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Unraveling the genetic basis of general combining ability in CIMMYT elite bread wheat germplasm: implications for breeding strategies optimization

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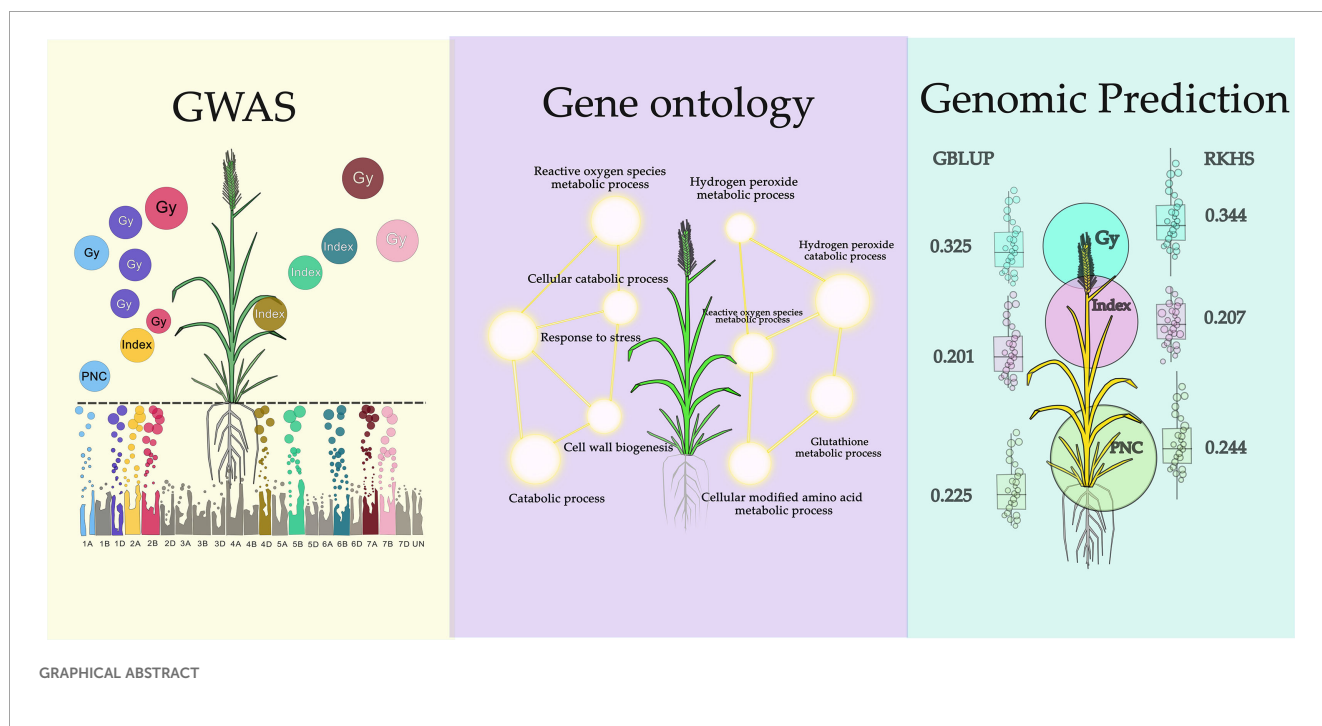
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In wheat breeding programs, several hundred crosses are performed annually, but only individuals from a few families advance to the final stages of the breeding pipelines. Therefore, a deeper understanding of the general combining ability (GCA) of wheat genotypes might enhance the breeding efficiency in selecting parents. For this reason, we tested the performance of the offspring of ~1200 parental elite lines. Using a genome-wide association study (GWAS), gene ontology (GO) analysis, and genomic prediction (GP), our objectives were to i) identify marker-trait associates (MTAs) and candidate genes, ii) assess temporal allele frequency dynamics of identified MTAs, and iii) estimate prediction accuracy (PA) for key traits: Progeny Number per-Cross (PNC), grain yield (GY), and a combined index incorporating these traits ("index"). Our findings revealed a total of 13 MTAs: eight for GY, four for the "index", and one for PNC. The GO analysis highlighted several genes involved in hydrogen peroxide metabolism and catabolism processes (H₂O₂), reactive oxygen species, response to oxidative stress, cell wall biogenesis, the metabolic process of modified amino acids at the cellular level, and glutathione metabolic process for the studied traits. Notably, allele frequency analysis over time indicated that most MTAs are under positive selection, likely reflecting indirect breeder-driven selection. The highest PA was reached by using the reproducing kernel Hilbert space (RKHS) model for the trait GY (0.34). The identification of MTAs for PNC and GY provided insight into the biological pathways underpinning combining ability and demonstrated the potential for predicting the ability of the genotypes to be crossed. These findings might contribute to the optimization crossing strategy saving costs and increasing the breeding program efficiency.

KEYWORDS

wheat breeding, general combining ability, GWAS, genomic prediction, cross-efficiency



1 Introduction

Wheat is one of the most important staple crop globally, serving as a primary source of nutrients for approximately 40% of the world's population (Giraldo et al., 2019; Reynolds and Braun, 2022). It is also the most widely cultivated crop worldwide, growing on over 217 million hectares of land (Erenstein et al., 2022). The breeding of new wheat varieties involves generating genetic variations through controlled crosses, self-fertilization, advanced generation selection, field trials, and several quality analyses, which entails a considerable investment of time (10 to 15 years) and resources (Haile et al., 2021).

Combining ability (CA) is the ability of the plants to combine with each other in order to transmit their desirable traits to their offspring; thus, by crossing one line with many others, it is possible to observe its average performance in all its crosses and highlight that the general combining ability (GCA) is described as the mean performance of a genotype across multiple crosses. By contrast, the specific combining ability (SCA) is characterized as deviations from the expected performance in certain combinations, either exceeding or falling short of the average performance of the parental inbred lines (Fasahat et al., 2016; Sprague and Tatum, 1942). CA has been widely studied in several crops, such as maize (Dermail et al., 2023; Ertiro et al., 2013; Makumbi et al., 2011; Ravikesavan et al., 2020; Run et al., 2013), cotton (Aishwarya et al., 2025; Anandan, 2010; Zeng and Pettigrew, 2015), sunflower (Habib et al., 2021; Ortis et al., 2005; Volotovitch et al 2008), alfalfa (Bhandari et al., 2007; Lawati et al., 2010), and rice (Azad et al., 2022; Shukla and Pandey, 2008; Verma and Srivastava, 2004). In wheat (*Triticum aestivum* L.), the presence of GCA has been statistically demonstrated using a 7×7

diallel set of bread wheat, also highlighting the potential to identify superior general combiners (Kumar et al., 2011). Furthermore, studies have shown that utilizing the GCA for parent selection can be an effective strategy to enhance wheat breeding programs (Gowda et al., 2012). In addition, the assessment of GCA and SCA in wheat germplasm from Pakistan has successfully identified superior combiners for grain yield and related traits (Iqbal, 2007). Despite these advancements, wheat breeding programs continue to produce hundreds of inefficient crosses each year. Understanding the genetic and molecular basis of GCA and SCA could facilitate efficient parent selection and crossing, accelerating the production of elite cultivars.

Genome-wide association studies (GWAS) aim to identify associations between single-nucleotide polymorphisms (SNPs) and phenotypic traits of interest, such as complex characters like yield. This allows for accelerated crop improvement through molecular marker-assisted and allele stacking selection. GWAS or quantitative trait loci (QTL) analyses have been performed in wheat for a variety of traits, such as grain yield and yield components (Eltaher et al., 2021; Li et al., 2019), phenology (Zhang et al., 2018), disease resistance (Singh et al., 2020; Tessmann et al., 2019), morphological traits (Sheoran et al., 2019; Vitale et al., 2021), and quality traits (Tadesse et al., 2015; Yang et al., 2020). Studies in maize have shown that the identification of key loci for GCA by GWAS could accelerate breeding and the selection of elite parents for the creation of hybrids (Liu et al., 2021; Lu et al., 2020). Association studies related to CA have been carried out in recent years, mainly in crops such as rice, corn, and cotton, in the case of rice, it was found that the accumulation of superior GCA and SCA alleles contributes to heterosis and that significant QTLs favor combinatorial ability, which could accelerate the selection of the

best parents for the development of hybrids (Chen et al., 2019; Li et al., 2022; Sarfraz et al., 2021).

Additionally, several QTLs have been linked to CA in rice, along with their pleiotropic effects on other agronomic traits (Qu et al., 2012). Notably, Lu et al. (2020) also identified numerous QTLs associated with per se performance and corresponding GCA effects for yield-related traits, which hold potential for improving maize hybrid breeding.

However, for a deeper analysis, it is necessary to complement them with other tools, such as gene ontology (GO) analysis, used to represent biological functions over genes, using a standardized vocabulary (Ashburner et al., 2000; Meng et al., 2009). Indeed, GO analyses have been performed following GWAS studies to investigate CA in rice (Chen et al., 2019; Ullah Zaid et al., 2019).

Genomic prediction (GP) is a technique for estimating phenotypic values from genotypic data, utilizing molecular information from the entire genome. Its use has increased significantly due to the low cost and incorporation of all or most of the markers, making it an essential tool in breeding programs for predicting traits of interest (Bernardo, 2008; Crossa et al., 2017; Meuwissen et al., 2001). GP has been applied for predicting a wide range of traits across several crops, such as wheat (Crossa et al., 2014; Lado et al., 2013), rice (Bartholomé et al., 2022; Labroo et al., 2021), maize (Crossa et al., 2013; Technow et al., 2014) and soybean (Jarquín et al., 2014; Zhang et al., 2016). In corn, GP has been used to predict CA in order to evaluate the performance of lines and hybrids more efficiently (Zhang et al., 2022). Furthermore, the effectiveness of predicting GCA using genomic prediction models has been evaluated. The study also compared GP application with phenotyping methods, concluding that the application of GP is a more effective and efficient approach for predicting the GCA of maize lines and their hybrid performance (Vélez-Torres et al., 2018). Finally, Werner et al. (2018) evaluated the accuracy of genomic prediction for various agronomic traits in oilseed rape. Their analysis utilized ridge-regression best linear unbiased prediction (BLUP) and three Bayesian alphabet models, considering both GCA and SCA.

The objectives of our study are: 1) to identify the marker traits associations for three traits related to the GCA (GY, “index”, and PNC) using GWAS analysis; 2) to uncover biological pathways or metabolic processes overrepresented among the identified genes through genetic enrichment analysis; 3) assess temporal allele frequency dynamics of identified MTAs over 10 years of data, and 4) to evaluate the genomic prediction accuracy for these traits, enabling practical implementation in wheat breeding programs.

2 Materials and methods

2.1 Plant material and combining ability-related traits

The phenotypic dataset comprised 1203 CIMMYT (International Maize and Wheat Improvement Center) elite breeding lines used as parents in the CIMMYT bread wheat breeding program crossing

block over five years (from the 2013/14 to 2017/18 seasons). The offspring of these breeding lines that reached the grain yield (GY) evaluation stages were tested across multiple years (from 2017/18 to 2022/2023) at the CENEB (Campo Experimental Norman E. Borlaug) research station (27°20' N, 109°54' W). The yield trials were conducted following a raised bed planting system, under optimal irrigated conditions (B5IR) (approximately 500 mm of water supplied across five irrigation events) and an optimal sowing date (late November to mid-December). They were arranged in an alpha lattice design with two replicates, using a 4.48 m² plot size and a seeding rate of 120 kg ha⁻¹. At maturity, whole plots were harvested to assess GY. GY was standardized to a moisture content of 12%. Finally, weather parameters including solar flux, temperatures, humidity, precipitation, wind pressure and soil properties were downloaded for each crop season from the NASAPOWER website (<https://www.nasa.gov/>) and shared in the open repository Figshare (<https://doi.org/10.6084/m9.figshare.30025069.v1>).

2.2 Statistical analysis and combining ability

The GY phenotypic performances of the offspring produced by the 1203 parents were analyzed using the lme4 package (Bates et al., 2015) in R software (Team, 2016), using the following statistical model (Equation 1):

$$y_{ijk} = \mu + r_j + b_{k(j)} + g_i + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} is the observed value, where μ is the general mean, r_j is the random effects of the replicates ($j = 1, \dots, 3$), g_i is the random effect of the wheat genotype, assumed to be identically and independently normally distributed (IID) with mean zero and variance σ_g^2 , and $b_{k(j)}$ represents the random effects of the incomplete blocks ($k = 1, \dots, 5$) nested within replicate, and it is assumed (IID) with mean zero and variance σ_b^2 . The term ε_{ijk} is a random residual assumed to be IID with mean zero and variance σ_e^2 . Then, we fit the same model but now with g_i as fixed effects to estimate adjusted means (Best Linear Unbiased Estimates, BLUEs).

To estimate GCA, the GY of each elite parental line was calculated as the average of its progeny. Before the calculation of the CGA, the GY BLUEs of each progeny were expressed in terms of the cultivar Borlaug 100 (common check across all the GY trials). Therefore (Equation 2),

$$g_i = \bar{F}_i - \bar{F} \quad (2)$$

Where g_i is the GCA effect of inbred lines i ; $\bar{F}_i$ is the average value of the progeny involving the inbred line i as parent, and $\bar{F}$ is the average value of all progenies.

Additionally, the number of progenies from each elite parental line was tallied and divided by the number of crosses in which the parental line participated (PNC). Finally, GY and PNC were expressed using a scale from 1 to 2 (1 minimum value and 2 maximum value), and they were used to calculate an “index” using the following formula (Equation 3):

$$\text{index} = (\text{GY} * 0.6) * (\text{PNC} * 0.4) \quad (3)$$

Phenotypic dataset was public shared in the open access repository Figshare (<https://doi.org/10.6084/m9.figshare.30024511.v1>).

2.3 Genotyping value

The genotypic data comprised a total of 18,239 SNP markers, generated using the Genotyping-by-Sequencing (GBS) approach. This process was carried out on an Illumina HiSeq2500 sequencer at Kansas State University, following the protocol described by (Poland et al., 2012). Data quality was ensured through meticulous filtering conducted with TASSEL v5.0 software (<https://tassel.bitbucket.io>) (Bradbury et al., 2007). Markers with a minor allele frequency (MAF) below 5% and those with over 50% missing data were excluded during the initial processing. The filtered HapMap was used to perform GWAS analysis. Subsequently, the HapMap was converted into a numerical matrix, ensuring compatibility with genomic prediction tools. Using the curated marker dataset, a genomic relationship matrix (G) was calculated with the AGHmatrix v2.1.4 R package (Amadeu et al., 2023). In addition, linkage disequilibrium (LD) was assessed by calculating the squared correlation coefficient (r^2) of allele frequencies for all pairwise SNP combinations within each chromosome using the TASSEL v5.0 software. To examine LD decay, r^2 values were plotted against the physical distance (in Mb) between marker pairs on each chromosome. Additionally, Kinship matrix and Principal Component Analysis (PCA) were also performed in TASSEL environment. The resulting filtered HapMap was made publicly available on figshare (<https://doi.org/10.6084/m9.figshare.29669330.v1>).

2.4 Genome-wide association study

We conducted the GWAS analysis using, as input, the GCA values, PNC values, and the selection index described in the “Data Analysis and Combining Ability” section, along with the filtered marker dataset outlined above. For the GWAS study, we used R software using the GAPIT v3.0 library (Genome Association and Prediction Integrated Tool) (Lipka et al., 2012); two methods of analysis were used; Bayesian information and Linkage disequilibrium Iteratively Nested Keyway (BLINK) (Huang et al., 2019) and Fixed and random model Circulating Probability Unification (FarmCPU) (Liu et al., 2016). In addition, a principal component parameter (PCA = 3) was incorporated to detect associations between markers and phenotypes (GY, PNC, and “index”). Finally, the significance threshold for the MTAs was identified using a Bonferroni correction $\alpha = 0.05$ with a $p = 5.0561 \times 10^{-6}$ ($-\log_{10} p = 5.30$). Following the identification of significant MTAs, the favorable allele for each locus was determined by inspecting allele-specific phenotypic distributions via boxplots. The allele associated with the higher average value of the target trait was classified as favorable. Based on these classifications, we generated Favorable Allelic Combinations (FACs) for GY

and the selection “index” by compiling all observed multi-locus combinations of favorable alleles across the respective sets of MTAs. These FACs were then used to evaluate the additive effect of multiple favorable alleles within genotypes, offering a composite view of their contribution to trait expression.

2.4.1 Candidate genes, gene ontology, and changes in favorable allele frequency

Flanking sequences covering ($\pm$)1 Mb of the significant markers from the GWAS analysis results, were analyzed in comparison with the wheat reference genome (IWGSC RefSeq v1.0) (Consortium et al., 2018). For this purpose, the Linux operating system was used, utilizing tools for manipulating genomic data, such as “gawk” (Robbins, 2004) and “bedtools” (Quinlan and Hall, 2010). For the genetic enrichment analysis, all the candidate genes for each trait were taken and analyzed using the tool ShinyGO v0.741, with a significance threshold p-value (FDR) of 0.05 (Ge et al., 2020). Following the identification of significant marker-trait associations, favorable alleles were determined by comparing the mean phenotypic values associated with each allele using boxplots. For each of the 13 MTAs, the allele linked to the more desirable phenotypic performance was classified as favorable. To evaluate changes in favorable allele frequencies over time, we leveraged a historical dataset from the CIMMYT wheat breeding program spanning ten consecutive crop seasons. Specifically, data from ten years of Elite Yield Trials (EYT), covering breeding cycles from 2013–2014 to 2022–2023, were used to monitor the temporal trends in favorable allele frequencies. For a detailed description of the EYT dataset and its structure, refer to Vitale et al. (2025).

2.5 Genomic prediction

For GP analysis, the same input data as for GWAS was used, with exception of the numerical SNPs conversion (1, 0, and 2) to meet software requirements. Markers were scaled and centered, and the matrix of genomic relationships was calculated (G) proposed by Van Raden (VanRaden, 2008). The five-fold cross-validation process was carried out (CV) 10 times. Prediction accuracy was assessed by correlating the predicted values with the observed phenotypes. The genomic best linear unbiased prediction (GBLUP) model was performed as follows (Equation 4):

$$y = \mu 1 + Zg + \varepsilon \quad (4)$$

where y is the vector of the phenotypes, μ corresponds to the intercept, Z corresponds to the design matrix of random effects, g is the vector of genomic breeding values, and ε is the vector of random errors. It is also assumed that $g \sim N(0, G\sigma_g^2)$, where G is the genomic relationship matrix, and σ_g^2 is the additive genetic variance (VanRaden, 2008).

Subsequently, RKHS with kernel averaging was applied as follows (Equation 5):

$$y = \mu + \sum_{l=1}^L u_l + \varepsilon \quad (5)$$

Where y , μ , and ε have been reported in Equation 4, and $u_l \sim N(0, K_l \sigma_{u_l}^2)$ is the additive genetic effect with K_l corresponding to the Gaussian reproducing kernel evaluated at l th of bandwidth parameters and $\sigma_{u_l}^2$ is the additive genetic variance as reported in the package BGLR (Pérez and de Los Campos, 2014). Both GBLUP and RKHS models were run as single strings of 12,000 iterations, of which the first 5,000 were discarded as burnIn in order to ensure that the model had reached convergence. The analysis was carried out using the BGLR library (Bayesian Generalized Linear Regression).

3 Results

3.1 Population phenotypic analysis

Descriptive statistics showed variations for all variables. The average grain yield was 95.3%, with a minimum of 70.0% and a maximum of 117.5% of Borlaug 100, and a standard deviation (SD) of 6.2, corresponding to a coefficient of variation (CV) of 6.5%. The “index” variable had values from 1.3 to 3.2, with a mean of 1.8, a CV of 11.3%, and an SD of 0.2. Finally, PNC exhibited an average of 4.0, with minimum values of 1.0 to a maximum of 29.0; its CV was 86.3%, and its SD was 3.5 (Figure 1).

3.2 Genome-wide association study

After filtering, 1190 genotypes and 9889 markers were identified. Supplementary Figure S1 displays the distribution of the markers along the three wheat genomes. LD ranged from 1.8 Mb to 15.1 Mb for the chromosome 6D and 2D, respectively (Supplementary Figure S2). Kinship and PCA were also reported in Supplementary Figures S3, S4, respectively. A total of 13 significant associations between markers and phenotypic traits were detected; the BLINK model identified 62.5%, while FarmCPU identified 37.5% of all associations. (Figure 2) We identified eight markers associated with GY, four to “index”, and one associated with PNC. The majority of associations were located on chromosome 1. Two associations were found on chromosome 1A and three on chromosome 1D (5 associations), while chromosomes 2 and 7 showed 3 and 2 associations, respectively. Chromosome 2 had one association on 2A and two on 2B, while chromosome 7 showed an association in 7A and another in 7B. Chromosomes 4D, 5B, and 6B showed only one association each. It is worth mentioning that the BLINK and FarmCPU models identified the same marker on chr 1D at ~432 Mb, on chr 2A at ~15 Mb, and on chr 7B at ~44 Mb. In addition, the *S1A_9565863* marker contributed 2.68% of the phenotypic variation explained (PVE) for the PNC variable (Table 1).

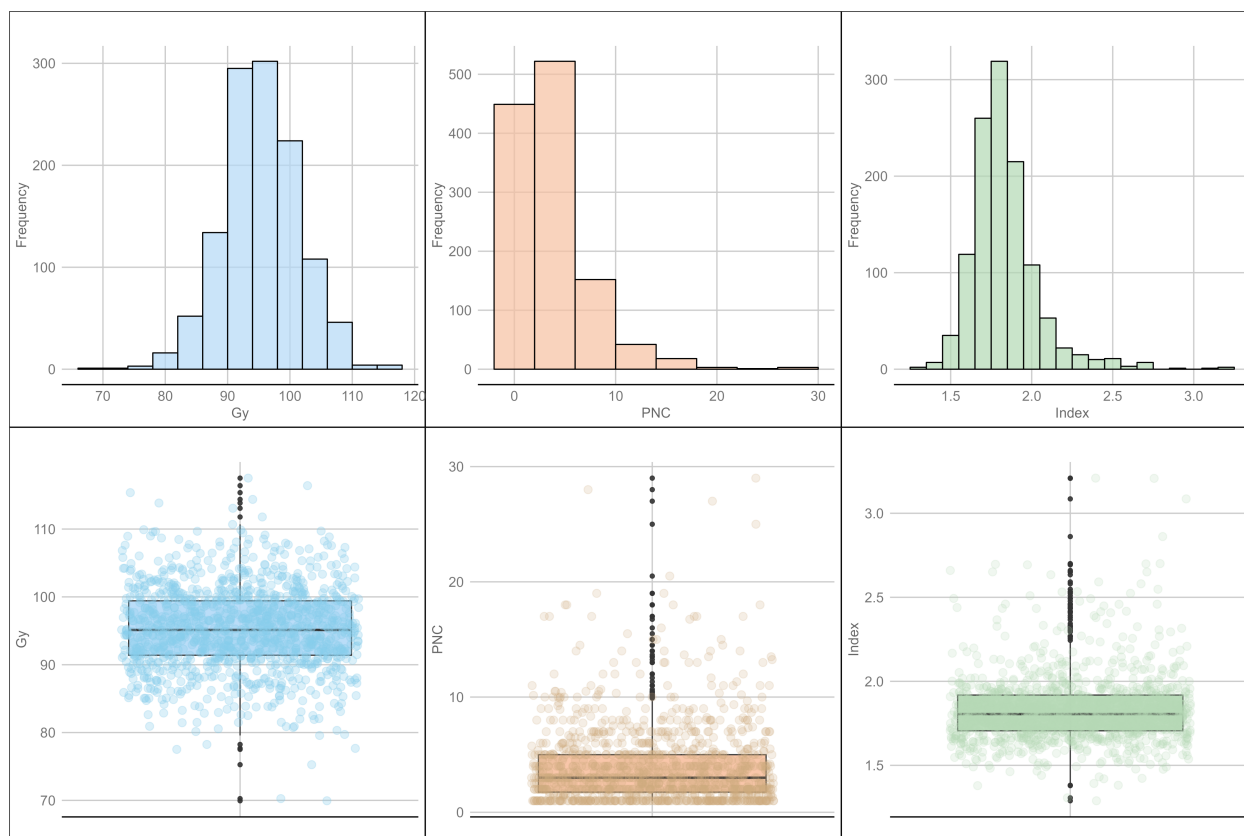


FIGURE 1
Distribution and box plots for combining ability-related traits: grain yield (GY), Progeny Number per-Cross (PNC), and the “index”.

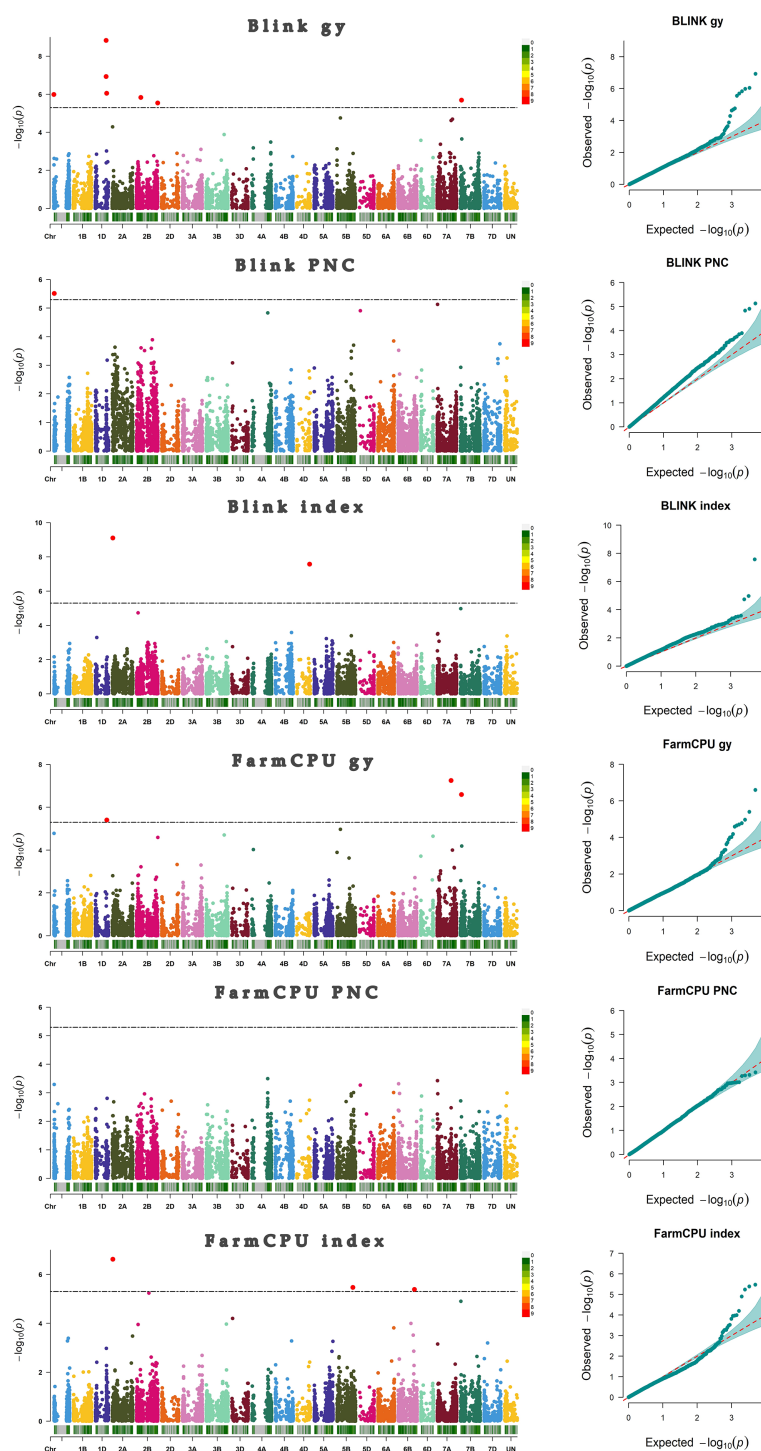


FIGURE 2

Manhattan and qqplot plots representing markers-traits associations. The red points above the black line (LOD ≥ 5.30) represent the significant markers associated with grain yield (GY), Progeny Number per-Cross (PNC), and the “index”.

To identify favorable allele combinations, we examined boxplots showing the phenotypic distribution associated with each allele across the 13 significant MTAs and their respective target traits. For each MTA, the allele contributing to superior phenotypic performance was designated as favorable. Based on this, we generated all observed combinations of favorable alleles, referred to as FACs for GY

(Figure 3) and the “index” (Figure 4). FACs were not computed for PNC, as only a single significant MTA was identified for this trait. For GY, we identified 187 unique FACs in the dataset and observed a clear gradient in mean phenotypic performance across combinations. The mean GY ranged from 93.60 to 102.58. The lowest-performing combination was composed of the favorable alleles at markers

TABLE 1 List of significant markers associated with grain yield (GY), Progeny Number per-Cross (PNC), and the “index”.

Number	Traits	SNP	Chr	Pos (Mbp)	Model	LOD	PVE	Ref/Pos (Mpb)
1	GY	S1A_1158042	1A	1.2	BLINK	5.98	3.89	
2	GY	S1D_411724142	1D	411.7	BLINK	6.93	2.98	(488.6–493.0) (Liu et al., 2020a)
3	GY	S1D_412504493	1D	412.5	BLINK	8.83	4.39	(488.6–493.0) (Liu et al., 2020a)
4	GY	S1D_432638693	1D	432.6	BLINK	6.05	3.35	(488.6–493.0) (Liu et al., 2020a)
	GY	S1D_432638693	1D	432.6	FarmCPU	5.4	0	
5	GY	S2B_161419325	2B	161.4	BLINK	5.83	2.25	
6	GY	S2B_800784801	2B	800.8	BLINK	5.54	3.48	(White et al., 2022) (766.2)
7	GY	S7A_533059303	7A	533.1	FarmCPU	7.25	NA	
8	GY	S7B_44882828	7B	44.9	BLINK	5.69	3.75	
	GY	S7B_44882828	7B	44.9	FarmCPU	6.59	3.65	
9	“Index”	S2A_15755581	2A	15.8	BLINK	9.1	5.75	(Liu et al., 2020a) (32.0 –32.9); (Li et al., 2019) (27.3–32.0); (White et al., 2022) (18.6)
	“Index”	S2A_15755581	2A	15.8	FarmCPU	6.62	0.3	
10	“Index”	S4D_481167093	4D	481.2	BLINK	7.57	4.87	(Khan et al., 2022) (465.8)
11	“Index”	S5B_610327124	5B	610.3	FarmCPU	5.47	0	(692.7–700.9) (Li et al., 2019)
12	“Index”	S6B_616781941	6B	616.8	FarmCPU	5.39	0.28	
13	PNC	S1A_9565863	1A	9.6	BLINK	5.51	2.68	

S1D_411724142, *S1D_412504493*, *S1D_432638693*, *S2B_800784801*, and *S7A_533059303*. In contrast, the highest-performing FAC included favorable alleles at *S1A_1158042*, *S1D_411724142*, *S1D_412504493*, *S2B_161419325*, and *S7B_44882828*.

In addition, FACs derived from the four MTAs associated with the “index” did not exhibit a clear trend. Mean “index” values ranged only slightly, from 1.85 to 1.94. The highest mean “index” value was associated with the combination of favorable alleles at *S2A_15755581*, *S4D_481167093*, *S5B_610327124*, and *S6B_616781941*.

3.3 Candidate genes, gene ontology, and changes in allele frequency

The 13 significant markers led to the detection of 430 genes (Supplementary File 1). The results of the genetic enrichment analysis for grain yield revealed antioxidant processes, including hydrogen peroxide metabolism, catabolism, and reactive oxygen species, among others (Fold Enrichment > 12). In turn, the “index” traits presented routes related to cell wall biogenesis, whereas for PNC, the most relevant functions were involved in cellular amino acid metabolism and glutathione metabolism (Figure 5).

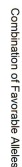
To identify favorable alleles, we examined the allelic effect distributions using boxplots, defining as favorable those alleles associated with increased phenotypic values for key traits. We then tracked the frequency of these favorable alleles across ten breeding cycles, from crop season 2013–2014 to 2022–2023, for the 13 significant MTAs (Figure 6).

Our results indicate that several favorable alleles have undergone directional shifts in frequency over time, consistent with positive selection pressure, which is likely applied indirectly by breeders. In particular, markers such as *S1A_1158042*, *S1D_432638693*, *S2A_15755581*, and *S4D_481167093* exhibited marked and sustained increases in favorable allele frequency. These trends suggest that these loci may be linked to traits that have been recurrently targeted by selection, intentionally or unintentionally, during parental advancement and recycling in the CIMMYT wheat breeding program. Notably, the favorable allele at *S4D_481167093* rapidly approached fixation within the population, which may indicate strong selection or linkage to a major-effect gene.

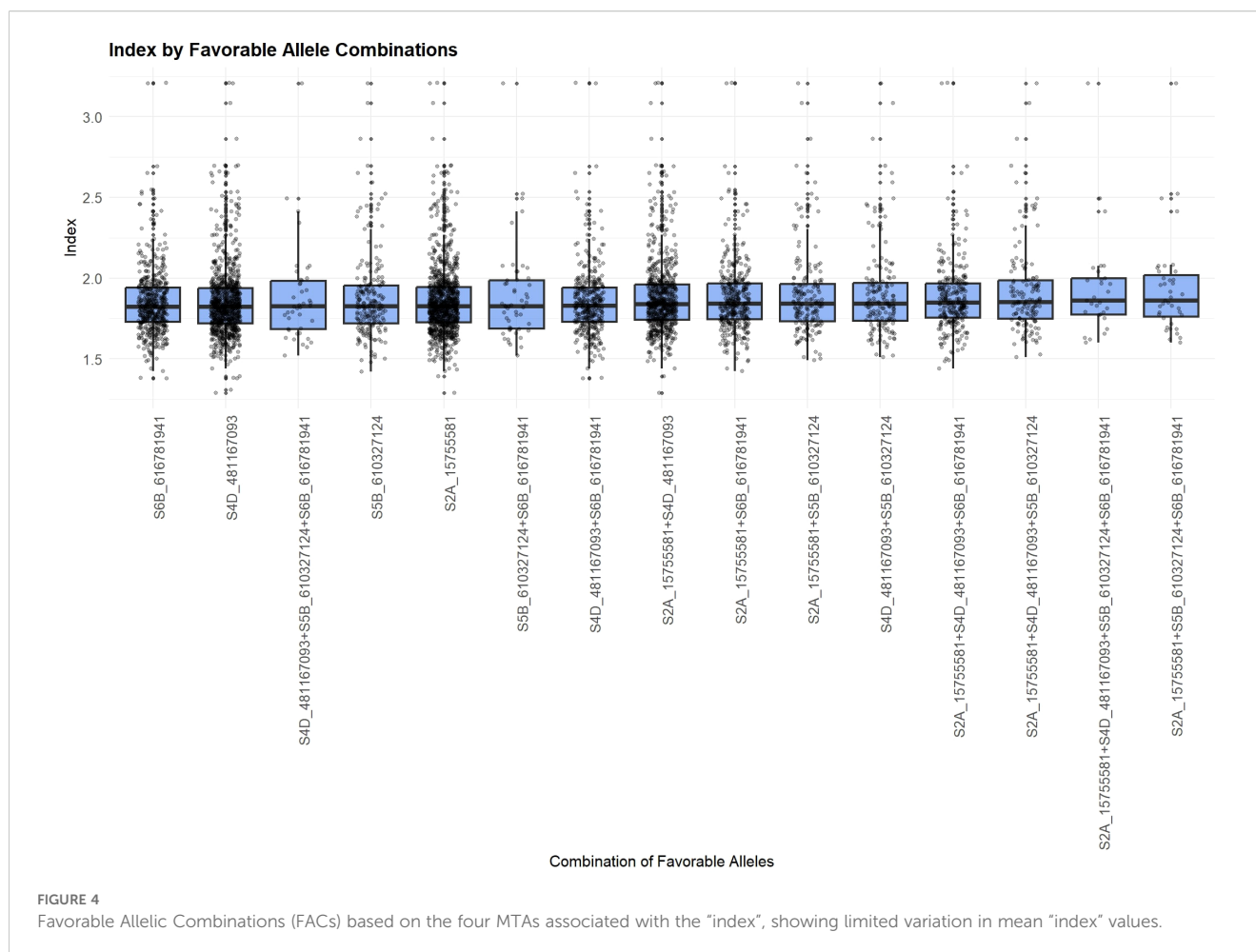
In contrast, other loci (e.g., *S2B_161419325*, *S5B_610327124*, *S7A_533059303*) displayed more erratic patterns in allele frequency, with no consistent directional trend. These irregular fluctuations may reflect genetic drift, the absence of strong selection, or linkage to traits with lower selection intensity or inconsistent value across environments.

3.4 Genomic prediction

In general, GY showed a higher prediction accuracy compared to the “index” and PNC for both models that have been used. The results of the genomic prediction analysis demonstrated that the RKHS model showed a slightly better prediction than GBLUP for the variables “index”, GY, and PNC, increasing the prediction



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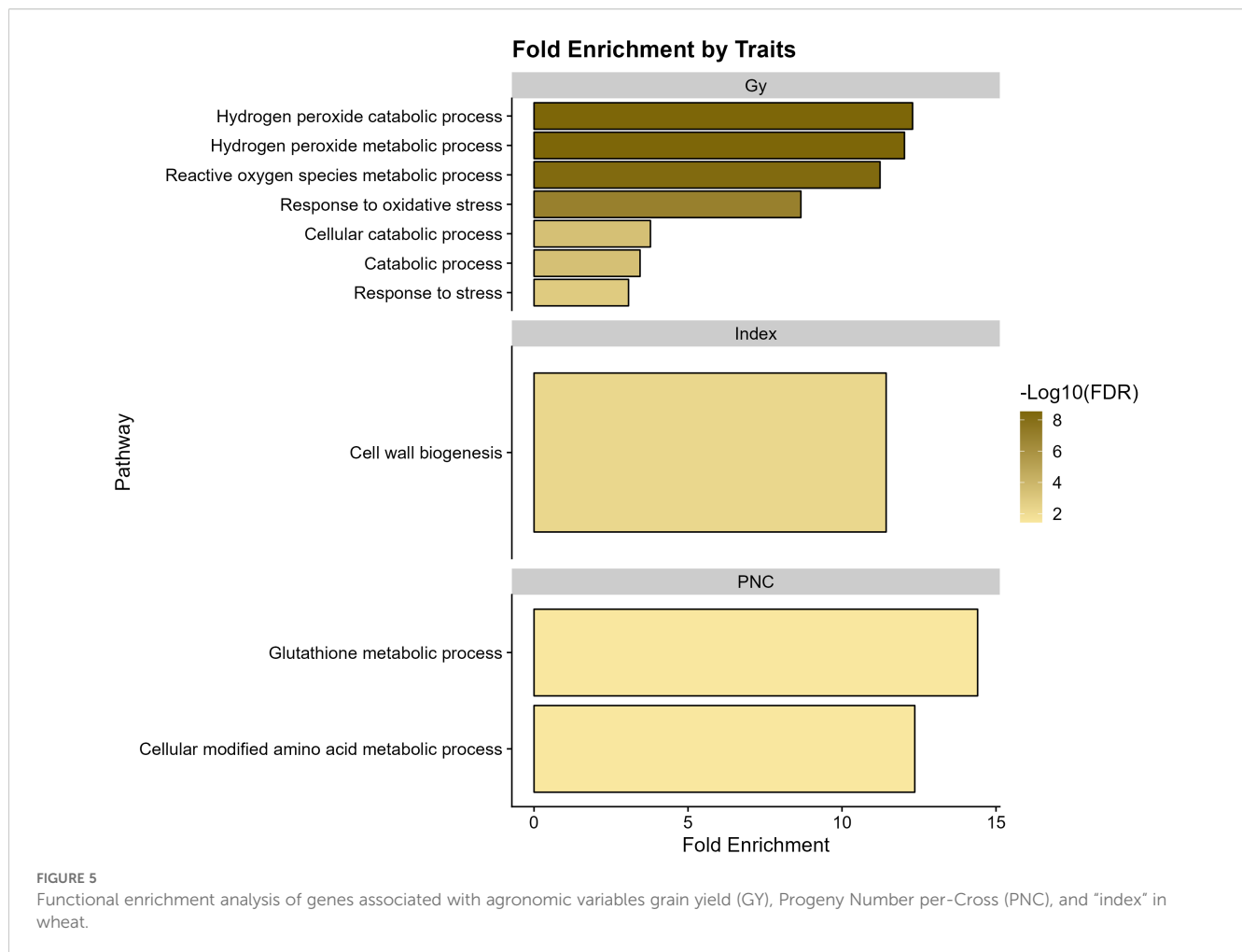
accuracy by 3.0%, 5.8%, and 8.3%, respectively. The best predictions were obtained with the RHKS model, with a value of 0.344 for GY, followed by 0.244 for PNC. Finally, the prediction of the “index” showed the lowest accuracy, with a value of 0.207 (Figure 7).

4 Discussion

Although association studies have been performed for CA in crops such as maize and rice (Chen et al., 2019; Liu et al., 2021; Lu et al., 2020); to date, no GWAS analysis has been reported in the literature for CA-related traits in wheat. Our studies identified eight significant markers associated with grain yield present in chromosomes 1A, 1D, 2B, 7A, and 7B (see Figure 2; Table 1). Previously, several studies have identified significant markers related to wheat yield on all 21 chromosomes (Akram et al., 2021; Guan et al., 2018; Luján Basile et al., 2019; Pinto et al., 2010; Sukumaran et al., 2015; Turuspekov et al., 2017; Yang et al., 2021). For grain yield, the highest number of significant MTAs occurred on chromosome 1D in positions 411.7, 412.5, and 432.6 Mbp. These locations are close to those reported by Liu (Liu et al., 2020a), which found flanking markers, in wheat for variables associated with yield (*D_contig32020_138–D_GDEEGVY01DD44S_389*; chr1D: 488.6–493.0 Mbp), close to our positions. We similarly identified four

MTAs associated with “index” related to yield, located in chromosomes 2A, 4D, 5B y 6D. One of the markers associated with “index” (*S2A_15755581*), was identified near the region described by White et al. (2022), which reported the marker *RAC875_c48625_182* in wheat for grain yield at 2.8 Mbp from our location; this raises an important relationship between the two regions, highlighting the need to study them in more depth. It is also important to note that SNPs *S1D_432638693*, *S7B_44882828*, and *S2A_15755581* were consistently identified by both statistical models (BLINK and FarmCPU). This suggests more confidence in the relevance of these MTAs. Other SPNs associated with “index” and GY, such as *S1A_1158042*, *S1D_432638693*, *S2B_161419325*, *S7A_533059303*, *S7B_44882828*, and *S2A_15755581* require further research due to the limited literature available at this time. In relation to PNC, we identified one MTA (*S1A_9565863*) located on chromosome 1A at position 9.6 Mbp. This finding highlights the importance of continuing studies due to its impact on the selection of elite genotypes.

Additionally, we observed clear differences in grain yield performance across the various FACs (Figure 3). The overall mean grain yield of the population was 95.3, whereas the highest-performing FAC reached a mean of 102.58, representing a 7.64% increase relative to the population mean (see Results section). This finding suggests a cumulative additive effect resulting from the



combination of specific favorable alleles. Such information is particularly valuable for breeding applications, as it can guide the selection of superior parental combinations. By targeting genotypes that carry optimal FACs, either as general combiners or complementary combiners, breeders can increase the likelihood of producing high-performing progeny.

The utility of FACs has also been documented in previous studies. For example, Wang et al. (2023) identified beneficial FACs for several yield component traits, including kernel number, kernel weight, and thousand-kernel weight, in a panel of 81 wheat varieties. They found that specific FACs could increase kernel weight by 0.34 or 0.26 g per thousand kernels. The strategy of pyramiding favorable alleles with additive effects is not new in wheat breeding and has proven effective for traits with monogenic or oligogenic control, such as grain quality and disease resistance (Liu et al., 2020b; Tyagi et al., 2014).

The results of the genetic enrichment analysis revealed implications of several key biological processes related to the different traits in wheat (see Figure 5). The metabolic and catabolic processes of hydrogen peroxide (H_2O_2), reactive oxygen species, and oxidative stress response are related to grain yield response. The processes of production and elimination of hydrogen peroxide are involved in plant physiological processes and

particularly resistance to stress; however, an excessive accumulation of hydrogen peroxide can activate autophagy in chloroplasts, peroxisomes, and programmed cell death (Quan et al., 2008; Smirnov and Arnaud, 2019). Similarly, Ullah Zaid et al. (2019) conducted a GWAS analysis to identify MTAs for general CA in rice, focusing on 11 yield-related traits. Their subsequent GO analysis revealed that many of these traits were significantly associated with stress response, metabolic, and biosynthetic processes. We also identified genetic enrichment for indices associated with cell wall biogenesis, highlighting the importance of cell wall functional and structural processes for the development and functionality of the crop (Mehdi et al., 2019). Interestingly, Chen et al. (2021) conducted transcriptome profiling to investigate GCA in barley, with a focus on yield-related traits. Through GO analysis, they identified several differentially expressed genes (DEGs) associated with cellular components, including cell parts and organelles.

On the other hand, PNC showed overexpression of genes in pathways related to the cellular-modified amino acid metabolic process and glutathione metabolic process. The latter plays an important role in biosynthetic pathways, conjugation, and detoxification of xenobiotics as well as reduction of reactive oxygen species (ROS) and is important for better stress tolerance

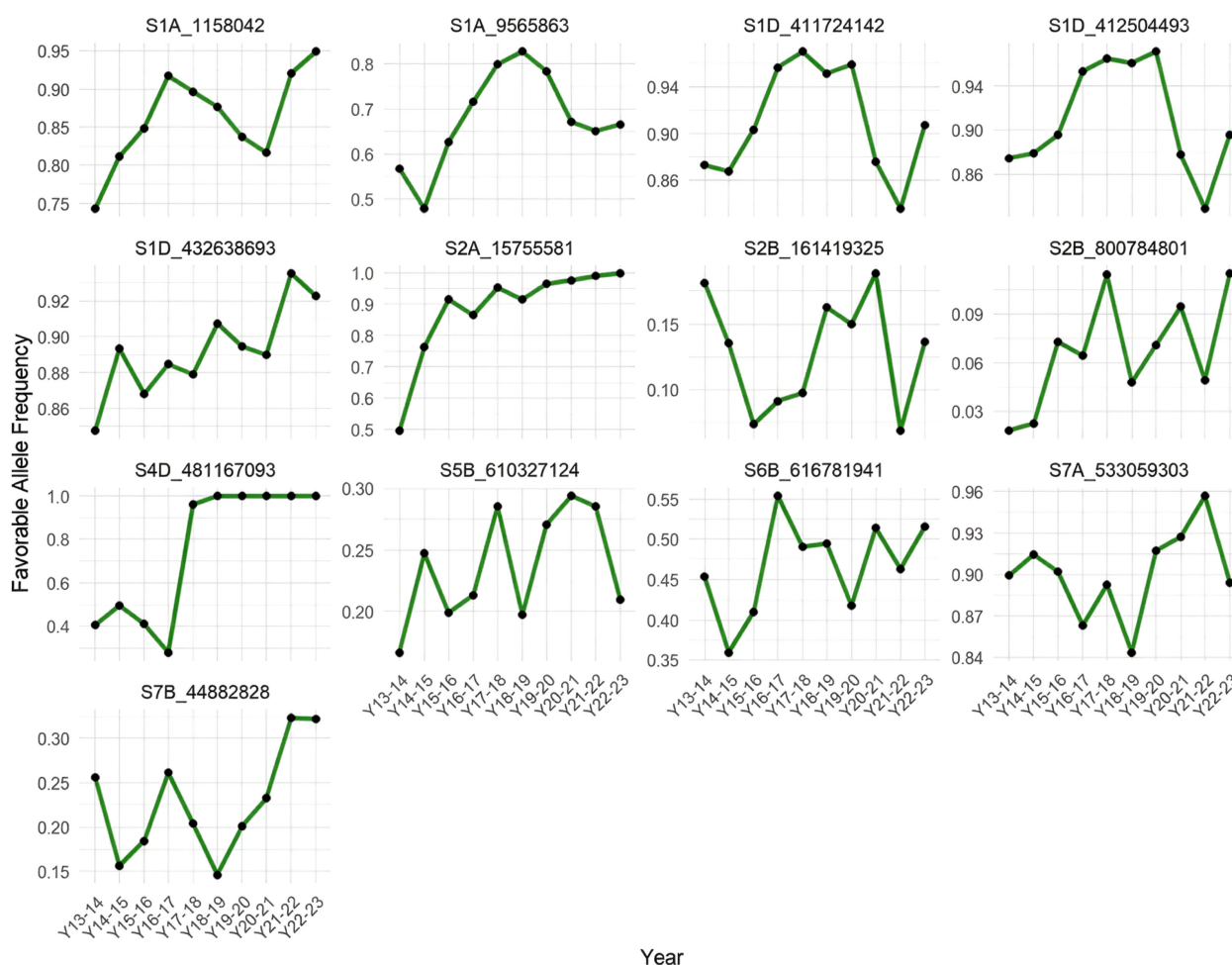


FIGURE 6
Temporal dynamics of favorable allele frequencies across 10 years in the CIMMYT wheat program (2013–2014 to 2022–2023).

(Dixon et al., 2002; Hasanuzzaman et al., 2019; Noctor et al., 2012; Sahoo et al., 2017).

The analysis of favorable allele frequency dynamics over ten breeding cycles revealed evidence of indirect positive selection acting on several MTAs, notably *S1A_1158042*, *S1D_432638693*, *S2A_15755581*, and *S4D_481167093* (see Figure 6). The consistent increase in their frequency suggests that these loci are linked to traits routinely favored during parent and line selection, such as yield or yield-related traits. In particular, the near fixation of some alleles points to strong selection pressure, likely reflecting their importance in breeding progress. In contrast, other MTAs exhibited no consistent temporal trend, possibly due to weak or environment-dependent selection, genetic drift, or association with traits of lower priority.

We acknowledge that GCA in wheat, as in other species, is a highly complex trait. Its genetic basis is governed by the cumulative effects of thousands of small-effect loci, rather than a few major genes (Rojas and Sprague, 1952; Walejko and Russell, 1977). While FACs offer a useful framework for identifying promising complementary crosses, their practical application may be limited by the polygenic nature of the trait. As such, the predictive utility of

individual GWAS-derived markers remains constrained in the context of routine selection pipelines. From a genomic prediction perspective, the emphasis shifts from identifying individual significant loci to modeling the entire genomic architecture of the trait. GP approaches, which are based on the infinitesimal model, circumvent the statistical limitations of GWAS by avoiding multiple testing and leveraging genome-wide marker information. While GWAS remains valuable for generating biological insights and identifying occasional candidate regions, its utility for improving complex traits like GCA is limited compared to the predictive accuracy and integration offered by GP models such as GBLUP and RKHS.

Indeed, this is the first study that assessed genomic prediction accuracy for GCA-related traits in wheat. The genomic prediction results showed values ranging from 0.325 and 0.344 for GY, 0.201 and 0.207 for the “index” (see Figure 7), similar results were found by Poland and colleagues (2012) for variables associated with wheat yield in a panel of 254 lines, with precisions from 0.28 to 0.45. Our results are also in line with another genomic prediction study in wheat in different environments and years that exhibited prediction accuracies from 0.27 to 0.59 for yield (Gill et al., 2021). The

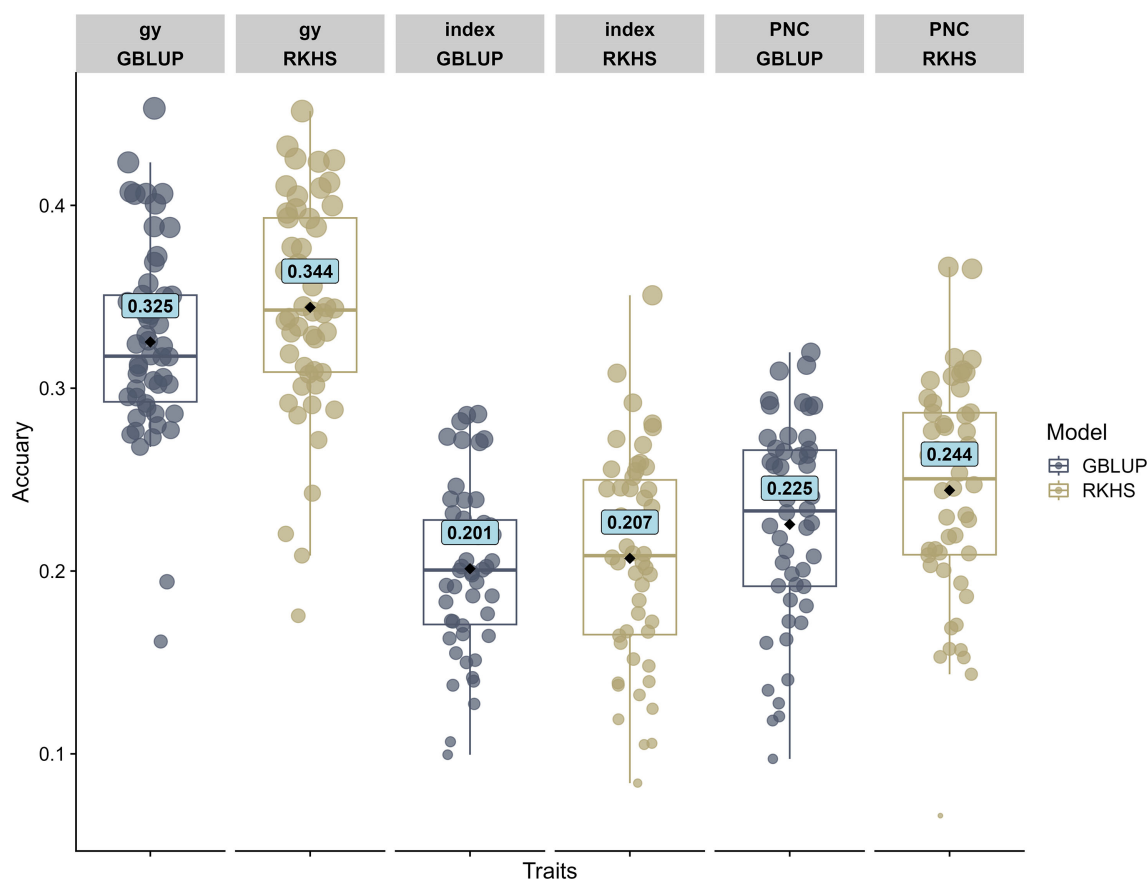


FIGURE 7

Boxplot of the predictions for the grain yield (GY), Progeny Number per-Cross PNC, and “index” variables using the reproducing kernel Hilber space (RKHS) and genomic best linear unbiased prediction (GBLUP) models.

predictive results for PNC had values of 0.225 and 0.244, indicating a moderately limited prediction. In other crops, such as maize, predictions related to GCA ranged from 0.49 to 0.61 (Vélez-Torres et al., 2018). However, due to the limited information related to the prediction of CA in wheat (Basnet et al., 2019; Zhao et al., 2013), our research could be a starting point for future analyses to improve the prediction of PNC in wheat. Interestingly, our results showed that the RKHS model only slightly outperformed GBLUP for grain yield and other traits, though the differences were not statistically significant. These findings align closely with those reported in durum wheat for grain yield and yield-related traits (Vitale et al., 2024). However, it is important to emphasize that improving prediction accuracy may require integrating diverse data sources and exploring alternative models, such as machine or deep learning (CROSSA et al., 2024).

5 Conclusions

To date, traits related to combining ability in wheat have not been thoroughly investigated and often fail to produce high-performing offspring despite originating from promising parents. Therefore, a deeper understanding of the combining ability of

parental lines is crucial for optimizing the parental selection process. In our study, we identified 13 MTAs, eight of which are related to grain yield (GY), four of which are associated with the “index”, in addition to a marker associated with the number of progeny per cross (PNC). Gene ontology analyses revealed major functions related to hydrogen peroxide metabolism and catabolism, cell wall biogenesis, and amino acid and glutathione metabolism for the traits of interest. Interestingly, several MTAs were found to be under positive selection pressure, indirectly driven by breeders’ decisions over successive cycles. This finding highlights the functional relevance of these loci and reinforces their importance within the wheat breeding pipeline. Likewise, the best predictions were obtained using the RKHS model, with values of 0.344, 0.207, and 0.244 for the traits GY, “index”, and PNC. These findings advance the current understanding of combining ability in wheat, shedding light on MTAs that could serve as valuable tools for developing new markers to better characterize candidate parents. Moreover, this study highlights novel insights into the biological pathways underlying traits related to combining ability. Applying genomic prediction to evaluate the combining ability of candidate parents offers a promising strategy to enhance the efficiency of parental selection. This approach can ultimately improve offspring yield performance and enhance the genetic gain.

Data availability statement

The Hapmap file was shared in the figshare repository: <https://doi.org/10.6084/m9.figshare.29669330.v1>. Additionally, the phenotypic dataset was publicly shared in the open access repository Figshare (<https://doi.org/10.6084/m9.figshare.30024511.v1>).

Author contributions

JS-Á: Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. GG: Conceptualization, Funding acquisition, Methodology, Visualization, Writing – review & editing. SE: Methodology, Supervision, Writing – review & editing. VG: Conceptualization, Writing – review & editing. JH: Conceptualization, Writing – review & editing. ZT: Conceptualization, Writing – review & editing. SD: Conceptualization, Writing – review & editing. CS-P: Conceptualization, Writing – review & editing. AP: Conceptualization, Writing – review & editing. FT: Conceptualization, Writing – review & editing. KG: Conceptualization, Writing – review & editing. LC-H: Conceptualization, Writing – review & editing. JC: Conceptualization, Writing – review & editing. PV: Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1675993/full#supplementary-material>

References

- Aishwarya, B., Patil, B., Patil, R., Hegde, M., and Rao, M. (2025). Assessment of combining ability and genetic parameters for yield and fiber traits in upland cotton (*Gossypium hirsutum* L.) using Linx Tester analysis. *Electronic J. Plant Breed.* 16, 133–142. doi: 10.37992/2025.1601.010
- Akram, S., Arif, M. A. R., and Hameed, A. (2021). A GBS-based GWAS analysis of adaptability and yield traits in bread wheat (*Triticum aestivum* L.). *J. Appl. Genet.* 62, 27–41. doi: 10.1007/s13353-020-00593-1
- Amadeu, R. R., Garcia, A. A. F., Munoz, P. R., and Ferrão, L. F. V. (2023). AGHmatrix: genetic relationship matrices in R. *Bioinformatics* 39, btad445. doi: 10.1093/bioinformatics/btad445
- Anandan, A. (2010). Environmental impact on the combining ability of fiber traits and seed-cotton yield in cotton. *J. Crop Improv.* 24, 310–323. doi: 10.1080/15427528.2010.497959
- Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., et al. (2000). Gene ontology: tool for the unification of biology. *Nat. Genet.* 25, 25–29. doi: 10.1038/75556
- Azad, A. K., Sarker, U., Ercisli, S., Assouguem, A., Ullah, R., Almeer, R., et al. (2022). Evaluation of combining ability and heterosis of popular restorer and male sterile lines for the development of superior rice hybrids. *Agronomy* 12, 965. doi: 10.3390/agronomy12040965
- Bartholomé, J., Prakash, P. T., and Cobb, J. N. (2022). Genomic Prediction: Progress and Perspectives for Rice Improvement. In: Ahmadi, N., and Bartholomé, J. (eds) *Genomic Prediction of Complex Traits. Methods in Molecular Biology* 2467. Humana, New York, NY. doi: 10.1007/978-1-0716-2205-6_21
- Basnet, B. R., Crossa, J., Dreisigacker, S., Pérez-Rodríguez, P., Manes, Y., Singh, R. P., et al. (2019). Hybrid wheat prediction using genomic, pedigree, and environmental

covariables interaction models. *Plant Genome* 12, 180051. doi: 10.3835/plantgenome2018.07.0051

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., et al. (2015). Package 'lme4'. *convergence* 12, 2. doi: 10.18637/jss.v067.i01

Bernardo, R. (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Sci.* 48, 1649–1664. doi: 10.2135/cropsci2008.03.0131

Bhandari, H., Pierce, C., Murray, L., and Ray, I. (2007). Combining abilities and heterosis for forage yield among high-yielding accessions of the alfalfa core collection. *Crop Sci.* 47, 665–671. doi: 10.2135/cropsci2006.06.0398

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., and Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23, 2633–2635. doi: 10.1093/bioinformatics/btm308

Chen, J., Zhou, H., Xie, W., Xia, D., Gao, G., Zhang, Q., et al. (2019). Genome-wide association analyses reveal the genetic basis of combining ability in rice. *Plant Biotechnol. J.* 17, 2211–2222. doi: 10.1111/pbi.13134

Chen, X., Zhao, B., Ji, C., Zhu, B., and Wang, R. (2021). Transcriptome profiling analysis of two contrasting barley genotypes in general combining ability for yield traits. *Braz. J. Bot.* 44, 117–123. doi: 10.1007/s40415-020-00696-3

Consortium, I. W. G. S., Appels, R., Eversole, K., Stein, N., Feuillet, C., Keller, B., et al. (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361, eaar7191. doi: 10.1126/science.aar7191

Crossa, J., Beyene, Y., Kassa, S., Pérez, P., Hickey, J. M., Chen, C., et al. (2013). Genomic prediction in maize breeding populations with genotyping-by-sequencing. *G3: Genes Genom. Genet.* 3, 1903–1926. doi: 10.1534/g3.113.008227

Crossa, J., Montesinos-Lopez, O. A., Costa-Neto, G., Vitale, P., Martini, J. W., Runcie, D., et al. (2024). Machine learning algorithms translate big data into predictive breeding accuracy. *Trends Plant Sci.* 30(2), 167–184. doi: 10.1016/j.tplants.2024.09.011

Crossa, J., Perez, P., Hickey, J., Burgueno, J., Ornella, L., Cerón-Rojas, J., et al. (2014). Genomic prediction in CIMMYT maize and wheat breeding programs. *Heredity* 112, 48–60. doi: 10.1038/hdy.2013.16

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquin, D., De Los Campos, G., et al. (2017). Genomic selection in plant breeding: methods, models, and perspectives. *Trends Plant Sci.* 22, 961–975. doi: 10.1016/j.tplants.2017.08.011

Dermail, A., Lübberstedt, T., Suwarno, W. B., Chankaew, S., Lertrat, K., Ruanjaichon, V., et al. (2023). Combining ability of tropical × temperate maize inducers for haploid induction rate, R1-nj seed set, and agronomic traits. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1154905

Dixon, D. P., Laphorn, A., and Edwards, R. (2002). Plant glutathione transferases. *Genome Biol.* 3, 1–10. doi: 10.1186/gb-2002-3-3-reviews3004

Eltaher, S., Baenziger, P. S., Belamkar, V., Emara, H. A., Nower, A. A., Salem, K. F., et al. (2021). GWAS revealed effect of genotype × environment interactions for grain yield of Nebraska winter wheat. *BMC Genomics* 22, 2. doi: 10.1186/s12864-020-07308-0

Erenstein, O., Jaleta, M., Mottaleb, K. A., Sonder, K., Donovan, J., and Braun, H. J. (2022). Global Trends in Wheat Production, Consumption and Trade. In Reynolds, M. P., and Braun, H. J. (eds) *Wheat Improvement* (Cham: Springer International Publishing Cham). doi: 10.1007/978-3-030-90673-3_4

Ertiro, B. T., Zeleke, H., Friesen, D., Blummel, M., and Twumasi-Afriye, S. (2013). Relationship between the performance of parental inbred lines and hybrids for food-feed traits in maize (*Zea mays* L.) in Ethiopia. *Field Crops Res.* 153, 86–93. doi: 10.1016/j.fcr.2013.02.008

Fasahat, P., Rajabi, A., Rad, J. M., and Derera, J. (2016). Principles and utilization of combining ability in plant breeding. *Biom. Biostat. Int. J.* 4, 1–24. doi: 10.15406/bbij.2016.04.00085

Ge, S. X., Jung, D., and Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* 36, 2628–2629. doi: 10.1093/bioinformatics/btz931

Gill, H. S., Halder, J., Zhang, J., Brar, N. K., Rai, T. S., Hall, C., et al. (2021). Multi-trait multi-environment genomic prediction of agronomic traits in advanced breeding lines of winter wheat. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.709545

Giraldo, P., Benavente, E., Manzano-Agugliaro, F., and Gimenez, E. (2019). Worldwide research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy* 9, 352. doi: 10.3390/agronomy9070352

Gowda, M., Longin, C. F. H., Lein, V., and Reif, J. C. (2012). Relevance of specific versus general combining ability in winter wheat. *Crop Sci.* 52, 2494–2500. doi: 10.2135/cropsci2012.04.0245

Guan, P., Lu, L., Jia, L., Kabir, M. R., Zhang, J., Lan, T., et al. (2018). Global QTL analysis identifies genomic regions on chromosomes 4A and 4B harboring stable loci for yield-related traits across different environments in wheat (*Triticum aestivum* L.). *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00529

Habib, S. H., Akanda, M. A. L., Hossain, K., and Alam, A. (2021). Combining ability analysis in sunflower (*Helianthus annuus* L.) genotypes. *J. Cereals Oilseeds* 12, 1–8. doi: 10.5897/CO2020.0221

Haile, T. A., Walkowiak, S., N'Diaye, A., Clarke, J. M., Hucl, P. J., Cuthbert, R. D., et al. (2021). Genomic prediction of agronomic traits in wheat using different models and cross-validation designs. *Theor. Appl. Genet.* 134, 381–398. doi: 10.1007/s00122-020-03703-z

Hasanuzzaman, M., Bhuyan, M. B., Anee, T. I., Parvin, K., Nahar, K., Mahmud, J. A., et al. (2019). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants* 8, 384. doi: 10.3390/antiox8090384

Huang, M., Liu, X., Zhou, Y., Summers, R. M., and Zhang, Z. (2019). BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. *Gigascience* 8, 1–12. doi: 10.1093/gigascience/giy154

Iqbal, M. M. (2007). Combining ability analysis in wheat. *Pak. J. Agric. Sci.* 44, 1.

Jarquín, D., Kocak, K., Posadas, L., Hyma, K., Jedlicka, J., Graef, G., et al. (2014). Genotyping by sequencing for genomic prediction in a soybean breeding population. *J. BMC Genomics* 15, 1–10. doi: 10.1186/1471-2164-15-740

Khan, H., Krishnappa, G., Kumar, S., Mishra, C. N., Krishna, H., Devate, N. B., et al. (2022). Genome-wide association study for grain yield and component traits in bread wheat (*Triticum aestivum* L.). *Front. Genet.* 13. doi: 10.3389/fgene.2022.982589

Kumar, A., Mishra, V., Vyas, R., and Singh, V. (2011). Heterosis and combining ability analysis in bread wheat (*Triticum aestivum* L.). *J. Plant Breed. Crop Sci.* 3, 209–217. doi: 10.5897/PBCS.9000075

Labroo, M. R., Ali, J., Aslam, M. U., de Asis, E. J., dela Paz, M. A., Sevilla, M. A., et al. (2021). Genomic prediction of yield traits in single-cross hybrid rice (*Oryza sativa* L.). *Front. Genet.* 12. doi: 10.3389/fgene.2021.692870

Lado, B., Matus, I., Rodríguez, A., Inostroza, L., Poland, J., Belzile, F., et al. (2013). Increased genomic prediction accuracy in wheat breeding through spatial adjustment of field trial data. *G3: Genes Genomics Genet.* 3, 2105–2114. doi: 10.1534/g3.113.007807

Lawati, A. A., Pierce, C., Murray, L., and Ray, I. (2010). Combining ability and heterosis for forage yield among elite alfalfa core collection accessions with different fall dormancy responses. *Crop Sci.* 50, 150–158. doi: 10.2135/cropsci2008.09.0549

Li, F., Wen, W., Liu, J., Zhang, Y., Cao, S., He, Z., et al. (2019). Genetic architecture of grain yield in bread wheat based on genome-wide association studies. *BMC Plant Biol.* 19, 168. doi: 10.1186/s12870-019-1781-3

Li, Z.-s., Dong, Y., Li, T., Feng, Z.-q., Duan, Y.-x., Yang, M.-x., et al. (2022). Genome-wide association analysis of yield and combining ability based on maize hybrid population. *Sci. Agric. Sinica* 1695–1709. doi: 10.3864/j.issn.0578-1752.2022.09.001

Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., et al. (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics* 28, 2397–2399. doi: 10.1093/bioinformatics/bts444

Liu, X., Hu, X., Li, K., Liu, Z., Wu, Y., Feng, G., et al. (2021). Identifying quantitative trait loci for the general combining ability of yield-relevant traits in maize. *Breed. Sci.* 71, 217–228. doi: 10.1270/jsbbs.20008

Liu, X., Huang, M., Fan, B., Buckler, E. S., and Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genet.* 12, e1005767. doi: 10.1371/journal.pgen.1005767

Liu, R., Lu, J., Zhou, M., Zheng, S., Liu, Z., Zhang, C., et al. (2020b). Developing stripe rust resistant wheat (*Triticum aestivum* L.) lines with gene pyramiding strategy and marker-assisted selection. *Genet. Resour. Crop Evol.* 67, 381–391. doi: 10.1007/s10722-019-00868-5

Liu, H., Mullan, D., Zhang, C., Zhao, S., Li, X., Zhang, A., et al. (2020a). Major genomic regions responsible for wheat yield and its components as revealed by meta-QTL and genotype-phenotype association analyses. *Planta* 252, 1–22. doi: 10.1007/s00425-020-03466-3

Lu, X., Zhou, Z., Yuan, Z., Zhang, C., Hao, Z., Wang, Z., et al. (2020). Genetic dissection of the general combining ability of yield-related traits in maize. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00788

Luján Basile, S. M., Ramírez, I. A., Crescente, J. M., Conde, M. B., Demichelis, M., Abbate, P., et al. (2019). Haplotype block analysis of an Argentinean hexaploid wheat collection and GWAS for yield components and adaptation. *BMC Plant Biol.* 19, 1–16. doi: 10.1186/s12870-019-2015-4

Makumbi, D., Betrán, J. F., Bänziger, M., and Ribaut, J.-M. (2011). Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. *Euphytica* 180, 143–162. doi: 10.1007/s10681-010-0334-5

Mehdi, C., Virginie, L., Audrey, G., Axelle, B., Colette, L., Hélène, R., et al. (2019). Cell wall proteome of wheat grain endosperm and outer layers at two key stages of early development. *Int. J. Mol. Sci.* 21, 239. doi: 10.3390/ijms21010239

Meng, S., Brown, D. E., Ebbola, D. J., Torto-Alalibo, T., Oh, Y. Y., Deng, J., et al. (2009). Gene Ontology annotation of the rice blast fungus, *Magnaporthe oryzae*. *BMC Microbiol.* 9, 1–6. doi: 10.1186/1471-2180-9-S1-S8

Meuwissen, T. H., Hayes, B. J., and Goddard, M. (2001). Prediction of total genetic value using genome-wide dense marker maps. *genetics* 157, 1819–1829. doi: 10.1093/genetics/157.4.1819

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., et al. (2012). Glutathione in plants: an integrated overview. *Plant Cell Environ.* 35, 454–484. doi: 10.1111/j.1365-3040.2011.02400.x

Ortiz, L., Nestares, G., Frutos, E., and MaChado, N. (2005). COMBINING ABILITY ANALYSIS FOR AGRONOMIC TRAITS IN SUNFLOWER (*Helianthus annuus* L.). *Helia* 28, 125–134. doi: 10.2298/hel0543125o

Pérez, P., and de Los Campos, G. (2014). Genome-wide regression and prediction with the BGLR statistical package. *Genetics* 198, 483–495. doi: 10.1534/genetics.114.164442

Pinto, R. S., Reynolds, M. P., Mathews, K. L., McIntyre, C. L., Olivares-Villegas, J.-J., and Chapman, S. C. (2010). Heat and drought adaptive QTL in a wheat population

designed to minimize confounding agronomic effects. *Theor. Appl. Genet.* 121, 1001–1021. doi: 10.1007/s00122-010-1351-4

Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S., Manes, Y., et al. (2012). Genomic selection in wheat breeding using genotyping-by-sequencing. *Plant Genome* 5:103–113. doi: 10.3835/plantgenome2012.06.0006

Qu, Z., Li, L., Luo, J., Wang, P., Yu, S., Mou, T., et al. (2012). QTL mapping of combining ability and heterosis of agronomic traits in rice backcross recombinant inbred lines and hybrid crosses. *PLoS One* 7, e28463. doi: 10.1371/journal.pone.0028463

Quan, L. J., Zhang, B., Shi, W. W., and Li, H. Y. (2008). Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J. Integr. Plant Biol.* 50, 2–18. doi: 10.1111/j.1744-7909.2007.00599.x

Quinlan, A. R., and Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842. doi: 10.1093/bioinformatics/btq033

Ravikesavan, R., Suhasini, B., Yuvaraja, A., and KumariVinodhana, N. (2020). Assessment of combining ability for yield and yield contributing traits in sweet corn. *Electronic J. Plant Breed.* 11, 224–229. doi: 10.37992/2020.1101.038

Reynolds, M. P., and Braun, H.-J. (2022). *Wheat improvement: food security in a changing climate* (Cham: Springer Nature).

Robbins, A. D. (2004). *Gawk: effective AWK programming* Vol. 3 (Boston: Free Software Foundation).

Rojas, B. A., and Sprague, G. F. (1952). A comparison of variance components in corn yield trials: III. General and specific combining ability and their interaction with locations and years. 44, 462–466. doi: 10.2134/agronj1952.00021962004400090002x

Run, L., XIAO, L.-h., Jing, W., LU, Y.-l., RONG, T.-z., PAN, G.-t., et al. (2013). Combining ability and parent-offspring correlation of maize (*Zea mays* L.) grain β -carotene content with a complete diallel. *J. Integr. Agric.* 12, 19–26. doi: 10.1016/S2095-3119(13)60201-4

Sahoo, S., Awasthi, J. P., Sunkar, R., and Panda, S. K. (2017). Determining glutathione levels in plants. In: Sunkar, R. (eds) *Plant Stress Tolerance. Methods in Molecular Biology*. 1631. New York, NY: Humana Press. doi: 10.1007/978-1-4939-7136-7_16

Sarfaraz, Z., Iqbal, M. S., Geng, X., Iqbal, M. S., Nazir, M. F., Ahmed, H., et al. (2021). GWAS mediated elucidation of heterosis for metric traits in cotton (*Gossypium hirsutum* L.) across multiple environments. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.565552

Theoran, S., Jaiswal, S., Kumar, D., Raghav, N., Sharma, R., Pawar, S., et al. (2019). Uncovering genomic regions associated with 36 agro-morphological traits in Indian spring wheat using GWAS. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00527

Shukla, S., and Pandey, M. (2008). Combining ability and heterosis over environments for yield and yield components in two-line hybrids involving thermosensitive genic male sterile lines in rice (*Oryza sativa* L.). *Plant Breed.* 127, 28–32. doi: 10.1111/j.1439-0523.2007.01432.x

Singh, S., Sehgal, D., Kumar, S., Arif, M., Vikram, P., Sansaloni, C., et al. (2020). GWAS revealed a novel resistance locus on chromosome 4D for the quarantine disease Karnal bunt in diverse wheat pre-breeding germplasm. *Sci. Rep.* 10, 5999. doi: 10.1038/s41598-020-62711-7

Smirnov, N., and Arnaud, D. (2019). Hydrogen peroxide metabolism and functions in plants. *New Phytol.* 221, 1197–1214. doi: 10.1111/nph.15488

Sprague, G. F., and Tatum, L. A. (1942). General vs. specific combining ability in single crosses of corn. *J. Am. Soc. Agron.* 34, 923–932. doi: 10.2134/agronj1942.00021962003400100008x

Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P., and Reynolds, M. (2015). Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. *Theor. Appl. Genet.* 128, 353–363. doi: 10.1007/s00122-014-2435-3

Tadesse, W., Ogbonnaya, F. C., Jighly, A., Sanchez-Garcia, M., Sohail, Q., Rajaram, S., et al. (2015). Genome-wide association mapping of yield and grain quality traits in winter wheat genotypes. *PLoS One* 10, e0141339. doi: 10.1371/journal.pone.0141339

Team, R. C. (2016). *R: A language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available online at: <https://www.R-project.org/>.

Technow, F., Schrag, T. A., Schipprack, W., Bauer, E., Simianer, H., and Melchinger, A. E. (2014). Genome properties and prospects of genomic prediction of hybrid performance in a breeding program of maize. *Genetics* 197, 1343–1355. doi: 10.1534/genetics.114.165860

Tessmann, E. W., Dong, Y., and Van Sanford, D. A. (2019). GWAS for Fusarium head blight traits in a soft red winter wheat mapping panel. *Crop Sci.* 59, 1823–1837. doi: 10.2135/cropsci2018.08.0492

Turuspekov, Y., Baibulatova, A., Yermekbayev, K., Tokhetova, L., Chudinov, V., Sereda, G., et al. (2017). GWAS for plant growth stages and yield components in spring wheat (*Triticum aestivum* L.) harvested in three regions of Kazakhstan. *BMC Plant Biol.* 17, 1–11. doi: 10.1186/s12870-017-1131-2

Tyagi, S., Mir, R., Kaur, H., Chhuneja, P., Ramesh, B., Balyan, H., et al. (2014). Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). *Mol. Breed.* 34, 167–175. doi: 10.1007/s11032-014-0027-1

Ullah Zaid, I., Tang, W., He, J., Ullah Khan, S., and Hong, D. (2019). Association analysis uncovers the genetic basis of general combining ability of 11 yield-related traits in parents of hybrid rice. *Aob Plants* 11, ply077. doi: 10.1093/aobpla/ply077

VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. *J. Dairy Sci.* 91, 4414–4423. doi: 10.3168/jds.2007-0980

Vélez-Torres, M., García-Zavala, J. J., Hernández-Rodríguez, M., Lobato-Ortiz, R., López-Reynoso, J. J., Benítez-Riquelme, I., et al. (2018). Genomic prediction of the general combining ability of maize lines (*Zea mays* L.) and the performance of their single crosses. *Plant Breed.* 137, 379–387. doi: 10.1111/pbr.12597

Verma, O., and Srivastava, H. (2004). Genetic component and combining ability analyses in relation to heterosis for yield and associated traits using three diverse rice-growing ecosystems. *Field Crops Res.* 88, 91–102. doi: 10.1016/S0378-4290(03)00080-7

Vitale, P., Fania, F., Esposito, S., Pecorella, I., Pecchioni, N., Palombieri, S., et al. (2021). QTL analysis of five morpho-physiological traits in bread wheat using two mapping populations derived from common parents. *Genes* 12, 604. doi: 10.3390/genes12040604

Vitale, P., Laidò, G., Dono, G., Pecorella, I., Ramasubramanian, V., Lorenz, A., et al. (2024). Univariate and multivariate genomic prediction for agronomic traits in durum wheat under two field conditions. *PLoS One* 19, e0310886. doi: 10.1371/journal.pone.0310886

Vitale, P., Montesinos-López, O., Gerard, G., Velu, G., Tadesse, Z., Montesinos-López, A., et al. (2025). Improving wheat grain yield genomic prediction accuracy using historical data. *G3: Genes Genomes Genet.* 15, jka038. doi: 10.1093/g3journal/jka038

Volotovitch, A., Silkova, T., Fomchenko, N., Prokhorenko, O., and Davydenko, O. (2008). Combining ability and heterosis effects in sunflower of byelorussian origin. *Helia* 31, 111–118. doi: 10.2298/hel0848111v

Walejko, R., and Russell, W. (1977). Evaluation of recurrent selection for specific combining ability in two open-pollinated maize cultivars 1. *Crop Sci.* 17, 647–651. doi: 10.2135/cropsci1977.0011183X0017000400043x

Wang, K., Shi, L., Zheng, B., and He, Y. (2023). Responses of wheat kernel weight to diverse allelic combinations under projected climate change conditions. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1138966

Werner, C. R., Qian, L., Voss-Fels, K. P., Abbadi, A., Leckband, G., Frisch, M., et al. (2018). Genome-wide regression models considering general and specific combining ability predict hybrid performance in oilseed rape with similar accuracy regardless of trait architecture. *Theor. Appl. Genet.* 131, 299–317. doi: 10.1007/s00122-017-3002-5

White, J., Sharma, R., Balding, D., Cockram, J., and Mackay, I. (2022). Genome-wide association mapping of Hagberg falling number, protein content, test weight, and grain yield in UK wheat. *Crop Sci.* 62, 965–981. doi: 10.1002/csc.220692

Yang, Y., Amo, A., Wei, D., Chai, Y., Zheng, J., Qiao, P., et al. (2021). Large-scale integration of meta-QTL and genome-wide association study discovers the genomic regions and candidate genes for yield and yield-related traits in bread wheat. *Theor. Appl. Genet.* 134, 3083–3109. doi: 10.1007/s00122-021-03881-4

Yang, Y., Chai, Y., Zhang, X., Lu, S., Zhao, Z., Wei, D., et al. (2020). Multi-locus GWAS of quality traits in bread wheat: mining more candidate genes and possible regulatory network. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.01091

Zeng, L., and Pettigrew, W. T. (2015). Combining ability, heritability, and genotypic correlations for lint yield and fiber quality of upland cotton in delayed planting. *Field Crops Res.* 171, 176–183. doi: 10.1016/j.fcr.2014.10.004

Zhang, X., Chen, J., Yan, Y., Yan, X., Shi, C., Zhao, L., et al. (2018). Genome-wide association study of heading and flowering dates and construction of its prediction equation in Chinese common wheat. *Theor. Appl. Genet.* 131, 2271–2285. doi: 10.1007/s00122-018-3181-8

Zhang, A., Pérez-Rodríguez, P., San Vicente, F., Palacios-Rojas, N., Dhliwayo, T., Liu, Y., et al. (2022). Genomic prediction of the performance of hybrids and the combining abilities for line by tester trials in maize. *Crop J.* 10, 109–116. doi: 10.1016/j.cj.2021.04.007

Zhang, J., Song, Q., Cregan, P. B., and Jiang, G.-L. (2016). Genome-wide association study, genomic prediction and marker-assisted selection for seed weight in soybean (*Glycine max*). *Theor. Appl. Genet.* 129, 117–130. doi: 10.1007/s00122-015-2614-x

Zhao, Y., Zeng, J., Fernando, R., and Reif, J. (2013). Genomic prediction of hybrid wheat performance. *Crop Sci.* 53, 802–810. doi: 10.2135/cropsci2012.08.0463