system main effects and their interaction. Furthermore, individual studies were scrutinized by assessing conclusions and interpretations about the effects of different traits (phenology and morphology) of species in mixtures to identify the general mechanisms responsible for cereal/legume intercropping yield advantage.

Different variables were extracted from each study (**Table 1**) in the core set of publications. Information, like intercropping design (design of the mixing system, i.e., substitutive or additive or intermediate), country of the experiment, number of genotypes, and other related variables, was extracted from each publication. Significance (or non-significance) of “genotype” effect, “cropping system” effect (pure vs. mixed stand), and “genotype” × “cropping system” interaction effect on yield data was extracted from ANOVA tables of the articles. This was done by extracting results from the ANOVA of each article; any differences among articles regarding the structure of statistical analysis (e.g., fixed vs. random effects) were disregarded. The mechanisms of intercropping performance were extracted from the description of results, and the full article was consulted if needed. Some studies reported various types of mixtures, from different species of either cereals or legumes. In addition, in these cases, data were extracted from all combinations in which at least two genotypes of at least one of the partners were evaluated.

The LER (Equation 1) of each genotype combination was extracted from the subset of articles reporting them, either directly when represented numerically, or in figures. Data from figures were digitalized using a web-based plot digitizer (Rohatgi, 2020), an online system used to extract data from images efficiently and accurately (Burda et al., 2017; Cramond et al., 2019). LER was reported in figures only in five articles (Rao and Willey, 1983; Odo, 1991; Watiki et al., 1993; Kontturi et al., 2011; Pappa et al., 2012; Barillot et al., 2014). The majority of the studies reported mean LER per genotype combination across multiple environments. However, in some cases, the studies reported data individually from each environment. If the mean LER across different environments was not reported, this mean was computed for each genotype combination of the species in the intercrop from the individual environments. When a study reported only the partial land equivalent ratio (PLER), the total LER was calculated for each genotype combination of the species in intercropping by summing the PLERs:

$$\text{LER}_{c+l} = \text{PLER}_{c/l} + \text{PLER}_{l/c} \quad (1)$$

TABLE 1 | Variables extracted from different studies.

Variables	Definition	Data type/Units
Title	Title of the publication	Text
Authors	Authors in publication	Text
Year	Publication year	Text
Journal	The journal in which the article was published	Text
Country	The country where the experiment was conducted	Text
Precipitation	The total rainfall during the growing period	Numerical
Soil texture	The texture of the soil in the experimental area	Categorical
Species and genotypes	The names of species and genotypes used in the experiment	Text
Number of genotypes	The number of genotypes of each species studied in the experiment	Numerical
Design	Plant density (additive/replacement/intermediate)	Categorical
Response variable	The response variable investigated	Text
Replication	How many times the treatment was replicated	Numerical
Number of locations	Number of the site where the experiment was conducted	Numerical
Number of seasons	Number of seasons during which the experiments were conducted	Numerical
Genotype, cropping system, and interaction effects	The statistical significance of interaction, cropping system, and genotype effect	Categorical
Interaction traits	List of traits/mechanisms highlighted as causal in crop interactions and intercropping performance	Categorical
LER	Land equivalent ratio	Numerical

where LER_{c+l} is the LER of the cereal genotype c with the legume genotype l ; and $PLER_{c/l}$ is the partial LER of genotype c in mixture with legume genotype l (and reciprocally for $PLER_{l/c}$). This genotype combination-specific LER was used in further analysis. If neither LER nor PLER was reported, LER for each genotype in a given cereal–legume combination was calculated from yields in mono-cropping and intercropping.

When other treatments were applied (such as different row spacing, and sowing density or proportion), LERs were extracted or calculated from only one treatment. If different levels of N were used, data for each level of fertilizer were considered and averages computed for each genotype combination. In one study, results from two species of cereals or legumes were reported. Thus, data were recorded from each genotype combination from each species and analyzed. Therefore, at least 2 data points from each article (depending on number of genotypes of cereals and legumes) were extracted. In this way, we obtained 262 LER data points.

Since only few (10%) LER data points were reported from forage legume species combinations with cereals (2 articles with oats, 1 article with finger millet, and 2 articles with maize) all data from forage and grain legumes were combined and analyzed together.

Data Analysis

The main effects of genotype and intercropping and their interaction effects were assessed by counting and calculating the proportion of articles that reported significant or non-significant effects on yields. In addition to the analysis of LER, a fixed-effects ANOVA model was used to test the effect of cereal species, design, and interaction effect on LER across cereal species by categorizing the dataset by cereal species. Because the number of data points of wheat was low ($n = 5$), and data records

from barley and rice were only from replacement design, we excluded these three from the analysis. The number of data records per cereal species varied from 25 (finger millet) to 131 (maize). Similarly, a fixed-effects ANOVA model was used to test the effect of legume species, design, and interaction on LER across legume species by categorizing the dataset by legume species. However, faba bean, grass pea, guar and hairy vetch, berseem clover, and bitter vetch were excluded because the number of data points (two to four) was low. The mean comparison was done by Tukey's honestly significant difference (HSD) test.

To assess the potential of genotype choice for optimizing LER, we calculated three indices using the extracted data from the articles (averages across the site years); to obtain these indices, we first calculated the maximum, median, and minimum LER across different genotype combinations for each article. Then (i) the difference between maximum and median LER was used as a measure for the potential of combined genotype choice to improve LER in comparison to a random choice; similarly, (ii) the difference between minimum and median LER was taken as a measure for the risk to choose an inappropriate genotype combination in comparison to a random choice; and (iii) the range, i.e., the difference between maximum and minimum LER from an article was used to characterize the maximum genotype combination effect within a study. The median used to calculate all three statistics were calculated from each individual article. The three statistics are equivalent when only two genotypes were evaluated. Because of sampling effects, it is expected that all three differences would tend to increase (in absolute terms) with increasing number of genotype combinations tested within a study (Schwarz, 2011); therefore, we plotted the indices against the number of genotype combinations. The extracted LER data were subjected to descriptive statistics; all analyses were conducted with R

(R CoreTeam, 2020), and figures were produced using the R package ggplot2 (Wickham, 2016).

RESULTS

Geographical Distribution and Characteristics of Studies

From about 4,000 search hits using all search terms, only 69 articles fulfilled the inclusion criteria (Table 2). The reported research studies were conducted in 28 different countries (Supplementary Table 2). The majority of data came from Africa (37%) followed by Europe (24%) and Asia (18%). The included studies considered different contrasting characteristics of genotypes of cereals and legumes evaluated.

Overall, 9 cereal crop species and 19 legume species were evaluated in 69 publications with maize as the most frequently evaluated cereal species followed by oat and wheat. Common bean was the most frequently evaluated legume followed by

cowpea and soybean. In the considered studies, common bean was only intercropped with maize. A single genotype was used in 62% of the studies for one of the partner species, i.e., in these studies, genotypic variation was only investigated in the other partner. On average, 4 cereal genotypes or 3 legume genotypes were compared per study, when excluding the single genotype studies (Figure 1). The most diverse comparison included 8 genotypes of cereal (*Avena sativa*) and 7 genotypes of legume species (*Trifolium alexandrinum*), in a total of 56 cereal–clover combinations.

The majority of studies (55) evaluated grain legumes, whereas eight studies evaluated forage legumes, and a small proportion (6) of studies evaluated both forage and grain legumes together. The number of genotypes used in the studies varied, with similar numbers of studies reporting on (i) combinations of two or more cereal genotypes with two or more legume genotypes, (ii) one cereal genotype combined with two or more legume genotypes; or (iii) one legume genotype combined with two or more cereal genotypes (Table 3).

Effect of Cropping System and Genotypes of Cereal/Legume on Intercropping Performance

Genotype × Cropping System Interaction

The extracted genotype × cropping system interaction effects on yield were reported in 49 (71%) studies out of 69 publications. Out of this, genotype × cropping system interaction effects were significant in 37 (75%) of the studies, whereas 12 (25%) of the studies reported non-significant interactions. The remaining studies did not report the effects of genotype × cropping system. In addition, intercropping main effects were reported in 38 (55%) studies. Out of this, the effect was significant in 27 (71%) and non-significant in 11 (29%) of the publications. Genotype main effects were reported in 37 (53%) studies; out of this, the genotype effect was significant in 25 (67%) and non-significant in 12 (33%) of the publications. The remaining studies did not mention the effects of cropping system and genotype effects.

Land Equivalent Ratio as Metric to Gauge Yield Advantage of Genotypes in Intercropping

From the 69 studies used for data extraction, 35 studies yielded 36 datasets (one study used two cereal species) and either reported the LERs directly or allowed calculation from the reported yield data. From these 36 datasets, 262 data points (cereal/legume genotype combinations) were extracted, based on a total of 85 cereal and 126 legume genotypes, with a number of cereal/legume combinations (LER) ranging from 2 to 22 per study.

The calculated mean and the median LER were 1.26 and 1.24, respectively (Figure 2), and LER was greater than 1.0 in 85% of the single cases. Although the number of data points for some cereals, especially wheat, may not be sufficient to compare the median LER with other cereals, the overall outcome was robustly > 1 with the highest median LER of 1.38 ($n = 25$) found in finger millet. The strikingly high variation in maize is in part due to the number of studies. In barley-based cropping

TABLE 2 | List of cereal and legume species in the 69 selected studies investigating genotype effects in intercropping; because some studies tested more than two species, the sum of studies across all crop species (152) is greater than $2 \times 69 = 138$.

Common name	Scientific name	No. of studies
Cereals		
Maize	<i>Zea mays</i>	30
Oat	<i>Avena sativa</i>	8
Wheat	<i>Triticum aestivum</i>	8
Finger millet	<i>Eleusine coracana</i>	6
Sorghum	<i>Sorghum bicolor</i>	6
Barley	<i>Hordeum vulgare</i>	5
Rice	<i>Oryza sativa</i>	5
Naked oat	<i>Avena nuda</i>	1
Durum wheat	<i>Triticum durum</i>	1
Legumes		
Common bean	<i>Phaseolus vulgaris</i>	17
Cowpea	<i>Vigna unguiculata</i>	13
Soybean	<i>Glycine max</i>	8
Pigeon pea	<i>Cajanus cajan</i>	7
Pea	<i>Pisum sativum</i>	7
Faba bean	<i>Vicia faba</i>	7
Berseem clover	<i>Trifolium alexandrinum</i>	5
Groundnut	<i>Arachis hypogaea</i>	3
White clover	<i>Trifolium repens</i>	2
Bitter vetch	<i>Vicia ervilia</i>	2
Common vetch	<i>Vicia sativa</i>	2
Hairy vetch	<i>Vicia villosa</i>	2
Guar	<i>Cyamopsis tetragonoloba</i>	1
Grass pea	<i>Lathyrus sativus</i>	1
Snail clover	<i>Medicago truncatula</i>	1
Serradella	<i>Ornithopus sativus</i>	1
Runner bean	<i>Phaseolus coccineus</i>	1
Caribbean stylo	<i>Stylosanthes hamata</i>	1
Subterranean clover	<i>Trifolium subterraneum</i>	1

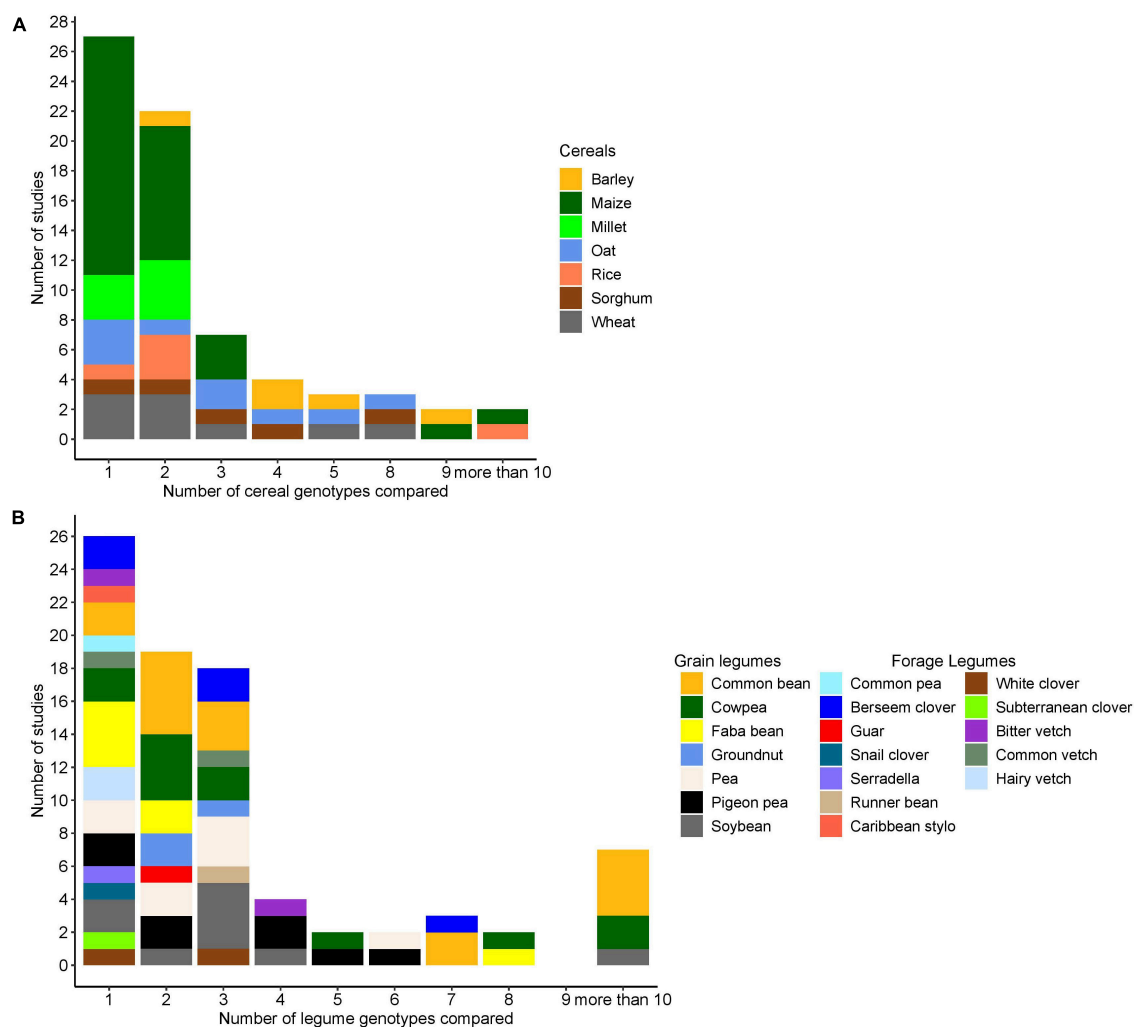


FIGURE 1 | (A) Number of cereal genotypes evaluated in combination with legume species (each combination was categorized based on the cereal species). **(B)** Number of legume genotypes evaluated in combination with cereal species (each combination was categorized based on the legumes species). In both cases, if one genotype of one partner is evaluated, the other partner had at least 2 genotypes.

systems, all of the LER data were greater than 1 ($n = 22$, range 1.05–1.48) (**Figure 3**).

The ANOVA resulted in highly significant differences across cereal species and design ($p < 0.01$). In addition, the interaction effect was significant ($p < 0.05$). The pairwise means comparison revealed that finger millet reached higher LERs in additive designs as compared to replacement designs, whereas no effect of design was found in maize and sorghum (see **Supplementary Table 3** for ANOVA and **Figure 4A**). The ANOVA, across legume species and design, resulted in highly significant differences across legume species with pigeon pea and soybean exceeding other species but non-significant effects of design and interaction effect ($p > 0.05$) (see **Supplementary Table 4** for ANOVA) (**Figure 4B**).

The Potential of Genotype Choice for Intercropping

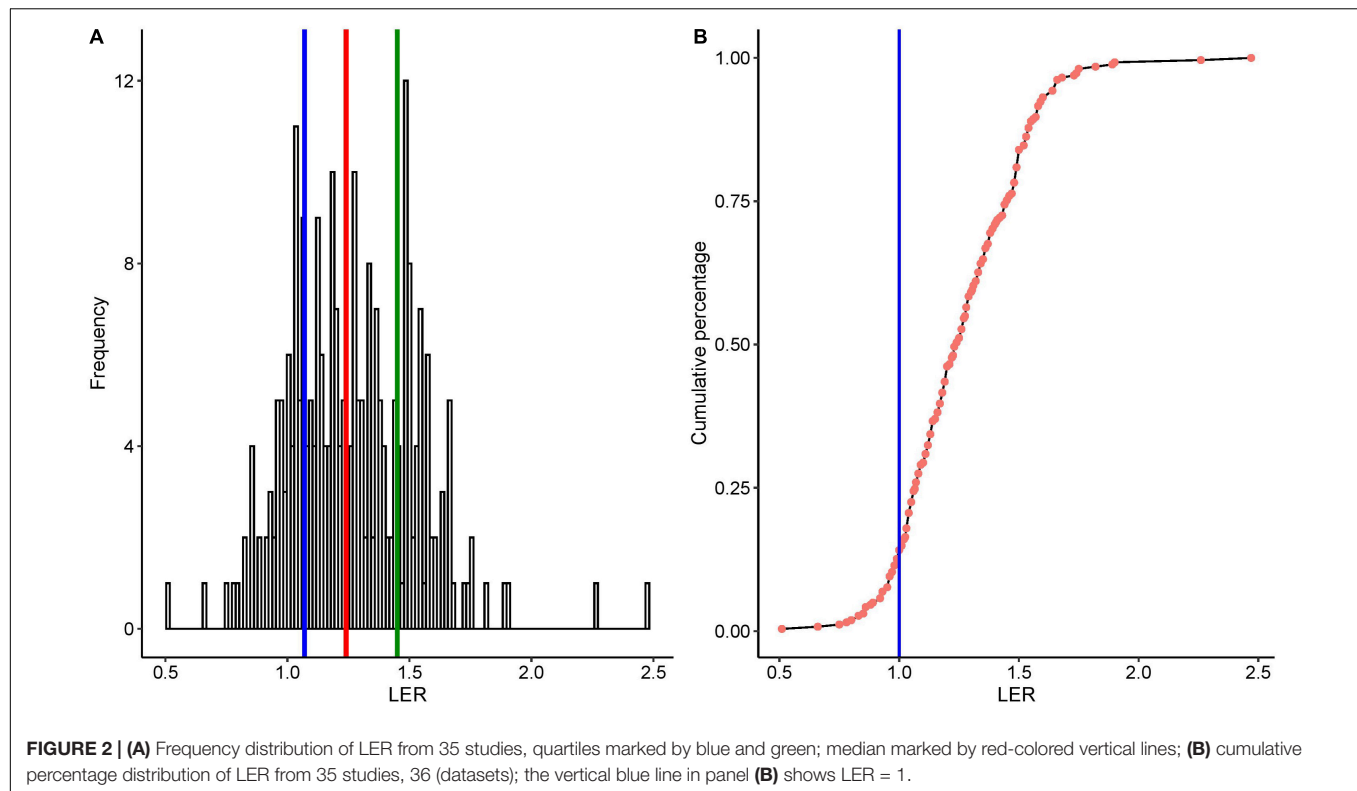
The distribution of the LERs within the studies around the median (**Figure 5**) indicates that genotype-specific effects play

a role in the performance of mixtures in comparison to sole crops. Overall, the range (i.e., difference between maximum LER and minimum LER within a study) varied between 0 and 1.98, showing the potential of large genotype effects in intercropping. Conversely, there was a risk to obtain low LERs by non-appropriate genotype choice (i.e., as indicated by the difference of minimum LER and median LER, red points in **Figure 5**); the

TABLE 3 | Number of studies with one or more than one genotype of cereal and/or legume (*not included in this review) from 69 studies.

	1 cereal genotype	> 1 cereal genotype
1 legume genotype	*	16
> 1 legume genotype	27	27

One article evaluated two cereal species resulting in a total of 70 datasets (out of one publication, two datasets were extracted).



difference between minimum and median ranged from -0.55 to 0 . The largest LER range (1.96) was found in a study with 20 different genotypes combinations (10 bean and two maize genotypes) (Santalla et al., 2001); in the only other study with 20 genotypes combination (Hauggaard-Nielsen and Jensen, 2001), the range was 0.27 , i.e., quite moderate (Table 5).

To elaborate the effect of genotypes on intercropping performance in terms of LER, the studies from maize-based were analyzed in detail. A total of 16 studies reported LER in maize-intercropping system and yielded 138 LER data records. The analysis shows that with the increasing number of maize genotypes included in the study, the LER range (maximum–minimum) increased significantly with an R^2 of 0.58 ($p = 0.00063$) and 0.47 ($p = 0.0046$) in the regression of LER against number of genotype combinations, when the study of Santalla et al. (2001) that represents an outlier in terms of the number of genotypes combination tested (20 compared to 2–15) was included or excluded, respectively (Figure 5B).

Mechanisms Underlying the Interactions Between Genotypes and Cropping System

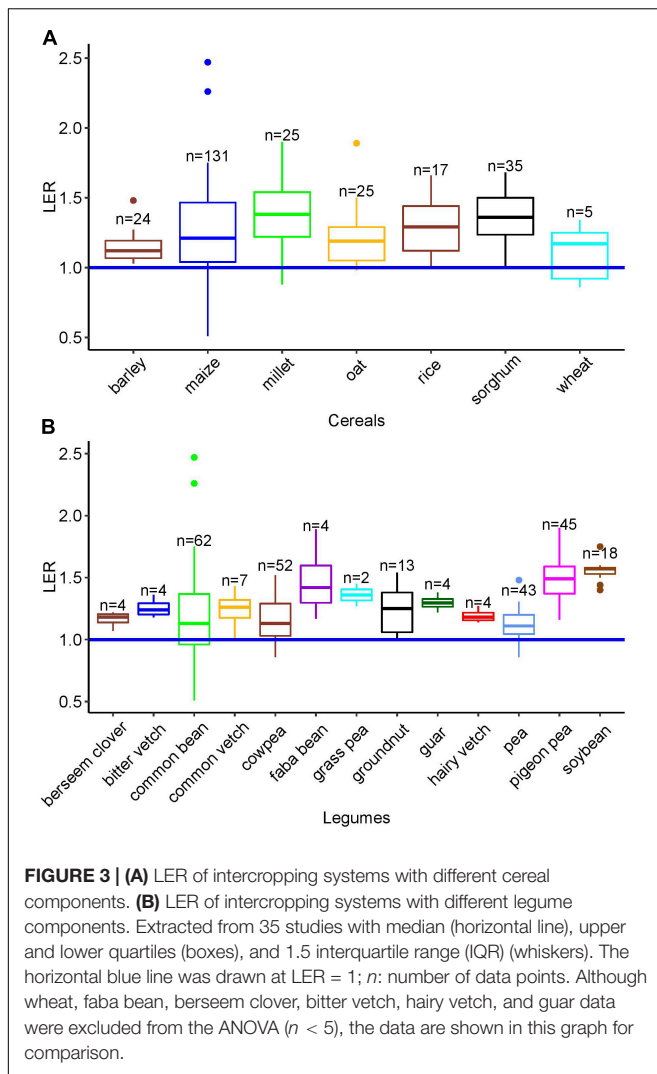
In 20 out of the 69 studies, contrasting phenological or architectural characteristics of cereal and/or legume genotypes were highlighted, suggesting that the temporal and spatial differences among genotypes contributed to intercrop performance. These traits were broadly categorized into phenological and morphological traits (Table 6).

The phenological traits include growth duration (days to maturity, days required from emergence to flowering, and harvesting time), whereas morphological traits include shoot architecture (plant height) and growth habit (determinate/indeterminate growth) of the genotypes of each species. The reported phenological legume traits that affect intercropping, growth habit, and growth duration were reported more often than the morphological traits (long/short straw and climbing/bushy beans). However, no trend can be extracted from the provided information. In case of the cereals, only the phenological trait growth duration and the morphological trait plant height were reported. Three studies reported a better intercropping performance for early maturing cereals (maize, barley, and sorghum), whereas three others for late-maturing cereals (sorghum, oat, and maize). In case of plant height, five out of six studies reported improved intercropping performance for shorter cereal genotypes. Thus, besides a tendency for higher intercropping performance in case of short cereal genotypes, no conclusion can be drawn.

DISCUSSION

Evaluation of the Performance of Different Cereal/Legume Species and Genotypes

The systematic assessment of LER from 35 independent studies showed the mean and median values of 1.26 and 1.24 (Figure 2A). This result is not far from the previously published meta-analysis

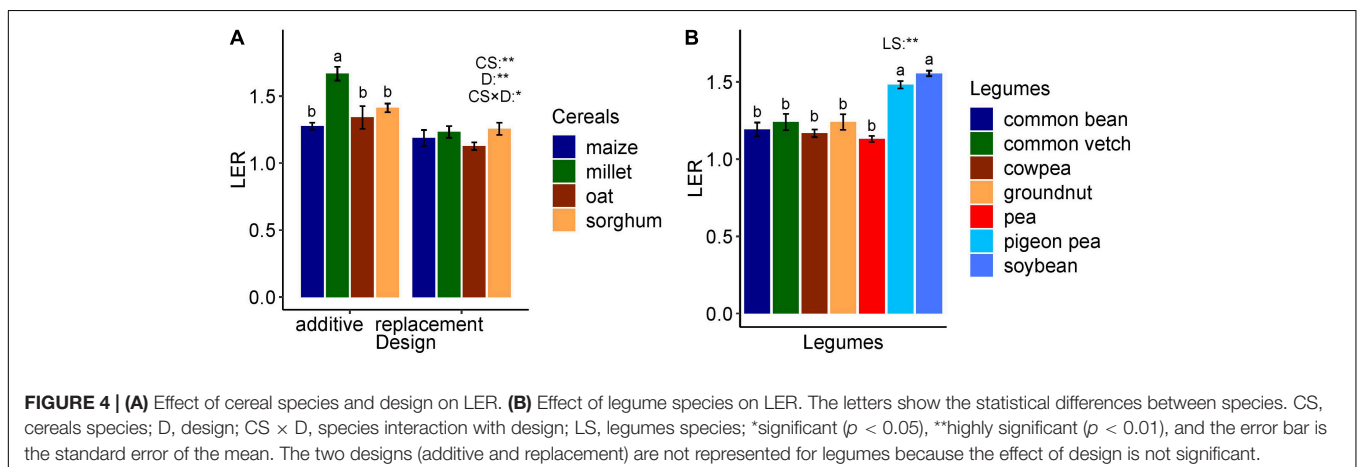


result median values of 1.17 (Yu et al., 2015), 1.16 (Yu et al., 2016), and 1.3 (Martin-Guay et al., 2018). These studies focus on the yield performance of crop species mixtures regardless of

genotype. The median LER of 1.24 across 16 maize-based studies in our study is in line with a meta-analysis from 43 studies of maize/soybean of intercropping that reported an LER of 1.32 (Xu et al., 2020). Although the mean and median varied among different cereals, median LER was above one in all cereals.

The species and design effects were highly significant ($p < 0.01$) (Figure 4A), with a significant interaction ($p < 0.05$), mainly due to the higher LER of finger millet (1.66) compared to other species in additive designs. However, in replacement designs, no differences were observed among species. The overall LER was higher in additive designs compared to replacement designs. In an additive design, the planting density of both species in the mixture may be equivalent or somewhat reduced compared to their sole stand resulting in planting densities leading to density equivalent ratios > 1 and up to 2. For example, pea-oat mixtures may be composed of 100% peas and 20% oats compared to the pure stand densities (Gronle et al., 2015) or wheat-winter pea mixtures of 70% wheat with 50% pea (Timaues et al., 2022). In replacement designs, the density of one sole crop species is proportionally (based on sole crop densities) replaced by the other species resulting in a density equivalent ratio of 1. For example, they may be composed of 50% barley and 50% pea compared to pure stand densities (Pappa et al., 2012). Although the planting proportion has an effect on LER, the range of effects depends on the species in the mixture because tillering in the case of cereals can compensate variable sowing densities (e.g., Finckh and Mundt, 1992; Finckh et al., 1999).

Compared to other cereal crops, millet was intercropped with short legumes, such as cowpea and pigeon pea. Intercropping the tall millet and sorghum cereals with shorter legumes permits better radiation use efficiency (Marshall and Willey, 1983; Matthews et al., 1991). Due to less resource competition by spatial segregation, yield in mixture and mono-cropping is comparable for both species which increased LER in additive compared to replacement designs. Nevertheless, a meta-analysis by Raseduzzaman and Jensen (2017) reported that in intercropping, replacement designs lead to higher yield stability compared to additive designs. The ANOVA across legume species (excluding faba bean, grass pea, guar and hairy vetch, berseem clover, and bitter vetch with $n < 4$ data points) resulted in significant



differences. However, the effects of design and interaction were not significant ($p > 0.05$) (see **Supplementary Table 5**) with greater LER for pigeon pea and soybean compared to other legume species (see **Supplementary Table 6**). These two legume species are frequently intercropped with C4 cereals, such as maize, millet, and sorghum, which may increase the LER due to temporal niche differentiation (Yu et al., 2015; Xu et al., 2020).

The interaction between different cereal and legume genotypes and different cropping systems was significant in 75% of the studies that reported interaction effects of genotype × cropping system. This implies that in many studies, genotypes behave differently in sole vs. intercropping, often resulting in changes in the performance ranking of varieties between the sole crop and mixture (Woolley and Rodriguez, 1987; Baxevanos et al., 2017). The analyses of variation of different genotypes of cereal/legume intercrops within each selected study (**Figure 5**) revealed that the choice of the specific genotype combination could result in positive or negative yield effects compared to the median of all genotype combinations within each study. The largest LER range was found in a study with 20 different genotypes combinations (10 bean and two maize genotypes) (Santalla et al., 2001). This indicates the potential for high LER in case of appropriate

genotype choice and highlights the potential for genotype or trait combination to optimize intercropping systems. However, this finding also emphasizes the need to develop a more general understanding of the mechanisms underlying these differences.

Concept of Cereal/Legume Intercropping Niche Complementarity

Out of 20 studies assessing the mechanisms underlying the intercropping performance, 10 studies reported that intercropping performance was improved by cereal genotype, whereas the remaining 10 studies reported that the improvement was by legumes genotype. In some studies, however, a relatively high number of genotypes did not affect the intercropping performance. For instance, in the study of Hauggaard-Nielsen and Jensen (2001), none of the five barley genotypes affected LER, whereas pea genotype affected intercropping performance in terms of LER (**Table 6**).

In an intercropping system with annual species, the niche differentiation is a general mechanism underlying the yield advantage and better resource use efficiencies (Lithourgidis et al., 2011). Niche differentiation improves the use of resources according to species complementarity for light interception and the use of both soil mineral N and atmospheric N (Bedoussac et al., 2015). The selection of cereal and legume genotypes for better complementarity is important because the traits required for intercropping are those which enhance the complementary effects between the partners (Davis and Woolley, 1993). Niche differentiation among plant species occurs for the various environmental resources, such as light, water, and nutrient availability. It is driven by plant phenology and morphology that allows for partitioning of resources over time and space that facilitates coexistence (Silvertown, 2004). The trait differences in genotypes of cereals and legumes result in differences in phenology and morphology of the plants. Therefore, in cereal/legume mixtures, both species could have similar phenology but contrasting morphology or contrasting phenology and morphology, resulting in temporal and/or spatial niche complementarity (Gaudio et al., 2019). The contrasting characteristics of the genotypes play an important role in the complementarity of the species in intercropping (Hauggaard-Nielsen and Jensen, 2001; Gebeyehu et al., 2006).

The ecological niche separation concept describes the fact that different species involved may have different resource requirements at different times, as well as different sources of nutrition, e.g., root exploitation of top subsoil layers by one component vs. deeper exploitation by the other component, different growth patterns, or different affinities for the same nutrient (Malézieux et al., 2009). The temporal and spatial segregation of species in intercropping is useful in two ways: better resource capture, hence utilization of more resources, and enhanced resource use efficiency in a given unit of resource (Willey, 1990). The maturity rate and the growth habit of cereal and legumes define either the domination or suppression of one of the species in the mixture (Baxevanos et al., 2021). However, besides niche separation, additional

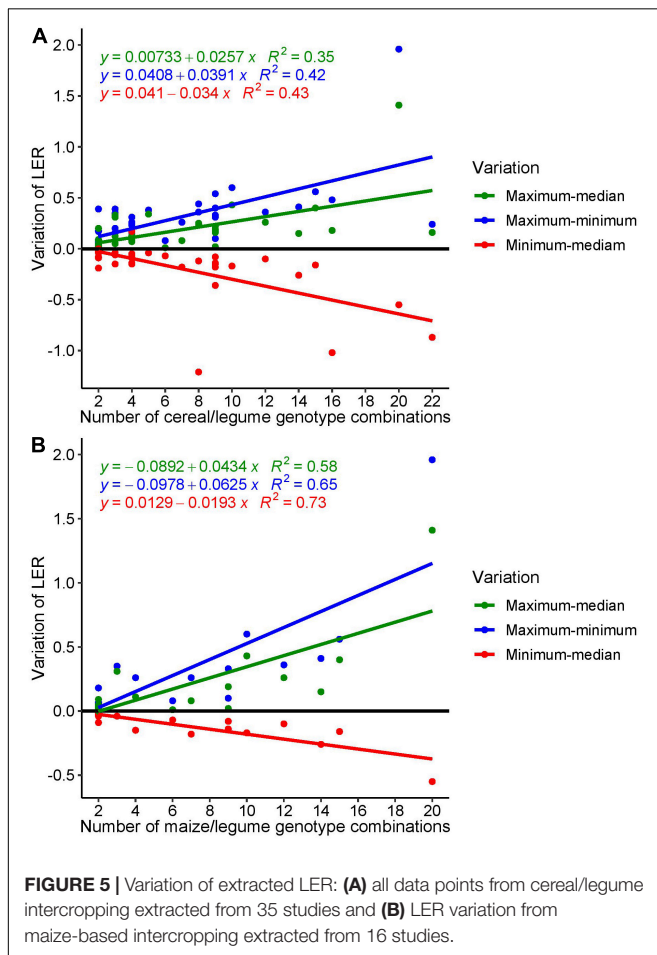


TABLE 4 | Number of studies reporting significant and non-significant genotype, cropping system, and interaction effects, categorized by cereals.

Cereal	Cropping system effect			Genotype effect			Interaction effect		
	sig.	n.s.	n.r.	sig.	n.s.	n.r.	sig.	n.s.	n.r.
Barley	2	1	2	2	1	2	2	1	2
Maize	12	8	9	12	9	9	19	7	4
Millet	2	0	5	1	0	5	5	0	1
Oat	5	0	4	3	0	6	6	0	3
Rice	1	0	4	2	0	3	2	0	3
Sorghum	1	0	4	2	0	3	1	0	4
Wheat	4	2	3	3	2	4	2	4	3
Total	27	11	31	25	12	32	37	12	20

sig., significant; n.s., not significant; n.r., not reported.

mechanisms, such as mutual beneficial interactions *via* the soil microbiome, including biological N fixation, have to be considered (Hauggaard-Nielsen et al., 2009). Thus, in cereal legume mixtures, the contribution of biological N fixation through the leguminous partner is affected by the mineral N-supply level with strong effects on the competitive interactions and overall biological N fixation by the legume (Wang et al., 2015; Li et al., 2021).

Temporal Niche Complementarity of Cereal/Legume Intercropping

A trend for enhanced intercrop performance due to a specific trait related to phenology or temporal combination cannot be identified from the evaluated studies. Days required for maturity is one of the important factors for complementarity of species in intercropping. In this review, out of 20 studies reported that phenological and morphological traits affected intercropping performance with 12 studies indicated that the difference of days of maturity of different genotypes of cereals and/or legumes had an effect on the intercropping performance. However, it also varies in some cases, with a late-maturing genotype of either of the species meeting better the aim of cultivation compared to an early maturing genotype. In contrast, early genotypes could also be better compared to late maturing genotypes of one of the species (Table 6). In the study of Ntare (1989), intercropping an early maturing cowpea genotype with a relatively late-maturing millet genotype performed better by reducing the co-growth period to escape moisture scarcity and minimizing all components not affected equally in drought-prone areas. Another example of temporal complementarity is the combination of determinate field peas with a cereal where peas started maturing and releasing N from the roots around the time when the cereal flowers and requires increased N to fill its grains (Jensen et al., 2020; Timaeus et al., 2021b). The rate of development and time between sowing and harvesting of the components in intercropping provide the opportunity of temporally complementary use of incident radiation, thereby improving intercropping performance (Keating and Carberry, 1993). Tefera and Tana (2002) reported that the temporal niche complementarity of different genotypes in sorghum/groundnut intercropping influences the general

performance of intercropping: partners that have a lower co-growth period produced higher yields compared to genotypes that have equal or higher co-growth period. Similar temporal niche complementarity was reported for millet/cowpea (Ntare, 1990), maize/cowpea systems (Egbe et al., 2010), and bean/maize systems (Gebeyehu et al., 2006). Depending on the aim of cultivation, the selection of cereal and legume genotypes with contrasting maturity periods will increase the intercropping yield advantage (Ross et al., 2004).

Spatial Niche Complementarity of Cereal/Legume Genotypes

Spatial niche complementarity can be exploited by the spatial arrangement of one component to maintain its full population, whereas allowing more space (and thus more resources) for another component (Willey, 1990). The spatial arrangement for better resource use efficiency could be classified as above-ground (canopy structure of both components) and below-ground (root system) (Gaudio et al., 2019). Canopy structure has considerable implications for intercropping systems. The erect open canopy of one component allows more transmission of radiation to shorter crops and enables more radiation use efficiency (Willey, 1990). The use of abiotic resources is improved according to species complementarity for light interception and the use of both soil mineral and atmospheric N.

In this review, 11 studies reported morphological differences of the genotype of either cereal or legumes to be involved in intercropping complementarity (Table 6). In most of these articles (7), plant height was observed. Whether the taller or the shorter genotype performed better varied. However, a tendency toward higher intercropping performance was observed with short cereal genotypes. Plant height and branching of long cycle pea genotypes varied between the sole and mixed cropping systems. This reveals the importance of the pea genotype choice in terms of morphology for intercropping systems (Barillot et al., 2014). The study by Hauggaard-Nielsen and Jensen (2001) revealed that pea genotypes with determinate growth absorbed more radiation under the barley canopy, which enhanced the intercropping performance compared to intercropping systems with indeterminate pea genotypes.

The growth habit of different genotypes of one species significantly affects the performance of other species, and thereby intercropping performance mainly by affecting radiation interception. Ramakrishna and Ong (1994) reported that the indeterminate pigeon pea genotype with indeterminate growth habit reduces the yield of rice by half due to the competitive advantage for radiation. In the barley/pea intercropping system, spatial complementarity due to pea genotypes has resulted in better N use efficiency of barley. An indeterminate pea genotype resulted in a greater proportion of peas in the intercrop yield due to high competitiveness, whereas a determinate pea genotype with normal leaves caused the highest degree of complimentary use of N sources by allowing barley to exploit the soil N sources efficiently, and they contribute with fixed N. However, indeterminate pea genotypes caused a reduced N uptake and yield of barley (Hauggaard-Nielsen and Jensen, 2001; Pappa et al., 2012). Based on the analyzed studies, we

TABLE 5 | Deviations of LER from the median in cereal/legume intercropping extracted from 35 studies including between 2 and 20 mixtures, i.e., different genotype combinations (N).

Author	Cereal species	Legume species	No. cereal genotypes	No. legume genotypes	Range (max–min)	Max–median	Min –median	Design	(N)
Klimek-Kopyra et al., 2015	<i>Avena nuda</i>	<i>Vicia faba</i>	1	2	0.39	0.2	–0.19	add	2
Li et al., 2020	<i>Avena sativa</i>	<i>Vicia sativa</i>	1	3	0.2	0.05	–0.15	add	3
Baxevanos et al., 2017	<i>Avena sativa</i>	<i>Pisum sativum</i>	3	3	0.31	0.16	–0.15	repl	9
Ross et al., 2004	<i>Avena sativa</i>	<i>Pisum sativum</i>	2	2	0.25	0.11	–0.14	repl	4
Kontturi et al., 2011	<i>Avena sativa</i>	<i>Pisum sativum</i>	1	3	0.16	0.12	–0.04	add	3
Baxevanos et al., 2021	<i>Avena sativa</i>	<i>Vicia sativa</i>	4	1	0.17	0.11	–0.06	repl	4
Pappa et al., 2012	<i>Hordeum vulgare</i>	<i>Pisum sativum</i>	2	1	0	0	0	repl	2
Hauggaard-Nielsen and Jensen, 2001	<i>Hordeum vulgare</i>	<i>Pisum sativum</i>	4	6	0.24	0.16	–0.87	repl	22
Sanou et al., 2016	<i>Eleusine coracana</i>	<i>Vigna unguiculata</i>	2	2	0.13	0.08	–0.05	repl	4
Reddy et al., 1990	<i>Eleusine coracana</i>	<i>Vigna unguiculata</i>	3	3	0.4	0.22	–0.18	repl	9
Yadav and Yadav, 2001	<i>Eleusine coracana</i>	<i>Cyamopsis tetragonoloba</i>	2	2	0.16	0.085	–0.075	repl	4
Rao and Willey, 1983	<i>Eleusine coracana</i>	<i>Cajanus cajan</i>	2	4	0.44	0.25	–1.21	add	8
Ramakrishna and Ong, 1994	<i>Oryza sativa</i>	<i>Vigna unguiculata</i>	2	2	0.23	0.13	–0.09	repl	4
		<i>Arachis hypogaea</i>		2	0.13	0.07	–0.06	repl	4
		<i>Cajanus cajan</i>		2	0.31	0.13	0.17	repl	4
Rahlakrishna et al., 1992	<i>Oryza sativa</i>	<i>Cajanus cajan</i>	1	5	0.38	0.34	–0.04	repl	5
Tefera and Tana, 2002	<i>Sorghum bicolor</i>	<i>Arachis hypogaea</i>	3	3	0.54	0.18	–0.36	add	9
Odo, 1991	<i>Sorghum bicolor</i>	<i>Vigna unguiculata</i>	2	1	0.17	0.08	–0.09	repl	2
de Queiroz et al., 1988	<i>Sorghum bicolor</i>	<i>Vigna unguiculata</i>	8	1	0.36	0.24	–0.12	repl	8
Rao and Willey, 1983	<i>Sorghum bicolor</i>	<i>Cajanus cajan</i>	4	4	0.48	0.18	–1.02	add	16
Barillot et al., 2014	<i>Triticum aestivum</i>	<i>Pisum sativum</i>	1	3	0.39	0.33	–0.06	repl	3
Haymes and Lee, 1999	<i>Triticum aestivum</i>	<i>Vicia faba</i>	2	1	0.17	0.09	–0.08	add	2
Egbe et al., 2010	<i>Zea mays</i>	<i>Vigna unguiculata</i>	1	10	0.6	0.43	–0.17	repl	10
Watiki et al., 1993	<i>Zea mays</i>	<i>Vigna unguiculata</i>	1	15	0.56	0.4	–0.16	add	15
Goshime et al., 2020	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	9	1	0.33	0.19	–0.14	add	9
Gebeyehu et al., 2006	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	2	7	0.41	0.15	–0.26	add	14
Javanmard et al., 2020	<i>Zea mays</i>	<i>Lathyrus sativus</i> ,	2	1	0.06	0.03	–0.03	add	2
		<i>Vicia villosa</i>		1	0.09	0.04	–0.04	add	2
		<i>Vicia ervilia</i> ,		1	0.04	0.02	–0.02	add	2
		<i>Trifolium alexandrinum</i>		1	0.05	0.02	–0.02	add	2
		<i>Phaseolus vulgaris</i>		2	0.18	0.09	–0.09	add	2
Molatudi, 2012	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	1	2	0.18	0.09	–0.09	add	2
Pierre et al., 2017	<i>Zea mays</i>	<i>Glycine max</i>	1	3	0.35	0.31	–0.04	add	3
Zaeem et al., 2019	<i>Zea mays</i>	<i>Glycine max</i>	2	3	0.08	0.01	–0.07	add	6
Yang et al., 2018	<i>Zea mays</i>	<i>Glycine max</i>	3	3	0.1	0.02	–0.08	add	9
Javanmard et al., 2009	<i>Zea mays</i>	<i>Vicia ervilia</i>	2	1	0.07	0.03	–0.03	add	2
		<i>Trifolium alexandrinum</i>		1	0.04	0.02	–0.02	add	2
		<i>Vicia villosa</i>		1	0	0	0	add	2
		<i>Phaseolus vulgaris</i>		1	0.07	0.03	–0.03	add	2
		<i>Phaseolus vulgaris</i>		7	0.26	0.08	–0.18	add	7
Nassary et al., 2020a	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	1	2	0.09	0.05	–0.04	add	2
Muraya et al., 2006	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	2	2	0.26	0.11	–0.15	add	4
Dasbak and Asiegbu, 2009	<i>Zea mays</i>	<i>Cajanus cajan</i>	2	6	0.36	0.26	–0.1	add	12
Santalla et al., 2001	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	2	10	1.96	1.41	–0.55	repl	20
Nassary et al., 2020b	<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	1	2	0.07	0.03	0.03	add	2

In some studies, more than one legume species was evaluated; add, additive; repl, replacement design.

TABLE 6 | Mechanisms of genotypes (G) complementarity in cereal/legume intercropping as mentioned in the consulted literature.

Cereal/legume	No. of G	Phenological and morphological traits that improve intercropping performance		References
		Cereals	Legumes	
Barley/pea	1 × 2		Long straw > short straw pea	Pappa et al., 2012
Barley/pea	5 × 6		Determinate > indeterminate pea	Hauggaard-Nielsen and Jensen, 2001
Barley/berseem clover	4 × 3	Early > late mature barley shorter > tall stature barley		Ross et al., 2004
Sorghum/groundnut	3 × 3	Late > early maturing sorghum when intercropped with early maturing groundnut	Late > early maturing groundnut when intercropped with early maturing sorghum	Tefera and Tana, 2002
Sorghum/cowpea	2 × 1	Short > tall stature sorghum		Odo, 1991
Sorghum/cowpea	4 × 4	Early > late mature sorghum		Rao and Willey, 1983
Rice/pigeon pea	2 × 2		Determinate > indeterminate pigeon pea	Ramakrishna and Ong, 1994
Millet/cowpea	2 × 2		Early > late mature cowpea	Ntare, 1990
Millet/cowpea	2 × 8		Early > early mature cowpea when intercropped with late mature millet	Ntare, 1989
Oat/faba bean	1 × 2		Indeterminate > determinate faba bean	Klimek-Kopyra et al., 2015
Oat/common vetch	3 × 3		Medium > late mature common vetch	Li et al., 2020
Oat/common vetch	4 × 1	Late > early mature oat and short > tall oat		Baxevanos et al., 2021
Wheat/faba bean	2 × 1	Tall > short straw of the oat		Haymes and Lee, 1999
Maize/cowpea	1 × 10		Early > late mature cowpea	Egbe et al., 2010
Maize/bean	2 × 7	Late > early mature of maize		Gebeyehu et al., 2006
Maize/bean	2 × 10	Short > tall maize		Davis and Garcia, 1983
Maize/common bean	1 × 2		Climbing > bushy bean	Clark and Francis, 1985
Maize/cowpea	3 × 2	Early > late mature maize		Ewansiha et al., 2014
Maize/bean	2 × 1	Short > tall maize		Munz et al., 2014
Maize/faba bean	1 × 3		Late > early mature faba bean	Fischer et al., 2020

The empty cells are in the case no traits were mentioned. The first and second number in the second column ("No. of G") refers to the number of genotypes on the first and of the second species mentioned in the first column ("Cereal/legume").

cannot draw a conclusion. In two articles, the intercropping performance was higher in case the growth of the legume partner was determinate, whereas in one study, it was higher for the indeterminate genotype.

Gaps of Genotype and Trait Evaluation in Cereal/Legume Intercropping

Even though ample research reported on cereal legume intercropping, the number of publications that evaluated cereal/legume genotypes for complementarity in intercropping systems was very limited. Among the studies analyzed (69), only 20 (29%) articles indicate the contrasting traits of genotypes that contribute to intercropping performance. From those, the general mechanisms underlying the genotype cropping system were broadly classified as phenological and morphological heterogeneity of cereal and/or legume genotypes. However, in most of the studies, the contrasting characteristics of genotypes of either cereal or legumes and/or both of the species were not described well. The phenology of the crops has an impact on resource use over time (Gaudio et al., 2019). Consequently, cultivating genotypes with different phenological characteristics

results in different temporal niche complementarity. The latter can increase the land use efficiencies, especially if N is released after grain filling of the legumes benefiting the cereals. Nevertheless, in most of the studies, sufficient information on phenology was not provided, and none of the studies reported the differences in the phenological stages of the genotypes.

Root growth and thus water and nutrient uptake are some of the most important factors in temporal and spatial heterogeneity (Hauggaard-Nielsen and Jen Yin et al., 2020). Root system distribution in time and space can partly explain competition. For instance, barley roots grow faster than pea roots (Hauggaard-Nielsen et al., 2001) and start nutrient acquisition earlier. Different genotypes of either the cereals or the legumes could have different root characteristics, which influence the competitive ability of the species. Streit et al. (2019) reported that mixtures of winter faba bean and winter wheat over yielded more below- than above-ground. The authors concluded that genotype differences in root biomass and over-yielding indicate the breeding potential of winter faba bean cultivars for mixed cropping. Legumes provide N to the agroecosystem through their exclusive capability to fix atmospheric N in a symbiotic relationship with soil rhizobia,

but different genotypes of a legume species might have different capabilities in nodulation (Rodríguez et al., 2011). Only a very limited number of studies considered the nutrient acquisition of different genotypes of cereals and legumes in intercropping. Different species have temporal niche differentiation in nutrient acquisition (Zhang et al., 2017). The symbiotic association of different legume genotypes and their rhizobia could also differ. The spatial complementarity of the genotypes in the nutrient acquisition is therefore important to increase the performance of intercropping. Hence, future research needs to address how different genotypes respond to nutrient competition, with a particular focus on below-ground traits.

Pest and disease resistance is one of the most important advantages of intercropping (Finckh et al., 2021). However, there are only a limited number of studies, which have considered genotype differences concerning pest and disease resistance in cereal/legume intercropping. Recent work has highlighted the importance of plant–plant interactions, either direct by mechanical, physical, or chemical cues, or mediated through soil/air microbiota, and the way they can affect plant immune system or other functions (Subrahmaniam et al., 2018; Khashi u Rahman et al., 2019; Zhu and Morel, 2019; Péliissier et al., 2021). Life cycle assessment (LCA) is a convenient, effective, and rarely used [but see Naudin et al. (2014)] approach for analyzing the environmental impact of cereal/legume intercropping, especially on the N cycle.

There are only a few studies considering the socio-economic importance of genotypes of both cereals and legumes species. Goshime et al. (2020) involved the farmers in the evaluation of genotypes. Different quality parameters of the genotypes not included in most of the articles hence could affect the acceptance of intercropping by farmers. The forage quality differences of legume genotypes were mostly ignored, and the number of studies on this topic is very limited. The consumer and market preference of different genotypes of cereals and/or legumes is also important in the selection of genotypes for intercropping. Therefore, in addition to morphological and phenological traits, other traits (roots, water and nutrient acquisition, and quality) and advantages in pest and weed suppression deserve attention to understand the mixing ability of different genotypes. Future research should consider pedigree analysis, functional genes, or key traits when selecting varieties tested in intercropping.

SUMMARY AND CONCLUSION

We evaluated the observations of studies that included at least two genotypes of one species in cereal/legume intercropping. While the number of studies is inadequate for obtaining a comprehensive and reliable insight, our results point to the potential of genotype selection in intercropping, and future research should therefore emphasize genotype × cropping system interaction in cereal/legume intercropping. In total, the majority of the studies reported that there was a significant genotype–cropping system interaction revealing the importance of genotype selection for intercropping for more land productivity. Among the 69 analyzed studies, only 35

studies reported LER values. We determined a median LER of 1.24, which indicated that a combination of specific genotype cereals and legumes improves the land productivity by 24% on average. In addition, 85% of the LER data points of cereal/legume intercropping were greater than 1. On the other hand, 15% of the specific cereal/legume genotype combinations resulted in $LER < 1$ revealing that judicious choice of genotype combination in cereal/legume is indispensable.

Furthermore, the ANOVA across cereal species and design indicated that different species have different land-use efficiency in the different design types with finger millet having higher land-use efficiency than other crops in additive designs, whereas no difference was observed between the species in replacement designs. The number of studies, which report LER from different wheat genotypes, was very limited [but see Timaeus et al. (2022)]; because of the high importance of wheat for global food security, we suggest that more research is needed to investigate the performance of different wheat genotypes in intercropping. Conversely, the effect of design on land use efficiency in legumes is not significant, whereas species effect is significant. Temporal and spatial heterogeneity between the genotypes of the cereals and those of the legumes was mentioned in the selected studies as the main mechanism enhancing the overall performance of cereal–legume intercropping. However, the spatiotemporal heterogeneity of genotypes was not described sufficiently in most of the studies to allow a detailed analysis. Hence, future research studies should consider and report the genotypes' traits more comprehensively, including root growth, soil nutrient and water acquisition, and diseases, among others. In most studies, only some agronomic traits of genotypes were emphasized ignoring other genotypic functional traits. Furthermore, we recommend that future research needs to evaluate a higher number of genotypes and their traits on various sites and under different climate and management conditions. It is impossible to test all possible combinations (genotype × genotype × environment × management) of intercropping in field trials. The complex interactions in intercropping can be disentangled by process-based agroecological models, which can help to identify the relevant influencing factors of intercrop performance. However, the prerequisite is an understanding of the basic mechanisms.

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All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.846720/full#supplementary-material>

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Mixing Ability of Intercropped Wheat Varieties: Stability Across Environments and Tester Legume Species

N. Moutier^{1*}, A. Baranger¹, S. Fall¹, E. Hanocq², P. Marget³, M. Floriot⁴ and A. Gauffreteau⁵

¹ INRAE, Institut Agro Agrocampus Ouest, Université de Rennes 1, UMR 1349 IGEPP, Le Rheu, France, ² INRAE, UE 0972 GCIE, Estrées-Mons, Péronne, France, ³ INRAE, UE 115 Epoisses, Bretenière, France, ⁴ Agri-Obtentions, Orsonville, France, ⁵ INRAE, AgroParisTech, Université Paris Saclay, UMR 211 Agronomie, Thiverval-Grignon, France

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*Correspondence:

N. Moutier
nathalie.moutier@inrae.fr

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Cereal-legume intercrops are developed mainly in low input or organic farming systems because of the overyielding and numerous ecosystem services they provide. For this management, little advice is available for varietal choice and there are almost no specific breeding programs. Our study aimed to evaluate the mixing ability of a panel of bread wheat genotypes in intercropping and to assess the impact of environment and legume tester choice on this ability. We used partial land equivalent ratios (LERs) to assess the mixing ability of a genotype defined as the combination of its ability to maintain its own yield in intercropping (producer effect, LER_w) and to let the mixed species produce (associate effect, LER_i). Eight wheat genotypes and 5 legume testers (3 pea and 2 faba bean varieties) were grown in sole crop and in all possible binary intercrops in nine contrasting environments. A mixed model was used to evaluate the effects of wheat genotypes, legume testers, environments, and all the interactions among these 3 factors on LER_w and LER_i. The chosen wheat genotypes presented contrasting mixing ability, either in terms of producer effect (LER_w) or associate effect (LER_i). A strong negative correlation was observed between these two components of genotype mixing ability, with an increase in producer effect being generally associated with similar decrease in associate effect, except for three genotypes. The impact of environment on the producer and associate effects was limited and similar between genotypes. Legume tester had a significant effect on both LER_w and LER_i, making the choice of tester a major issue to reveal the producer or associate effects of wheat genotype. Although the 5 testers showed no significant differences in wheat genotype order for both producer or associate effects, they showed different competitiveness and ability to discriminate genotypes: faba bean was very competitive, resulting in low LER_t and low capacity to discriminate wheat genotypes for their mixing ability. On the contrary, pea was less competitive, resulting in higher LER_t and better capacity to discriminate wheat genotypes. In particular, the Hr varieties (Geronimo and Spencer) discriminated best the wheat genotypes. Consequences on the implementation of breeding programs for wheat varieties adapted to intercropping are discussed.

Keywords: cereal, pea, faba bean, breeding, G × G × E interactions, land equivalent ratio (LER), producer/associate concept, mixtures

INTRODUCTION

During the last 60 years, agriculture in industrialised countries has become more intensive, focusing on reduced numbers of main crops in shorter rotations on increasingly large sole crop plots. Higher yields were obtained thanks to the intensive use of mineral fertilisers and synthetic pesticides, which strongly impacted the environment (environmental degradation, resources depletion), agrosystem biodiversity, and human health (Tilman et al., 2002). The acknowledged need to move towards more sustainable and responsible agriculture and design more resilient arable cropping systems was advocated by Altieri et al. (2017) through agroecological principles, among which are (1) the diversification of the agroecosystem by increasing the biodiversity at landscape, farm, and field levels, over time and space, and (2) the optimised use of beneficial biological interactions that are naturally available in the agrosystem to maximise ecological services.

Intercropping, which corresponds to simultaneous cultivation of two or more crop species in the same field (Willey, 1979), strongly contributes to these principles. It is an old and widespread practice in many areas of the world (Anil et al., 1998). It has been largely abandoned in Europe following intensification but arouses a renewed interest in the context of transition from intensive to low-input systems (Malézieux et al., 2009).

Intercropping can provide higher, more secure, and stable yields than sole crops (Raseduzzaman and Jensen, 2017; Stomph et al., 2020), with less or no external inputs. It also improves soil conservation and fertility, and grain protein concentration of a cereal when intercropped with a legume (Gooding et al., 2007). It allows for better control of pests and weeds (Banik et al., 2006; Boudreau, 2013; Lopes et al., 2015) and reduces lodging (Chen et al., 2020). Intercropping derives these advantages from the ecological principles of complementarity, cooperation, competition, and compensation between crops, the so-called “4C approach” (Bedoussac et al., 2015; Justes et al., 2021). Intercropping usually brings together two (or more) species affected by different pests, showing contrasted root and/or aerial systems, displaying different sensitivities to low or high temperature and complementary requirements for natural resources (light, water, and/or nutrients) in time and/or space. An obvious example is cereal-legume intercrops: in which the use of soil mineral nitrogen by non-leguminous crops is complemented by atmospheric nitrogen fixation by leguminous crops.

In spite of these many potential advantages, the adoption of intercrops stays at low levels in Europe (apart in conservation agriculture and organic farming) due to remaining technical, economic and policy barriers to wider dissemination, as recently shown in the case of bread wheat and field pea intercrops (Mamine and Farès, 2020).

Just on the field scale, many factors may indeed influence intercrop services and performances: environmental conditions (rainfall, temperatures, soil fertility, etc.), crop management practices, such as species choice (Wendling et al., 2017), sowing densities and dates (Neumann et al., 2007; Pöttsch et al., 2019),

spatial designs (Ndzana et al., 2014), fertilisation strategies (Yu et al., 2016), and availability of machinery settings (sowing, harvesting, and sorting).

Although the varietal choice within each species is likely to affect canopy traits, resource access, provided ecosystemic services and performance of the mixtures, there are only few publications on the varietal effect on intercropping. They often focused on specific performance or service of one of the species, and/or integrated a limited number of varieties from intercropped species in a limited number of environments: response to nitrogen application (Hauggaard-Nielsen and Jensen, 2001), nitrogen use efficiency (Tsialtas et al., 2018), disease control (Kinane and Lyngkjaer, 2002), quality and yield performance (Barker and Dennett, 2013; Baxevanos et al., 2017; Kammoun et al., 2021), biomass production (Streit et al., 2019; Li et al., 2020). Recently, reports considering larger varietal diversity are emerged (Haug et al., 2021).

Since very little data are available to date to assess the mixing ability of a given variety in intercrop, either for its capacity to produce (producer effect) or its ability to make the associated species produce (associate effect), farmers base their varietal choice on traits and performances evaluated in sole crop. This practice may be risky, since some of these traits and performances are not always predictive of those observed in mixtures (Hauggaard-Nielsen and Jensen, 2001; Moutier et al., 2018, 2021; Annicchiarico et al., 2019).

To consider adapted breeding methodologies targeting this cultivation practice (Annicchiarico et al., 2019; Sampoux et al., 2020), breeders need to be able to assess the mixing ability of genotypes belonging to a focal species both across environments and companion species/varieties. These companion species/varieties are below called testers for their potential to reveal the mixing ability of a genotype, by analogy to testers used to identify superior germplasm in hybrid-oriented breeding programs (Hallauer et al., 2010; Fasahat et al., 2016). Since an intercropped tester may also influence the mixing ability of a genotype because of its competitive and discriminatory power, breeders also need to find adequate testers to reduce the number of combinations studied.

The purpose of our study was to (i) identify the potential impact of wheat varietal choice on the productive and associated performances when wheat is mixed with different tester varieties from two grain legumes species (pea and faba bean), (ii) evaluate if the varietal mixing ability is stable across environments and testers, (iii) compare the capacity of contrasted tester legume species to discriminate stably wheat genotypes for their suitability for intercropping.

MATERIALS AND METHODS

Plant Material and Experimental Design

Eight bread winter wheat (*Triticum aestivum*) genotypes, all early maturing (to ensure joint harvest with legumes), resistant to lodging, and partially resistant to main diseases (especially to yellow rust), were chosen according to yield potential (high vs. low), earliness in heading stage (early vs. mid early-mid late),

and canopy height at heading stage (short vs. tall) in sole crop (SC). The genotypes covered all 8 possible combinations of the 3 previous traits (Table 1A).

Five legume testers, i.e., 2 faba bean (*Vicia faba*) and 3 afila field pea (*Pisum sativum*) varieties, including 2 “Hr” varieties needing a minimal photoperiod to initiate flowering and 1 “hr” variety whose flowering initiation does not depend on photoperiod, were chosen according to their phenological (flowering starting date) and architectural (plant height at harvest and soil coverage power) traits to create different competition conditions with wheat in time and space (Table 1B). All the legume testers were late maturing in sole crop to ensure joint harvest with wheat.

All possible binary mixtures of the eight wheat genotypes with the five legume testers were considered, leading to 40 intercrop (IC) and 13 sole crop modalities.

Each trial contained two parts: one with the wheat SC, the pea SC, and the wheat-pea IC, i.e., a total of 35 treatments; the other with the faba bean SC and IC, i.e., a total of 18 treatments. This spatial distribution is aimed at suppressing the neighbouring effects of faba bean (in SC and IC) on the other species plots. In these two parts, the treatments (35 vs. 18) were distributed into 8–10 m² microplots according to a

complete randomised block design with three blocks and one replicate per block.

Environments and Management Practices

Nine trials (3 locations × 3 years) were conducted by INRAE (French National Research Institute for Agriculture, Food and Environment) from 2016/17 to 2018/19 seasons, in organic farming (Rennes, RE) or very low input systems (Estrées-Mons, EM and Dijon, DI; Figure 1).

The 3 locations were known to be contrasting in their soil characteristics (type and depth) and climatic conditions over the 10 years preceding the implementation of the trials (cumulative rainfall and distribution of precipitation over the growing season: minimum, medium, and maximum temperatures; Table 2).

The microplots were sown in the fall (from 21 October in DI and EM to 9 November in RE in autumn 2016) with a 6- to 8-row grain seed drill, spacing 13.5–20 cm between the rows, just after ploughing, and with grains fully mixed in the row for IC.

The wheat genotypes were sown at 300, 150, and 210 seeds/m² in SC, IC with pea, and IC with faba bean, respectively. The

TABLE 1 | Phenological, architectural, and agronomic traits in sole crop (SC) of (A) the 8 winter bread wheat genotypes and (B) the 5 field pea and faba bean varieties involved in binary mixtures.

A. Wheat genotype							
	Yield potential		Earliness at heading stage		Height at heading stage		
	Expected ¹	Observed ² q/ha	Expected ¹	Observed ²	Expected ¹	Observed ² cm	
Flamenko	high	50	early	May 14	short	80	
Geny		49		May 14	tall	83	
Attlass		48	mid early–mid late	May 18	short	75	
RE13003		49		May 21	tall	87	
Forcali/Rebelde ³	low	37	early	May 12	short	74	
CF14336		44		May 16	tall	88	
Renan		41	mid early–mid late	May 19	short	80	
Ehogold		41		May 19	tall	102	

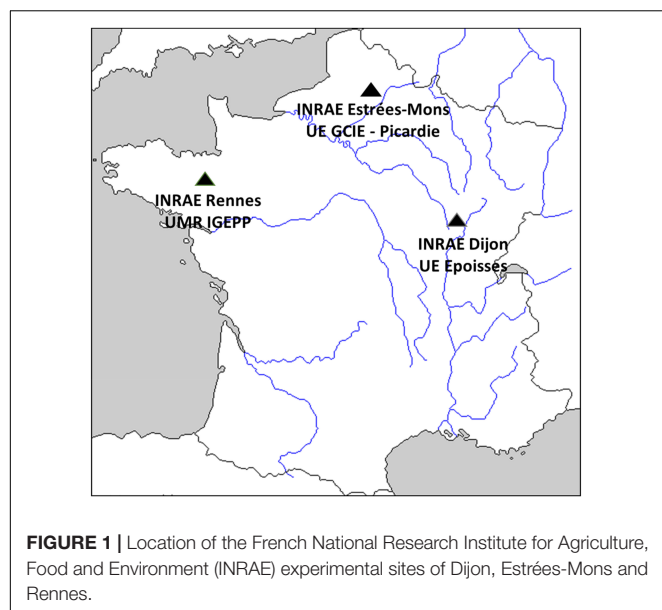
B. Legume varieties							
	Type ¹ /Species	Flowering starting date (SC)		Plant height at harvest (SC)		Soil coverage power (SC)	
		Expected ¹	Observed ²	Expected ¹	Observed ² cm	Expected ²	Observed ³ %
Fresnel	hr field pea	early	April 22	high	81	high	37 (early stages) – 99 (late stages)
Geronimo	Hr field pea	late	May 19	low	75	low (early stages) to high (late stages)	20 (early stages) – 83 (late stages)
Spencer		late	May 20	low	75	low (early stages) to high (late stages)	19 (early stages) – 85 (late stages)
Irena	Faba bean	early	April 15	low	102	low	30 (early stages) – 70 (late stages)
Olan		late	April 21	high	120	high	33 (early stages) – 79 (late stages)

(A) ¹Expected trait from pre- or post-certification trial data and/or from breeder/expert communication. ²Mean observed trait under SC conditions across 9 environments (this issue, organic or low inputs systems). ³The cultivar Forcali was replaced by Rebelde, showing the same varietal trait combination, in 2018 and 2019.

(B) ¹Hr field pea varieties are highly responsive to photoperiod for their floral initiation, whereas hr field pea varieties are not.

²Expected trait from pre- or post-certification trial data and/or from breeder/expert communication.

³Mean observed trait under SC conditions across 9 environments (this issue).



hr pea genotype was sown at 80 and 60 seeds/m² in SC and IC, respectively, while the Hr pea genotypes were sown at 40 seeds/m² both in SC and IC, and the faba bean genotypes were sown at 28 and 21 seeds/m² in SC and IC, respectively. The relative higher pea and faba bean sowing densities (75–100% of SC density) than that of wheat (50–70% of SC density) in the ratio was justified by higher competitiveness of wheat expected in the ICs, together with the aim of an expected balance of species in the harvest (Lithourgidis et al., 2011).

There were no or few chemical controls, and no fertiliser was spread on the crops (except for 30 units of nitrogen supplied at the end of March 2018 in EM). Weeds were mainly managed by mechanical weeding when needed. The harvest occurred in mid-July. The harvested grains of the two species were separated mechanically.

Variables Under Study

Each year, for each sorted sample from the 159 microplots and for each of the species, the weight and the moisture were measured, and the gross yield was calculated.

The performance of each mixture component (either wheat or legume) in each trial and block was evaluated from the observed

wheat or legume yields in SC and IC by partial land equivalent ratio (Crookston and Hill, 1979) as follows:

$$\text{LER}_w = Y_{w(\text{IC})}/Y_{w(\text{SC})} \text{ and } \text{LER}_l = Y_{l(\text{IC})}/Y_{l(\text{SC})}, \quad (1)$$

where LER_w and LER_l are the partial land equivalent ratios for wheat and legume, $Y_{w(\text{IC})}$ and $Y_{w(\text{SC})}$ are the yields of wheat in IC and SC, $Y_{l(\text{IC})}$ and $Y_{l(\text{SC})}$ are the yields of legume in IC and SC.

The performance of each mixture was then evaluated by its total land equivalent ratio (LER_t) by the sum of the partial wheat and legume LER values as follows:

$$\text{LER}_t = \text{LER}_w + \text{LER}_l, \quad (2)$$

Total land equivalent ratio measures the total land area under SC (in ha) required to produce the same amount of grain as the wheat-legume IC in 1 ha.

This index allows comparing performances in IC relative to SC. If LER_t > 1, environmental resources are used more efficiently by IC than by SC.

In our study, the mixing ability of a wheat genotype was defined both by its capacity to maintain its SC potential yield, i.e., to limit the loss of yield in IC compared to SC, and its ability to make the associated species produce. In each trial and block, the ability of a wheat genotype to maintain its yield when intercropped with legumes (producer effect) was estimated by the ratio of its yield in IC to its yield in SC, corresponding to LER_w. Similarly, the ability of a wheat genotype to maintain the yield of the associated legume genotype (associate effect) was estimated by the ratio of legume yield when intercropped with this particular wheat genotype to the legume yield in SC, corresponding to LER_l.

Statistic Model

In order to identify the terms to be included in the analysis, the following model was first considered:

$$Y_{ijk} = \mu + G_i + T_j + E_t + GT_{ij} + GE_{it} + TE_{jt} + EB_{tk} + GTE_{ijt} + \varepsilon_{ijk}, \quad (3)$$

where the LER_w or LER_l of the wheat genotype i intercropped with the legume tester j in the block k of the environment t , and the block k (Y_{ijk}) is decomposed in an overall

TABLE 2 | Agronomic and environmental characteristics (means in the 2006–2016 period) of the 3 French National Research Institute for Agriculture, Food and Environment (INRAE) experimental sites (Dijon, Estrées-Mons, and Rennes).

Location	Climatic zone	Temperature (°C)			Cumulated rainfall (mm)	Soil type	Soil depth
		Mean	Min	Max			
		On the growing season (October 21–July 20)					
INRAE Dijon (DI)	semi-continental	9.3 (8.2 to 10.6)	−10.1 (−20.2 to −4.7)	34.1 (31.8 to 37.7)	560 (470 to 660)	clay-loam to clay	moderately deep
INRAE Estrées-Mons (EM)	oceanic	9.0 (7.8 to 10.3)	−8.6 (−15.8 to −2.5)	31.8 (27.9 to 34.6)	490 (370 to 620)	loamy	moderately deep
INRAE Rennes (RE)	oceanic	10.3 (9.5 to 11.1)	−5.9 (−8.6 to −3.3)	32.1 (27.8 to 35.8)	590 (440 to 770)	loamy, beating	deep

mean (μ) and the effects of the wheat genotype i (G_i), legume tester j (T_j), environment t (E_t) and all possible interactions between these three factors. The experimental design was also considered through the block effect in each environment (EB_{tk}). A preliminary analysis of variance showed that all the terms of the model, except the triple interaction GTE, had a significant effect on both LERw and LERl.

As the environment has a great impact on both LERw and LERl but is not predictable before sowing (mainly because of the effect of year), we considered it as a random factor. Both producer and associate effects were then analysed by the following mixed model (model 1):

$$Y_{ijtk} = \mu + G_i + T_j + E_t + GT_{ij} + GE_{it} + TE_{jt} + EB_{tk} + \varepsilon_{ijtk}(1), \quad (4)$$

where Y_{ijtk} is the LERw or LERl obtained when the wheat genotype i was intercropped with the legume tester j in the environment t and the block k . μ is the overall mean, G_i is the main effect of i -th wheat genotype, T_j is the main effect of j -th legume tester, E_t is the main effect of t -th environment, GT_{ij} is the ij -th wheat genotype $\times$ legume tester interaction, GE_{it} is the it -th wheat genotype $\times$ environment interaction, TE_{jt} is the jt -th legume tester $\times$ environment interaction, EB_{tk} is the effect of k -th block in the t -th environment, and ε_{ijtk} is the $ijtk$ -th residue. Wheat genotype and legume tester were regarded as fixed factors, while environment was as a random factor. With this assumption, the effects of wheat genotype (G_i), legume tester (T_j), and their interaction (GT_{ij}) were considered as fixed, whereas all the other effects were considered as random. The random effects E_t , EB_{tk} , GE_{it} , and TE_{jt} , were assumed to be independently distributed with zero mean and variances σ_E^2 , σ_{EB}^2 , $\sigma_{GE(i)}^2$, and $\sigma_{TE(j)}^2$. We assumed the heteroscedasticity of the model, i.e., for the t -th environment, $\varepsilon_{ijtk} \sim N(0, \sigma(t)^2)$. The model was fit by maximising the restricted log-likelihood with the R package nlme (version 3.1-152; Pinheiro J. et al., 2021).

Test of Random and Fixed Terms

Each random term was tested by comparing model 1 with another one obtained by dropping the term under study (Supplementary Table 1). For instance, to test the effect of wheat genotype $\times$ environment interaction (GE_{it}), model 1 was compared to the following model:

$$Y_{ijtk} = \mu + G_i + T_j + E_t + GT_{ij} + TE_{jt} + EB_{tk} + \varepsilon_{ijtk}, \quad (5)$$

Three indicators were considered for comparison: Akaike information criterion (AIC), Bayesian information criterion (BIC), and the result of a log-likelihood-ratio test between the two models. The lower the AIC and the BIC, the better the model was, and we considered a random term as significant only if the p -value associated with the log-likelihood-ratio test was below 5%. Once the random terms were set, the fixed

terms of the model were tested by classic analysis of variance and Fisher tests.

Comparing Producer and Associate Effects of Wheat Genotypes When Intercropped With a Legume

The mean varietal performance of the i -th wheat genotype in intercropping (either producer or associate effect) is given by G_i . The stability of this performance across the environments is given by $\sigma_{GE(i)}$. When the genotypes had a significant effect on producer or associate effects, their performances were compared by pair comparisons implemented using the package emmeans (Version 1.6.1¹). The p -values of the 28 possible comparisons were adjusted with the Tukey method.

Comparing the Legume Testers for Their Ability in Classifying and Discriminating the Wheat Genotypes for Their Producer or Associate Effects

The effects of legume testers on the mean producer or associate effects were measured by T_j and their stability across environments by $\sigma_{TE(j)}$. Therefore, these parameters gave information about the competitiveness of legumes against wheat.

If the impact of wheat genotype $\times$ legume tester interaction was significant on producer or associate effects, genotype rankings obtained with each tester were compared graphically. Particular attention was paid on pairs of genotypes that each tester was able to discriminate significantly at 5%.

RESULTS

Variability of Environmental Conditions

Environmental conditions (temperature, rainfall) were close to the means recorded across the 10 previous years in the 3 locations, apart from rainfall through the growing season (21 October–20 July) in EM17, with the lowest rainfall recorded over the previous 10 years period, denoting a particularly dry season, and in DI18, with the highest rainfall recorded in the 10 previous years, denoting a particularly wet season (Table 3).

At all three sites, the years 2017 and 2019 were characterised by rather dry and cold winters, followed by warm springs; 2018 presented low temperatures in February, very wet winter and spring (Supplementary Figure 1).

The storm Miguel passed through the RE19 trial in June, causing early and heavy lodging of almost all the sole peas and intercropped wheat-pea combinations.

The previous crops were straw cereals in Dijon and Estrées-Mons every year, and maize or grassland in Rennes depending on the year.

The soil types were clay-loam to heavy clay in Dijon, and loamy in Estrées-Mons and Rennes. The nitrogen available in the soil at the end of winter was 70 U N ha^{-1} on average in

¹<https://declaredesign.org/r/estimatr/articles/emmeans-examples.html?msclid=f0d2191fc0f311ecb250231c4530e485>

TABLE 3 | Agronomic and environmental conditions of the 9 trials testing 8 wheat genotypes for their ability to be cropped in binary mixtures with 5 legume (pea, faba bean) tester varieties.

Location (system)	Year	Trial/ Environment	Sowing date	Harvest date	Temperature (°C)			Rainfall (mm)- Cumulated on the growing season	Previous crop	Soil type	Soil depth	Available N Units (end of winter)
					Mean	Min	Max					
					On the growing season							
NRAE Dijon (DI) (very low input)	2017	DI17	2016/10/21	2017/07/12	9.4	-9.6	36.2	460	Spring oat	clay-loam to clay	moderately deep	107 U
	2018	DI18	2017/10/24	2018/07/09	10.0	-11.6	34.1	740	Spring oat	clay-loam to clay	moderately deep	31 U
	2019	DI19	2018/10/24	2019/07/17	9.9	-6.0	37.6	420	Spring oat	clay-loam to clay	moderately deep	
NRAE Estrées- Mons (EM) (very low input)	2017	EM17	2016/10/21	2017/07/18	9.4	-6.2	34.4	300	Wheat	loamy	moderately deep	117 U
	2018	EM18	2017/10/27	2018/07/15	9.8	-8.6	31.7	540	Wheat	loamy	moderately deep	23 U
	2019	EM19	2018/10/25	2019/07/17	9.5	-4.5	33.3	430	Wheat	loamy	moderately deep	66 U
NRAE Rennes (RE)	2017	RE17	2016/11/09	2017/07/21	10.6	-7.4	35.1	430	Grassland	loamy, beating	deep	61 U
(organic farming)	2018	RE18	2017/10/31	2018/07/18	10.9	-7.2	31.2	530	Maize	loamy, beating	deep	64 U
	2019	RE19	2018/11/06	2019/07/17	10.8	-3.6	35.7	430	Maize	loamy, beating	deep	74 U

the nine environments, ranging from 23 U N ha⁻¹ in EM18 (completed with 30 units of nitrogen at the end of March) to 117 U N ha⁻¹ in EM17.

The delay between sowing and emergence varied between environments and species: from 13 to 19 days for wheat, 15 to 31 days for pea, and 15 to 37 days for faba bean, with a maximum delay of 6 days between genotypes of the same species when considering a single environment. Depending on the environment, pea emerged 2–12 days and faba bean 2–18 days after wheat.

Wheat and Grain Legume Yields in Sole Crop and Intercrop

Yields were highly variable between environments. Wheat yields in SC, averaging 45 q/ha over the 8 genotypes and the 9 environments, ranged from 23 q/ha in DI18 to 61 q/ha in EM17 and RE19 (**Supplementary Figure 2A**). This corresponds to average to high yields for wheat sole crops in very low input or organic farming systems, where between 20 and 30 q/ha are usually expected². On average, over the 3 years, Dijon showed the lowest SC wheat yield potential (34 q/ha), systematically lower than the average, while Rennes and Estrées-Mons showed higher yield potentials, each close to 50 q/ha. Average wheat yields across environments for genotypes with high yield potential in SC were, as expected, higher (+10 q/ha) than those of genotypes with lower potential (**Supplementary Figure 2A**). The average wheat yield obtained in IC across environments was 26 q/ha, with EM showing better wheat yield potential over the 3 years (36 q/ha) when compared to Dijon and Rennes, which had lower wheat yield potential (20 and 22 q/ha, respectively).

Pea yields in SC over the 3 testers averaged 36 q/ha across environments (**Supplementary Figure 2B**), and ranged from 14 q/ha in RE18 to 54 q/ha in DI17 and EM19, with, globally, higher pea yield potentials in Dijon and Estrées-Mons (41 and 48 q/ha, respectively) than in Rennes (20 q/ha). The average pea yield obtained in IC across environments was 24 q/ha with, globally, the same pea yield potential over the 3 years at the 3 locations (±1 q/ha).

Faba bean yields in SC over the 2 testers varied from 25 q/ha in RE18 to 43 q/ha in EM19. The average faba bean yield in SC over the 8 environments was 33 q/ha with similar faba bean yield potential over the 3 years for the 3 locations (±2 q/ha). The average faba bean yield obtained in IC across environments was 21 q/ha. Dijon and Rennes showed better yield potential over the 3 years (24 and 25 q/ha, respectively) when compared to Estrées-Mons, which had lower yield potential (16 q/ha).

Partial Land Equivalent Ratios

Across all environments and intercrops, LERw varied between 0.08 and 1.23 and LERl between 0.15 and 2.6 (**Figure 2**), with high variations between environments. In mean, Estrées-Mons showed the highest LERw, comprised between 0.64 in 2019 and 0.82 in 2018 (**Figure 2A**), together with the lowest LERl (**Figure 2B**). Dijon, where wheat yields were lowest both in SC

²<https://www.agencebio.org/vos-outils/les-chiffres-cles/>

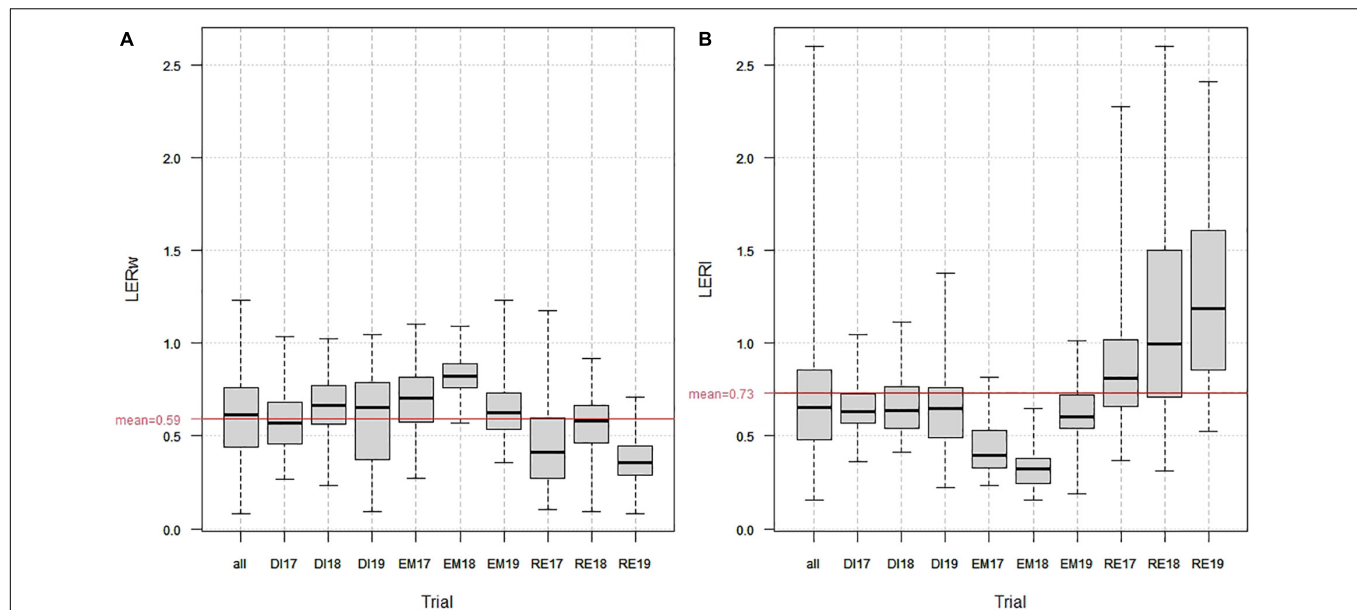


FIGURE 2 | Distribution of partial Wheat **(A)** and Legume **(B)** Land Equivalent Ratios over the 9 environments (all) and for each environment (DI, Dijon; EM, Estrées-Mons; RE, Rennes; 17, 2016/2017 trial; 18, 2017/2018 trial; 19, 2018/2019 trial). The red bars show the LERw **(A)** and LERl **(B)** means across all environments.

and in IC, showed medium LERw, presumably stable between years, ranging from 0.54 in 2017 to 0.65 in 2018, together with medium, and, presumably stable between years, LERl. Rennes, with high wheat yields in SC and low wheat yields in IC, showed lowest LERw (from 0.37 in 2019, linked to high lodging in IC, to 0.56 in 2018), together with high and very variable LERl (due to very low pea yields obtained in SC over the 3 years), sometimes over 1 (meaning that in some cases, the legume yields obtained in IC were higher than those obtained in SC).

Impact of Environment, Wheat Genotype, and Legume Tester on Wheat Mixing Ability

Partial land equivalent ratios across wheat genotypes, environments, and testers averaged 0.59 for LERw and 0.73 for LERl (Figure 2). The estimates of standard deviations and confidence intervals of LERw (SD = 0.04, CI = 0.51–0.68) and LERl (SD = 0.1, CI = 0.53–0.93) showed that if LERl was, on average, higher than LERw, it was also less precisely estimated.

The observed LERw and LERl were highly dependent on trial. Indeed, environmental effects on both variables were significant at 0.1% (Table 4), with standard deviations of 0.1159 and 0.2933 for the models on LERw and LERl, respectively (Table 5). Therefore, tester legume varieties were subjected to 2.5 times higher variations of their partial LER between environments than wheat genotypes.

Environment also significantly influenced the effects of wheat genotypes and legume testers on both LERw and LERl (p -values of GE and TE terms below 0.01 as shown in Table 4). However, the impact of environment on wheat genotype effect remained moderate on both variables, with standard deviations

of the GE term below 0.05 (Table 5), whereas it was much higher on legume testers effect, with standard deviations of the TE term over 0.15 on LERl, and for faba bean testers on LERw (Table 5).

Mean residual standard deviations of the models on LERw and LERl, i.e., 0.1104 and 0.0987, respectively (Table 5), showed that experimental variance remained significant compared to other sources of variability. Furthermore, this experimental variance clearly differed from one environment to another (p -values < 0.001 as shown in Table 4). The residual standard deviations indeed ranged from 0.0774 in RE19 to 0.198 in RE17 for the model on LERw and from 0.0824 in EM18 to 0.3818 in RE18 for the model on LERl, which indicates that some trials were more precise than the others.

TABLE 4 | p -Values of log-likelihood-ratio tests between reference and test models (see Supplementary Table 1) compared to assess the relevance of environment (E), block (EB), wheat genotype $\times$ environment interaction (GE), and legume tester variety $\times$ environment interaction (TE) random effects on partial wheat genotypes and legume tester varieties land equivalent ratios (LERs), and the relevance of estimating residual variance by environment [$\sigma_{(t)}^2$], GE variance by genotype [$\sigma_{GE(t)}^2$], and TE variance by legume tester [$\sigma_{TE(t)}^2$].

Term	p -value LERw	p -value LERl
E	$<10^{-16}$	$<10^{-16}$
EB	3.4×10^{-12}	2.0×10^{-8}
GE	$<10^{-16}$	0.0043
TE	$<10^{-16}$	$<10^{-16}$
$\sigma_{(t)}^2$	$<10^{-16}$	$<10^{-16}$
$\sigma_{GE(t)}^2$	0.9317	0.7112
$\sigma_{TE(t)}^2$	0.0193	0.3885

TABLE 5 | Standard deviations estimated for residues on random terms [environment (E), wheat genotype $\times$ environment interaction (GE), legume tester variety $\times$ environment interaction (TE), and block (EB)].

Term	LERw	LERI
E	0.1159	0.2933
EB	0.0392	0.0493
GE	0.0498	0.0270
TE (Fresnel)	0.0640	
TE (Geronimo)	0.0601	
TE (Irena)	0.1600	
TE (Olan)	0.1480	
TE (Spencer)	0.0006	0.1723
ε	0.1104	0.0987

As TE variance differed significantly in legume tester only for LERw, standard deviation of the TE term was estimated by legume tester only for the model on LERw.

TABLE 6 | p -Values of Fisher test on wheat genotype (G), legume tester variety (T) main effects and their interaction (GT) from an ANOVA analysis.

Term	LERw	LERI
G	3.9×10^{-5}	3.5×10^{-13}
T	0.0023	0.0331
GT	0.0296	0.1069

TABLE 7 | Mean partial and total land equivalent ratios (LER) across 9 environments for the 8 wheat genotypes intercropped with the 5 legume testers.

	LERw		LERI		LERt
Mean (μ)	0.59		0.73		1.32
Wheat genotype					
Ehogold	0.67	a	0.69	cd	1.36
Attlass	0.61	ab	0.70	cd	1.31
Renan	0.60	abc	0.72	bcd	1.32
Flamenko	0.59	abc	0.67	d	1.26
RE13003	0.59	abc	0.73	bcd	1.32
CF14336	0.59	abc	0.77	ab	1.36
Geny	0.57	bc	0.75	bc	1.32
Forc-Reb	0.52	c	0.82	a	1.34
Legume tester					
Spencer	0.65	a	0.80	ab	1.45
Fresnel	0.65	a	0.71	ab	1.35
Geronimo	0.62	a	0.83	a	1.45
Irena	0.59	ab	0.57	b	1.16
Olan	0.45	b	0.75	ab	1.21

LERw, partial wheat LER; LERI, partial legume LER; LERt, total LER.

Genotypes with the same letter are not significantly different at the 5% threshold.

Wheat genotype had a significant effect on both LERw and LERI (p -values of G term below 0.001 as shown in **Table 6**), meaning that some wheat genotypes are likely to have better producer and/or associate effects on intercropping than others. As the standard deviation of GE interaction did not depend on the genotype under study (p -value > 0.7 for LERw and LERI as shown in **Table 4**), we were not able to identify wheat genotypes whose effect on LERw or LERI was more stable between environments than others.

Legume tester also had a significant effect on both LERw and LERI (p -values of T term below 0.05 as shown in **Table 6**). The effect of TE interaction on LERw depended on legume tester (p -value < 0.05 as shown in **Table 4**). Therefore, the

effects of the pea tester varieties (Spencer, Fresnel, and Geronimo, $0.0001 < sd < 0.065$ as shown in **Table 5**) were at least twice more stable between environments than the effects of faba bean testers (Irena and Olan, $0.148 < sd < 0.16$ as shown in **Table 5**). This difference in stability between legume testers was not observed for LERI (p -values > 0.38 as shown in **Table 4**), with all the testers' effects being strongly impacted by environments in this case ($SD = 0.172$ as shown in **Table 5**).

Finally, the interaction between wheat genotype and legume tester variety had a significant effect on LERw (p -value of GT term below 0.05 as shown in **Table 6**) and a less but still likely significant effect on LERI (p -value = 0.1069 as shown in **Table 6**). Thus, it appeared that the producer or associate effect of a wheat genotype on intercropping may depend on legume tester, making the choice of this tester a key issue both in screening and in breeding.

Classification of Wheat Genotypes Based on Their Average Producer and Associate Effects

On average, over the 9 environments and 5 legume testers, LERw estimates ranged from 0.52 for Forcali/Rebelde to 0.67 for Ehogold, with an average of 0.59; and LERI estimates ranged from 0.67 for Flamenko to 0.82 for Forcali/Rebelde, with an average of 0.73 (**Table 7**). LERt ranged from 1.26 (for Flamenko) to 1.36 (for Ehogold and CF14336), with the five other genotypes having a LERt close to the 1.32 mean.

Among the 28 possible comparable pairs of wheat genotypes, only 3 showed significant differences in their producer effect (LERw) across all legume testers at 5%, i.e., Ehogold vs. Geny, Ehogold vs. Forcali/Rebelde, and Attlass vs. Forcali/Rebelde (**Table 8A**). Renan, Flamenko, RE13003, and CF14336 formed a homogeneous group, with an LERw very close to the 0.59 average across legume testers (**Table 7**). A larger set (10) of pairs of wheat genotypes showed significant differences in their associate effect (LERI) at 5%, i.e., Forcali/Rebelde vs. all the other genotypes except for CF14336, CF14336 vs. Attlass, Ehogold, and Flamenko, and Geny vs. Flamenko (**Table 8B**).

Each wheat genotype can, thus, be characterised by its effect on both LERw (producer effect) and LERI (associate effect). Both effects appear to be negatively linked, with a slope not significantly different from -1 , meaning that improving the producer effect generally leads to a similar decrease in the associate effect (**Figure 3**). The wheat genotypes Attlass, Renan, RE13003, Geny, and Forcali/Rebelde are all located very close to the 1.32 LERt mean, equal to a 1.32 mean but with different LERw and LERI contributions to LERt. The 3 other genotypes deviate from this mean line, with Flamenko having the lowest LERI and average LERw, thus leading to lowest LERt; Ehogold showing the highest LERw but a rather low LERI, and CF14336 showing a high LERI together with an average LERw, both leading to highest LERts.

The correlation coefficients between genotypes' producer or associate effects and their yields, height or lateness at heading stage estimated in SC (**Table 1**) are presented in **Table 9**. Producer

TABLE 8 | Significant differences between pairs of wheat genotypes intercropped with legume tester varieties for their **(A)** producer and **(B)** associate effects on average over all testers and for each of the 5 testers studied.

A - LERw		Legume testers varieties					
		Mean	Fresnel	Geronimo	Spencer	Olan	Irena
Wheat genotype 1	Wheat genotype 2						
Ehogold	Flamenko					(+)	
Ehogold	RE13003			++	(+)		
Ehogold	CF14336	(+)					
Ehogold	Geny	++		+++	+		
Ehogold	Forc-Reb	+++	+	+++	+++	+++	(+)
Attlass	Forc-Reb	+	+		+		
Renan	Forc-Reb	(+)					
Flamenko	Forc-Reb				(+)		
Geny	Forc-Reb					(+)	

B - LERI		Legume testers varieties					
		Mean	Fresnel	Geronimo	Spencer	Olan	Irena
Wheat genotype 1	Wheat genotype 2						
Forc-Reb	Geny	+		+			
Forc-Reb	RE13003	+++		+++			
Forc-Reb	Renan	+++		+++	+		
Forc-Reb	Attlass	+++	+	+++	+++		
Forc-Reb	Ehogold	+++	++	+++	+++		
Forc-Reb	Flamenko	+++		+++	+++	+	
CF14336	Renan			(+)			
CF14336	Attlass	+			(+)		
CF14336	Ehogold	++	(+)	+	+		
CF14336	Flamenko	+++		+++	++		
Geny	Flamenko	+		++	+		

The wheat genotypes are ranked from top to bottom according to decreasing LER in question.

Pairs showing no significant difference are not shown.

+++ , p -value < 0.001 and genotype 1 > genotype 2.

++ , p -value < 0.01 and genotype 1 > genotype 2.

+ , p -value < 0.05 and genotype 1 > genotype 2.

(+), p -value < 0.1 and genotype 1 > genotype 2.

effects (LERw) were positively and significantly correlated to lateness and height at the heading stage but had a low correlation to yield. Height, lateness and yield were all negatively correlated to associate effects (LERI) but the correlations were not significant at 15%. These results must be considered cautiously as the number of wheat genotypes under study was small and the correlations were influenced by some genotypes like Ehogold and Forcali/Rebelde that are among the genotypes presenting the lowest and highest producer and associate effects.

Legume Testers Competitiveness and Ability to Discriminate Wheat Genotypes

On average, over the nine environments and across the eight wheat genotypes, Olan was the most competitive tester with a LERw of 0.45, whereas Spencer, Fresnel, and Geronimo were the least, showing the highest LERw of 0.65, 0.65, and 0.62, respectively (Table 7).

Wheat genotype $\times$ legume tester interaction was significant for LERw (p -value < 0.05 as shown in Table 6) and less significant but still possible for LERI (p -value = 0.11 as shown in Table 6). This type of interaction may be qualitative (inversion in wheat genotype classification between legume testers) or quantitative

(without modification of wheat genotypes classification), or both. Depending on the tester considered, the wheat genotypes were not always classified in the same order, either for LERw or LERI, indicating possible qualitative interactions (Figure 4). On all the testers, 9 and 11 pairs of wheat genotypes among the 28 possible combinations showed significant differences in their LERw (Table 8A) and LERI (Table 8B). When classification inversions occurred between testers, they did not give rise to significant differences between wheat genotypes. Indeed, when a significant difference was observed between two genotypes for one tester, they were either ranked the same way or not significantly different when intercropped with the other testers. Therefore, wheat genotype $\times$ legume tester interaction was mainly quantitative.

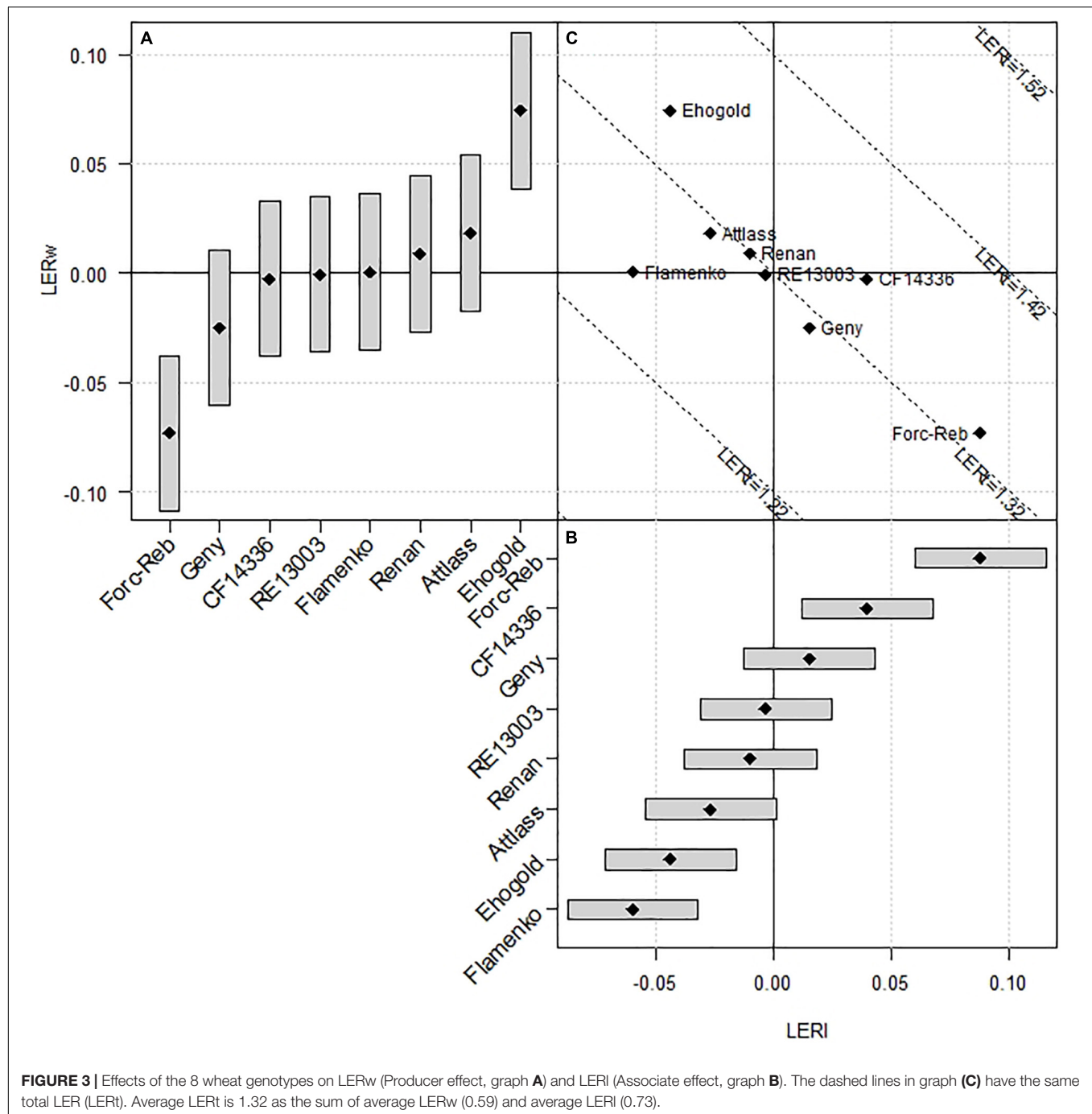
The ability of a legume tester to discriminate wheat genotypes for their producer (LERw) and associate (LERI) effects can be approached graphically or by the number of significantly different pairs of genotypes it can dissociate. All the legume testers did not seem to have the same discrimination potential for LERw and/or LERI (Figure 4). Individual tester ability to distinguish wheat genotype pairs differed greatly between testers (Table 8): at a 5% threshold, Irena did not distinguish any pair of genotypes both for LERw and LERI, Olan only distinguished the most different pairs (Ehogold vs. Focali/Rebelde for LERw and Forcali/Rebelde vs. Flamenko for LERI), and Fresnel distinguished only two pairs for both LERw and LERI. Interestingly Spencer and Geronimo distinguished the highest number of wheat genotype pairs for both LERw (3 and 3, respectively) and LERI (7 and 9, respectively).

DISCUSSION

Approaches to Varietal Mixing Ability Assessment

Studying the impact of varieties and species on the performance of binary intercropping raises a methodological problem, since all possible combinations (m genotypes of the target species A $\times$ n genotypes of tester B) cannot be considered in all environments. The experimental effort in identifying varietal mixing abilities may be reduced by (i) testing genotypes with contrasting traits, assuming these traits are likely to impact performance (Hauggaard-Nielsen and Jensen, 2001; Baxevanos et al., 2017), and mixing them with chosen tester representatives of the possible mixed species and genotypes, (ii) reducing experimental investment using incomplete designs, making it possible to maintain sufficient precision and statistical power to identify differences between genotypes (Haug et al., 2021), or (iii) coupling experimental designs with agronomic modelling (Gaudio et al., 2019).

We chose the first strategy, assuming that the producer and associate effects of a species involved in intercropping could depend on choices of both genotypes in the species and the mixed tester species or variety. Our results on wheat-grain legume intercrops confirmed this hypothesis by showing that (1) contrasting wheat genotypes have different producer and associate effects and (2) the choice of grain legume tester has an



important impact on our ability to differentiate wheat varieties for their producer and associate effects.

A Wide Range of Environments Strengthen the Robustness of the Results

The yield potential of each species in sole crop or intercropping can be significantly impacted by the genotype under study, genotype of the mixed crop, cropping practices such as

sowing densities, and by intercrop pedo-climatic conditions, i.e., environments. Indeed, all factors directly or indirectly impact the so-called 4Cs (Justes et al., 2021), mainly competition and complementarity. Therefore, the robustness and genericity of the resulting relative contributions of each intercropped species to yield highly depend on the range of environments under study. The multi-environment trial network described in this report indeed integrated both conventional (DI and EM) and organic (RE) cropping conditions and a large range of environments, including extreme and unusual ones. EM17

TABLE 9 | Estimates and *p*-values (pv) of Pearson correlation coefficients between lateness at heading stage, height at heading stage and yield in SC, and producer (LERw) and associate (LERl) effects of the 8 wheat genotypes.

	LERw	LERl
Lateness at heading stage (SC)	0.67 (pv = 0.07)	-0.45 (pv = 0.26)
Height at heading stage (SC)	0.73 (pv = 0.04)	-0.34 (pv = 0.40)
Yield (SC)	0.15 (pv = 0.73)	-0.54 (pv = 0.17)

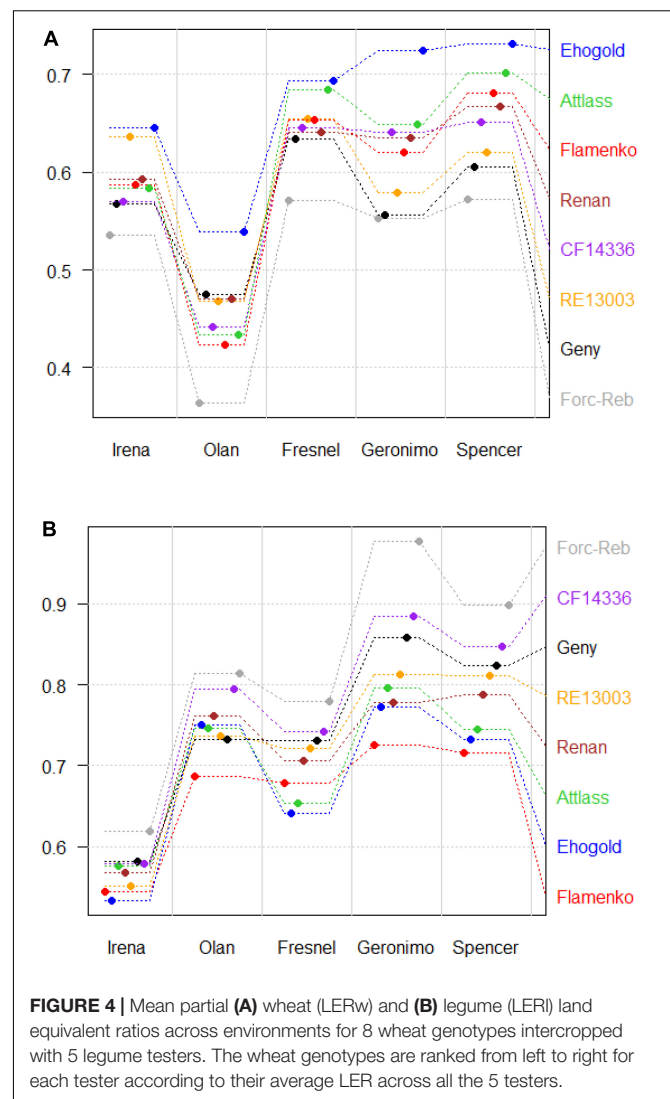
presented a lack of rainfall throughout the season, RE19 experienced an important storm that resulted in high and variable rate of lodging for all SC and IC pea plots, and DI18 presented particularly high rainfall and low levels of N by the end of winter, which clearly altered wheat and legume tester yields (**Supplementary Figure 2**). As a whole, both LERw and LERl differed significantly between environments. The relative contributions of these partial LERs to total LER differed greatly between environments, showing that the chosen environments cover a large range of biotic and abiotic stress patterns and competition situations between mixed species/varieties, some conducive for both species and some conducive for one of the species and not the other.

Sowing Densities for Balanced Mixtures

Relative seeding rates in mixtures are considered an important parameter in the performance of mixtures (Neumann et al., 2007; Barker and Dennett, 2013; Pötzsch et al., 2019). They influence the competition between species throughout the crop cycle and can thus impact the estimation of the producer and associate effects of tested wheat varieties. For practical reasons (number of microplots per trial), we could not test several relative seeding densities per type of mixture and chose densities allowing balanced production of each species. Indeed, balanced mixtures allow to discriminate between varieties on both their capacity to produce and to make produce. The objective of balanced production between wheat and legume was globally reached with partial LERs generally higher than 0.5 and similar for each species. However, it appears that the partial LERs of Hr peas are higher than those of wheat, and this would probably have justified lowering slightly their seeding rate in IC compared to the sole crop. The partial LERs of the Olan tester are significantly higher than those of the associated wheat, arguing for a significant reduction in the seeding rate of faba bean in IC for this tester. Finally, we cannot exclude that the ability to discriminate between wheat varieties would be different with very unbalanced seeding rates of the intercropped species.

Partial Land Equivalent Ratios Are Adapted to Identify Genotypes Adapted to Intercrop

Many indices may be used to assess species interactions in intercrops for growth and/or yield, including ratios (such as partial and total LERs), simple differences in performances (Haug et al., 2021), or even differences between ratios (aggressivity). Some indices (such as relative efficiency index or comparative



absolute growth rate) take into account the dynamics of competitive interactions in growth. Others finally tend to separate interspecific from intraspecific interactions (reviewed in Bedoussac and Justes, 2011). Since our purpose was to assess the final relative yield outcome of the interaction rather than compare relative growth dynamics or analyse separately intraspecific from interspecific interactions, it looked sound to compare wheat varieties for their producer and associate effects based mainly on their partial LERs for yield. Indeed, this index that allows to quantify mixture productivity compared to the sole crops was acknowledged as relevant and versatile (Bedoussac and Justes, 2011), and it was widely used and adapted for large meta-analyses (reviewed in van Der Werf et al., 2021). The choice to use yield ratios (partial LERs) rather than yield differences to compare the mixing abilities of wheat genotypes was also supported by their fixed sowing density in IC, set as a percentage of their sowing density in SC. Therefore, we expected that their yield in IC would also depend on their yield potential in SC, which may vary from one environment to another, and should be

expressed as a percentage of the yield in SC rather than through a yield loss between SC and IC. The chosen wheat genotypes also differed significantly in terms of yield potentials in SC (for instance, the Flamenko and Ehogold genotypes presented a mean yield of 50 and 40 q/ha in SC, respectively), so using the yield loss between SC and IC to qualify mixing ability would have suffered a possible confusion with productivity. The low and non-significant correlation coefficients between LERw and yield in SC for the 8 wheat genotypes under study (**Table 9**) validate this choice *a posteriori*. Finally, the application of models (this issue) to differences led to much higher $G \times E$ interactions (data not shown). Using yield ratio instead of yield loss between IC and SC, therefore, makes it possible to differentiate the mixing ability of varieties from their productivity in SC and to compare properly the varieties for their mixing ability in very different environments.

We did not correct partial LERs for initial sowing densities in IC, because the sowing ratios were all identical between wheat genotypes for a given mixture and chosen according to a potential farmer's objective aiming at harvesting a balanced quantity of both species.

Wheat Genotypes Show Contrasted Profiles of Mixing Ability

The observed significant differences between wheat genotypes in their ability to produce (producer effect) and make their mixed tester produce (associate effect) in intercrop confirm the relevance of the traits considered in the choice of wheat varieties (i.e., potential yield, earliness, and height at heading stage in SC). Indeed, the traits were previously reported to impact significantly the competitive ability of a species in general (Annicchiarico et al., 2019), and particularly of cereals (Haug et al., 2021; Kammoun et al., 2021) in IC. Considering both productive and associate effects of the genotypes, it is likely that complementation for resource use took place in most situations, since mean LERt values across environments were all above 1.26 (**Figure 3**), which confirms a large consensus from previous results on intercropping binary mixtures (Bedoussac et al., 2015; Stomph et al., 2020). In most cases, competition between species also took place, since reduction in the partial LER of one species is generally compensated by a rise in the partial LER of the mixed species. Therefore, while some genotypes showed higher producer or associate effects, they were hardly different in terms of their global mixing ability (producer + associate effects). Noticeable exceptions were observed for the wheat genotype Flamenko, whose competitiveness does not let the legume produce at an expected level, and wheat genotypes Ehogold and CF14336, which generated higher mean LERt than the others (**Figure 3**) probably because of stronger facilitation effects.

Using the mean values of yield, earliness and height of each wheat genotype in SC, we have identified a possible impact of earliness or height on producer effects; late and high genotypes showing higher LERw. However, these relationships are particularly influenced in our study by the behaviour of the Ehogold and Forcali/Rebelde genotypes and should be confirmed on a larger set of genotypes. No other obvious links between

these traits and producer or associate effects were detected. This can be explained both by the small number of genotypes under study that does not allow for a clear relationship to be established, and by other plant and canopy traits that may impact competition and facilitation between species in IC. Indeed, in addition to height, earliness, and productivity, plant and canopy traits likely to be involved are numerous, such as early vigour, light interception, leaf area index (LAI), tillering ability, canopy architecture, crop ground cover, nutrient use efficiency, lodging, and disease resistance. Canopy height, lodging, and maturity date were, for instance, shown to be important determinants of forage yield and quality when oat was intercropped with vetch species (Assefa and Ledin, 2001). Furthermore, these traits interact strongly with cropping management, so their expression in SC is likely not to predict their expression in IC (Moutier et al., 2018, 2021; Kammoun et al., 2021). Indeed, the plasticity in traits initially identified in SC is a key issue to understanding cultivar adaptation to IC (Gaudio et al., 2019). Therefore, a study is in progress to define the plasticity of varietal and canopy traits in IC, test a larger range of variations of these traits, and try to identify other traits possibly correlated that may impact competitive ability in a complex way. It is likely, for instance, that different dynamics of canopy closure in IC among Ehogold, Flamenko, Forcali/Rebelde, and CF14336 may explain a part of their significant different competitiveness schemes (data not shown).

Grain Legume Tester Varieties Differ in Their Ability to Discriminate Wheat Genotypes

A significant impact of a mixed species on the performance of a target species has often been shown (Wendling et al., 2017). On the contrary, a recent study focusing on the general mixing (GMA) and specific mixing (SMA) abilities of barley genotypes showed that SMA is very low compared to GMA, which led to the conclusion that the performance of a genotype in intercropping hardly depends on mixed tester genotype, and that tester choice is not a key issue (Haug et al., 2021). Our study partially confirms these results, as there are no significant differences in the ranking of wheat genotypes according to their mixing abilities when the genotypes were intercropped with different grain legume testers. However, we report that the grain legume testers have different abilities to discriminate wheat genotypes, with some having a more stable impact on LERw than the others.

Among the grain legume testers, Geronimo and Spencer (Hr pea varieties) significantly discriminate the largest number of wheat genotype pairs for their mixing ability (both for producer and associate effects; **Table 8**), and they allow the wheat genotypes both to produce and to maintain associate effects, leading to highest LERt (**Table 7**). Fresnel (hr pea variety) only discriminates wheat genotypes that are extreme for their LERw and/or LERl (**Table 8**), and may be slightly less competitive than Geronimo and Spencer (not significant; **Table 7**). The faba bean grain legume testers Olan and Irena both fail in discriminating wheat genotype pairs for their mixing ability (except Olan with Forcali/Rebelde vs. Ehogold for LERw and Forcali/Rebelde vs.

Flamenko for LERl; **Table 8**). They also display lower LERw (although this effect is not stable between environments), leading to lower LERt, showing better competitiveness towards wheat (**Table 7**). Olan is the most competitive, probably because of its height and soil coverage power (**Table 1**), while Irena is less competitive (it is shorter and covers the soil more slowly; **Table 1**).

The number of representatives in each of the three cultivated types (pea Hr, pea hr, and faba bean) is far from being enough to definitively make a conclusion on potential interest on them as testers. Differences between Geronimo and Spencer on one side, and between Olan and Irena on the other side, show that there may be a variation in the cultivated types. We can, however, hypothesise that their different development dynamic cycles known from sole cropping probably affect differently their competitiveness. For the Hr pea testers, weak development at early stages allows for the wheat to establish during the winter, and then strong development from ramifications at the end of the cycle makes it possible to build up their own production (Lejeune-Hénaut et al., 2008). Stronger development during the whole cycle for hr pea testers or very strong development from the very early steps of the cycle during the winter for the faba bean testers do not help wheat to build up its own production. It is likely that discrepancies in both competitiveness and ability to discriminate wheat genotypes also result from traits impacting the relative use of resources (light interception and water and nutrient use from the soil due to differences in root development dynamics).

Although further confirmation may be needed, using one or two Hr pea testers, for both their competitiveness profile and their ability to select between wheat genotypes, may be the optimal way to assess and discriminate wheat genotype (or varieties) mixing abilities without checking for large sets of genotypes in the mixed species. A collateral benefit is the likely more synchronous ripening with wheat of this Hr pea than hr pea and faba bean.

Breeding for Mixing Ability Should Consider Both Producer and Associate Effects

There is a rather large consensus stating that since the higher performing genotypes in SC are not necessarily the higher performing in IC (Francis, 1981), specific breeding programs to optimise mutual cultivar adaptation to intercropping are needed (Nelson and Robichaux, 1997; Hauggaard-Nielsen and Jensen, 2001; Annicchiarico et al., 2019; Kammoun et al., 2021). This, however, includes choice of traits to select in SC and IC on different scales (plant, canopy) and rapid and cost-effective methods for their measurements, probably the combination of SC and IC evaluation on different steps of the selection process and recurrent intercrosses in each of the mixed species for recombination steps (Wright, 1985). This study opens the way to simplifying partly this process, since the preliminary choice of a tester variety in the mixed species may reduce the number of mixtures to be tested in the selection steps and limit the recombination steps to a target species. As usual, in breeding, developing breeding programs dedicated to adaptation to binary

IC depends on both the expected objective of a crop (balanced production between species or a priority on one or the other IC component, combined with a number of ecosystemic services) and whether the purpose is to create a variety that would be adapted to both SC and IC or specifically adapted to IC. We suggest that these two points are a prerequisite for defining primary screening traits, selection schemes (SC or IC at early and late generation levels), and procedures for variety certification. Our study does not definitely clarify these points but clearly confirms that both varietal choice, with some varieties moving away from the negative correlation between the producer and associate effects, and identification of an adequate tester species or variety are key points to move towards the expected objective of breeding for IC, and that, in most cases, breeding should consider both the producer and the associate effects.

CONCLUSION

Wheat genotypes, therefore, show various mixing abilities. Some lose more yield (in % of their SC yield) and/or cause greater yield loss to mixed species than others when intercropped. This confirms that the varietal factor is a key issue for farmers who need to consider the mixing ability of varieties when they choose to optimise crop yields as well as potential ecosystem services.

Considering that the impact of environment on wheat genotype effect on LERw and LERl remained moderate, that some genotypes seem to stand out from the negative correlation between the producer and associate effects, and, finally, that the ability to produce in intercropping of a variety does not seem to be correlated with its SC yield potential, developing breeding methods and procedures for mixing ability seems both possible and necessary. Among these, choice of tester, which seems to have a little impact on the ranking of the mixing ability of wheat genotypes but has an impact on genotype discrimination, can be helpful to breeders to reduce the number of combinations to be tested when screening large numbers of wheat genotypes for their mixing ability. A study is currently in progress, with a view to registering wheat varieties bearing the mention “adapted to intercropping”.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, on demand, without undue reservation.

AUTHOR CONTRIBUTIONS

NM, AB, and AG wrote the manuscript. NM, AB, EH, PM, and MF designed the experiments. PM, EH, and NM managed the Dijon, Estrées-Mons, and Rennes experiments, respectively. SF and AG wrote the R scripts for the analyses. All authors reviewed the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.877791/full#supplementary-material>

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EDITED BY

Diego Rubiales,
Institute for Sustainable Agriculture
(CSIC), Spain

REVIEWED BY

Sushan Ru,
Auburn University, United States
Niels P. R. Anten,
Wageningen University & Research,
Netherlands
Stefano Tavoletti,
Marche Polytechnic University, Italy

*CORRESPONDENCE

Cyril Firmat
cyril.firmat@inrae.fr

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Linking quantitative genetics with community-level performance: Are there operational models for plant breeding?

Cyril Firmat^{1,2*} and Isabelle Litrico²

¹AGIR, INRAE, University of Toulouse, Castanet-Tolosan, France, ²P3F UR 004, INRAE, Le Chêne RD150, Lusignan, France

Plant breeding is focused on the genotype and population levels while targeting effects at higher levels of biodiversity, from crop covers to agroecosystems. Making predictions across nested levels of biodiversity is therefore a major challenge for the development of intercropping practices. New prediction tools and concepts are required to design breeding strategies with desirable outcomes at the crop community level. We reviewed theoretical advances in the field of evolutionary ecology to identify potentially operational ways of predicting the effects of artificial selection on community-level performances. We identified three main types of approaches differing in the way they model interspecific indirect genetic effects (IIGEs) at the community level: (1) The community heritability approach estimates the variance for IIGE induced by a focal species at the community level; (2) the joint phenotype approach quantifies genetic constraints between direct genetic effects and IIGE for a set of interacting species; (3) the community-trait genetic gradient approach decomposes the IIGE for a focal species across a multivariate set of its functional traits. We discuss the potential operational capacities of these approaches and stress that each is a special case of a general multitrait and multispecies selection index. Choosing one therefore involves assumptions and goals regarding the breeding target and strategy. Obtaining reliable quantitative, community-level predictions at the genetic level is constrained by the size and complexity of the experimental designs usually required. Breeding strategies should instead be compared using theoretically informed qualitative predictions. The need to estimate genetic covariances between traits measured both within and among species (for IIGE) is another obstacle, as the two are not determined by the exact same biological processes. We suggest future research directions and strategies to overcome these limits. Our synthesis offers an integrative theoretical framework for breeders interested in the genetic improvement of crop communities but also for scientists interested in the genetic bases of plant community functioning.

KEYWORDS

community genetics, eco-evolutionary dynamics, breeder's equation, agroecology, anticipatory predictions, general mixing ability (GMA), specific mixing ability (SMA), genetic variance

Introduction

Ongoing agricultural intensification, which started during the last century, has successfully increased yields by relying on large-scale monoculture. But it is now generally accepted that this form of agriculture is not sustainable and does not ensure stable food supplies in the current context of high environmental variability (Lin et al., 2008). Field experiments on grassland communities in the two last decades have established that species diversity is a strong determinant of yield, yield stability, and other agroecosystem functions (Hector et al., 1999; Tilman et al., 2001; Isbell et al., 2015). Congruent benefits have been obtained for cereal–grain legume intercrops (Hauggaard-Nielsen et al., 2008; Bedoussac et al., 2015; Annicchiarico et al., 2019; Demie et al., 2022). Together, these results suggest that, compared to monoculture, multispecies cover crops could be a way to maintain and stabilize high yields while reducing the demand for chemical inputs. Overall, growing several crop species in the same plot, either through intercropping, the use of companion crops, or agroforestry, is now among the most promoted strategies to develop sustainable and efficient farming systems (Beillouin et al., 2019).

Today, most efforts invested in plant breeding focus on improving a single species, ignoring the effect of ecological interactions with any other species potentially sown together. This dominant breeding strategy only considers ecological interactions of the improved cultivars in the field among conspecifics. This is questionable for two main reasons. First, well-established positive relationships between plant species richness and agroecosystem functioning (synthesis in Isbell et al., 2017) point to the fact that adding the right species to a monoculture can increase yield and other agronomically relevant parameters. Second, going on improving crops in pure stands if the target environment is a diversified cropping system is expected to substantially reduce the efficiency of breeders' actions (Annicchiarico et al., 2019). In these cases, continuing breeding in the same way as today might cause a lock-in in the transition toward more agroecological practices, making the situation problematic for the plant breeding sector.

Litrico and Violle (2015) proposed that redesigning breeding methods accounting for species interactions and their underlying traits could enable an upward shift of the typical saturating curve that links ecological functions and species richness in natural communities (refer to Tilman et al., 2014). Thus, the plant breeder's usual problem, i.e., “*What is the best way to improve the performance of this species from this gene pool?*” should become “*What is the best way to improve the performance of this plant community* (refer to Box 1) *by selecting within several gene pools?*” (refer to Box 2 for some illustrative examples). Regarding biological knowledge, this is far from a minor transition, as the second question requires coupling two biodiversity scales, the population genetic scale and the ecological community scale. The properties of a complex and

composite structure cannot be trivially extrapolated from the properties of its components (Anderson, 1972). Typically, the properties of a plant community cannot be trivially extrapolated from the genetic composition of its individual component species. Such an extrapolation remains a key challenge in applied evolutionary and ecological research (Levin, 1992).

As a professional practice rooted in quantitative genetics, the original objective of plant breeding was and continues to be population-level variation. The quantitative genetic bases of species interactions and community properties were thus broadly excluded from the rich history of modeling efforts (refer to e.g., Walsh and Lynch, 2018). Although the implications of ecological interactions among individuals within species have been well studied by quantitative geneticists (Griffing, 1967; Moore et al., 1997; Wolf et al., 1999; review in Bijma, 2014), this does not address the coupling between two nested diversity scales as interspecific indirect genetic effects (hereafter IIGE, refer to Box 1). The evolutionary dynamics of IIGE do not involve the same processes as indirect genetic effect *within* species as the latter is assumed to account for individual relatedness (Queller, 2014). The study of IIGE needs to assume that individual relatedness is null and its effects can be treated as an external environmental influence whose variability is partly determined by genes (Goodnight, 1991).

The scope of the present article (Figure 1) is to explore recent modeling efforts conducted under the umbrella of eco-evolutionary dynamics (Hendry, 2016) from a plant breeding perspective. This exploration can be an inspiring source of concepts and models to extend the practice of plant breeding to the crop community scale, i.e., the minimum scale of interest from an agroecological point of view. While evolutionary biology (as well plant breeding) was for many years focused on the effect of natural (or artificial) selective factors on evolution (or “genetic improvement”), eco-evolutionary approaches have also investigated the effects of evolutionary changes in the environment. Eco-evolutionary thinking addresses the problem of the relationship between populations and their environment as a feedback loop between ecology and evolution (Schoener, 2011). It is therefore potentially relevant for the challenge facing community-level plant breeding today. The first task is to provide breeders with genetic models to help them to identify the range of community-level breeding problems. In this review, we postulate that the “evo-to-eco” half of the eco-evolutionary causal circle can provide some of the missing elements required to renew plant breeding practices and orient them toward more integrative and sustainable objectives.

As stressed by Casler and Van Santen (2010), plant breeders often have to make difficult choices based on incomplete scientific knowledge. Current plant breeding practice mostly pursues continuous improvement of predefined value criteria of a given cultivar (i.e., through the notions of ideotype and breeding indices). Accounting for genetic diversity and species interactions to improve mixed cropping systems requires

BOX 1 Definitions of specific terms used in the text.

Plant community: Synonym for intercropping systems or mixtures of species used in the context of this review. Assemblage of plant species (two or more) growing in the same place and interacting through processes such as competition, facilitation or resource partitioning. These interactions and their variability generate functional properties measurable at the scale of the whole community (refer to examples in [Box 2](#)).

Community-level variable (c): The target of selection predefined by the breeder according to agronomic objectives. Its variation is expected to result from both *direct (DGE)* and *interspecific indirect (IIGE) genetic effects* that occur within the *plant community*. It is modeled as a linear selection index. For two species (A and B) in the mixture, each with n traits: $c = (z_{A1} + z_{A2} + \dots + z_{An}) + (z_{B1} + z_{B2} + \dots + z_{Bn})$. Specific cases of c can be simplified to the yield measurements of a single *associated species* of economic interest in the mixture (thus, e.g., $c = z_{A1}$). The phenotype z_{A1} of this species is expected to be affected by *IIGE* from the genotypes of one or several species in the mixture.

Indirect genetic effect (IIGE): The expression of genes in one species affecting the phenotypic expression in individuals of another species and potentially the value of the functional trait (i.e., the *community-level variable c*) at the whole community level. It is therefore distinct from the usual direct genetic effects (DGE) qualifying the expression of genes on the phenotype of the individual bearing these genes.

Focal species: A species for which genetic relatedness is experimentally known to measure the variation in its indirect genetic effect (i.e., the *IIGE*) on a *community-level variable (c)* or on the phenotype of an *associated species*. This makes it possible to select genotypes of this focal species based on their *IIGE* of interest.

Associated species: A species grown in interaction with a *focal species*. Phenotypes of this species are measured but genetic relatedness among its individuals may remain unknown in the experimental design.

BOX 2 Some examples of agronomic goals that could be achieved using breeding approaches that account for interspecific indirect genetic effects.

To connect the model variables used in the text with agronomic problems, below we use letters in parentheses to identify: the community-level variable as a final goal for the breeder [c] and the candidate traits [z] assumed to affect it, as measured in a species of the sown community (see main text for details).

Persistence of legumes in sown grasslands. In low input multispecies grasslands, the persistence of legume species [z] in the cover is a major determinant of the effects of biodiversity on yield ([Brophy et al., 2017](#)), and legumes are generally the most sensitive component. Persistence is a heritable trait that can be improved by selection ([Smith and Kretschmer, 1989](#); [Casler and Van Santen, 2010](#); [Annicchiarico et al., 2019](#)). Although community performance such as multiyear biomass production and quality [c] is the final objective of selection, we have no direct indication of the effectiveness of breeding programs for legume persistence at the grassland community level. Accounting for *IIGE* linked to the legume persistence trait at the community level and above (e.g., N release that enhances the overall performance of the cropping system) could improve the agronomic relevance of such breeding efforts.

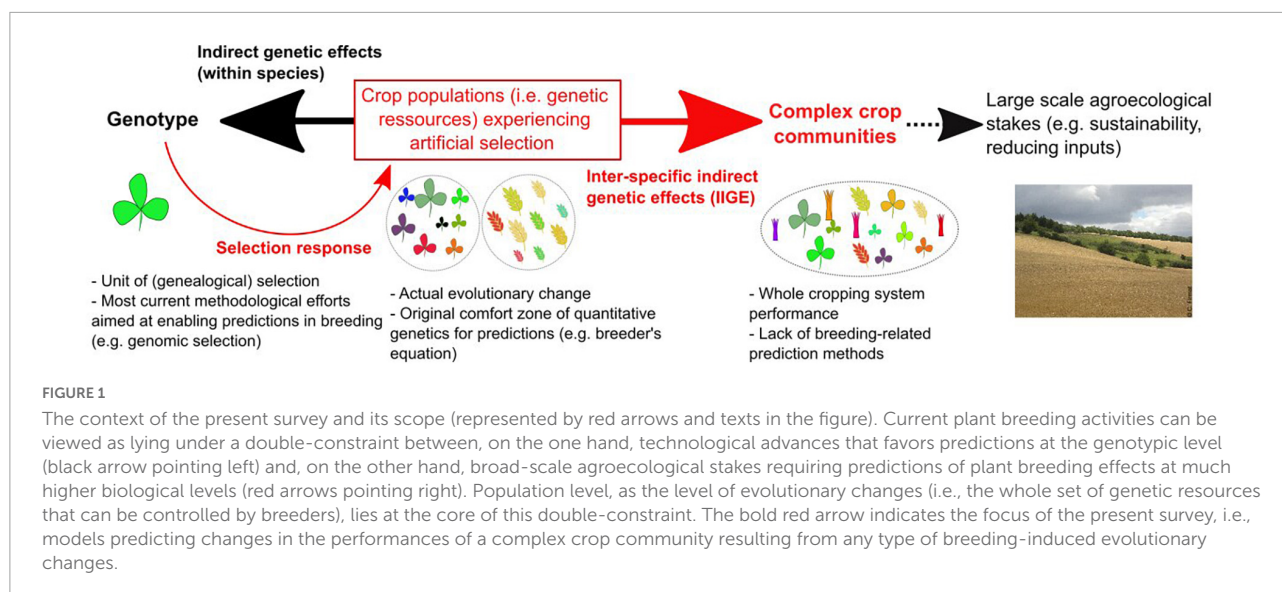
Weed suppression in faba bean. Faba bean is among the most promising species in temperate areas to develop to increase protein autonomy, but is quite sensitive to competition with weeds. Intercropping faba bean with cereals is a sound management strategy to solve this problem ([Jensen et al., 2010](#)). In barley, resistance to weed competition is known to vary among cultivars ([Dhima et al., 2000](#)). Consequently, breeding cereals for increased weed suppressing effect [z] can be an *IIGE* to target to improve faba bean grain yield in no herbicide systems [c].

Lodging reduction in sensitive grain legumes. Lentil or pea is intercropped by farmers with cereals or with Brassicaceae to avoid legume lodging and to improve their mechanical harvestability ([Cowell et al., 1989](#); [Viguié et al., 2018](#)). The rigidity of the stem of the non-legume companion species [z] thus becomes a trait of interest for legume performance and harvestability [c]. Stem rigidity is known to be genetically variable and, accordingly, performance in intercropping can differ among cultivars (e.g., [Cowell et al., 1989](#)). Selecting for this trait can produce a positive response at the scale of the intercrop, through an *IIGE* on the legume component. In addition, the lodging resistance traits of the legume lose their relevance under intercropping ([Hauggaard-Nielsen and Jensen, 2001](#)), allowing breeders to invest efforts in other traits more closely related to yield performance.

accounting for the distributions of traits to identify the best performing genotypes ([Litrico and Violle, 2015](#)). The breeder's choices become even more difficult and knowledge even more incomplete as the biological system to be managed becomes substantially more complex. We consider that selection theory is a powerful tool for objectivizing daily breeding practice and appraising its future orientations ([Cobb et al., 2019](#)). In the present context, we need to further extend this theory to shed light on the community-level consequences of choosing certain genotypes within a range of genetic resources. We thus aim to describe models that provide guidance for choosing the best strategy when breeding targets include interacting species. To guarantee the practical relevance of our survey, we focused on models based on the breeder's equation or its parameters ([Walsh and Lynch, 2018](#); see details at the end of this section). Modeling approaches based on more complex models that are usually designed for the purpose of disentangling eco-evolutionary causal feed-back in the wild

(typically: [Ellner et al., 2011](#)) are consequently not relevant for our purpose here.

We first distinguished “evo-to-eco” approaches in the literature according to the concepts they use to interface the two biodiversity scales: the population-level response to selection and a community-level variable. Thus, for each approach, we start by providing the assumed underlying statistical equation for interfacing the community variable with quantitative genetic effects. We then discuss whether each approach is potentially “practically operational” when used by practitioners to manage crop genetic resources (i.e., breeders, but also certain farmers and crop conservationists). Here, we define “practically operational” as fulfilling three criteria: first, model parameters are expected to be estimated or approximated from data routinely obtainable by practitioners or with reasonable additional experimental efforts and no loss of effectiveness in their breeding activities; second, these models should be meaningful in the sense that they rely on biologically realistic



assumptions and make predictions based on a theoretically sound framework (sensu Houle et al., 2011), here, the breeder's equation; third, we expect the modeling approaches to provide practitioners with *anticipatory predictions*.

Description of the modeling approaches

Epistemological remark

Below we follow Maris et al. (2018)'s enlightening distinction between anticipatory and corroboratory predictions. Corroboratory predictions are hypothesis-derived predictions. Their goal is to be compared to observations for the *purpose of understanding*. They are necessary for corroborating or invalidating a hypothesis. In this paper, we analyze how quantitative genetic models of selection across two levels of diversity can provide operational anticipatory predictions. Anticipatory predictions consist in the application of extant knowledge. They can be used to achieve a *transformative goal* (Maris et al., 2018), i.e., the essence of plant breeding. Indeed, breeders usually have to act on a genetic system before knowing exactly how the system they are acting on will react (i.e., before knowing its response to selection). In this context, anticipatory predictions are expected to fuel a decision-making process by improving the intelligibility of the potential effects of breeders' practices on complex, multispecies genetic systems. For this purpose, the three criteria mentioned in the previous section are tightly interlinked.

Notation

The breeder's equation and its equivalent forms (Walsh and Lynch, 2018) predict the mean population change in a trait z from the product of a measure of genetic variation (such

as heritability) by a measure of selection strength (such as a selection differential, S):

$$\Delta \bar{z} = h^2 S \quad (1)$$

with the heritability $h^2 = \frac{\sigma_G^2}{\sigma_P^2}$, i.e., the ratio of the (additive) genetic to total phenotypic variance of the measured trait. This simple formula is widely used by breeders and evolutionary biologists but under several equivalent forms (cf. Walsh and Lynch, 2018; Bijma, 2020). Breeders emphasize the selection intensity $\bar{i}$ and the accuracy of selection h :

$$\Delta \bar{z} = \sigma_G \left(\frac{\sigma_G}{\sigma_P} \right) \left(\frac{S}{\sigma_P} \right) = \sigma_G h \bar{i} \quad (2)$$

while the equivalent version by Lande (1979) is most commonly used in evolutionary biology:

$$\Delta \bar{z} = \sigma_G^2 \left(\frac{S}{\sigma_P^2} \right) = \sigma_G^2 \beta \quad (3)$$

with β the linear selection gradient, i.e., the slope of the linear regression of the relative fitness w on trait z ($\beta = \text{cov}[z, w] / \text{var}[z]$). This last version (eq. 3) can be extended to predict the change in the multivariate case through the concepts of G-matrix and multivariate selection gradient β (Lande, 1979):

$$\Delta \bar{\mathbf{z}} = \mathbf{G} \beta \quad (4a)$$

or, under a developed matrix form for l traits:

$$\Delta \bar{\mathbf{z}} = \begin{bmatrix} G_{11} & G_{12} & \dots & G_{1l} \\ G_{21} & G_{22} & \dots & G_{2l} \\ \vdots & \vdots & \ddots & \vdots \\ G_{l1} & G_{l2} & \dots & G_{ll} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_l \end{bmatrix} \quad (4b)$$

with G_{ii} the genetic variance for the i^{th} trait and G_{ij} its genetic covariance with the j^{th} trait, and β_i the selection gradient for

the i^{th} trait. By focusing on the case of artificial selection for plant breeding, we assume that selection is strong, and that β is controlled by the breeder, therefore known with high accuracy. Consequently below, the error due to the incidental influence of natural selection on potentially unmeasured traits (synthesis in Walsh and Lynch, 2018, Chapter 20) is treated as negligible.

In the following, depending on the approach described, we refer to each or all of the three equivalent formulations mentioned above (eqs. 1, 2, 3, or 4) to extend the effects of genetic evolution (in response to artificial selection) on a community-level variable. Below, this *community-level variable* is labeled c (refer to Box 1 for a definition and refer to Box 2 for biological examples). c potentially encompasses several components of community functions such as the total biomass yield of mixtures of perennial forage crops, or the yield of cereal grain when a cash crop and a companion forage legume are selected together, or any other ecological functions such as weed suppressing effects among grain legumes when a cereal species is intercropped for this purpose (refer to the example of faba bean in Box 2). As we will see below, c can be a community-level index including weighted performance components from several species and functions.

Our focus is on the links between the population and the community levels. Thus, we do not distinguish between the type of breeding scheme or methods, which are well summarized elsewhere (cf. Walsh and Lynch, 2018). We thus made our analytical framework as general as possible, so it can be used for different artificial (i.e., intentional) multispecies selection approaches based on randomized trials with available estimates of genotypic or phenotypic values for selection candidates to produce the next generation. It is therefore able to cover a wide range of breeding strategies and tools depending on how the quantitative genetic parameters are estimated (e.g., mass, family-based, genomic selection). Our analytical framework can therefore be easily adapted to account for breeding cycle length, plant reproductive systems (self- and cross-pollinated crops), or any other parameters that affect breeding efficiency, for instance in simulation approaches (Bančič et al., 2021).

Approach 1: Estimating the heritability of interspecific indirect genetic effects

Generalities and theoretical grounding

The most straightforward and intuitive approach to examine how genetic variation affects a variable c at the community level is to apply the standard quantitative genetic expression to the variable concerned:

$$c_{ik} = \mu + g_{Bi} + e_{ik} \quad (5)$$

This assumes that the value of c for the i^{th} community comprised of associated species (refer to definition in Box 1) will deviate from its mean μ , under the influence of the genotype

g_{Bi} of a focal species B (Box 1) and under the influence of environmental effects e_{ik} (which might include genetic effects originating from other species not controlled for here, see below). This is the basic rationale in community genetics: genetically related individuals belonging to a focal species (also termed “foundation species” in the ecological literature) will favor similar patterns and similar values for the community-level variable (Whitham et al., 2003).

The value of c is treated as a “community phenotype” or a community-level variable (Box 1): the phenotype of one or several associated species with a genetic variance σ_g^2 and “community heritability” $H_c^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$ (Fritz and Price, 1988; Whitham et al., 2006). We use uppercase H to denote the broad sense heritability usually estimated in community genetics. Community genetics usually investigates the effect of the genetic variation in a focal plant species on its associated arthropod community. The experimental settings typically consist in a common garden with randomized replicated clones of the focal species and measurements taken at the community level such as arthropod abundance or species richness (reviews in Haloin and Strauss, 2008; Genung et al., 2011; Tack et al., 2012).

In such biological systems, the variable c only considers the IIGE on the arthropod community traits, excluding DGE on the plant species. Community heritability is therefore a measure of IIGE variance (which would not be true if c was, e.g., the total biomass of the whole plant-arthropod system). Thus, we now refer to IIGE heritability, H_{IIGE}^2 . The notion of IIGE heritability as presented above presents an analogy with population-level heritability without assuming that communities can be selected as a whole (Collins, 2003). It is thus a matter of some debate. Could H_{IIGE}^2 be predictive of the response to selection of the community trait according to the breeder's equation, i.e., $\Delta \bar{c} = H_{IIGE}^2 S_c$? (with S_c a hypothetical selection differential on c). Community ecologists who quantified this parameter warned against such interpretation (Whitham et al., 2006; Genung et al., 2011). Whitham et al. (2006) asserted that this approach does not imply that communities have a fitness in the wild or evolve as populations do. Rather, they called for an interpretation of H_{IIGE}^2 as an integrative measure of the cascading effects of genes of the focal species at the community level. This means that H_{IIGE}^2 should be taken as a measure of an association to estimate how much of the variance in the community variable results from genetic variation in the focal species.

Practical application for breeding

The notion of IIGE heritability was designed to investigate the strength of IIGE of host plants on other taxa. To what extent could it be useful for breeders interested in improving plant communities? The first practical advantage over a more fine-grained approach (see below) is that it is trait free: it does not investigate the effect of the phenotype of the focal species on the community variable (Figure 2A) and instead quantifies

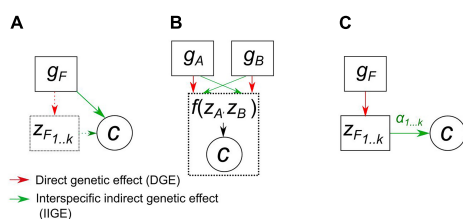


FIGURE 2

Causal pathways between genetic effects, g , and a community variable, c , as assumed in the quantitative genetic models described in the main text. (A) IIGE heritability approach: a genetic variance component of a focal species (g_F) is used to quantify the genetic basis of variation in a community trait. The direct genetic effect on the focal species trait are ignored (dotted line). (B) Joint phenotype approach: the genetic variation of two species is treated symmetrically, considering for each their respective direct and indirect genetic effects. This is a variance component analysis that might either decompose on each species phenotype or directly on the community variable, the joint phenotype, if the two species are harvested together and not sorted. (C) Community-level index for traits: the community variable is regressed on the l traits of the focal species. The regression coefficients are then used to project genetic changes of these traits onto the community variable using a standard linear selection index approach.

IIGE as a whole, thus limiting the need for trait phenotyping. For estimating IIGE heritability, a breeder would first have to choose a focal species and control for its genetic variation within a standard range of experimental crop communities. H^2_{IIGE} would provide insights into the expected efficiency of selection of the focal species with respect to the improvement in the community variable.

The only example we found of H^2_{IIGE} estimates in a crop community in the literature was provided by Maamouri et al. (2015). These authors grew 46 contrasted lucerne genotypes (*Medicago sativa*) with a grass (*Festuca arundinacea*) grown as a companion crop. While they found strong average broad-sense heritability for the direct genetic effect on lucerne biomass ($H^2 = 0.76$, range across sampling rounds: [0.64 – 0.83]), the broad-sense heritability on the grass biomass was much lower ($H^2_{IIGE} = 0.05$ [0.00 – 0.17]). This suggests that selection on lucerne biomass would have at most minor consequences for the grass biomass.

Heritability estimates of IIGE or at the community level are exposed to the same flaws as heritability estimates at the population level (Hansen et al., 2011), including high dependency on the study context and assumptions and computation preferences (Wilson, 2008; Firmat et al., 2017). When the community variable is on an arithmetic scale such as yield, this can be partly improved by computing the coefficient of genetic variation for IIGE (i.e., the IIGE standard deviation standardized by the mean value of c , refer to Hansen et al., 2011). Such mean standardized estimates could help to perform meaningful comparisons across breeding populations and testing sites to adjust community-level selection strategies.

Although in nature, the value of c is not directly associated with a value of fitness (genetically based selection does not act at the community level), this might be the case in a breeder's field if artificial selection is performed among isolated and genetically controlled communities. In this case, H^2_{IIGE} estimates might become interpretable within the breeders' equation. However, DGE and IIGE can sometimes be selected as a whole. This is the situation addressed by the breeder's version of the joint phenotype approach described in the following section.

Approach 2: Joint phenotype

Generalities and theoretical grounding

Summing the genetic contributions of several species in a community trait is an alternative approach to interfacing responses to selection between the population and the community-level. The simplest is to sum the genotypic values of each interacting species (Queller, 2014):

$$c_{ijk} = g_{Ai} + g_{Bj} + e_{ijk} \quad (6)$$

where g_{Ai} and g_{Bj} are the breeding values for individual i of species A and individual j of species B , respectively. According to this statistical expression, the joint phenotype c is defined by Queller and Strassmann (2018) as "a trait or outcome that can be affected, and potentially evolve, under the influence of two or more parties." Both species are treated symmetrically and the joint phenotype results from the potential interaction between species, but the details of these interactions (values of interacting traits, complex feedbacks, etc.) are treated as a black box (Figure 2B). The evolutionary properties of joint phenotypes were investigated by Queller (2014) by applying the Robertson-Price equation (Frank, 2012). Queller (2014) showed that the change in c is predicted by the sum of the genetic variance for each species, respectively multiplied by their selective effects on c , i.e., the covariances $\sigma(w_A, c)$ and $\sigma(w_B, c)$. A non-zero covariance between fitness w and c indicates that natural selection acts on species' traits that affect the community variable (Johnson et al., 2009). When the two covariances for each species are of opposite signs, a change in c results from an evolutionary conflict between the two species: an increase in fitness in one species is constrained by increased fitness in the other species.

If c is the amount of light captured within a plant community (Queller and Strassmann, 2018), an increase in size in species A will increase its competitive effect by negatively affecting the fitness of species B , and vice versa. The conflict can be resolved and c increased further if, for instance, one species evolves a strategy of shade tolerance, generating niche differentiation with respect to light. Recent studies in multispecies grasslands showed that niche differentiation and complementary resource use can evolve in a single generation (van Moorsel et al., 2018; Meilhac et al., 2020). As such, the

concept of joint phenotype developed by Queller (2014) might provide a relevant theoretical framework to investigate the genetic determinants of such patterns. However, the analysis by Queller (2014) assumes regulation processes under natural selection in both species, which is of limited relevance for guiding artificial selection at the community level, which requires more specific modeling.

An analog version was coined for plant breeding many years ago. Wright (1985) proposed a selection model relying on the variance decomposition of a joint phenotype summing the “economic yield” of each species. This approach was inspired by factorial designs used to improve the parental population for breeding schemes for hybrid maize. The scale shift here relies on an analogy between the genetic combining ability of alleles (i.e., within a genome) from inbred lines and the ecological combining ability of genotypes (i.e., among distinct genomes) from different species.

Practical application for breeding

Wright (1985) approach aimed at modeling genetic gain on the joint phenotype when selection is performed at the scale of the population of bispecific communities that differ in their genotypic composition. Fully dissecting the genetic variation in the joint phenotype across a population of genetically controlled artificial communities requires a factorial design including a large number of combinations of each genotype of species *A* sown with each genotype of species *B*. Accordingly, two sorts of variance components of *c* can be partitioned: (1) the average or general effect of a genotype of one species on the joint phenotypes *c*, i.e., the general mixing ability (GMA) including both DGE and IIGE of this genotype on *c*; (2) the specific effect resulting from genotypic interactions deviating from additivity and quantifying the specific mixing ability (SMA) of individual pairs of genotypes on *c* (for more details see: Annicchiarico et al., 2019; Sampoux et al., 2020). Put simply, the GMA component of variance includes both additive direct genetic effects and effects of additive ecological interactions on *c* (average IIGE of a genotype across the factorial), whereas the SMA component of variance quantifies the contribution of non-additive effects on *c*.

Today, 35 years after Wright's publication, no prediction based on a full decomposition of the variance of *c* in a bispecific community has yet been made. This is likely due to the demanding experimental design required for such a decomposition. For two species and a minimum of 30 candidate genotypes each and three replicates per pair, the number of plots would be $3 \times 30^2 = 2,700$, corresponding to a huge single site experimental design beyond the reach of most breeding programs. With a third species, the required experiment becomes completely unrealistic (81,000 plots) (but refer to Haug et al., 2021).

Specifically targeting the GMA variance of interacting species (Hill, 1990) can substantially reduce experimental requirements. This involves parallel selection of the GMA of the

two species, each with a specific (recurrent) selection process. For each of the two species, candidate genotypes of the selected species (let's say species *A*) are sown in mixture with a mixture of representative genotypes from the other (unselected) species (let's say species *B*), used in the mixture as a tester for genotypes of *A*. In parallel, the same procedure is performed for species *B* with a mixture of representative genotypes of species *A* used as testers (i.e., selection in parallel for GMA, refer to Figure 2 in Sampoux et al., 2020). According to Sampoux et al. (2020), when targeting GMA variance for *c* by selecting on, e.g., species *A*, the measurable component of the joint phenotype *c* is as follows:

$$c_{Aij} = \mu_A + \mu_B + v_{Ai} + a_{Ai} + A_{ij} \quad (7)$$

with μ_A and μ_B the mean value of species *A* and *B* contributing to the community value *c*. Here, the controlled genetic variance of *c* in the design is only due to species *A*, with v_{Ai} and a_{Ai} the direct genetic (DGE) and the indirect genetic effects (i.e., IIGE, *a* for “associated effect” in breeding terms) of species *A*, respectively. Together, these terms model the GMA of the *i*th genotype of species *A*. From this expression, improving *c* by selecting on species *A* only gives the following expected response for the joint phenotype:

$$\Delta \bar{c} = \frac{\bar{i}_c}{\sigma_c} [\sigma_{v_A}^2 + 2\sigma_{v_A, a_A} + \sigma_{a_A}^2] \quad (8)$$

$\bar{i}_c$ is the selection intensity on the value of *c* and σ_c the total phenotypic variance for *c*. Under the procedure of selection in parallel for GMA described above, the analogous expression can be derived for species *B* (with *A* as a tester), also contributing to genetic gain for *c*. The value between brackets is the variance of the joint phenotype caused by genetic variation only in species *A* on which selection is performed. This variance expression is the sum of two components: the direct response to selection on *c* for the contribution of species *A* ($\sigma_{v_A}^2 + \sigma_{v_A, a_A}$) and the correlated response on the value of species *B* ($\sigma_{v_A, a_A} + \sigma_{a_A}^2$) [for a detailed argument including a release from the assumption of the equal species weights, refer to Sampoux et al. (2020)]. Both components include the covariance between the DGE and the IIGE of the selected species. Interestingly, eq. 8 parallels the results obtained by Griffing (1967) for the response to group selection at the intraspecific level, when assuming unrelated interacting individuals.

The main advantage of this joint phenotype approach is that it emphasizes the constraining role of this covariance term for community-scale genetic improvement. If $\sigma_{v_A, a_A} < 0$, improving the contribution of species *A* (DGE) to the mixture while not accounting for its IIGE on *B* (a_A) is not possible without weakening the contribution to the mixture of species *B* (Wright, 1985). This typically happens when selection for yield in *A* increases its competitive effect, thus weakening the contribution of *B*. Breeders that has to cope with an evolutionary conflict (*sensu* Queller, 2014) and manage a trade-off between species vs. community performance. Performing two selection

processes in parallel (one for each species), as proposed by [Sampoux et al. \(2020\)](#) and described above, could be a promising way to deal with such an evolutionary conflict.

By allowing integrative decomposition of the genetic interactions underlying the variation in a community variable, the joint phenotype approach provides quantitative genetic expression of the among-species conflicts the plant breeder will have to deal with. However, this approach enables prediction across biological scales (from species quantitative traits to c) through estimates of covariances (i.e., σ_{v_A, a_A}) between traits measured in two different species (as the IIGE of species A affects, e.g., the yield of species B). The nature of this parameter exposes quantitative predictions to strong limitations of both biological and methodological origins, as we will see below (refer to section headed “Common limitations and links between the three approaches”).

Approach 3: Community-trait genetic gradient

Generalities and theoretical grounding

In contrast to the two previous approaches, this approach relies on the functional trait assumption: a set of functional traits, and not genotypes, affects the community variable ([Figure 2C](#)). Investigating how a set of traits measured in a single species affects community functioning requires a multivariate approach linking the evolution of genetically correlated functional traits to a community-level phenotype. The multivariate selection model proposed by [Johnson et al. \(2009\)](#) relies on the notion of a “community-trait gradient”: a multiple regression of a single community trait c on a set of l evolving traits for a focal species. Variation in the community variable is therefore modeled as follows:

$$c_{ik} = \mu_c + \sum_{j=1}^l \alpha_j g_{ij} + e_{ik} \quad (9)$$

where α_j is the partial regression coefficients for the j^{th} trait and g_{ij} its genetic value for the i^{th} genotype at trait j . The estimated vector of coefficients makes it possible to project the multivariate evolutionary change in a focal species on the ecological variable. This is a way of modeling the IIGE of a complex, multivariate phenotype on a predefined community variable. This “matrix projection model” therefore builds on [Lande’s \(1979\)](#) formulation of the breeder’s equation to extend it to a community variable ([Johnson et al., 2009](#)):

$$\Delta \bar{c} = [\alpha_1, \alpha_2, \dots, \alpha_l] \begin{bmatrix} G_{11} & G_{12} & \dots & G_{1l} \\ G_{21} & G_{22} & \dots & G_{2l} \\ \vdots & \vdots & & \vdots \\ G_{l1} & G_{l2} & \dots & G_{ll} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_l \end{bmatrix} \quad (10a)$$

or, for a more synthetic notation:

$$\Delta \bar{c} = \alpha^T G \beta \quad (10b)$$

The second and last terms are the elements of the standard multivariate breeder’s equation, respectively, the G-matrix, and the linear gradient of selection ([Lande and Arnold, 1983](#)), i.e., the vector of partial regression coefficients of each j trait on fitness, whose product gives the expected response to selection in the vector of traits. The first term is the vector of partial regression coefficients (estimated independently in eq. 9), allowing the projection the genetic changes on the community variable (the subscript T denotes the transpose of this vector). This projection of evolutionary change corresponds to the sum of the evolutionary changes for the l traits, weighted by the α_j coefficients (refer to [Appendix 1](#), for a derivation of this expression using the Robertson theorem). In ecological terms, these coefficients represent the level to which each j trait is “functional” (refer to [Litrico and Violle, 2015](#)), i.e., interact with the species of the targeted community and affect the community-level variable.

[Johnson et al. \(2009\)](#) originally used this model to investigate the effect of functional trait variation in a wild species *Oenothera biennis* (Onagraceae) on the associated arthropod community. The model described above helped these authors formalize a set of conditions for the evolution of IIGE on the focal plant species: the trait j should be genetically variable ($G_{jj} > 0$), selection should affect the trait ($\beta_j \neq 0$), and the trait should cause variation in the community variable ($\alpha_j \neq 0$).

Practical application for breeding

[Johnson et al. \(2009\)](#)’s approach is strictly analogous to a linear selection index: it weights the sum of change in each dimension of a multivariate phenotypes to project it on a single variable, i.e., the selection index. The only – but nevertheless significant – difference is that the coefficients of the index are estimated statistically (eq. 9, refer to [Appendix 1](#)). Selection indexes are widely used by breeders and their properties are well known ([Hazel et al., 1994](#)). According to the properties of the linear selection index ([Lin and Allaire, 1977](#); [Nordskog, 1978](#)) and using [Johnson et al. \(2009\)](#)’s notation, the heritability of the community variable described by k underlying traits is given by:

$$h_c^2 = \frac{\alpha^T G \alpha}{\alpha^T P \alpha}, \quad (11)$$

with P the phenotypic variance–covariance matrix among traits. Thus, the response to selection in equation (10b) can be expressed in the selection intensity form, which is more familiar to breeders:

$$\Delta \bar{c} = \bar{i}_c \frac{\alpha^T G \alpha}{\sqrt{\alpha^T P \alpha}}, \quad (12)$$

with $\bar{i}_c$ the univariate intensity of selection applied to the community variable.

From a practical point of view, this approach includes two independent steps. The first step aims to estimate how the phenotypic trait values of the focal species affect the community variable (i.e., estimating α). The second step aims to quantify the genetic architecture of the ecologically relevant traits previously identified (i.e., estimating G).

This approach has two potential advantages. First, by explicitly modeling ecological functions in the first step, it avoids the potentially problematic use of covariances between IIGE and DGE, which play a central role in joint phenotype approaches, as this covariance is suspected of strong variability among experimental contexts (discussed in the following section). Second, the main practical advantage of this trait-centered approach is that it makes it possible to discard traits that have no influence on the target community variable c and to focus on the traits that have the main effects. From a multiple traits-community variable regression (eq. 9), a standard model selection procedure using information theoretical approaches (review in Grueber et al., 2011) could be used in the first step to discard traits that do not deserve to be taken into account for the improvement of c . This would make it possible to considerably reduce the size of the sample required for further quantitative genetic predictions in the following prediction step (eq. 10). Estimates of α could rely on biological and experimental knowledge from functional (agro)ecology (Garnier and Navas, 2012). The analysis of α would provide an innovative way to design for the focal species what we call “community-ideotypes,” i.e., ideotypes that not only target the focal species’ own performance but also the expected performance generated through IIGE by such an ideotype at the scale of the community. However, one should keep in mind that a trait j with no community-level effect ($\alpha_j \approx 0$) may still be useful to define the selection index due to its genetic correlation with other ecologically relevant traits.

Another key advantage of this approach is that it allows breeders to map the consequences of genetic constraints (i.e., genetic covariances among the focal species’ traits) in terms of response at the community scale. Let us assume a simple situation where the genetic covariance among two traits is positive ($G_{12} > 0$) and both α_1 and α_2 for these traits are also positive. Then, selecting for trait 1 alone is expected to improve the contribution of both traits to the community performance (i.e., as $\Delta\bar{c} = \alpha_1 G_{11}\beta_1 + \alpha_2 G_{12}\beta_1$). But if α_1 and α_2 are of opposite signs ($\alpha_1 > 0$, denoting, e.g., a direct genetic effect on total yield and $\alpha_2 < 0$, denoting, e.g., a negative competitive effect on a companion legume that supplies nitrogen), selecting for trait 1 alone is expected to reduce the value of trait 2 due to the positive genetic covariance. In other words, this could enable breeders to easily objectivize the consequences of their usual (i.e., species-centered) work at the community scale, by generating anticipatory predictions at this level. For instance, by extending the standard breeding indices based on a series of traits measured in a pure stand to predict

performance in a mixed stand (e.g., Annicchiarico, 2003), this approach would make it possible to predict the consequences of this species-specific performance at the community scale. This includes the quantitative genetic exploration of the promising notion of “biological interaction function” coined by Haug et al. (2021). To sum up, Haug et al. (2021) suggest correlating both direct and indirect genetic effects with measured traits that generate different types of species interactions in the mixture, leading to the identification of traits that produce favorable biological interactions at the community scale. To identify suitable cultivars for cereal–legume mixtures, Kammoun et al. (2021) argue for a simple statistical approach that predicts reliable mixtures based on pure stand performances for both components combined with a description of their interaction function. We believe that such a basic set of variables would be a reliable starting point for designing selection criteria for this type of simple crop mixture.

This approach is based on the predictions of c from genetic (co)variation in a single species. It therefore assumes that one species needs to receive more attention than any other species from the breeder. We observed that this is the most frequently reported case in the plant breeding literature (for recent examples: Annicchiarico et al., 2021; Ergon and Bakken, 2022; Moutier et al., 2022).

Common limitations and links between the three approaches

Integrative expressions for community-level selection response

The three approaches we identified are distinct in several conceptual, statistical, and practical dimensions (Table 1). These approaches rely on distinct strategies for modeling causal pathways between genetic variation and community-level variation (Figure 2). However, a general model linking all three approaches is possible. The community-trait genetic gradient approach could theoretically be extended to more than one species. The evolution of c affected by two species A and B represented by l traits each (kept equal for the sake of simplicity) can also be modeled as the evolution of a linear index:

$$\Delta\bar{c} = \sum_{j(A)=1}^l \alpha_{Aj} \Delta\bar{z}_{Aj} + \sum_{j(B)=1}^l \alpha_{Bj} \Delta\bar{z}_{Bj} \quad (13)$$

This equation simply sums the ecologically weighted effects of each evolving trait for each species. But note that changes in traits in species A and B are not independent, but are linked through genetic covariance across each pair of species traits, caused by genetic interactions among species (IIGE) mediated by interaction traits (*sensu* Litrico and Violle, 2015). The sum of the trait’s specific α for each species corresponds to their respective weights regarding their contribution to c . The

TABLE 1 Main features of the three modeling approaches described in the main text.

Modeling approaches:	(1) Heritability of IIGE	(2) Joint phenotype	(3) Community-trait genetic gradient
Analogy/similarity with standard breeding concepts	Heritability	Factorial designs for hybrid maize breeding schemes	Linear selection index
Focal species-centered	Yes	No	Yes
Trait-based	No	No	Yes
Application to multivariate phenotypes	By dimensional reduction	Difficult	Designed intentionally
Approach for partitioning genetic variance of c	Components of variance	Components of variance	Regression on species phenotypic traits associated with an estimate of the genetic (co)variances of the trait.
Key informative parameter for breeding	Strength of IIGE for c (σ_{aB}^2 or $\sigma_{IIGE_A}^2$ for species B cf. eq. 5)	Sign of the genetic covariance between DGE and IIGE (σ_{v_A, a_A} or $\sigma_{DGE_A, IIGE_A}$, i.e., the genetic covariance of traits measured among interacting species)	Sign of the product between the expected genetic change for trait j and its community-scale effect ($\alpha_j \Delta \bar{z}_j$)

generalization of equation (13) for m interacting species with l traits each is:

$$\Delta \bar{c} = \sum_{i=1}^m \sum_{j=1}^l \alpha_{j(i)} \Delta \bar{z}_{ij} \quad (14)$$

with the first and second sigma summing effects over species and over traits, respectively. Interestingly, obvious links exist between this general expression and the integrative functional parameters used to describe the trait-based diversity of functional types in a plant community (refer to [Violle et al., 2007](#); [Garnier and Navas, 2012](#)). The issue, however, is that full predictions of selection response for such an index require a complex set of interacting parameters involving both species-specific G-matrices and reciprocal cross-species genetic covariance matrices (CSG), describing the interspecific, among trait genetic relations between DGE and IIGE between pairs of species ([Figure 3](#)). To our knowledge, the only estimate for such a combined G-CSG-matrix, i.e., including both the direct and indirect genetic effects for a focal species, was provided by [Riedel et al. \(2018\)](#). However, these authors did not report estimate uncertainties and did not compute the effect on an integrative, index-like, community-level variable.

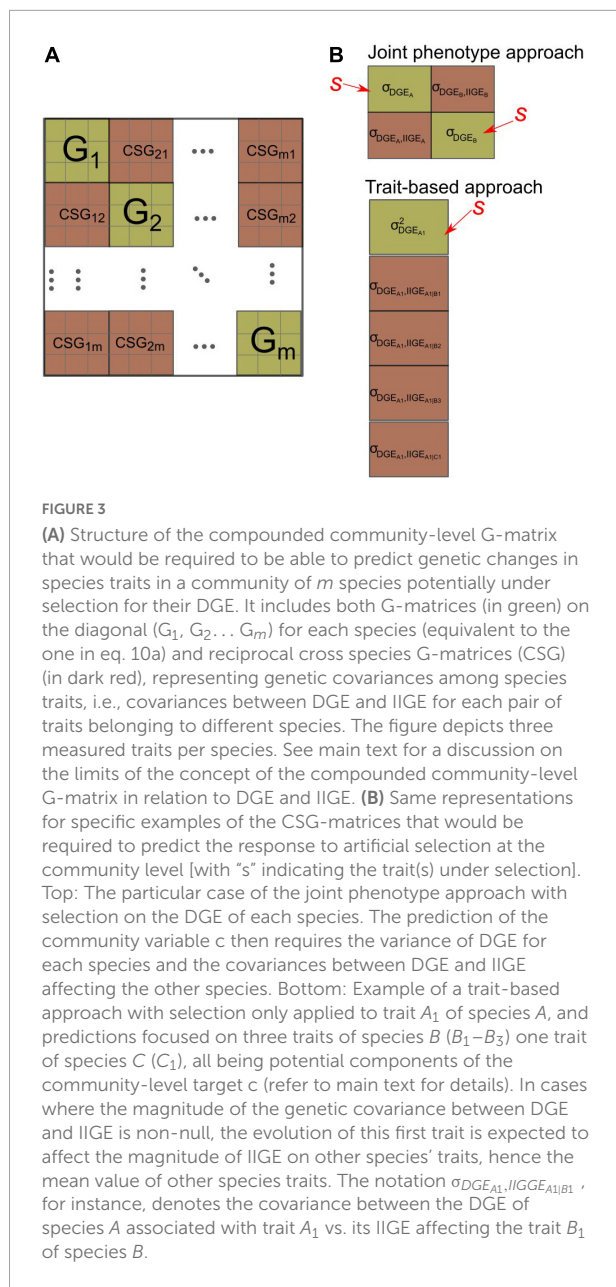
A CSG-matrix combines the covariance between the DGE of a given species A with its IIGE (originating from A) on the partner species' traits. It thus involves the association between traits measured in two different species (e.g., a biomass trait measured in a forage crop species A [DGE], that through IIGE, affects a grain yield trait measured in an associated cash crop species B), i.e., in totally unrelated individuals. This type of covariances is therefore distinct from standard genetic covariances among traits measured in the same series of individuals as well as for DGE and IGE measured in interacting individuals of the same species ([Griffing, 1967](#)). These genetic covariances among species' traits are complex parameters that

include both genetic and ecological (i.e., species interactions) causal pathways. They are pivotal parameters in the joint phenotype approach ([Wright, 1985](#); [Sampoux et al., 2020](#)) and in some versions of multitrait approaches to community genetics ([Riedel et al., 2018](#)) as they make it possible to link population genetics to the community scale.

If the capacity of the general equation cited above (eq. 14) to provide reliable predictions should be considered with great caution, it is nevertheless useful for clarifying the statistical and conceptual links between the three approaches described above. With l measured traits in a single species ($m = 1$) eq. (14) reduced to Johnson et al.'s. (2009) community-trait gradient approach (eqs. 10a, 10b, and 12). With two species ($m = 2$), each with a single measured trait ($l = 1$) and no estimated ecological gradient ($\alpha_A = \alpha_B = 1$), this becomes [Wright's \(1985\)](#) style joint phenotype approach (eq. 8). If the latter assumption is further reduced to one species, then the last remaining parameter is a variance component analogous to IIGE H^2 approaches. This is also equivalent to a joint phenotype approach with only the IIGE effect measured (i.e., in eq. 6, only $\sigma_{a_A}^2$ is assumed to be non-zero), underlining the fact that community H^2 approaches are a special case of the joint phenotype, with neglected direct effects. Breeding based on the community H^2 approach would then be possible if the community is taken as the unit of selection (i.e., the breeder assigns a fitness value – whether or not the genotype is selected – to the community-level performance of a genotype).

Cross-species genetic correlations and inaccurate quantitative predictions

It will be recalled that when restricted to traits measured in the same species, genetic correlations (a standard measure for genetic covariances) are often the poor predictors of the actual



correlated response to selection (Gromko, 1995), and obtaining reliable predictions of the correlated response to recurrent artificial selection in plants seems very challenging (Pélabon et al., 2021). It has also been shown that estimates of species-specific G-matrices are environment-dependent (Wood and Brodie, 2015), i.e., the environment in which they are measured influences their estimation, which can introduce a strong bias in predictions that were made for another environment. Taken together, what precedes might explain why below a few hundred measured individuals, it seems reasonable to draw qualitative instead of quantitative conclusions (Lynch and Walsh, 1998),

that is, to determine whether correlations are significantly negative, positive, or non-different from zero.

These limitations are expected to be even stronger for the covariances between DGE and IIGE, by definition measured on traits from two ecologically interacting species. First, the IIGE includes ecological interactions that are not explicitly accounted for in quantitative genetics models. This may introduce a strong bias resulting from external environmental factors that affect both species. Second, the evaluation and selection of genotypes within experimental plant communities typically requires reducing intra-plot genetic diversity for the selected species to the single genotype under evaluation. It has been shown that when genetic diversity is low, the traits of bi-specific experimental communities are more strongly exposed to experimental stochasticity and are hence less reliable (Milcu et al., 2018). Third, in certain experimental cases (e.g., in perennials), natural selection may co-select genotypes among species (van Moorsel et al., 2018). If not accounted for, this could bias the level of genetic covariance between DGE and IIGE due to processes analogous to linkage disequilibrium (cf. Wade, 2003).

Consequently, as it includes ecological causalities, the genetic (co)variances among species’ traits (the elements of CSG) are not of the same nature as species-specific covariances (the triangle elements of G-matrices) and are not exposed to exactly the same sources of bias. Thus, they should neither evolve nor drive the evolution of c according to the same processes. We suggest that this is the main limitation of the biological scale-coupling approaches from a quantitative genetic viewpoint. To sum up, accurately predicting selection response at the community scale might be challenging as prediction is contextual at this level. This suggests that breeders looking for anticipatory predictions will have to choose the “least bad” assumptions for their breeding context and targets.

Future directions

Our analysis of extant models underlines the fact that predicting the effects of artificial selection performed simultaneously on more than one species is technically challenging. This is first due to the size of the factorial experimental designs required (but refer to suggestions from Haug et al., 2021), especially if several functional traits need to be measured in each species. However, the need for estimates of genetic covariances between traits measured both within and among species (for IIGE) is a further obstacle. Reliable parameter estimations (e.g., for genomic selection) might therefore require an unusually large number of test environments (refer to Annicchiarico et al., 2021).

Mobilizing the modeling approaches for community-level breeding strategies

The complexity of mixed ecological and genetic systems reminds us that these different modeling approaches should probably not be merged into an ideal, all-purpose integrative modeling approach. Instead, we suggest that they should serve as an adaptable toolkit for breeders to obtain the anticipatory predictions they need. We now present some situations and strategies in which these modeling approaches could be used.

A frequent pattern observed in plant communities is that all species do not contribute equally to community-level performance (Mahaut et al., 2020), which is, as pointed above, often assumed in the plant breeding literature for mixture. The respective contribution of each species is contextual and depends on both the agronomic context and the breeding objectives (Box 2). Once the species composition of the target community to be improved is established, a reasonable breeding strategy should start by identifying the key species to breed as a priority to achieve the highest possible community-level genetic gain with the minimum breeding effort (note that the relevance of the focal species might not be determined by relative biomass of each species in the cover). Such focal species should be economically relevant and/or should have a major ecological effect on community performance, and the genetic variance in the indirect genetic effects should be strong (typically $H^2_{IGE} > 0.5$). The breeding potential of this species could be identified through gross estimates of IIGE heritability (“Approach 1” above) for the available genetic resources and within the targeted community.

If genetic improvement requires the simultaneous selection of several species (i.e., if the breeder assumes there is no obvious single “focal species” to reach a breeding goal), the joint phenotype approach (“Approach 2”) could be used to identify the strength of negative correlations between DGE and IIGE and to select the appropriate breeding scheme to overcome these constraints (Sampoux et al., 2020). This strategy then resembles the intra-specific breeding context where the magnitude of the negative (hence constraining) correlation between DGE (i.e., individual genotype performance) and IGE is estimated and accounted for (e.g., at the population level : Costa e Silva et al., 2013).

If a focal species has been clearly identified, one possible strategy is to identify the key traits of this focal species for performance at the community level. The community gradient approach of Johnson et al. (2009) described above (“Approach 3”) appears to be a suitable integrative theoretical framework for this purpose. In practical terms, the goal was to identify the traits of the focal species with both: (1) high absolute values for their α coefficients (eq. 10a), i.e., with strong (or non-negative) effects on the community-level performance and (2) substantial standing genetic variation in the pool of available breeding

candidates. However, the limitation of this trait-based approach is that it does not account for reciprocal interactions with other species potentially under selection. Reciprocal interactions may bias estimates of the partial regression coefficients α , as shown with the interaction coefficient of models for intra-specific genetic effects (Bijma, 2014). This could be overcome by coupling this quantitative genetic model with recently developed of ecophysiological models of crop mixtures (Louarn et al., 2020). These models could be adapted to provide the values of the α coefficients for a given context and with respect to a given breeding target, while accounting for reciprocal species interactions (for further discussion on this topic, see Bourke et al., 2021 in this special issue).

If the reciprocal interactions are shown to have negligible effects on the selection process, this trait-based approach is similar to basic selection index theory extended to the community scale and could be readily implemented by breeders (e.g., leading to the design of “community level ideotypes” for the focal species). Conversely, under strong reciprocal interactions, the best pair(s) of the few best performing genotypes identified in the previous selection efforts could be finally selected using a full factorial design of reasonable size, with a limited number of genotype entries (e.g., design of Moutier et al., 2022), which makes it possible to account for both GMA and SMA (cf. Wright, 1985) (however, note that there is no clear evidence for the importance of SMA variance in the experimental literature; Annicchiarico et al., 2019). Such an optional final selection step could be particularly useful if fine-tuned co-adaptation of genotypes is required (i.e., requiring well-defined and stable growing conditions, which might not be much frequent in a low input agroecological future).

The realized response to selection: Assessing the efficiency of modern breeding for crop mixtures

Assessing and predicting the efficiency of current breeding practices is of major concern to adapt breeding schemes to agroecological objectives. Breeding efforts in the recent decades aimed at pure stand performance might retain some efficiencies for the focal species in mixtures (Annicchiarico et al., 2019). However, intensive selection for monocropping performance that has led to current elite varieties is suspected to have reduced the relevant genetic variation for important traits at the community level, with potentially negative IIGE resulting from overcompetitive genotypes.

Several common garden experiments comparing cultivars according to their registration years have documented important genetic gains for monocropping yield traits (e.g., Sampoux et al., 2011; Laidig et al., 2014; Rose and Kage, 2019; Voss-Fels et al., 2019; Herrera et al., 2020). Although some of these studies investigated the effects of management conditions

(e.g., low vs. high nitrogen fertilization, conventional vs. organic management) on historical genetic trends, to our knowledge, none estimated the extent to which pure stand breeding in a focal species has affected community-level performance. Thus, we do not know the long-term effects of modern, monoculture-oriented plant breeding on the performance of crop mixtures.

Filling this knowledge gap would require a randomized experimental design comprising dozens of registered varieties (with known registration years) for a given focal species, each sown in a pure stand (experimental control) and in a mixture (experimental treatment). This would make it possible to measure (1) how genetic gain in pure stands is sustained in mixtures (direct genetic effect) as well as (2) the effects of the focal species on the community performance, i.e., IIGE on yield or yield stability. Cereal–legume intercrops or forage legume–grass mixtures would be the ideal candidate systems for such a test.

Estimating the realized selection response of the target species would be the ultimate test of the relevance of current breeding strategies for agroecological practices and their effect on plant–plant interactions. Having the realized selection response to selection on a single focal species for a community-level variable would exceptionally corroborate anticipatory predictions (Maris et al., 2018) useful for breeding [e.g., the parameters of Johnson et al.'s. (2009) model]. In addition, comparing genetic gain in a pure stand and in crop mixtures would help to identify cultivars and design ideotypes that combine both species-level performance and good ecological abilities. The community gradient approach proposed by Johnson et al. (2009) is a relevant framework for interpreting the results of such an experiment. Community-scale performance

could be formalized in terms of interaction traits (sensu Litrico and Violle, 2015) by estimating the magnitude of their respective α coefficients.

Investigating a blind spot: Genetic variation in the sensitivity to species interaction

Joint phenotype approaches assess genetic variation in mixtures for two or more species. The resulting statistical concepts such as GMA (Wright, 1985) make it possible to avoid distinguishing between the direct genetic effect of a species and its sensitivity to the IIGE of a companion species: its performance corresponds to its (experimental average for GMA) expression within a mixture of species, as measured in the experimental design. However, to date, most experimental breeding approaches used for mixtures have been focused on a single focal species in which genetic variation is assessed (review in Annicchiarico et al., 2019), likely due to the complex implementation of full factorial designs. Such approaches assume that IIGE variation only results from variation in the interaction trait of the focal species, i.e., all genotypes of the associated species affected by the IIGE react in the same way to the influence of the selected species. In the model proposed by Johnson et al. (2009), for instance, this is reflected in the fact that the α_i coefficients (eq. 10a) are constants, a feature inherited from pioneering models on intraspecific indirect genetic effects (Moore et al., 1997). *A priori*, this assumption only holds if the IIGE of the focal species is estimated against a single genotype of the associated species (i.e., there is no genetic variation in the companion species, thus no genetic variation to its sensitivity to

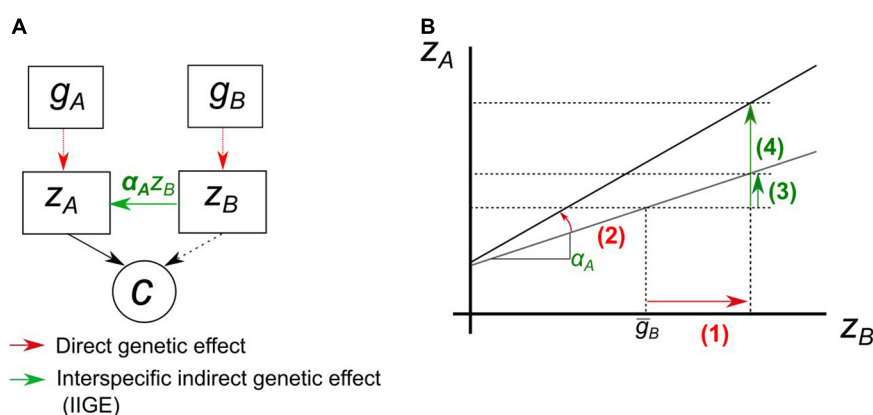


FIGURE 4

Schematic representation of the reaction norm approach for a focal species A with an agronomic trait, z_A , regressed on the interaction trait of a second species B , z_B . (A) Each species is influenced by its own genotype. z_A deviates under the influence of z_B times its sensitivity to this trait α_A , i.e., slope of the reaction norm to z_B effect. The slope α_A is itself a trait of species A , can be genetically variable and respond to selection. Both traits can potentially contribute to a community variable c . (B) Graphical representation of the path diagram. Selection on z_B (1) involves a change in z_A (3) through IIGE at α_A constant. With the same amount of change in the interaction trait z_B , if selection now also targets the genetic value of the slope (2), the magnitude of the IIGE is modified, here increased (4).

the effect of the focal species). If the focal species interacts with several associated genotypes, they may be more or less sensitive or robust to this interaction and a relevant source of variation in IIGE would be ignored in this case.

This variation can be modeled by assuming that α coefficients (in eq. 10a) are genetically variable. The graphical model in **Figure 4** illustrates how the distinction made by Litrico and Violle (2015) between agronomic and interaction traits can be made operational to explore such an assumption (refer to Navas and Violle, 2009). Instead of estimating species interaction as a factorial design combining individual genotypes, a trait of major agronomic value for species *A* can be regressed against a trait of species *B* involved in species interaction (e.g., biomass or an estimate of competitive effects). Assuming that the sensitivity of *A* to this interaction is genetically variable makes it possible to investigate specific genetic effects in a crop mixture (refer to section headed “Approach 2” above), while avoiding the need for a huge factorial design.

We suggest that the sensitivity of *A* can be modeled using a reaction norm approach, taking the value of the interaction trait of *B* as the environmental predictors for the agronomic trait of *A*. The properties of reaction norms under artificial selection have been well modeled (Kolmodin and Bijma, 2004). Modern statistical tools and concepts to analyze the genetics of reaction norms in plants, such as genetically informed random-regression models (Arnold et al., 2019), could easily be extended to interspecific interactions. This would make it possible to compute the genetic variance for the slope $G(\alpha_A)$ and its genetic covariance with the genetic value of the agronomic trait $G(\alpha_A, g_A)$. As the ecological interaction is modeled by a genetic-by-functional trait(s) interaction, this strategy would help to avoid the huge factorial experiments (Wright, 1985) required to estimate IIGE variance when the species genetic background interacts. In situations where the slope of the reaction norm is genetically variable, breeding for increasing (or decreasing, if IIGE is dominated by, for instance, negative competitive effect) the value of this slope could be a relevant target to achieve breeding goals by acting on the “leverage effect” of the slope illustrated in **Figure 4B**.

Conclusion

It is now clear that major chapters of quantitative genetic theory must be adapted to align current plant breeding efforts with increasing sustainability challenges. Quantitative genetics provided plant breeders with a scientific framework depicting the complex systems they needed to transform by both managing genetic variation and selection intensity. In the future, plant breeders will have to manage the consequences of their efforts for the improved functioning of complex crop communities. While breeding and evolutionary ecology evolved from the same theoretical background (Bijma, 2020), most

relevant models to link the population to the community level of biological diversity have been designed in evolutionary ecology. Our review stresses that inputs from evo-to-eco models have the potential to shed light on the relevant properties of this scale shift to guide the development of future breeding activities. We now conclude by underlining the main practical implications of our survey:

- (1) Identifying species with major effects on the breeding target will facilitate the prediction procedure. However, when the total number of species to be considered at once increases, quantitative genetic modeling will quickly reach its limits in providing meaningful anticipatory predictions, as the system becomes poorly controllable by artificial selection.
- (2) Context dependence is expected to increase with diversity, rendering any anticipatory prediction of the response to artificial selection extremely unreliable. Furthermore, for such complex systems, empirical approaches based on evolutionary processes might be more cost-effective (Annicchiarico et al., 2019) and could represent valuable “stopgaps” (Hill, 1996) in the absence of efficient breeding schemes that can be implemented at the community level. The models we reviewed here could serve as baselines for ex-post interpretation of these empirical results and help to made them more reproducible in practice.
- (3) Each of the three types of models we identified can be treated as a particular case of a multispecies-multitrait selection index approach. However, shifting the scale of quantitative genetics toward community performance involves dealing with genetic covariance among traits measured both within (DGE vs. DGE) and among species (DGE vs. IIGE), which have an intrinsically distinct biological nature. They consequently evolve and constrain community-level genetic change according to different processes that are not fully accounted for in the estimation procedure, which could finally drive anticipatory predictions away from reality.

We have provided a first synthesis of extant models linking quantitative genetics to community variation, identified knowledge gaps and inherent limitations, and suggested some directions for future research. We focused on the operability of the modeling approaches to provide anticipatory predictions (Maris et al., 2018) for breeders. This review emphasizes the potential complexity of fine-grained interactions among genotypes of different crop species. Coupled with the generally low predictability of short-term selection response in plants (Pélabon et al., 2021), this should encourage the search for robust qualitative evidence to facilitate the choice of breeding strategies. The overview we provided (summarized in **Table 1**) of the available models and their parameters should be useful in this regard. Such models could be incorporated in the

framework of the complex experimental pipelines currently being developed to articulate farmers' field experiments and plant breeding stations (Wolfe et al., 2021). This would facilitate the critical assessment and monitoring of such large-scale strategies in the future. More generally, plant breeders, possibly in interaction with evolutionary ecologists, could use this theoretical framework to design appropriate experimental settings and community-level breeding strategies. We have no doubt that this scenario has the potential to improve plant breeding practice to cope with current agroecological challenges.

Author contributions

CF and IL provided the original idea. CF did the literature search, framed and wrote the manuscript with inputs from IL. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

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Appendix

Appendix 1 | Using selection theory to recover the extended breeder's equation proposed by Johnson et al. (2009) for a community variable

Johnson et al. (2009) provided a theoretical formulation to extend the breeder's equation to a community variable, with selection affecting l traits of a focal species. The authors did not provide a formal derivation. As we will show, this equation can be derived from the standard multiple regression of traits on fitness on the one hand, i.e., the definition of the multivariate linear selection differential describing the individual fitness (Lande, 1979; Lande and Arnold, 1983):

$$w = \mu_w + \sum_{j=1}^l \beta_j z_j + e_w$$

And, on the other hand, the “evo-to-eco” multiple regression of the community variable c on the population traits:

$$c = \mu_c + \sum_{j=1}^l \alpha_j z_j + e_c$$

The response to the selection of traits on the community variable can be derived from the Robertson (1968) secondary theorem of natural selection (refer to Walsh and Lynch, 2018), i.e., by developing $\Delta \bar{c} = \text{cov}_A(w, c)$. For the sake of clarity, we keep the linear notation, as follows:

$$\Delta \bar{c} = \text{cov}(\beta_1 g_1 + \beta_2 g_2 + \dots + \beta_l g_l,$$

$$\alpha_1 g_1 + \alpha_2 g_2 + \dots + \alpha_l g_l)$$

Then,

$$\Delta \bar{c} = \alpha_1 \beta_1 G_{11} + \alpha_2 \beta_2 G_{22} + \dots + \alpha_l \beta_l G_{ll}$$

$$+ \alpha_1 \sum_{j \neq 1} \beta_j G_{1j} + \alpha_2 \sum_{j \neq 2} \beta_j G_{2j} + \dots + \alpha_k \sum_{j \neq l} \beta_j G_{lj},$$

with G_{jj} the (co)variance elements of the G-matrix. The first line encompasses direct response in the trait while the second line encompasses the correlated selection responses. Factoring out by α_j gives:

$$\Delta \bar{c} = \alpha_1 \left[\beta_1 G_{11} + \sum_{j \neq 1} \beta_j G_{1j} \right] + \alpha_2 \left[\beta_2 G_{22} + \sum_{j \neq 2} \beta_j G_{2j} \right] + \dots + \alpha_l \left[\beta_l G_{ll} + \sum_{j \neq l} \beta_j G_{lj} \right],$$

which is the developed form of Johnson et al.'s. (2009) equation. The terms in parentheses are the sum of the direct and correlated genetic changes due to directional selection on the j^{th} trait of the focal species, i.e., its predicted response to multivariate directional selection. This means that:

$$\Delta \bar{c} = \alpha_1 \Delta \bar{z}_1 + \alpha_2 \Delta \bar{z}_2 + \dots + \alpha_l \Delta \bar{z}_l$$

$$\Delta \bar{c} = \sum \alpha_j \Delta \bar{z}_j$$

This exercise clarifies the assumptions behind Johnson et al.'s. (2009) evo-to-eco model. It also illustrates that genetically-based change in a community variable under the influence of a focal species corresponds to the sum of changes of each trait weighted by its respective contribution to the community variable α_j . This is a basically equivalent to a linear selection index (cf. Lin and Allaire, 1977; Nordskog, 1978) except that the values of $[\alpha_1, \alpha_2, \dots, \alpha_l]$ are IIGE parameters estimated with error from data and not fixed *a priori* as in selection indices.

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