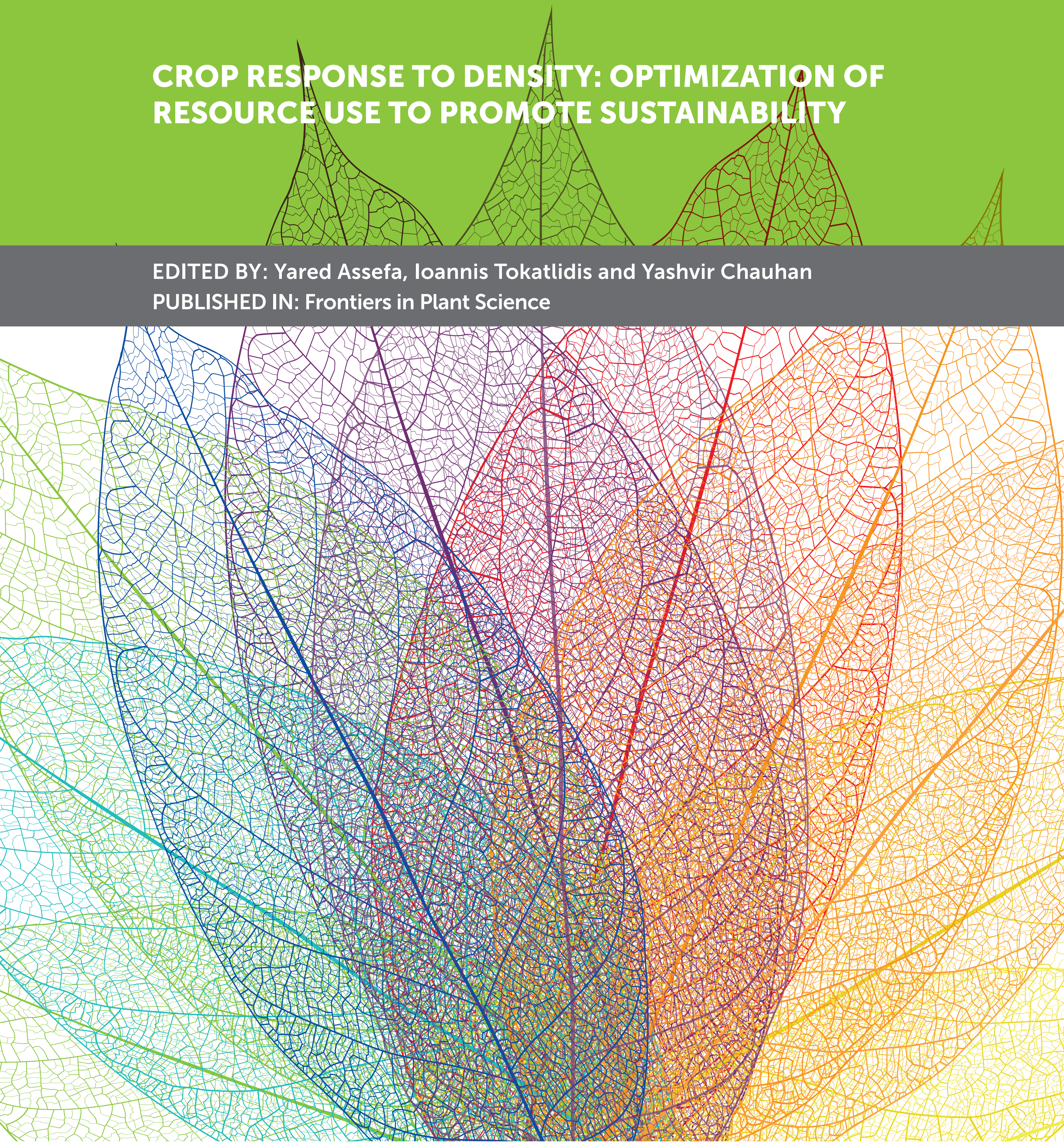
CROP RESPONSE TO DENSITY: OPTIMIZATION OF RESOURCE USE TO PROMOTE SUSTAINABILITY



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CROP RESPONSE TO DENSITY: OPTIMIZATION OF RESOURCE USE TO PROMOTE SUSTAINABILITY

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Editorial: Crop response to density: Optimization of resource use to promote sustainability

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crop resilience, competition, grain yield efficiency, low-input agriculture, yield gap

Editorial on the Research Topic
Crop response to density: Optimization of resource use to promote sustainability

This issue centered around plant population density and related topics; those were stem lodging and kernel abortion, decline and variability in solar radiation, leaf area index and the amount of intercepted photosynthetically active radiation, variability in optimum density and other topics for maize and other crops (sweet corn, wheat, grain sorghum, and barley).

To prevent a general food crisis, one of the major challenges facing agricultural research today is bridging a considerable yield gap. The yield gap is due to inefficient use of natural resources, resulting in harvested yield that lags behind the attainable yield. Among several factors contributing to the yield gap, the inability of individual plants to sufficiently capture inputs is a radical source. Low "plant yield efficiency" drastically affects the required number of plants per area, i.e., the plant population density (crop population); since water is the most crucial input, the phenomenon is more pronounced in rainfed crops. There is a consensus that under stressful conditions (i.e., drought), optimal resource use is accomplished only in low crop populations. On the other hand, due to the inability of individual plants to respond to additional inputs, modern varieties may reach the attainable yield of favorable environments at high crop populations. As conditions of growing seasons are difficult to predict during the growing period, the established plant population density may deviate from the one suitable for the season, and farmers may sustain a yield penalty. Crop modeling is helping to overcome this limitation but incorporating greater resource use plasticity could be a way forward.

The maize (*Zea mays* L.) collapse events of 2012 in Iowa and 2018 in Germany indicate that crop adaptation to spacing (low populations) is imperative to avoid crop failure in dry seasons without compromising the attainable grain yield during favorable seasons. As one of the topic editors previously emphasized (Tokatlidis, 2013, 2014, 2017), substantial benefits arise from crop adaptation to spacing: mitigation of the acquired plant-to-plant variability to optimize further the resource use; better compensation in both the common situation of missing plants and when multiline or open pollinated varieties are preferred to counteract unpredictable acute stresses (in both cases, individual plants would be able to utilize the input share of missing neighbors); adaptation to crop spacing would also expand the optimal planting date; adopting low-input cropping where necessary would prevent soil degradation and protect natural resources and the environment.

The objective of our collection was to attract articles from the fields of breeding, agronomy, physiology, soil science, molecular and genomic approaches related to the crop by population interaction, and in particular: crop response to population across varying environments; plant physiological response to crop population; interplant competition within a crop and breeding to mitigate the consequent intra-crop variation; soil water and soil biological and physicochemical properties that may relate to plant response to crop population; yield components related to crop population; environmental indicators for the optimal crop population; molecular and genomics that relate to crop population.

In this collection, most of the accepted submissions (7 out of 11) concerned maize, a crop whose average crop population has increased over the years across the globe (Assefa et al., 2018). A significant grain yield increase was associated with increased crop population, mainly in high- to medium-yielding environments (Assefa et al., 2016). The main questions addressed are the consequences of increased crop population and possible solutions. One of the articles (Shah et al.) deliberates that stem lodging and kernel abortion are major constraints in maize grain yield production as the crop population increases. Therefore, it is crucial to overcome stem lodging and kernel abortion, and Shah et al. review address that concern. The other shortcoming with increasing the crop population is a decline and variability in solar radiation that reaches each plant due to shading, affecting the crop productivity drastically; the issue is addressed by Yang et al. Zhang et al. reported a significant increase in leaf area index and the amount of intercepted photosynthetically active radiation with increased crop population. However, increased plant population reduced photosynthetic capacity, stomatal conductance, leaf chlorophyll content, and other responses, which are vital for crop productivity and yield stability. Capitalizing on the point that a high crop population aggravates competition among plants and harms plant growth and productivity, Liu et al. (2022) presented results proposing that nitrogen application and chemical control may improve plant growth and increase grain yield in a high crop population.

Crop population is a function of row spacing and plant spacing. An optimal combination could result in the same crop population but better resource use efficiency and higher productivity achieved through better planting configuration. Row- and plant-spacing should also be considered in conjunction with different soil and crop management. Indeed, Haarhoff and Swanepoel address the issue and accomplish increased light interception in a no-till semi-arid environment. Capitalizing on the same subject, Winans et al., beyond row spacing, explored other agronomic inputs such as P-S-Zn fertility, K-B fertility, N fertility, and foliar protection that could alleviate density-induced stress in a high crop population. In light of this, one might look for the key to identifying maize hybrids that tolerate high crop populations. Larrosa and Borrás answer that there is a relationship between density tolerance and radiation reduction around flowering.

Other publications of the collection discuss the issue of crop population in sweet corn (Dhaliwal et al.), wheat (Jaenisch et al.), grain sorghum (Zhou et al.), and barley (Tsivelikas et al.). Similar to points raised in maize, these papers also discuss planting density trends, yield component compensation, yield and quality response, and possible hybrid selection tools for high crop populations. From the breeding perspective, Tsivelikas et al. deal with interplant distance as a factor affecting the efficiency of single-plant selection, suggesting the absolute absence of inter-genotypic competition. We believe the information compiled delivers important Research Topics regarding crop response to planting density and raises new breeding challenges. However, we expected greater contribution from a breeding point of view, particularly in adapting crops to lower densities, stabilizing optimum density and creating varieties capable of effective resource use in variable environments, and reducing the yield gap (Tokatlidis, 2017; Fischer, 2020). The collection did not intensively cover historical trends in maize plant density reliance and plant density relations, which we assumed are covered in prior publications. We encourage readers to look into previous publications of the editors related to these areas as further reading (e.g., Tokatlidis and Koutroubas, 2004; Tokatlidis et al., 2011; Tokatlidis, 2013, 2014; Assefa et al., 2016, 2018; Solomon et al., 2017; Mylonas et al., 2020). Tokatlidis (2017) argues that to reach crop adaptation to lower populations and resilience, breeding for density-independent varieties *via* improved plant yield efficiency is a viable option and imperative to bridge the yield gap. Also, the effects of plant density on root systems and its consequence on the efficiency of the use of below ground resources, including their influence on soil microflora, were not extensively covered in this collection, and we suggest further issues to cover these gaps.

Author contributions

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

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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ORIGINAL RESEARCH
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Solar Radiation Effects on Dry Matter Accumulations and Transfer in Maize

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Solar radiation is the energy source for crop growth, as well as for the processes of accumulation, distribution, and transfer of photosynthetic products that determine maize yield. Therefore, learning the effects of different solar radiation amounts on maize growth is especially important. The present study focused on the quantitative relationships between solar radiation amounts and dry matter accumulations and transfers in maize. Over two continuous years (2017 and 2018) of field experiments, maize hybrids XY335 and ZD958 were grown at densities of 4.5×10^4 (D1), 7.5×10^4 (D2), 9×10^4 (D3), 10.5×10^4 (D4), and 12×10^4 (D5) plants/ha at Qitai Farm (89°34′E, 44°12′N), Xinjiang, China. Shading levels were 15% (S1), 30% (S2), and 50% (S3) of natural light and no shading (CK). The results showed that the yields of the commonly planted cultivars XY335 and ZD958 at S1, S2, and S3 (increasing shade treatments) were 7.3, 21.2, and 57.6% and 11.7, 31.0, and 61.8% lower than the control yields, respectively. Also, vegetative organ dry matter translocation (DMT) and its contribution to grain increased as shading levels increased under different densities. The dry matter assimilation amount after silking (AADMAS) increased as solar radiation and planting density increased. When solar radiation was <580.9 and 663.6 MJ/m^2, for XY335 and ZD958, respectively, the increase in the AADMAS was primarily related to solar radiation amounts; and when solar radiation was higher than those amounts for those hybrids, an increase in the AADMAS was primarily related to planting density. Photosynthate accumulation is a key determinant of maize yield, and the contributions of the vegetative organs to the grain did not compensate for the reduced yield caused by insufficient light. Between the two cultivars, XY335 showed a better resistance to weak light than ZD958 did. To help guarantee a high maize yield under weak light conditions, it is imperative to select cultivars that have great stay-green and photosynthetic efficiency characteristics.

Keywords: maize, solar radiation, density, dry matter accumulation and translocation, photosynthates, leaf area duration

INTRODUCTION

Food shortage has long been a worldwide problem (Jia et al., 2011), but the recent COVID-19 pandemic, beginning in early 2020, has not only seriously affected public health but has also added significant uncertainty to national and global food supplies (Balwinder et al., 2020; Lamichhane and Reay-Jones, 2021). One of the most important crops globally, maize, provides food and protein for people, as well as raw material for industrial production (Gao et al., 2017). However, maize production is vulnerable to abnormal weather conditions, such as continuous rain, wet weather, and low-light levels caused by cloud cover, and that has been exacerbated due to worldwide climate change and environmental pollution (Wu et al., 2020). Solar radiation drives crop photosynthesis and yields, as well as the formation and development of plant organs (Ding et al., 2005; Zhang et al., 2007; Dordas, 2009; Ye et al., 2020). Studies have shown that global solar radiation has been decreasing by an average of 1.4–2.7% per decade, and the effective sunlight duration decreasing by 1.28% each decade over a period of time in China (Cui et al., 2015; Ren et al., 2016). For example, in the Huang-Huai Plain region, predicted maize yields could be reduced by 3–6% by rainy weather and insufficient light during the growing period, especially given the background of global climate change (Cui et al., 2012; Ren et al., 2016; Gao et al., 2017). Therefore, exactly how solar radiation changes affect maize production must be investigated to help guarantee maize yield under future climate change scenarios.

Dry matter production, accumulation, and transportation are important factors that determine maize yield (Hou et al., 2020; Liu et al., 2020a,b), which is significantly correlated with the continuous increase of dry matter accumulation after flowering (Zhang et al., 2016). Gao et al. (2017) suggested that ∼60% of the carbohydrates in maize grains come from post-flower photosynthetic products, whereas Yan et al. (2001) suggested that higher yielding cultivars have stronger post-flowering photosynthetic capacity but poor assimilate transfer to grain. Nevertheless, some studies posited that the main reason for higher maize yields is the accumulation of more dry matter at the pre-silking stage and a higher transport rate in the post-silking stage (Yang et al., 1999). Barnabás et al. (2007) demonstrated that maize grain yield is dependent on post-silking photosynthate accumulation, but the translocation of reserved carbohydrates in vegetative organs to grains cannot be ignored (Mu et al., 2010; Wang et al., 2020a; Ye et al., 2020). Maize yield may effectively be increased by increasing dry matter production capacity and then transferring as much of that accumulated dry matter to the grain as possible (Chen, 1994; Ding et al., 2005; Hou et al., 2012). Although aboveground dry matter accumulation, partitioning, and translocation have been well documented in rice (Yang et al., 1997), wheat (Dordas, 2009; Zhou et al., 2012), cotton (Ibrahim et al., 2010), and maize (Zhu et al., 2011; Pu et al., 2016), little is known about the effects of solar radiation on dry matter accumulation and translocation in maize.

Field shading, a common method used to study the effects of solar radiation on crop growth (Yang et al., 2001; Cui et al., 2015; Ren et al., 2016; Fan et al., 2018), shows how different shading periods have different effects on maize growth (Zhang et al., 2006; Cui et al., 2013a; Shi et al., 2015; Gao et al., 2017). Shading during the reproductive period of the maize decreases grain yield more than during the vegetative growth stages (Early et al., 1967; Zhang et al., 2007; Yang et al., 2019). Furthermore, different degrees of shading have different effects on maize growth and development (Cui et al., 2013a). The accumulation and distribution of dry matter in the stem, leaf, and sheath are important factors in maize grain yield (Karlen et al., 1987; Gao et al., 2017; Yang et al., 2021). Also, assimilates in the vegetative organs gradually move to the grain in the late growth stage (Yang et al., 1997; Ma et al., 2008; Gao et al., 2017). Modern maize grain yield improvements are highly dependent on increasing plant density while enabling the plants to intercept more solar radiation (Liu et al., 2017, 2021c; Hou et al., 2020), and planting density affects light quality and other environmental factors that influence the yield as well (Jin et al., 2020). Also, planting density has important effects on maize dry matter partitioning between vegetative and reproductive organs (Wei et al., 2019), as planting density increases, the numbers of vegetative organs increase while that of reproductive organs decrease (Liu et al., 2011). Previous studies have indicated that leaf area index (LAI) increases as plant density increases (Xu et al., 2017; Liu et al., 2020a), an overly high LAI may cause self-shading and has been noted for possible photosynthetic decrease and yield loss (Cui et al., 2013b; Liu et al., 2015, 2020a; Srinivasan et al., 2017), and the increase of leaf area duration (LAD) of maize was accompanied by the increase of photosynthetic rate, and finally significantly increased the total biomass (Liu et al., 2020a).

There have been many studies on shading (Andrade et al., 1993; Andrade and Ferreiro, 1996; Cerrudo et al., 2013), however, little is known about the interactive and quantitative relationships between solar radiation, planting density, and hybrids in maize. Additionally, because most of the previous studies were conducted in lower solar radiation areas in China (Jia et al., 2007; Cui et al., 2015; Ren et al., 2016), their findings were not closely connected to the actual production conditions after shading. In this study, we chose a farm in the Xinjiang region, the area with the most abundant solar radiation in China (Xue et al., 2016), and the two most widely planted maize genotypes were selected. We also established different shading and planting density treatments to re-create different solar radiation conditions so that we could study the quantitative relationships between maize dry matter accumulations and transfers and solar radiation. Our results provide a theoretical basis for cultivar breeding and improved field management as agronomists cope with climate change and dense planting.

MATERIALS AND METHODS

Experimental Design

We conducted field experiments in 2017 and 2018 at the Qitai Farm (43°49′27″N,89°48′22″E) in Xinjiang, China. A split block design was conducted with cultivars as the main factor, planting density as the subplot factor, and shading level as the secondary subplot factor, and all plots were arranged in a completely randomized design with three replications. We used maize hybrids Xianyu 335 (XY335) and Zhengdan 958 (ZD958) in both the years because they are widely grown in China, and the plant architecture of these two hybrids was different, such as leaf length and leaf angles (Ma et al., 2014; Hou et al., 2020). The experimental plots measured 11 × 10 m and adjacent plots were separated by a 1 m wide walkway. Different environmental solar radiation conditions were created by manipulating shading and planting density. The maize was planted at five different densities: 4.5×10^4 (D1), 7.5×10^4 (D2), 9×10^4 (D3), 10.5×10^4 (D4), and 12×10^4 (D5) plants/ha in 2018 and three planting densities (D2, D4, and D5) in 2017. Shading levels were 50 (S3), 30 (S2), and 15% (S1) of natural light and no shading (CK). We used nylon nets to build temporary shading sheds. The nets were 4.5 m above the ground, which were fixed in place ∼1.5 m above the maize canopy in order to maintain the same microclimatic conditions except for solar radiation as in the unshaded portions of the field. The shading period began at silking and lasted until maturity. Shading nets were designed and fabricated to have different shading strengths, and the incident light quality in the maize canopy was not affected by field shading (Andrade et al., 2000; Jia et al., 2011; Yang et al., 2020).

All experimental plots were irrigated (15 mm) on the 1st day after sowing, and starting from 60 days after sowing, single water applications of 58 mm were delivered at 9–10 day intervals throughout the growing season for a total of nine applications. The total irrigation amount was ∼540 mm (Zhang et al., 2017). All weeds, diseases, and pests were controlled. Base fertilizers were applied before sowing and included 150 kg/ha N from urea, 225 kg/ha P_2O_5 (super phosphate), and 75 kg/ha K_2O (from potassium sulfate). To ensure a non-limiting supply of nutrients, additional urea (300 kg/ha N) was applied *via* drip irrigation in alternate irrigations during the growing season.

Sampling and Measurement

In each plot, three adjacent plants from the same inside row were cut manually at silking and at physiological maturity. We assigned plant part categories as stalk (stalk, sheath, and tassel), leaf, cob, husk, and grain; and after harvest, the parts were oven dried (85°C) to a constant weight. At physiological maturity, a 3.3 × 5 m area [in an alternating narrow–wide (40:70 cm) row planting pattern] was manually harvested from the center of each plot and its grain weight was measured (Liu et al., 2020a). We determined grain moisture content using a PM8188 portable moisture meter (Kett Electric Laboratory, Tokyo, Japan), and grain yield and thousand kernel weights (TKW) were determined at 14% moisture content. The kernel rows per ear and kernel number per row were calculated using 10 selected ears. The kernel number per ear (KNP) was calculated as follows: KNP = kernel rows per ear × kernel number per row (Liu et al., 2019). In 2018, every 10 days after silking and until maturity, leaf area measurements [leaf length (L) and maximum leaf width (W) of all the leaves on each tagged plant) were taken from five marked, representative plants from each plot. Then leaf areas and LAIs were calculated as described by Xu et al. (2017).

$$\text{Leaf area} = \text{L} \times \text{W} \times 0.75 \tag{1}$$

$$\text{LAI} = \frac{\text{Leaf area per plant} \times \text{plant number per plot}}{\text{Plot area}} \tag{2}$$

Leaf area duration (LAD) was calculated as:

$$LAD = \frac{L1 + L2}{2} \times (t1 - t2) \tag{3}$$

where L1 and L2 are the leaf area per plant at time $t1$ (maturity) and $t2$ (silking), respectively (Liu et al., 2021a).

We obtained meteorological data for the 2017 and 2018 maize growing seasons from a WatchDog 2000 Weather Station data logger (Spectrum Technologies, Inc., Washington, DC, United States) located in the experimental field (the data were recorded at hourly intervals), and the measured PAR was averaged in the wide and narrow rows at the top and the bottom of the canopies at 13:00 and 15:00 hours (Xu et al., 2017) on clear days using a SunScan (Delta-T Devices, Cambridge, United Kingdom). The total intercepted PAR was calculated according to the following formula.

$$\text{Total intercepted PAR (MJ/m2)} = (1 - \frac{B}{A}) \times C, \tag{4}$$

where A is PAR above the canopy, B is the transmitted PAR at the bottom of the canopy, and C is total accumulated par from silking to maturity.

In 2018, ear leaves per plot were chosen for photosynthesis measurement during the grain filling stage (20 days after silking). First, gas exchange measures were made on clear days at 13:00 and 15:00 using an LI-6400 programmable, portable open-flow gas exchange system (Li-Cor Inc., Lincoln, NE, United States). We performed light induction by keeping the leaves in the leaf chamber with the CO_2 concentration controlled at 400 μmol CO_2 (per mol air) and under PAR = 2,000 μmol/m^2/s until the parameter readings were stable (Liu et al., 2020a). Dry matter translocation (DMT) of vegetative organs (stalk + leaf), contribution of pre-silking dry matter to grain (CDMG), and the amount of assimilated dry matter after silking (AADMAS) were calculated as described by Zhu et al. (2011) and all weights were measured as t/ha.

$$\begin{aligned}\text{DMT of vegetative organs} &= \text{Dry matter weight at silking}\\ &\quad - \text{Dry matter weight at maturity}\end{aligned} \tag{5}$$

$$\begin{aligned}&\text{CDMG of the vegetative organ (\%)}\\ &= \frac{\text{DMT of the vegetative organ}}{\text{Kernel dry matter weight at maturity}} \times 100\end{aligned} \tag{6}$$

$$\begin{aligned}\text{AADMAS} &= \text{Dry matter weight of grain at maturity}\\ &\quad - \text{DMT of vegetative organs}\end{aligned} \tag{7}$$

Statistical Analysis

Statistical calculations were performed and charts generated in Excel 2016 (Microsoft, Redmond, WA, United States) and Origin

2018 (OriginLab, Northampton, MA, United States). SPSS ver. 21.0 (IBM SPSS, Chicago, IL, United States) was used to conduct one-way ANOVA followed by Duncan's multiple range tests at $P < 0.05$ to test the differences between different treatments in the two study years. Treatment effects and interaction between treatments were analyzed by ANOVA using mixed models. Residuals were analyzed to corroborate the assumptions of the ANOVA. For all of the dependent variables analyzed, year, cultivar, density, and shading level were considered as fixed factors.

RESULTS

Different Shading Levels Affect Maize Yield, Yield Components, and Dry Weight of Organs Under Different Density Conditions

Shading affected maize yield, the decrease rate of yield was in the order S3 > S2 > S1, compared with CK (**Table 1**). Over the 2 years of the experimental period, the mean yields of five planting densities of XY335 were >ZD958; and compared with CK, yields of XY335 decreased <ZD958 after shading. Averaging all planting densities (D1, D2, D3, D4, and D5), and over both the study years, the yields of XY335 and ZD958 at S1, S2, and S3 were 7.3, 21.2, and 57.6%, and 11.7, 31.0, and 61.8% lower than CK, respectively. Also, the dry matter weight of vegetative organs at maturity were 8.7, 8.9, and 18.2%, and 4.5, 10.7, and 20.2% lower than CK, respectively (**Table 1**). Averaging all shading treatments, the yields of XY335 and ZD958 at D1, D2, D3, D4, and D5 were 21.9, 21.1, 30.0, 31.6, and 35.1% and 13.3, 18.2, 42.8, 40.4, and 39.9%, the vegetative organ dry matter weights were 18.6, 17.0, 17.8, 21.6, and 12.8% and 16.2, 13.4, 16.3, 14.0, and 8.2% lower than CK, respectively. The reduction of ear density, KNP, and TKW significantly increased with the increase of shade level (**Table 2**). The main effect of shading treatment on maize yield components was the decrease in KNP and TKW and therefore the shading mainly affected grain formation and filling after silking. For the cultivars, the KNP and TKW of XY335 were higher than that of ZD958.

Effects of Different Shading Levels on DMT of Vegetative Organs and CDMG Under Different Density Conditions

Both vegetative organ DMT and pre-silking CDMG increased as shading level increased under different densities (**Figure 1**). These results showed that over the 2 years and five planting densities, the mean DMTs in CK, S1, S2, and S3 were 0.68, 1.07, 1.91, and 2.01 *t*/ha, while the mean CDMGs were 3.52, 5.54, 11.28, and 41.25% (15.4% total), respectively. Shading increased DMT by averages of 56.5, 179.4, and 196.1%, and increased CDMG by averages of 0.6, 2.23, and 10.45% in S1, S2, and S3, respectively, compared with those measures in CK. We also showed that the 2-year DMT and CDMG averages of all shading treatments (CK, S1, S2, and S3) for D1, D2, D3, D4, and D5 were 0.16, 0.91, 2.52, 2.04, and 1.47 *t*/ha and 1.92, 9.83, 20.52, 20.21, and 20.33%, respectively. DMT and CDMG rates increased more for XY335 than for ZD958, thus indicating that XY335 transferred more photosynthetic products to grain than ZD958 under low–solar radiation stress. The XY335 and ZD958 DMTs in CK were 0.40 and 0.97 *t*/ha, respectively, but those measures increased significantly in S1, S2, and S3: by 123.9, 304.8, and 458.4% for XY335 and by 28.6, 127.6, and 215.9% for ZD958. Although DMTs increased with the increase of shade levels, the amplitude of the changes between them was not proportional. Likewise, the mean CDMG over both years and all planting densities of XY335 and of ZD958 under S1, S2, and S3 increased significantly (by 121.7, 354.2, and 1549.8% for XY335 and by 31.7, 162.9, and 813.9% for ZD958) compared with those measures for CK (2.11 and 4.63% for XY335 and ZD958, respectively).

Quantitative Relationships Between AADMAS and Planting Densities Under Different Solar Radiation Levels

As solar radiation increased so did AADMAS, which also decreased as planting density increased when solar radiation was low, but increased at the same planting densities when radiation was high (**Figures 2A,B**). For three-dimensional analysis, we used multiple linear regression to evaluate the interaction effects between planting density and solar radiation on AADMAS in XY335 and ZD958. Combined planting density and solar radiation explained 93% and 88% of the variations in AADMAS for XY335 and ZD958, respectively. When the solar radiation was <580.9 and 663.6 MJ/m^2, for XY335 and ZD958, respectively, increases in AADMAS were primarily related to the amount of solar radiation. When the solar radiation was higher than 580.9 and 663.6 MJ/m^2 for XY335 and ZD958, respectively, increases in AADMAS were primarily related to planting density. The XY335 and ZD958 AADMAS of CK were 19.7 and 19.2 *t*/ha, respectively. AADMAS decreased significantly in S1, S2, and S3 by 8.7, 22.7, and 80.3% for XY335 and by 7.8, 27.3, and 81.8% for ZD958. Averaging all shading leves and over both the study years, the AADMAS of XY335 and ZD958 D1, D2, D3, D4, and D5 were 26.0, 32.5, 36.6, 39.8, and 45.2%, and 26.6, 32.8, 35.4, 46.9, and 45.1% lower than CK, respectively. The fluctuations of AADMAS and shading level were not synchronous, and which also increased as planting density increased.

Influences of Shading on Photosynthetic Characteristics and LAD of Different Maize Cultivars

All ear leaf photosynthetic rates (*Pn*) changed significantly after shading (**Figure 3**) and they decreased as shading levels and planting densities increased (*Pn* of ZD958 was not decreased with increase in plant densities). As shown in **Figure 3**, *Pn*s were greater for XY335 than for ZD958, as the *Pn* under S1, S2, and S3 decreased significantly by 18.6, 19.54, and 28.1%, for XY335 and by 31.11, 32, and 33.82% for ZD958. This indicated that the net leaf *Pn* decreased as shading increased, and since the ratio of the decrease of XY335 was lower than that of

TABLE 1 | Effects of different shading levels (CK, natural light; S1, 15% natural light; S2, 30% natural light; S3, 50% natural light) and planting densities (D1, 4.5 × 10^4 plants ha^{-1}; D2, 7.5 × 10^4 plants ha^{-1}; D3, 9 × 10^4 plants ha^{-1}; D4: 10.5 × 10^4 plants ha^{-1}; D5, 12 × 10^4 plants ha^{-1}) on maize grain yields and dry weights of plant organs (dry matter of vegetative organs at silking [VS] and at maturity [VM]) in 2017 (Y1) and 2018 (Y2).

	XY335			ZD958		
Treatment	**Yield (t ha^{-1})**	**VS (t ha^{-1})**	**VM (t ha^{-1})**	**Yield (t ha^{-1})**	**VS (t ha^{-1})**	**VM (t ha^{-1})**
Y1D2CK	18.46 a	11.3 a	14.9 a	17.15 a	10.3 a	11.8 a
Y1D2S1	18.55 a	11.3 a	12.3 a	16.92 a	10.3 a	11.2 a
Y1D2S2	17.02 a	11.3 a	13.7 a	13.90 b	10.3 a	11.2 a
Y1D2S3	12.42 b	11.3 a	11.9 a	8.77 c	10.3 a	9.3 b
Y1D4CK	19.92 a	14.8 a	17.0 a	19.29 a	14.7 a	13.5 a
Y1D4S1	18.16 ab	14.8 a	13.5 b	16.66 ab	14.7 a	13.5 a
Y1D4S2	16.20 b	14.8 a	13.7 b	13.87 b	14.7 a	13.1 a
Y1D4S3	11.13 c	14.8 a	13.9 b	8.46 c	14.7 a	11.0 b
Y1D5CK	21.76 a	16.3 a	16.4 a	20.13 a	15.1 a	16.1 a
Y1D5S1	19.93 ab	16.3 a	15 b	17.34 b	15.1 a	15.3 a
Y1D5S2	16.99 b	16.3 a	13.3 c	14.14 c	15.1 a	15.0 a
Y1D5S3	10.29 c	16.3 a	12.8 c	7.55 d	15.1 a	15.0 b
Y2D1CK	18.39 a	7.9 a	10.9 a	14.74 a	7.5 a	9.7 a
Y2D1S1	17.12 a	7.9 a	9.1 ab	15.14 a	7.5 a	8.8 a
Y2D1S2	17.13 a	7.9 a	9.6 ab	15.20 a	7.5 a	8.6 a
Y2D1S3	8.84 b	7.9 a	7.9 b	8.01 b	7.5 a	7.1 b
Y2D2CK	19.29 a	12.0 a	11.3 a	17.61 a	11.6 a	12.1 a
Y2D2S1	16.85 a	12.0 a	11.7 a	16.63 a	11.6 a	11.0 a
Y2D2S2	16.76 a	12.0 a	12.6 a	10.97 b	11.6 a	10.6 a
Y2D2S3	7.96 b	12.0 a	11.4 a	7.28 c	11.6 a	8.2 b
Y2D3CK	19.21 a	14.0 a	12.8 a	17.77 a	12.7 a	12.1 a
Y2D3S1	17.69 b	14.0 a	10.7 a	14.98 b	12.7 a	11.6 a
Y2D3S2	15.05 c	14.0 a	10.8 a	10.12 c	12.7 a	9.5 b
Y2D3S3	7.58 d	14.0 a	10.2 a	5.40 d	12.7 a	9.3 b
Y2D4CK	20.06 a	13.6 a	12.1 a	18.16 a	16.2 a	14.3 a
Y2D4S1	17.98 a	13.6 a	13.2 a	13.89 b	16.2 a	12.8 ab
Y2D4S2	14.25 b	13.6 a	12.9 a	9.27 c	16.2 a	11.7 b
Y2D4S3	4.26 c	13.6 a	9.2 b	5.04 d	16.2 a	11.1 b
Y2D5CK	21.27 a	13.5 a	12.8 ab	18.99 a	15.0 a	10.4 ab
Y2D5S1	20.55 a	13.5 a	13.3 a	15.48 b	15.0 a	11.3 a
Y2D5S2	11.41 b	13.5 a	11.9 ab	11.74 c	15.0 a	9.7 bc
Y2D5S3	4.70 c	13.5 a	11.3 b	4.40 d	15.0 a	9.0 c

Different lowercase letters indicate significant differences between treatments at $P < 0.05$.
The 2017 yield data was published and cited in Yang et al. (2019).

ZD958, XY335 had better photosynthetic characteristics than did ZD958.

From silking to maturity, the LAD gradually decreased (**Figure 4**). Under CK, S1, S2, and S3, the LADs of XY335 were 44.3, 43.6, 40.6, and 38.6 m^2/day, respectively, and they were 52.6, 42.4, 44.5, 35.8, and 33.7 m^2/day, under D1, D2, D3, D4, and D5, respectively (**Figures 4A–E**). Under the CK, S1, S2, and S3, the LADs of ZD958 were 41.2, 37.5, 35.7, and 30.5 m^2/day, respectively, and were 42.6, 38.0, 36.0, 32.1, and 32.5 m^2/day, under D1, D2, D3, D4, and D5, respectively (**Figures 4F–J**). As shown in **Figure 4**, LADs were greater for XY335 than for ZD958, as the LAD under S1, S2, and S3 decreased significantly by 1.8, 7.9, and 12.4%, for XY335 and by 9.3, 13.6, and 24.6% for ZD958. This indicated that LAD decreased as shading increased. Since the decrease rate of XY335 was lower than that of ZD958, XY335 had better leaves anti-aging ability than ZD958 (**Figure 4**).

Relationships Between DMT and Accumulation and Leaf *Pn*, LAD, and Their Correlations With the Yield

DMT and AADMAS were significantly affected by both the *Pn*s and LAD (**Figures 5A–D**). First, leaf *Pn* and LAD were significantly negatively correlated with DMT and positively correlated with AADMAS, Specifically, when the *Pn* increased by 1 μmol $CO_2/m^2/s$, DMT decreased by 0.19 *t*/ha and AADMAS increased by 0.68 *t*/ha. Also, when the decreases in LAD increased

TABLE 2 | Yield components of maize under different shading levels and planting densities.

Treatment	XY335			ZD958		
	Ear density (10^3 ha^{-1})	KNP	TKW (g)	Ear density(10^3 ha^{-1})	KNP	TKW (g)
Y1D2CK	80 a	619 a	401.94 a	110 a	527 a	381.61 a
Y1D2S1	85 a	602 a	407.05 a	109 a	509 ab	341.18 b
Y1D2S2	88 a	636 a	395.36 a	97 ab	477 b	356.88 b
Y1D2S3	79 a	179 b	194.97 b	86 b	314 c	110.77 c
Y1D4CK	106 a	554 a	411.27 a	118 a	576 a	364.90 a
Y1D4S1	107 a	496 b	384.37 b	116 a	513 b	346.15 a
Y1D4S2	95 b	495 b	351.63 c	101 b	444 c	324.08 b
Y1D4S3	84 c	113 c	192.55 d	91 c	211 d	120.97 c
Y1D5CK	121 a	525 a	374.10 a	135 a	469 a	383.35 a
Y1D5S1	122 a	464 b	384.20 a	122 b	470 a	326.92 b
Y1D5S2	106 a	428 b	350.95 a	113 c	453 b	326.59 b
Y1D5S3	110 a	93 c	185.57 b	92 d	147 c	222.58 c
Y2D1CK	75 a	639 a	430.99 a	67 a	611 a	452.63 a
Y2D1S1	74 a	643 a	428.66 ab	66 a	602 a	432.69 ab
Y2D1S2	67 a	642 a	404.82 b	63 a	601 a	421.69 b
Y2D1S3	46 b	521 b	372.67 c	43 b	537 b	362.93 c
Y2D2CK	78 a	615 a	424.43 a	74 ab	604 ab	398.05 a
Y2D2S1	70 a	621 a	405.82 a	73 ab	616 a	389.39 a
Y2D2S2	73 a	563 a	398.83 ab	77 a	544 b	340.76 b
Y2D2S3	70 a	323 b	376.13 c	64 b	414 c	312.94 c
Y2D3CK	84 ab	577 a	420.73 a	90 a	576 a	369.37 a
Y2D3S1	89 a	596 a	395.79 b	86 a	581 a	341.73 b
Y2D3S2	74 b	526 a	387.74 b	89 a	525 a	319.84 bc
Y2D3S3	74 b	266 b	371.57 c	72 b	262 b	300.10 c
Y2D4CK	100 a	577 a	402.60 a	93 a	564 a	378.72 a
Y2D4S1	94 a	569 a	376.37 a	93 a	515 a	363.60 a
Y2D4S2	86 ab	454 b	377.54 a	94 a	548 a	342.60 ab
Y2D4S3	72 b	372 c	371.86 a	65 b	307 b	300.86 b
Y2D5CK	108 a	544 a	405.07 a	103 a	535 a	353.48 a
Y2D5S1	105 a	507 ab	383.54 ab	109 a	463 b	315.52 ab
Y2D5S2	93 ab	399 b	369.24 b	99 a	408 c	322.27 ab
Y2D5S3	73 b	248 c	366.41 b	81 b	221 d	262.83 b

*Different lowercase letters indicate significant differences between treatments at $P < 0.05$. KNP, kernel number per ear. TKW, thousand-kernel weight. See **Table 1** for planting density and shading treatment definitions.*

by 1 m^2/day, DMT decreased by 0.15 *t*/ha and AADMAS increased by 0.31 *t*/ha.

As shown in **Table 3**, vegetative organ dry matter at silking (VS), at maturity (VM), ear density, and TKW were significantly affected by the interaction of Y × C; VS, VM, and TKW were significantly affected by the interaction of Y × D; yield, KNP, and TKW were significantly affected by the interaction of Y × S; VS, ear density, and TKW were significantly affected by the interaction of C × D; yield, ear density, KNP, and TKW were significantly affected by the interaction of C × S and D × S (D × S was not significant for TKW); yield, VS, VM, ear density, KNP, and TKW were significantly affected by the interaction of Y × C × D and Y × C × S (Y × C × D was not significant for yield and Y × C × S was not significant for VS); the interaction of Y × D × S was significant for TKW, the interaction of C × D × S was significant for yield and KNP, and the interaction of Y × C × D × S was significant for VM and TKW. VS and VM, as well as the AADMAS were significantly positively correlated with the yield (**Figure 6**). However, DMT was significantly negatively correlated with the yield.

DISCUSSION

As a primary environmental factor of crop growth, light intensity importantly influences maize yield (Jia et al., 2011; Shi et al., 2013; Ren et al., 2016; Hou et al., 2021). Indeed, we found that both dry matter weight and yield under our S1, S2, and S3 treatments were lower than those measures in CK, a result that has been found in other maize shading studies (Shi et al., 2015; Guo et al., 2020; Yang et al., 2021). Also, maize grain

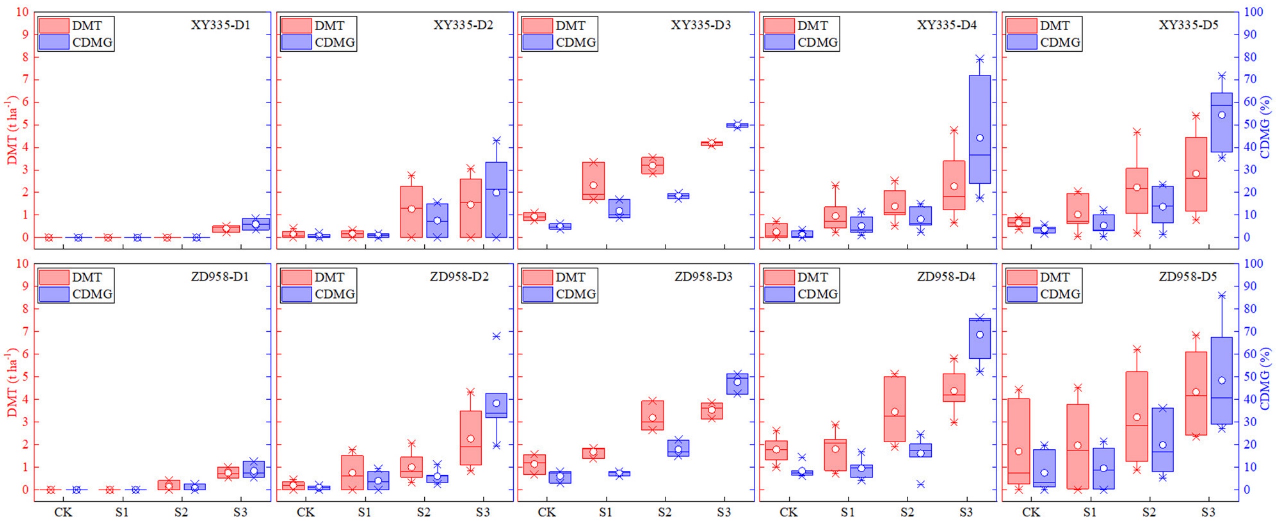

FIGURE 1 | Effects of different shading levels (CK, natural light; S1, 15% natural light; S2, 30% natural light, S3, 50% natural light) on vegetative organ dry matter translocation (DMT) and pre-silking dry matter contributions to grain (CDMG) under different planting densities (D1, 4.5 × 10^4 plants/ha; D2, 7.5 × 10^4 plants/ha; D3, 9 × 10^4 plants/ha; D4, 10.5 × 10^4 plants/ha; D5, 12 × 10^4 plants/ha). Maize hybrids: XY335, Xianyu 335, and ZD958, Zhengdan 958. Boxes, 25th and 75th percentiles; interior circles and bars, mean and median, respectively; bars, minimum and maximum values.

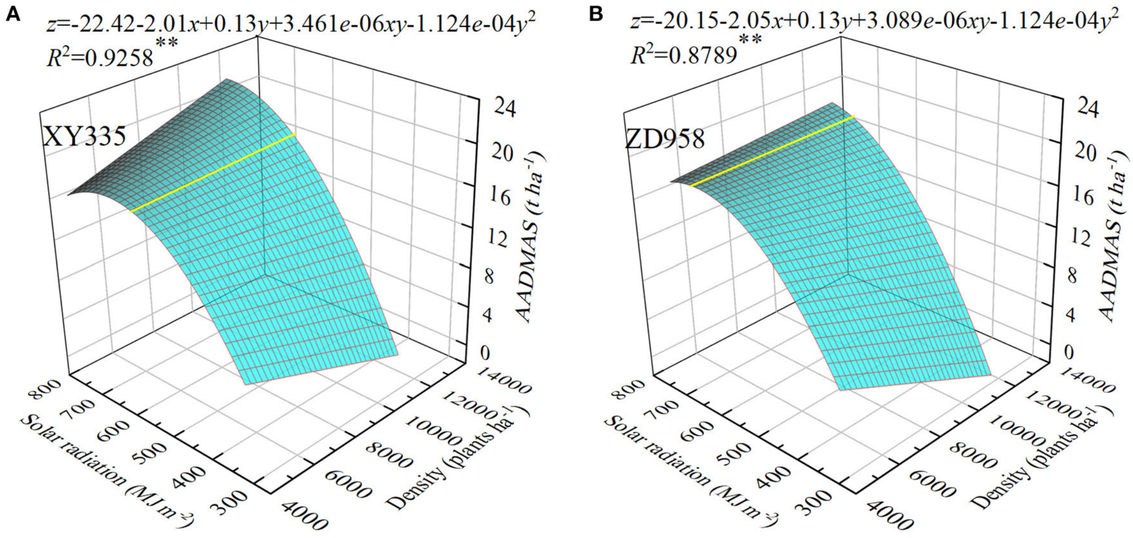

FIGURE 2 | Relationships between assimilation amount of dry matter after silking (AADMAS), planting density, and different totals of accumulated photosynthetically active radiation from the silking to the maturity stages in two maize hybrids (XY335 and ZD985). *x* is planting density, *y* is solar radiation, *z* is AADMAS. $^{**}P \leq 0.01$.

yield is dependent on post-silking photosynthate accumulation and on the translocation of the reserved carbohydrates in vegetative organs (Barnabás et al., 2007; Wang et al., 2020a). We found that DMT and CDMG increased as shading levels increased and differed under different planting densities. Those results confirm those of Wang et al. (2020a) who reported that the translocation of pre-silking assimilates in vegetative organs increased under shading (Wang et al., 2020a). In our study, DMT increased as solar radiation decreased (**Figure 1**), and it was significantly negatively correlated with AADMAS and yield (**Figure 6**), thus suggesting that vegetative organ dry matter transportation to the grain could not compensate for the yield loss received by AADMAS under insufficient light environments. DMT and AADMAS reduction due to shading does not correspond to the magnitude of radiation reduction. The reason for this phenomenon may be that compensatory photosynthesis occurred under mild low-light conditions, while more photosynthates were used for respiration under severe low-light conditions. Previous studies observed a similar result when grain dry matter that had been transferred from other organs was

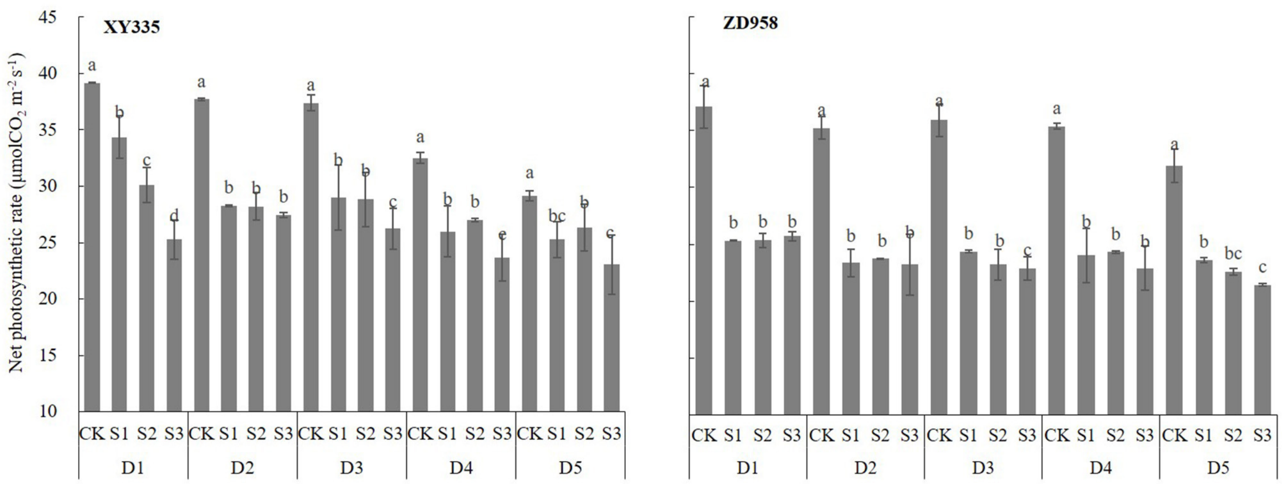

FIGURE 3 | Ear leaf photosynthetic rate (*Pn*) at the grain-filling stage of maize cultivars XY335 and ZD958 under low-light stress in 2018. See **Figure 1** for planting density and shading treatment definitions. Different lowercase letters of the same cultivar above the columns show significant differences between each shading treatment for each planting density at $P < 0.05$.

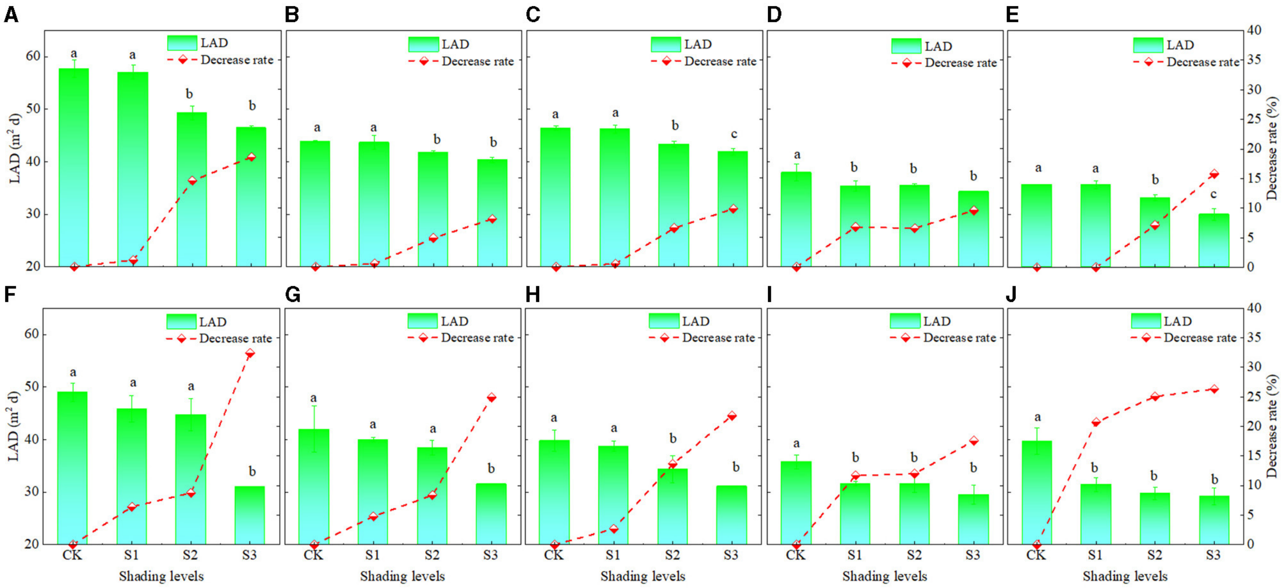

FIGURE 4 | The leaf area duration (LAD) and after silking under different shading levels and planting densities treatments and the decrease rate in LAD under each shading treatment compared to the CK. **(A–E)** show the cultivar XY335 results at densities D1, D2, D3, D4, and D5, respectively, and **(F–J)** show the ZD958 results at the same densities. See **Figure 1** for planting density and shading treatment definitions. Different lowercase letters of the same cultivar above the columns show significant differences between each shading treatment for each planting density at $P < 0.05$.

mainly from stems and leaves, but that amount was not large (Liang et al., 2015); and, during shading, it could not make up for the reduced post-silking biomass accumulation, thus resulting in lower yields (Mu et al., 2010; Wang et al., 2020a).

Modern maize grain yield improvement is highly dependent on increased plant density that intercepts more solar radiation than lower densities do (Antonietta et al., 2014; Liu et al., 2017; Hou et al., 2020). Previous studies have indicated that the ratio of transfer and contribution of dry matter in the stem increased when plant density increased, but that measure in leaves was the opposite (Han et al., 2008). Our results indicated that no such transfer occurred in CK, S1, and S2, whereas that transfer and contribution did occur in S3 planted at the lowest density (D1). This suggests that photosynthetic productivity after silking could supply yield formation demands and that transfer under low-density conditions is not needed.

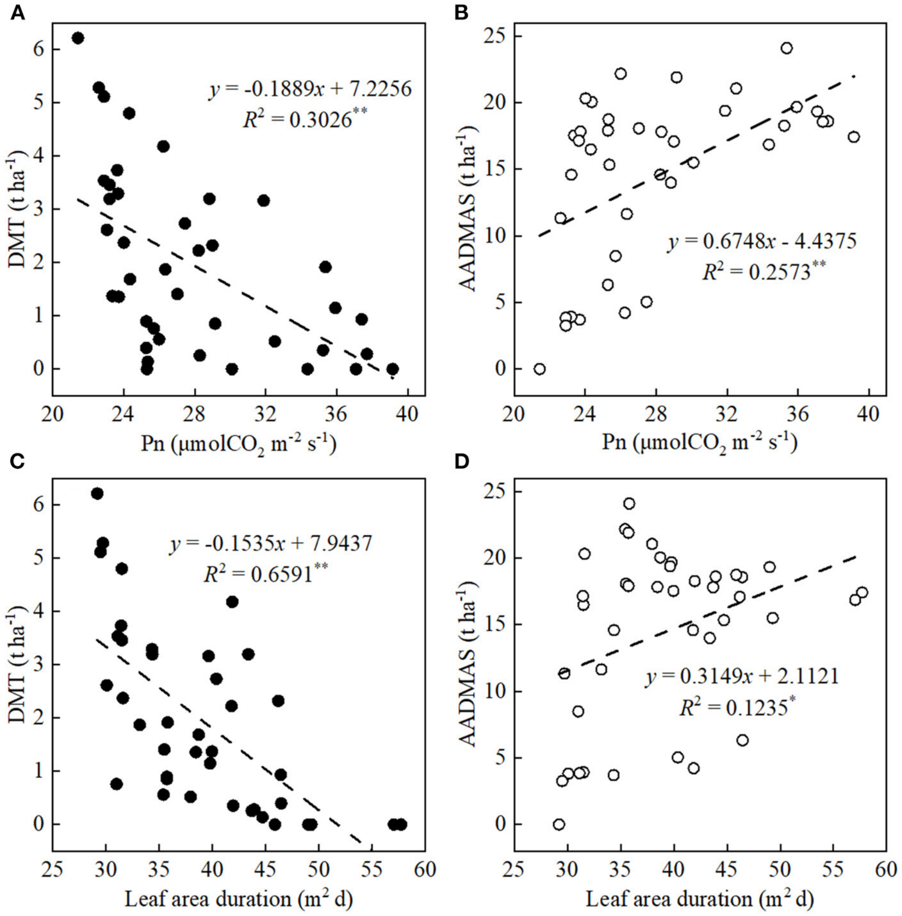

FIGURE 5 | Relationships between ear leaf photosynthetic rates (*Pn*) at the grain-filling stage and LAD with DMT and AADMAS. DMT and AADMAS are indicated by solid circles and empty circles, respectively. $^*P \leq 0.05$, $^{**}P < 0.01$.

TABLE 3 | ANOVA analysis for the effects of year, cultivar, planting density and shading level on the grain yields, yield components (KNP, kernel number per ear. TKW, thousand-kernel weight) and dry weights of plant organs (dry matter of vegetative organs at silking [VS] and at maturity [VM]).

Source	Yield	VS	VM	Ear density	KNP	TKW
Year (Y)	**	ns	**	**	**	**
Cultivar (C)	**	ns	**	**	ns	**
Density (D)	*	**	**	**	**	**
Shading level (S)	**	ns	**	**	**	**
Y × C	ns	**	*	**	ns	**
Y × D	ns	**	**	ns	ns	**
Y × S	**	ns	ns	ns	**	**
C × D	ns	**	ns	*	ns	**
C × S	*	ns	ns	*	**	**
D × S	**	ns	ns	**	**	ns
Y × C × D	ns	**	*	**	**	**
Y × C × S	**	ns	*	**	**	**
Y × D × S	ns	ns	ns	ns	ns	**
C × D × S	*	ns	ns	ns	**	ns
Y × C × D × S	ns	ns	*	ns	ns	**

** and ** indicate significant differences at $P < 0.05$ and $P < 0.01$ probability levels, ns indicates no significance, respectively.*

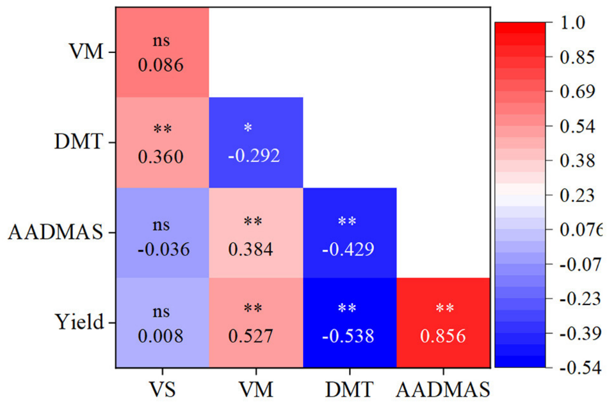

FIGURE 6 | Relationships of yield, vegetative organ dry matter at silking (VS) and at maturity (VM), and AADMAS and DMT, across all treatments, including different shading levels, cultivars, and planting densities. $^{*}P < 0.05$; $^{**}P < 0.01$; ns, not significant.

Furthermore, as density increased, transfer occurred in the shading treatments. Also, the higher the planting density the greater transfer need, thus indicating that the photosynthate produced under high density and weak light could not satisfy yield formation (**Figure 1**). In support of several studies (Cui et al., 2013a; Liu et al., 2021b), our results suggest that solar radiation intensity is the limiting factor for AADMAS, and that given sufficient light radiation, increased planting density fosters increased AADMAS and thus effectively increases yield (Yang et al., 2019) (**Figure 2**).

Photosynthesis, the main physiological process that drives plant growth, is very sensitive to light changes (Fan et al., 2018; Wu et al., 2020). Dry-matter production, especially post-silking dry matter accumulation, is closely related to photosynthetic capacity (Liu et al., 2020a). As the main photosynthetic organs, leaves (Chen, 1994; Ye et al., 2020) provide assimilates for grain development and directly affect the final yield (Tollenaar and Daynard, 1982; Barnabás et al., 2007; Zhang et al., 2007; Mu et al., 2010). Previous studies have shown that since shading likely hinders leaf photoprotective mechanisms and chlorophyll fluorescence properties, the result is decreased net photosynthetic capacity (Cui et al., 2013b; Gao et al., 2017). Decreased photosynthetic capacity was likely due to leaf senescence (Ye et al., 2020). We found that, for the physiological traits, the LAD decreased as shading increased (**Figure 4**), and that was accompanied by a decreasing net *Pn*, likely a consequence of leaf senescence. Based on previous research (Qian et al., 2021), the translocation of reserved carbohydrates in vegetative organs to grains was one of the important factors that determined maize yield. If the transport exceeded 20%, it would cause early senescence of maize leaves (Qian et al., 2021). However, in the present study, the average transport (CDMG) of both tested cultivars was 15.4% (**Figure 1**). As shown in **Figures 5** and **6**, LAD was significantly negatively correlated with DMT and positively correlated with AADMAS. It meant that in the shortage of light resources condition, the sink required more transport of nutrients from the vegetative organs which would deprive the strength of leaf photosynthetic capacity and affect the production of dry matter. This might be one reason for early senescence of leaves under low light conditions in this study. On the other hand, the main resource for grain yield was still from photosynthetic products formed after silking.

Differences in yield and photosynthate accumulation and translocation under light intensity changes vary among maize cultivars (Liang et al., 2015; Wang et al., 2020a). In comparing those differences in cultivars XY335 and ZD958, we found that the average yield of XY335 was higher than that of ZD958. The sink characters of KNP and TKW of XY335 were higher than that of ZD958, and DMT and CDMG rates increased more for XY335 than for ZD958, thus indicating that XY335 transferred more photosynthetic products to grain than ZD958. This indicated that the sink capacity of XY335 was better than that of ZD958 under low–solar radiation stress, and that may promote increased DMT in XY335 (Borrás et al., 2004). Also, the photosynthetic substances produced by ZD958 were used mostly for vegetative organ growth. Comparing the results of the quantitative relationship of the two cultivars (**Figure 2**), the corresponding demarcation value for XY335 was smaller than that for ZD958, which indicated that XY335 had better resistance than ZD958 to weak light. Additionally, under light stress, the range of *Pn* decrease in XY335was lower than that in ZD958, and the LAD decreased rate of ZD958was faster than that of XY335. XY335 has a greater leaf source duration and dry matter production capacity than ZD958. Hou et al. (2020) reported that XY335 had a compact canopy and became more compact at the high density. Other studies showed that the optimal spatial distribution of leaves contributed to delayed leaf senescence and intercept more solar radiation to improve the photosynthetic rate and promote the production potentials of maize at high planting density (Bai et al., 2020; Liu et al., 2021a). However, Antonietta et al. (2014) reported that delayed leaf senescence did not increase yields under high planting density, this may be due to different maize hybrids. So, in this study, XY335 was better able to adapt to weak light, have anti-aging ability, and maintain a higher photosynthetic ability compared to ZD958. Consequently, those superior low-light abilities may lower yield loss caused by light deficiency (Wang et al., 2020b; Wu et al., 2020).

CONCLUSIONS

This study of maize cultivars XY335 and ZD958 determined the differences in DMT contributions under different shading levels and planting densities, and the quantitative relationship between solar radiation, density, and the accumulation of dry matter. In conclusion, shading significantly reduced the *Pn* and LAD, which consequently reduced the amount of dry matter assimilated and thus lowered maize yield. Maize hybrid XY335 was better able to adapt to weak light, maintain a higher photosynthetic and anti-aging ability compared with the cultivar ZD958. These findings show the importance of selecting maize cultivars that have strong stay-green abilities that can guarantee good grain yields even under weak light conditions.

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

YY, PH, and SL designed the study. YY, XG, GL, and WL performed the study. YY, XG, GL, WL, JX, BM, RX, KW, PH, and SL analyzed data and performed the statistical analyses. YY wrote the paper. All authors contributed to the article and approved the submitted version.

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Historical Trends in Sweet Corn Plant Density Tolerance Using Era Hybrids (1930–2010s)

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Over the last six decades, steady improvement in plant density tolerance (PDT) has been one of the largest contributors to genetic yield gain in field corn. While recent research indicates that PDT in modern sweet corn hybrids could be exploited to improve yield, historical changes in PDT in sweet corn are unknown. The objectives of this study were to: (a) quantify the extent to which PDT has changed since introduction of hybrid sweet corn and (b) determine the extent to which changes over time in PDT are associated with plant morpho-physiological and ear traits. An era panel was assembled by recreating 15 *sugary1* sweet corn hybrids that were widely used at one time in the United States, representing hybrids since the 1930s. Era hybrids were evaluated in field experiments in a randomized complete block design with a split-plot arrangement of treatments, including hybrid as the main factor and density as the split-plot factor. Plant density treatments included "Low" plant density (9,900 plants/ha) free of crowding stress or "High" plant density (79,000 plants/ha) with crowding stress. On average, per-area marketable ear mass (Mt/ha) increased at a rate of 0.8 Mt/ha/decade at High densities, whereas per-plant yield (i.e., kg/plant) remained unchanged over time regardless of the density level. Crate yield, a fresh market metric, improved for modern hybrids. However, processing sweet corn yield metrics like fresh kernel mass and recovery (amount of kernel mass contributing to the fresh ear mass) showed modest or no improvement over time, respectively. Modern sweet corn hybrids tend to have fewer tillers and lower fresh shoot biomass, potentially allowing the use of higher plant density; however, plant architecture alone does not accurately predict PDT of individual hybrids.

Keywords: plant density tolerance, *Zea mays*, yield potential, hybrid era, factor analysis

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INTRODUCTION

Grain yield gains in field corn over the past six decades have attributed to genetic gains and improved management practices (Tollenaar et al., 1994; Duvick, 2001). An estimated 50–70% of yield gains are due to improved genetics, with the remaining attributed to superior management practices (Duvick, 2001). Genetic gains are associated with increased plant density tolerance (PDT, also known as crowding stress tolerance) in modern field corn hybrids (Tollenaar and Wu, 1999), as evidenced by increases in plant density at a rate of ~700 plants/ha/year from 1987 to 2016 (Assefa et al., 2018).

Previous research has shown variability for PDT among widely used sweet corn hybrids (Williams, 2015). Sweet corn hybrids with improved PDT, when planted at their optimum plant densities, outperform hybrids with poor PDT (Williams, 2012). Recent research has shown that both vegetable processors and sweet corn growers benefit from using higher (i.e., economic optimum) plant densities for PDT hybrids without changing other management practices (Dhaliwal and Williams, 2019). While there is evidence that improved PDT in sweet corn could be exploited in ways to increase profitability for the sweet corn industry, the extent to which PDT has changed since introduction of hybrid sweet corn remains an open empirical question.

Numerous studies have reported on plant morpho-physiological traits associated with improved PDT in field corn (Tetio-Kagho and Gardner, 1988; Sangoi et al., 2002; Duvick, 2005). More recent research evaluated 48 phenotypic traits from five categories (photosynthetic capability, plant architecture, growth responses, source–sink relationship, and general stress tolerance) in relation to PDT in field corn (Mansfield and Mumm, 2014). Williams (2016) reported two categories of traits, namely, photosynthetic capacity and source–sink relationships associated with PDT in modern *shrunken-2* processing sweet corn hybrids. However, traits involved with changes in PDT over time in sweet corn remain to be explored. This knowledge gap is significant, because not only are sweet corn hybrids unique, but the yield metric of field corn (i.e., grain) does not apply to critical yield metrics of fresh market or processing sweet corn.

Using an era panel of *sugary1* (*su1*) sweet corn hybrids, the objectives of this study were to: (a) quantify the extent to which PDT has changed since introduction of hybrid sweet corn and (b) determine the extent to which changes over time in PDT are associated with plant morpho-physiological and ear traits.

MATERIALS AND METHODS

Germplasm

An era panel of 15 *su1* sweet corn hybrids was created (**Table 1**). While some older hybrids are commercially available today (e.g., Golden Cross Bantam), many were not. Older, important hybrids no longer commercially available were recreated in-kind exclusively for this experiment by participating seed or processing companies. The entries represent some of the most widely used hybrids, by acreage, during their zenith since introduction of hybrid sweet corn in the 1930s.

Site Description

The study was conducted near Urbana, IL at the University of Illinois Vegetable Crop Research Farm (40°04′36.0′′N 88°14′35.7′′W) from 2018 to 2020. The predominant soil type is a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) with 5.8% organic matter. The previous crop for all years was soybean [*Glycine max* (L.)] in a sweet corn–soybean rotation. Growing season conditions for all three years are provided in **Supplementary Figure 1**.

Experimental Design

The experiment was a randomized complete block with four replicates and treatments assigned in a split-plot arrangement of treatments. The main plot factor was the hybrid line, and subplots were assigned plant density factor (9,900 and 79,000 plants/ha). Hereafter, the two levels of plant density will be simple referred to as “Low” and “High” plant density. Low plant density represents growing conditions free of crowding stress. High plant density was chosen to induce crowding stress based on previous research (Williams, 2015). The dimensions of main plots were 9.1 m by 6.1 m, and each four-row subplot (76 cm row spacings) measured 9.1 m by 3 m. The study was planted on a different field each year on May 15, June 1, and June 1 in 2018, 2019, and 2020, respectively. The seed bed was prepared by a single pass of a field cultivator prior to planting. The study was overseeded at planting to improve seedling recruitment, and subplots were thinned to the desired levels of plant density at the two-leaf stage. Tefluthrin {(2,3,5,6-tetrafluoro4-methylphenyl) methyl (*1R,3R*)-rel-3-[(*1Z*)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate} was applied in a t-band at planting to control corn rootworms (*Diabrotica* spp.) A pre-emergence treatment of s-metolachlor {2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy1-methylethyl acetamide} plus atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) was applied after planting. The study was kept weed-free by hand weeding and a post-emergence treatment of 1 kg/ha a.i. atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) in 2019 and 2020. The plots were irrigated using a linear irrigation system to avoid water deficit stress during periods of abnormally low rainfall.

TABLE 1 | Basic information about the *sugary1* sweet corn hybrids evaluated for plant density tolerance in field trials at Urbana, IL, in 2018–2020.

Hybrid	Year of release	Source
Golden Cross Bantam	1934	Charter Seed Company
IowaChief	1951	Charter Seed Company
NK199	1954	Charter Seed Company
Jubilee	1959	Syngenta
Silver Queen	1960	Syngenta
Merit	1961	Seminis
StylePak	1975	Harris Moran Seed Company
DMC2038	1984	DelMonte
Chase	1988	Seminis
Eliminator	1993	Crookham Company
Bonus	1995	Syngenta
Golden Beauty	1995	Charter Seed Company
GH6462	2004	Syngenta
SC1263	2010	Seminis
GH9394	2014	Syngenta

Data Collection

Mid-tassel (VT) and mid-silk (R1) dates were recorded for each subplot. Beginning at tassel emergence, the total number of plants with fully opened tassel branches was counted until at least 50% of the total plants in the center two rows of a subplot had fully opened tassel branches. Similarly, mid-silking date was recorded by counting the total number of plants with visible silks on primary ears until at least one-half of the total plants in center rows reached R1. The difference between mid-anthesis and mid-silking dates was used to determine the anthesis–silking interval (ASI). Cumulative growing degree days (GDD) using a base temperature 10°C and daily air temperature data were recorded from a weather station within 1 km of the experiment sites. Cumulative GDDs from planting to silking and GDD accumulation over the ASI were calculated. Growth degree days were calculated using the below equation:

$$GDD = [(T_{max} - T_{min})/2] - T_{base}$$

where T_{max} and T_{min} are the daily maximum and minimum air temperature, respectively, and T_{base} is the base temperature (here 10°C).

Plant Morphological Measurements

All plant morphological traits were measured at silking stage on two randomly selected plants from the center two rows of each subplot. Plant flag leaf height and primary ear height were measured from the soil surface. Leaf angle was measured on the 10th leaf of randomly selected plants using a clinometer smartphone application. Leaf angle was measured as the angle of leaf relative to the stalk; thereby, more upright leaf would have smaller angle. Leaf number and tillers per plant were recorded. Leaf area index (LAI) was estimated in full sun within 2 h of solar noon with a linear ceptometer (AccuPAR Linear Ceptometer; Decagon Devices, Pullman, WA, United States) for the center two rows of each subplot.

Physiological Data

Leaf gas exchange was measured at midday at silking on the leaf subtending the primary ear using four portable gas exchange systems (LI-6800, LICOR, Lincoln, NE, United States) with the leaf cuvette set to ambient conditions measured at the leaf subtending the ear: (CO_2) (410 mmol mol^{-1}), temperature (28.2–32.4°C), light level (750–1,500 μmol m^{-2} s^{-1}), vapor pressure deficit (1.1–1.8 kPa). The flow rate was set to 500 mmol s^{-1}. Within each year of measurement, all gas exchange systems were set to the same temperature and light levels to ensure consistency between measurements within a growing season. Leaf photosynthesis (A) and stomatal conductance to water vapor (g_s) were calculated using the equations of von Caemmerer and Farquhar (1981). Instantaneous water use efficiency was calculated as A/g_s.

Harvest Data

Plots were hand harvested at the milk stage (R3) of development, which was 18–21 days after mid-silk. Six meters of the center two rows for each subplot was harvested, and stand counts were recorded for the 6 m harvest length. Green ears with diameter >4.5 cm were considered "marketable" ears; smaller ears were considered "non-marketable." Marketable and non-marketable ear mass and number were recorded for each subplot. Marketable ear mass per plant was calculated as the total marketable mass divided by stand count over the harvest length for each subplot. Similarly, marketable ear number was calculated using marketable ear number and stand counts over the harvest length for each subplot. Marketable ear number was used to estimate crate yield (crates/ha)—a commonly used metric in the fresh-market industry, with each crate containing 48 ears. A subsample of 10 randomly selected marketable ears was measured for ear traits described below. Subsampled green ears were husked with a husking bed (A&K Development, Eugene, OR, United States). Husked ear mass, ear length, and filled ear length were recorded. Fresh kernels were cut from the cob using an industry-grade hand-fed corn cutter (A&K Development, Eugene, OR, United States). Cob mass was recorded. Kernel mass was calculated as the difference between husked ear mass and cob mass. Recovery was calculated as the percentage of green ear mass constituted by kernel mass. A subsample of kernel mass (∼100 g) was used to determine kernel moisture content gravimetrically at 55°C until dry. Kernel moisture was adjusted to 76%.

Statistical Analyses

Plant Morpho-Physiological and Yield and Ear Traits

All response variables were analyzed with an analysis of variance (ANOVA) model using the mixed procedure in SAS (version 9.4; Sas Institute, 2020). The Shapiro–Wilk test of normality and Brown–Forsythe test for homogeneity of variance were performed on ANOVA residuals to test model assumptions. As needed, the Box-Cox procedure (Box and Cox, 1964) was used to transform response variables to satisfy model assumptions. Plant density, hybrid, and their interactions were considered fixed effects. Year and replicates nested within year were treated as random effects. Mean comparisons for significant treatment effects were performed using Tukey's mean separation test ($\alpha = 0.05$).

Regression Analysis and Comparison of Slope Estimates

Simple linear regression models were constructed to quantify changes over time in response variables with significant plant density by hybrid interaction effects. Data were analyzed separately for each year (2018–2020).

$$\mathbf{Y}_{\mathbf{i}j} = \beta_{\mathbf{0}} + \beta_{\mathbf{1}}\mathbf{Y}OR_{\mathbf{i}} + \beta_{\mathbf{2}}\mathbf{D}_{\mathbf{j}} + \beta_{\mathbf{3}}\mathbf{Y}OR_{\mathbf{i}}\mathbf{D}_{\mathbf{j}} + \varepsilon_{\mathbf{i}\mathbf{j}}$$

$\mathbf{Y}_{\mathbf{i}j}$ is the response variable for $\boldsymbol{i}^{th}$ year of release and $\boldsymbol{j}^{th}$ plant density,

$\mathbf{Y}OR_{\mathbf{i}}$ is the $\boldsymbol{i}^{th}$ year of release for hybrid,

$\mathbf{D}_{\mathbf{j}}$ is the $\boldsymbol{j}^{th}$ plant density level, where

$$\begin{cases} j = 0, \ \textit{if plant density is } 9,900 \ \textit{plants/ha} \\ j = 1, \ \textit{if plant density is } 79,000 \ \textit{plants/ha} \end{cases}$$

$\mathbf{Y}OR_{\mathbf{i}}\mathbf{D}_{\mathbf{j}}$ is the interaction between $\boldsymbol{i}^{th}$ year of release for hybrid and $\boldsymbol{j}^{th}$ plant density

$\varepsilon_{\mathbf{i}j}$ is the random error term associated with response variable $\mathbf{Y}_{\mathbf{i}j}$, and $\varepsilon_{\mathbf{i}j} \sim \mathbf{N}[\mathbf{0}, \sigma^2]$.

A significant interaction term indicates slope estimates for Low and High plant density levels were different at $\alpha = 0.05$.

Factor Analysis and Factor Regression

A correlation matrix of plant morpho-physiological and ear traits was used for exploratory factor analysis to reduce dimensionality of data. Low and High plant density data were analyzed separately using *stats* package in R (R Core Team, 2020) with varimax rotation. Factors with eigenvalues >1 were retained, and the orthogonal factor loadings for each latent factor were interpreted similar to correlation coefficients. Factor scores matrix was obtained by multiplying factor loadings matrix and standardized plant morpho-physiological and ear trait variables used for factor analysis.

Partial correlation coefficients were obtained for factor scores and per-area marketable ear mass (Mt/ha), separately for Low and High plant densities. Factor scores for the latent variables and year of release for hybrid were used as independent variables to predict per-area marketable ear mass (Mt/ha) using separate linear regression models for Low and High plant densities.

RESULTS

Yield and Ear Traits

Plant density and hybrid had an interactive effect on yield traits including per-area marketable ear mass (Mt/ha), crate yield (crates/ha), per-plant marketable ear mass (kg/plant), and number of marketable ears per plant (**Table 2A**). High plant densities reported higher per-area marketable ear mass, while Low densities showed higher per-plant marketable ear mass. All ear traits except recovery were greater under Low densities.

Growth and Development Traits

Plant density influenced most growth and development traits (**Table 2B**). High plant density favored taller plants with higher position of the flag leaf and height of the primary ear from the soil surface. Plants in the High plant density treatment had fewer tillers per plant, lower fresh shoot biomass, but greater LAI compared to plants in the Low plant density treatment. Only a few variables (i.e., tiller number, LAI, and fresh shoot biomass) were influenced by an interactive effect of plant density and hybrid (**Table 2B**).

Physiological Traits

Plant density, not hybrid, had a main effect on all plant physiological variables (**Table 2C**). Plants at Low density showed higher photosynthetic CO_2 assimilation and stomatal conductance but lower instantaneous water use efficiency. This could be attributed to the presence of larger canopy gaps in Low density and complete canopy closure in High density. There was also an interactive effect of plant density and hybrid for photosynthetic CO_2 assimilation, and stomatal conductance.

Trends in Per-Area and Per-Plant Yields

Per-area marketable ear mass (Mt/ha) was unchanged over time for Low plant density; however, a significantly increasing trend was observed for High plant density (**Figure 1A**). Across years, per-area marketable ear mass (Mt/ha) increased by 0.8 Mt/ha for each decade for High density. In contrast, slope estimates for the two densities were similar for per-plant marketable ear mass (kg/plant) (**Figure 1B**).

Trends in Yield Metric for Fresh-Market and Processing Industry

Crate yield (crates/ha), a yield metric used in the fresh-market industry, increased over time only at High plant density. Crate yield increased by 35–51 crates/ha/decade at High plant density (**Figure 2A**). Kernel mass (Mt/ha), a yield metric used to evaluate the performance of processing sweet corn, showed slightly increasing trends at High plant density in 2019 and 2020; however, differences in slope estimates between plant densities were inconsistent (**Figure 2B**). Recovery, an important processor variable showed no trends over the period of 80 years for either density level (**Figure 3**). Regardless of yield metric used to assess hybrid performance, yield was unchanged over time at Low plant density.

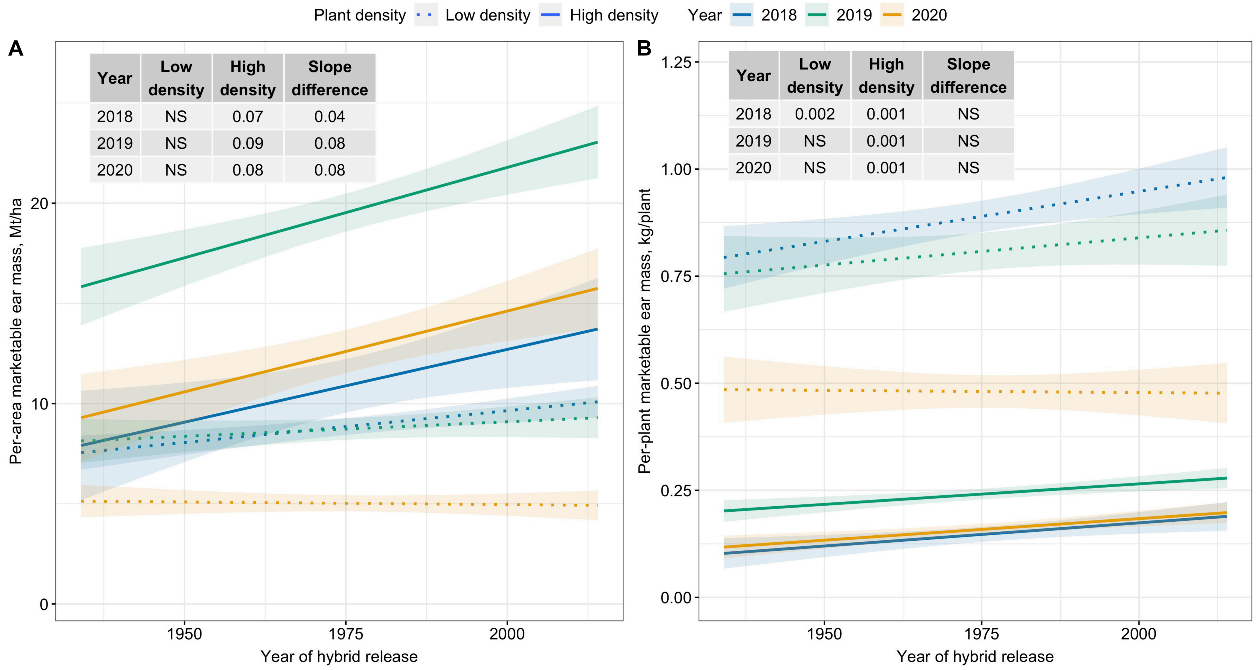
Trends in Plant Morphological and Ear Traits

Among plant morphological and ear traits measured, regression analyses for variables with significant plant density–hybrid interactions are illustrated in **Figure 4**. Ear length has not changed since the 1930s (**Figure 4A**). However, tillers per plant, LAI, and fresh shoot biomass per plant have generally decreased over time at Low plant density (**Figures 4B–D**).

Factor Analysis and Factor Regression

A multivariate approach was used to address the second objective—determine the extent to which changes over time in PDT are associated with plant morpho-physiological and ear traits. Since most of plant morpho-physiological and ear traits were highly correlated and posed issue of multicollinearity in a multiple linear regression model, factor analysis models were used to reduce dimensionality of plant morpho-physiological and ear traits (**Table 3**). Factors with eigenvalues >1 were retained, resulting in three latent factors for both plant density factor models. Factor models explained 58.6 and 62.0% of the total variability for Low and High plant density, respectively (**Table 3**). Interestingly, factor loadings of variables on latent factors were similar for both density levels. For instance, physiological variables including transpiration rate, photosynthetic CO_2 assimilation, stomatal conductance, and instantaneous water use efficiency loaded heavily on Factor 1 for both density levels. Factor 1 can be interpreted as "Physiological traits." Factor 2 had high loadings for tillers per plant, flag leaf height, LAI, and fresh shoot biomass for both the density levels, and can be inferred as "Plant architecture." However, Factor 2 for High plant density also had high loadings for ear traits, such as ear length and recovery, in addition to "plant architecture" variables. Factor 3 explained a significant amount of variation for both density levels; however, the factor loadings were from random variables and did not translate into any meaningful latent factor variable.

Separate multiple linear regression models were conducted for both density levels using factor scores from the factor model and year of release for hybrid as independent variables to predict per-area marketable ear mass (Mt/ha). For both plant densities, increasing scores for Factor 2 resulted in maximum increase in



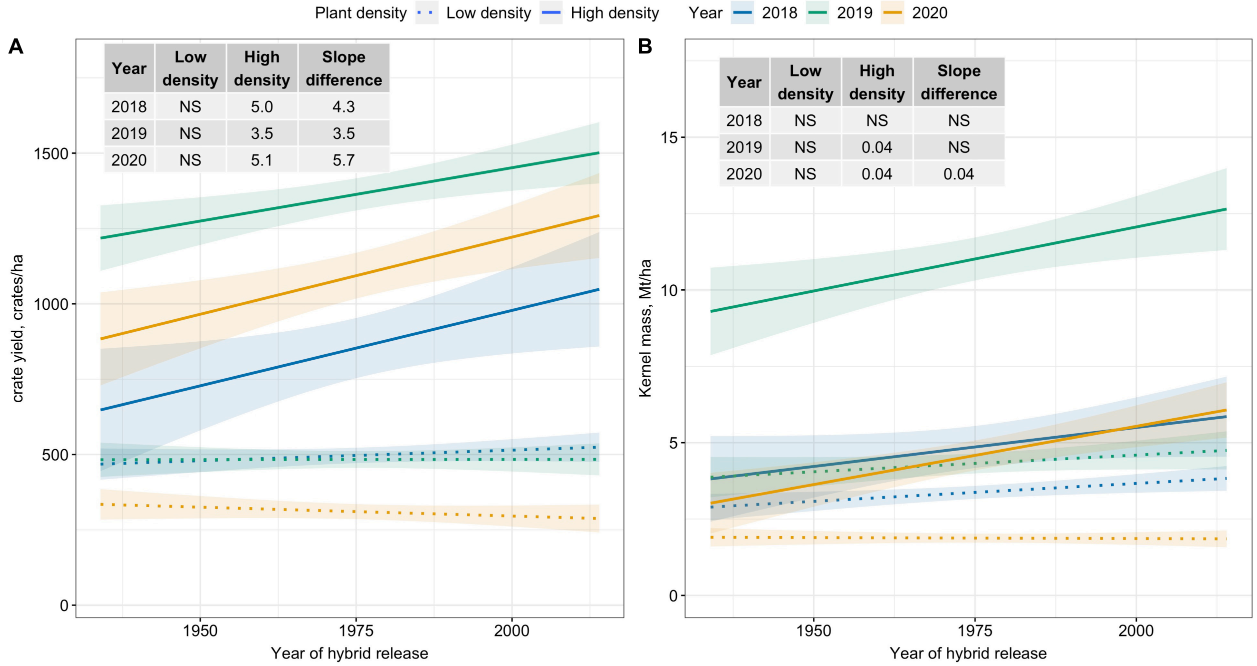
A

Year	Low density	High density	Slope difference
2018	NS	0.07	0.04
2019	NS	0.09	0.08
2020	NS	0.08	0.08

B

Year	Low density	High density	Slope difference
2018	0.002	0.001	NS
2019	NS	0.001	NS
2020	NS	0.001	NS

FIGURE 1 | Best fit line for relationship between year of hybrid release and **(A)** per-area marketable ear mass (Mt/ha), and **(B)** per-plant marketable ear mass (kg/plant) for two levels of plant density at Urbana, IL in 2018–2020. Ninety-five percent confidence intervals are shown by the shaded regions around the line of best fit. Slope estimates from linear regression analysis for Low plant density (9,900 plants/ha), High plant density (79,000 plants/ha), and difference between two plant densities are shown. Non-significant slope estimates are denoted by NS (α = 0.05).



A

Year	Low density	High density	Slope difference
2018	NS	5.0	4.3
2019	NS	3.5	3.5
2020	NS	5.1	5.7

B

Year	Low density	High density	Slope difference
2018	NS	NS	NS
2019	NS	0.04	NS
2020	NS	0.04	0.04

FIGURE 2 | Best fit line for relationship between year of hybrid release and **(A)** crate yield (crates/ha), and **(B)** kernel mass (Mt/ha) for two levels of plant density at Urbana, IL in 2018–2020. Crate yield is the total number of crates, each filled with 48 marketable ears, produced per hectare. Ninety-five percent confidence intervals are shown by the shaded regions around the line of best fit. Slope estimates from linear regression analysis for Low plant density (9,900 plants/ha), High plant density (79,000 plants/ha), and difference between two plant densities are shown. Non-significant slope estimates are denoted by NS (α = 0.05).

TABLE 2 | Significance of fixed effects and interactions for crop response variables as a function of plant density and sweet corn hybrid for **(A)** Yield and Ear traits, **(B)** Growth and Development traits, and **(C)** Physiological traits at Urbana, IL in 2018–2020.

(A) Yield (area-wise and per plant) and ear traits

		Area yield			**Yield per plant**		**Ear traits**		
Main effects		**Per-area marketable ear mass**	**Kernel mass**	**Crate yield**	**Per-plant marketable ear mass**	**Marketable ears**	**Ear length**	**Filled ear length**	**Recovery**
		Mt/ha	Mt/ha	Crates/ha	kg/plant	no./plant	cm	%	%
Plant density (D)		**	**	**	**	**	**	**	**
	Low	7.6	3.2	413	0.73	2.0	19.5	18.7	39.4
	High	14.4	6.9	1,064	0.18	0.7	18.3	16.7	41.1
Hybrid (H)		**	**	**	**	**	**	**	**
Interaction									
	DxH	**	NS	**	**	**	*	NS	NS

(B) Growth and development traits

Main effects		**Flag leaf height**	**Primary ear height**	**Tiller number**	**Leaf number**	**Leaf angle**	**Leaf Area Index**	**Shoot biomass**	**Days to silking**	**Anthesis-silking interval**
		cm	cm	no./plant	no./plant	degrees	–	g/plant	GDD	GDD
Plant density (D)		**	**	**	NS	**	**	**	**	NS
	Low	152.9	54.1	2.2	14.9	41.8	2.12	1.17	1,305	104
	High	165.9	61.6	0.8	14.8	38.3	4.14	0.37	1,324	104
Hybrid (H)		**	**	**	**	**	**	**	**	**
Interaction										
	DxH	NS	NS	**	NS	NS	**	**	NS	NS

(C) Physiological traits (leaf gas exchange measurements)

Main effects		**Photosynthetic CO_2 assimilation**	**Stomatal conductance**	**Instantaneous water use efficiency**
		μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	–
Plant density (D)		**	**	**
	Low	40.0	0.386	116.4
	High	36.1	0.320	124.7
Hybrid (H)		NS	NS	NS
Interaction				
	DxH	**	*	NS

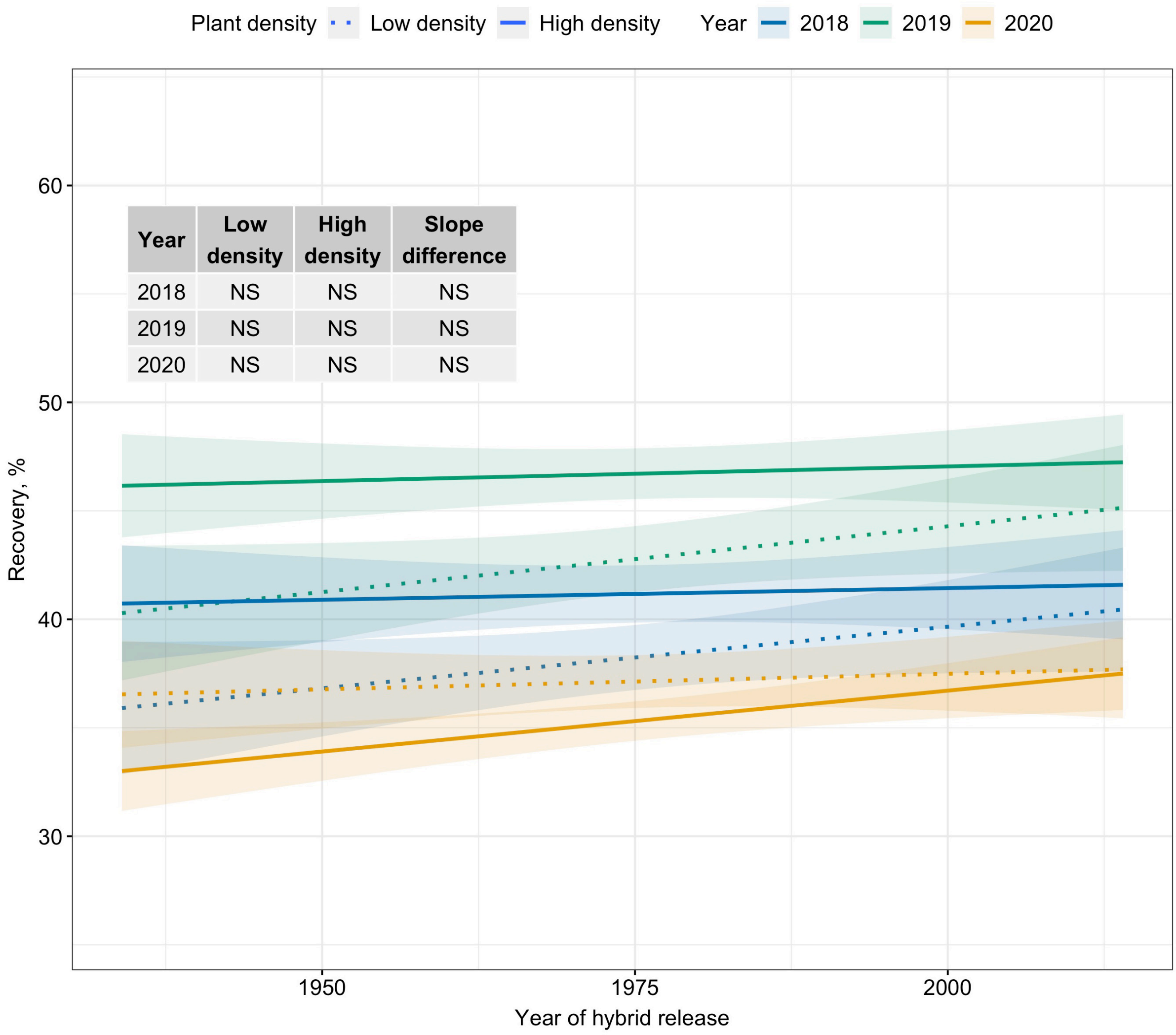
All crop response variables were recorded for 15 different sweet corn hybrids (H) at two levels of plant density (D), namely, Low (9,900 plants/ha) and High (79,000 plants/ha).

** and ** denote significant effects at $p < 0.05$ and $p < 0.01$, respectively. NS stands for a non-significant effect.*

per-area marketable ear mass (Mt/ha) (**Table 4**). However, the amount of variation in per-area marketable ear mass (Mt/ha) explained by Factor 2 was much higher for the Low plant density (50%) model than the High plant density model (12%) (**Table 4**). Year of release was positively correlated with per-area marketable ear mass (Mt/ha), but the amount of variation explained was low ($\leq$8%) for both density levels (**Table 4**).

DISCUSSION

Modern corn hybrids are plant-density dependent, i.e., yield gains are observed from using increased number of plants per unit area (Tokatlidis and Koutroubas, 2004). This is evident from increased optimal plant densities for modern field corn (Ciampitti and Vyn, 2012; Assefa et al., 2018) and certain crowding stress tolerant sweet corn hybrids (Dhaliwal and Williams, 2019). Our results using a sweet corn era panel show modern hybrids outperform old hybrids in per-area marketable ear mass at High plant density. These results are in agreement with previous findings utilizing field corn era panels, where yield gains were documented in modern hybrids at higher plant densities (Carlone and Russell, 1987; Duvick, 1997; Sangoi et al., 2002). Thus, gains in marketable ear mass observed in modern sweet hybrids are primarily due to increased PDT.

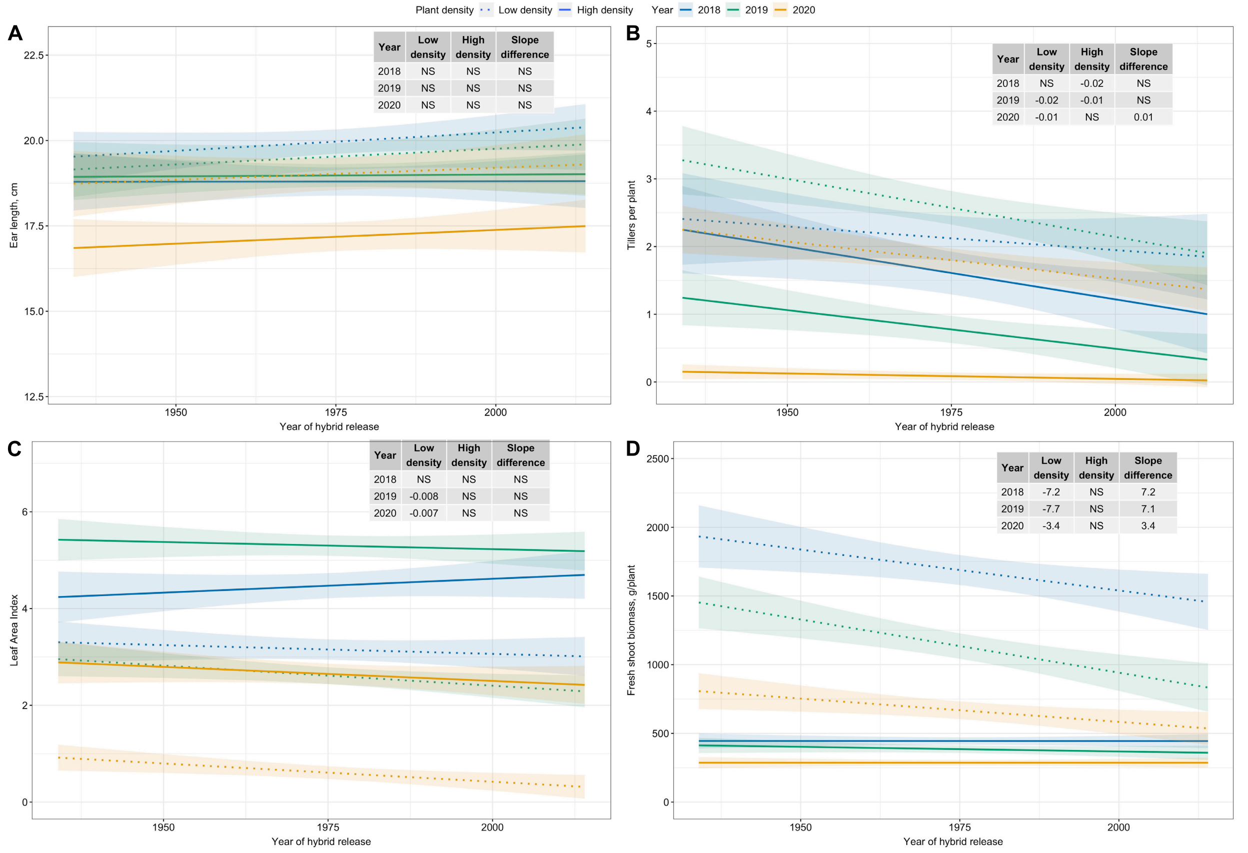


Year	Low density	High density	Slope difference
2018	NS	NS	NS
2019	NS	NS	NS
2020	NS	NS	NS

FIGURE 3 | Best fit line for relationship between year of hybrid release and recovery (%) for two levels of plant density at Urbana, IL in 2018–2020. Recovery is the percentage of green ear mass accounted by kernel mass. Ninety-five percent confidence intervals are shown by the shaded regions around the line of best fit. Slope estimates from linear regression analysis for Low plant density (9,900 plants/ha), High plant density (79,000 plants/ha), and difference between two plant densities are shown. Non-significant slope estimates are denoted by NS (α = 0.05).

On the contrary, yield potential per plant has not changed in hybrid sweet corn since inception in the 1930s. Modern sweet corn hybrids did not show any yield superiority when plants were grown under conditions free of crowding stress. Similar results were reported from the analysis of field corn era hybrids under low plant densities (Duvick, 1997; Sangoi et al., 2002). Since evidence suggests that yield potential per plant has not changed in modern hybrids, growing modern hybrids at plant densities higher than their predecessors is essential to realize the benefits from improved PDT.

The era panel evaluated in this study comprised fresh-market, processing-type, and dual-purpose sweet corn hybrids; therefore, trends in yield metrics relevant to both fresh-market (crate yield) and processing industry (kernel mass and recovery) were quantified. Unlike crate yield, kernel mass showed limited improvement in modern hybrids. Recovery, the single most important variable to vegetable processors, showed no improvement over time at either density. Traditionally, sweet corn breeding programs have used ear number and mass to assess the performance of sweet corn hybrids; response variables unrelated to recovery (Williams, 2014). Recovery is vitally important to the vegetable processing industry, because as recovery increases, the processor buys less ear mass to achieve their “pack”—a seasonal goal of cases of finished product. Furthermore, efficiency of the processing factory improves with higher recovery (e.g., less husk and cob waste is generated). Hence, recovery should not be overlooked in evaluating processing sweet corn germplasm for improved PDT. Fortunately, in the last decade, measuring kernel mass and recovery has become more widespread to



A

Year	Low density	High density	Slope difference
2018	NS	NS	NS
2019	NS	NS	NS
2020	NS	NS	NS

B

Year	Low density	High density	Slope difference
2018	NS	-0.02	NS
2019	-0.02	-0.01	NS
2020	-0.01	NS	0.01

C

Year	Low density	High density	Slope difference
2018	NS	NS	NS
2019	-0.008	NS	NS
2020	-0.007	NS	NS

D

Year	Low density	High density	Slope difference
2018	-7.2	NS	7.2
2019	-7.7	NS	7.1
2020	-3.4	NS	3.4

FIGURE 4 | Best fit line for relationship between year of hybrid release and **(A)** ear length (cm), and **(B)** tillers per plant, **(C)** leaf area index, and **(D)** fresh shoot biomass (g/plant) for two levels of plant density at Urbana, IL in 2018–2020. Ninety-five percent confidence intervals are shown by the shaded regions around the line of best fit. Slope estimates from linear regression analysis for Low plant density (9,900 plants/ha), High plant density (79,000 plants/ha), and difference between two plant densities are shown. Non-significant slope estimates are denoted by NS ($\alpha = 0.05$).

evaluate processing-type hybrids (M. Williams, pers. obs.; S. Grier, pers. com.).

Improved PDT is accompanied by changes in morphological traits that allow for use of more plants per unit area. Modern field corn hybrids have more compact plant architecture for reduced interference from neighboring plants at higher plant densities (Duvick, 2005; Ma et al., 2014). Our data show that modern sweet corn hybrids also tend to develop compact plant architecture under conditions free of crowding stress. For instance, modern sweet corn hybrids had fewer tillers per plant and lower fresh shoot biomass per plant. This modified plant architecture in modern sweet corn hybrids permits the utilization of more plants per unit area, and consequently higher LAI, and also ensures complete canopy closure.

Plant density tolerance is a complex trait in sweet corn. Choe et al. (2016) reported that the molecular basis of crowding stress tolerance in sweet corn is genotype specific, i.e., PDT hybrids have unique tolerance mechanisms. Gene expression studies identified a network of genes involved in biological functions including photosynthesis, glycolysis, cell wall structure, carbohydrate/nitrogen metabolic processes, chromatin, and transcription regulation-related processes as possible mechanisms of crowding stress tolerance in sweet corn. Our analysis of plant and ear traits showed that plant architecture—comprised of tillers per plant, LAI, and fresh shoot biomass per plant—predicted per-area marketable ear mass at Low density. Essentially, the more prolific sweet corn hybrids would yield higher per-area marketable ear mass under conditions free of crowding stress. However, morpho-physiological traits are poor predictors of PDT of specific hybrids, consistent with research on 26 modern *shrunken-2* hybrids (Williams, 2016). In short, modern hybrids with superior PDT cannot be identified from plant architecture alone.

Unlike field corn, morpho-physiological and ear traits in PDT sweet corn could not be structured into distinct categories like those previously identified by Mansfield and Mumm (2014). They classified 48 different plant morpho-physiological and ear traits into five categories: photosynthetic capability, plant architecture, growth responses, source–sink relationship, and general stress tolerance. The lack of explicit associations between underlying plant and ear traits, and PDT in sweet corn could be explained by inherently different breeding objectives for the two crops. Sweet corn breeders do not primarily select for yield, instead maintaining or improving eating quality and specific

TABLE 3 | Exploratory factor analysis results, based on varimax rotation, using the correlation matrix of select ear, growth and development, and leaf gas exchange traits measured at **(A)** Low and **(B)** High plant densities across all sweet corn hybrids at Urbana, IL in 2018–2020.

(A)

		Low density			
Variable	**Units**	**Factor 1**	**Factor 2**	**Factor 3**	**Communality**
Ear length	cm		0.199		0.04
Recovery	%			**−0.416**	0.18
Tillers	No. per plant	−0.208	**0.427**		0.23
Flag leaf ht.	cm	0.127	**0.649**		0.44
LAI	–		**0.793**	−0.232	0.69
Fresh shoot biomass	g/plant	0.111	**0.975**	0.177	1.00
Anthesis-silking interval	GDD	0.153	0.295	0.271	0.18
Photosynthetic CO_2 assimilation	$\mu mol\ m^{-2}\ s^{-1}$	**0.633**	−0.224	**0.681**	0.92
Stomatal conductance	$mol\ m^{-2}\ s^{-1}$	**0.934**		0.340	0.99
Instantaneous water use efficiency	-	**−0.903**	−0.181		0.86
Variance explained	**%**	**26.5**	**21.8**	**10.3**	**58.6**

(B)

		High density			
Variable	**Units**	**Factor 1**	**Factor 2**	**Factor 3**	**Communality**
Ear length	cm		**0.466**		0.23
Recovery	%	−0.276	**0.545**	−0.351	0.50
Tillers	No. per plant		**0.428**		0.19
Flag leaf ht.	cm		**0.763**	0.183	0.62
LAI	–	−0.228	**0.852**		0.79
Fresh shoot biomass	g/plant		**0.730**	0.142	0.55
Anthesis-silking interval	GDD		0.128	**0.460**	0.23
Photosynthetic CO_2 assimilation	$\mu mol\ m^{-2}\ s^{-1}$	**0.771**	−0.364	**0.437**	0.92
Stomatal conductance	$mol\ m^{-2}\ s^{-1}$	**0.978**	−0.116	0.102	0.98
Instantaneous water use efficiency	–	**−0.867**		0.343	0.87
Variance explained	**%**	**30.5**	**24.7**	**6.8**	**62.0**

Factor loadings from variables that were >0.400 in magnitude are in bold.

parameters for ear traits like ear length and girth and tip-fill. Sweet corn breeding also requires improving host plant resistance to common sweet corn diseases prevalent in the North America and focuses on post-harvest shelf life (Lertrat and Pulam, 2007; Pataky et al., 2011).

TABLE 4 | Regression parameters for per-area marketable ear mass (Mt/ha) as a response of year of hybrid release (YOR) and factor scores for Low and High plant densities across all sweet corn hybrids at Urbana, IL in 2018–2020.

Plant density	**Predictor variable**	***r******	**Slope estimate**	***p*-value**	**Variance explained (%)**
Low	Year of hybrid release	0.44	0.03	< 0.001	2.00
	Factor 1	−0.10	−0.17	0.19	–
	Factor 2	0.79	2.04	< 0.001	50.0
	Factor 3	−0.30	−0.53	< 0.001	4.00
			Adjusted R^2		**0.63**
High	Year of hybrid release	0.36	0.08	< 0.001	8.00
	Factor 1	−0.31	−1.66	< 0.001	8.00
	Factor 2	0.41	2.38	< 0.001	12.0
	Factor 3	−0.08	−0.44	0.35	1.00
			Adjusted R^2		**0.29**

**Partial correlations between predictor variables and per-area marketable ear mass (Mt/ha). Proportion of variance explained by each of the regression models is shown in bold.*

CONCLUSION

To our knowledge, this is the first study to examine an era panel in sweet corn. We used the panel to quantify changes in PDT and associations with plant and ear traits. Our results show that modern sweet corn hybrids are plant density dependent, i.e., hybrids benefit from increased PDT under crowding stress. The increase in per-area marketable ear mass at the rate of 0.8 Mt/ha/decade in sweet corn is primarily due to improved PDT. Yield potential per plant has remained unchanged. Recovery has not changed over the last 80 years, likely because it was not the target of a breeding objective. Modern sweet corn hybrids have been modified into a generally more compact plant architecture that supports more individual plants per unit area and less interference from neighboring plants. However, plant architecture alone is not predictive of PDT among modern hybrids.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the **Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MW conceptualized and designed the study. DD led the overall study, contributed to the data collection, analysis, and interpretation, and wrote the manuscript. EA and MW contributed to the data collection and interpretation. All authors read, contributed to the manuscript revisions, 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.2021.707852/full#supplementary-material

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REVIEW
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Combating Dual Challenges in Maize Under High Planting Density: Stem Lodging and Kernel Abortion

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High plant density is considered a proficient approach to increase maize production in countries with limited agricultural land; however, this creates a high risk of stem lodging and kernel abortion by reducing the ratio of biomass to the development of the stem and ear. Stem lodging and kernel abortion are major constraints in maize yield production for high plant density cropping; therefore, it is very important to overcome stem lodging and kernel abortion in maize. In this review, we discuss various morphophysiological and genetic characteristics of maize that may reduce the risk of stem lodging and kernel abortion, with a focus on carbohydrate metabolism and partitioning in maize. These characteristics illustrate a strong relationship between stem lodging resistance and kernel abortion. Previous studies have focused on targeting lignin and cellulose accumulation to improve lodging resistance. Nonetheless, a critical analysis of the literature showed that considering sugar metabolism and examining its effects on lodging resistance and kernel abortion in maize may provide considerable results to improve maize productivity. A constructive summary of management approaches that could be used to efficiently control the effects of stem lodging and kernel abortion is also included. The preferred management choice is based on the genotype of maize; nevertheless, various genetic and physiological approaches can control stem lodging and kernel abortion. However, plant growth regulators and nutrient application can also help reduce the risk for stem lodging and kernel abortion in maize.

Keywords: kernel abortion, maize, lodging, sugar metabolism, management, stem lodging, field management, grain yield

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INTRODUCTION

Maize is one of the most widely grown cereal crops and is important for human food, animal feed, industrial raw materials, and biofuel energy (Shiferaw et al., 2011; Anjum et al., 2017). The world population is increasing and is projected to surpass 9.8 billion by 2050 (FAO, 2012). Thus, the crop production must be increased by 50% to the current production level by 2050, to meet food demand of the burgeoning human population (Searchinger et al., 2019; Tanveer, 2020). How to increase maize yield has been a key research question for agronomists for many years (Aslam et al., 2015). The use of high-yield cultivars or hybrid varieties, along with efficient crop husbandry practices, contributes to the increase in maize production; however, there are some inevitable factors, such as climate change, that are still limiting the achievement of maximum yield potential of maize worldwide (Xiao et al., 2008; Martins et al., 2019; Hussen, 2020).

Managing plant density (PD) at the field level is one of the most effective practices and plays a key role in increasing maize yield per unit area (Maddonni and Otegui, 2006; Dhaliwal and Williams, 2020). A low PD results in lower yield production because of smaller number of productive plants per unit area and greater weed infestation (Sharifi et al., 2009; Lashkari et al., 2011; Zhang et al., 2016; Zhao et al., 2019). The use of high PD may provide a high yield (Tokatlidis et al., 2011; Van Ittersum and Cassman, 2013) due to increased leaf area index (LAI) and photosynthetically active radiation (Maddonni and Otegui, 2004; Novacek et al., 2013), and improved dry matter and nitrogen accumulation (Ciampitti and Vyn, 2011; Yan et al., 2017). Conflicting reports have indicated negative effects of high PD, such as mutual shading, intraspecific competition for resources, accelerated leaf senescence, and reduced photosynthesis (Edmeades et al., 2000; Sharifi et al., 2009; Li H. et al., 2010; Li S. K. et al., 2010; Antonietta et al., 2014). In maize, high PD decreased cob length, ear weight, the number of kernels per row, and stalk area by 10.8, 6, 10, and 20% (Testa et al., 2016). Therefore, proper management is required to optimize PD to increase the yield per unit area.

High PD also results in two major problems, stem lodging and kernel abortion, which are casually linked with each other in the context of maize yield. Under intensive crop management, high PD can cause plants to be more susceptible to lodging, which is mainly due to increased stem length and decreased diameter and wall thickness that could diminish flexural rigidity, breaking resistance, and Young's modulus (Wu et al., 2012; Wu and Ma, 2018). High PD increases plant height through greater internode length and smaller stem diameter, promoting the chances of stem lodging further (Wang et al., 2011; Shah et al., 2017). High PD increases competition for nutrients between individual plants, which results in thinner maize stems and a higher risk of lodging (Huang, 2008; Feng et al., 2010; Shah et al., 2017). Moreover, increasing PD also results in reduced quality and intensity of light reaching the maize canopy (Tokatlidis et al., 2011), thus inhibiting the photo-destruction of auxin. More auxin increases the rate of internode elongation, causing the length of internodes to increase (Chen et al., 2018). In addition, plant density influences the quality of light (ratio of red to far-red) during the early growing season and impacts internode length in the lower canopy (Rajcan et al., 2004). Xue et al. (2016a) reported that high PD increases the rate of rapid internode elongation and decreases the duration of rapid internode thickening, causing internodes to increase in length and decrease in diameter. Therefore, as plant density increases, the length of the basal internode significantly increases, whereas the diameter significantly decreases (Novacek et al., 2013). The length of internodes below the ear increases as plant density increases, causing ear and plant height to increase. Elevated ear height increases the center of gravity (Xu et al., 2017). These changes make maize plants more susceptible to bending by the wind. Moreover, under high PD, reduction in mechanical strength and alteration in sugar metabolism also induces stem lodging (Shah et al., 2017; Kamran et al., 2018a).

Kernel abortion is another negative impact of increasing PD in maize, as high PD induces the abortion of young kernels by reducing the ratio of ear growth rate to tassel growth rate (Vega et al., 2001a,b; Sangoi et al., 2002). This reduction favors ear and kernel abortion (Cárcova and Otegui, 2001; Vega et al., 2001a,b). Moreover, high PD increases leaf formation and plant-to-plant competition, accelerates leaf aging, and reduces photosynthesis and the net assimilation rate of individual plants because of decreased carbon and nitrogen supply to the ear, thereby causing kernel abortion (Edmeades et al., 2000; Hiyane et al., 2010; Yan et al., 2010; Antonietta et al., 2014). Under high PD, there is a series of consequences that are detrimental to ear ontogeny and results in barrenness. First, ear differentiation is delayed in relation to tassel differentiation (Sangoi, 2001). Later-initiated ear shoots have a reduced growth rate, resulting in fewer spikelet primordia transforming into functional florets by the time of flowering (Sangoi et al., 2002). Functional florets extrude silks slowly, decreasing the number of fertilized spikelets because of lack of synchrony between anthesis and silking (Sangoi, 2001; Testa et al., 2016). Thus, the risk of kernel abortion increases under high PD because of increased ear height and the development of stems with reduced diameters (Stanger and Lauer, 2007; Novacek et al., 2013). Therefore, kernel abortion and stem lodging in maize under high planting density were discussed in this review.

Regardless of high PD, stem lodging also causes kernel abortion, and at a high PD, it is very difficult to identify the key player that causes kernel abortion. Some preliminary studies indicated that the first week of pollination is crucial for seed set in maize, and the continuous availability of sucrose for the development of seeds is necessary during and after pollination. If stem bending/breakage occurs in dense maize, which may cause an inadequate supply of sucrose, the abortion of kernels could result, which may range from 20 to 50% (Zinselmeier et al., 1999; McLaughlin and Boyer, 2004; Hiyane et al., 2010). It is noticed that stem lodging in maize effects yields most when it occurs at the reproductive stage (Li et al., 2015), as lodging resulted in stalk breakage, which causes a reduction in nutrient flow for developing grains, inducing kernel abortion (Xue et al., 2020). To improve crop yield and ensure food security, we need to eradicate factors that cause complete crop failure. Along with other factors, kernel abortion is significantly noticed along with stem lodging, making stem lodging a prominent factor that needs to be addressed. This phenomenon has not been discussed before; therefore, in this review, we elucidated the link between stem lodging and kernel abortion in maize under high planting density. This study also reviewed recent knowledge of plant characteristics that are important for improving the resistance to stem lodging and kernel abortion (**Figure 1**). We also discussed further physiological, genetic, agronomic, and management approaches to reduce maize stem lodging and kernel abortion.

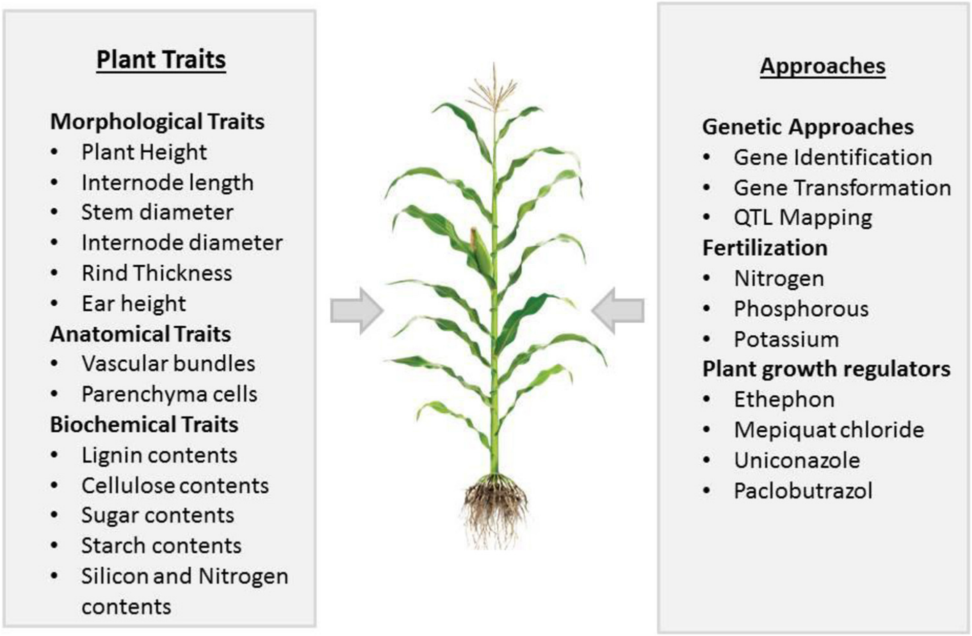

FIGURE 1 | Summary of different plant traits and approaches important in affecting stem lodging and kernel abortion in maize.

GRAIN YIELD LOSS CAUSED BY STEM LODGING AND KERNEL ABORTION

Stem lodging significantly reduces maize yield by up to 75% (Cheng et al., 2011; Wen et al., 2019); however, yield reduction caused by lodging depends on its timing and the stage of maize growth (Li et al., 2015). Lodging during the 12th leaf and grain-filling stages can reduce maize yield by 30–38 and 45–48%, respectively (Li et al., 2015; Jun et al., 2017). In addition to grain loss, lodging increases harvest cost and reduces grain quality (Huang et al., 2015; Jun et al., 2017; Xue et al., 2018). Lodging-induced yield reduction is significantly associated with reduced carbon assimilation and mineral translocation during the grain-filling stage, and improved respiration and chlorosis, i.e., loss of chlorophyll content and greater vulnerability to pests and diseases (Zhu et al., 2006; Foulkes et al., 2011; Shah et al., 2017). Stem lodging at the reproductive stage is more detrimental than during the vegetative stage because at an early development stage, the logged stem can be re-erected, whereas the stem cannot be re-erected during the anthesis/grain filling stage after lodging, resulting in greater yield reduction (Berry et al., 2000, 2004; Piñera-Chavez et al., 2016; Jun et al., 2017; Shah et al., 2017).

Kernel setting is associated with a source-sink relationship, which is an important determinant of maize yield (Borrás and Otegui, 2001; Borrás et al., 2003; Borrás and Gambín, 2010; Yu et al., 2015). Kernel abortion can account for 8–12% yield loss during the dry season (Cheng and Lur, 1996). During pollination, any biotic or abiotic stress exacerbates the abortion of kernels, which may reduce the number of final kernels and final yield by up to 95% (Rattalino et al., 2011; Novacek et al., 2013; Testa et al., 2016).

PHYSIOLOGICAL REGULATION OF STEM LODGING AND KERNEL ABORTION UNDER HIGH PD

Morphophysiological Traits

Plant morphological and physiological traits play important roles in determining stem lodging and kernel abortion under high PD. Stem lodging is one of the most severe constraints on the use of high PD in maize (Argenta et al., 2001).

The major and most important morphological feature associated with stem lodging and kernel abortion under high PD is plant height (Yan et al., 2010; Song et al., 2016; Sher et al., 2018). Tall cultivars may be more susceptible to lodging than shorter plant cultivars, which are more resistant to lodging stress (Li et al., 2011). Decreased plant height results in higher lodging resistance because of lower center of gravity and reduced fresh weight, which minimize the risks of lodging (Ransom, 2005; Echezona, 2007). Additionally, plants with lower height also have a small dry matter, and this decreases grain yield. Therefore, maize cultivars with high resistance to stalk lodging should have a lower ear position to decrease center of gravity, larger leaf spacing, and smaller leaf angle above the ear to allow for more light transport to the mid and lower canopy. This plant type has increased stalk lodging resistance and decreased kernel abortion.

Rind thickness is another morphological feature associated with stem lodging. In various studies, rind thickness, internode diameter, and internode length have been used as predictors of stem strength in sorghum (Teetor et al., 2017). Under high PD, rind strength decreased, as evidenced by a decrease in rind penetrometer resistance, which resulted in smaller diameters and weaker stems that broke easier (Stanger and Lauer, 2007). This

TABLE 1 | Plant traits of maize connected with stem lodging resistance and kernel abortion.

Traits	Crop	Connection	References
Morphological traits			
Plant height	Maize	Positive	Yokozawa and Hara, 1995; Dong et al., 2006; Wang et al., 2011
Basal internode length	Maize	Positive	Yang et al., 2009; Wang and Frei, 2012; Zhang et al., 2013
Stem diameter	Maize	Positive	Sellmer et al., 2001; Shah et al., 2017
Internodal diameter	Maize	Positive	Zuber et al., 1999; Shah et al., 2017
Rind thickness	Maize	Negative	Zuber and Grogan, 1961; Thompson, 1963; Zuber et al., 1999
Anatomical traits			
Vascular bundles	Maize	Positive	Wang et al., 2006
Ear height	Maize	Positive	Stanger and Lauer, 2007; Novacek et al., 2013
Parenchyma cells	Maize	Positive	Dunn and Briggs, 1989; Niklas, 1991; Spatz et al., 1993
Biochemical traits			
Lignin contents	Maize	Positive	Zhang et al., 2010; Chen et al., 2011; Loor et al., 2013
Cellulose contents	Maize	Positive	Jones et al., 2001; Tanaka et al., 2003; Shah et al., 2017
Hemicellulose contents	Maize	Positive	Jones et al., 2001; Tanaka et al., 2003; Shah et al., 2017
Sugar contents	Maize	Positive	Setter and Flannigan, 1986; Thomas and Howarth, 2000; Ruan et al., 2012; Shah et al., 2017
Starch contents	Maize	Positive	Hänggi and Fleming, 2001; Loor et al., 2013; Shah et al., 2017
Silicon and nitrogen contents	Maize	Positive	Zhang et al., 2010; Chen et al., 2011

was also caused by a decrease in the mechanical tensile strength of maize stems under high PD, which resulted in plant lodging and reduced both the yield and quality of maize (Fu et al., 2013). Additionally, positive correlations have been observed among plant height, stem diameter, internodal length, numbers, and lodging index, and it has been concluded that these traits are substantial plant characteristics that influence the vulnerability of plants to lodging (**Table 1**).

The leaf sheath that surrounds and protects the hollow internodes of a stem also provides plants with great physical support (Hale et al., 2021). In a study, it was found that, on average, the leaf sheath contributed 40, 68, and 38% of the overall stem bending strength, flexural rigidity, and safety factor, respectively, in oat, while it accounted for 11, 24, and 10%, respectively, in wheat plants (Wu and Ma, 2020). Any damage to leaf sheaths may result in the weakening of stem breaking resistance in plants (Wu et al., 2012). Moreover, the leaf sheath of rice varieties showing low stem breaking strength generally died down earlier than varieties having high stem breaking strength (Ookawa and Ishihara, 1992). These studies emphasized the importance of maintaining the vitality of leaf sheath for enhancing stem bending strength.

Leaf angle plays a vital role in the determining the amount of light intercepted by the canopy, and in maize leaves with different leaf angles receive different qualities of light (particularly enriched with far-red, FR) and reduced red (R) radiation under high PD (Lee and Tollenaar, 2007; Hammer et al., 2009). An erect leaf posture improves lodging resistance, and a prostrate leaf stature reduces resistance to lodging under dense planting (Wu and Ma, 2019). A high FR/R ratio triggers many morphophysiological changes in plant architecture, stimulating stem elongation, and favors apical dominance and decrease in stem diameter (Rajcan and Swanton, 2001). Nonetheless, stem lodging and kernel abortion increased, and stem diameter decreased because of mutual shading (Troyer and Rosenbrook, 1983; Valentinuz and Tollenaar, 2004). Such changes make maize stems more susceptible to falls and breakage before kernels reach physiological maturity (Li et al., 2011).

Anatomical and Biochemical Traits

The anatomical characteristics of plants have a significant effect on lodging and kernel abortion under high PD. Stem lodging occurs under high PD when plant-to-plant competition for light, nutrients, water, and carbohydrates increases between the stem (source) and the ear (sink) within the plant, ultimately reducing the vigor of the sclerenchyma cells around the vascular bundles in the stem (Nielsen, 2006; Stanger and Lauer, 2007).

To reduce lodging potential and ear falls, plants rely on their anatomical features to provide shape and strength, bond cells together, and provide rigidity for the whole plant (Wen et al., 2019). However, the development of anatomical features varies significantly with cells, species, and accessions within species (Brett and Waldron, 1990; Hazen et al., 2003). Pith parenchyma cells play a vital role in stabilizing the stem and reducing the risk of local buckling and collapse (Niklas, 1991; Spatz et al., 1993; Kong et al., 2013). Stem stand ability increases with the thickness of the parenchyma layer because parenchyma cells can absorb the effects of environmental forces, such as light, wind, and rain without heating or mechanical damage (Kokubo et al., 1989). About 50–80% of the strength of a maize stalk comes from its outer structure, the rind (Zuber et al., 1980). Wang T. et al. (2015) reported that the crush strength of maize stalks is significantly positively correlated with the ratio of mechanical tissue thickness to internode radius and the ratio of sclerenchyma thickness to internode radius. Xue et al. (2016a) reported that the number of mechanical cell layers, the thickness of mechanical tissue, and the ratio of cortex thickness to internode radius determine ∼79% of the Rind penetration strength (RPS) at the third internode. The mechanical strength of maize stalks depends primarily on the cell wall of mechanical tissues in the internode rind (Leroux, 2012). The main components of the cell wall are structural carbohydrates, such as cellulose, hemicellulose, and lignin (Li S. C. et al., 2003; Li Y. et al., 2003; Wang et al., 2006), and, therefore, stalk strength is significantly and positively related to the contents of these materials (Appenzeller et al., 2004; Chen et al., 2007). Sclerenchyma cells around the vascular bundles in the stem are responsible for mechanical strength, and reduction in sugar supplies can reduce the vigor of sclerenchyma cells, inducing stem lodging (Novacek et al., 2013).

In addition to the anatomical characteristics of maize plants, some basic biochemical properties, such as cellulose, hemicellulose, lignin, and soluble sugar content, are essential and have a significant effect on lodging resistance under high PD. When PD increases, it affects the vigor of sclerenchyma cells around vascular bundles and may reduce the synthesis of total non-structural carbohydrates and proteins, and potassium level in the stem, possibly causing stem lodging by altering the source-sink ratio (Wang et al., 2006). Furthermore, decreased stem protein and sugar levels cause senescence of pith tissue and increase stem lodging (Shah et al., 2017). In another study, Wang and Hu (1991) found that lodging-resistant maize varieties exhibited a higher accumulation of carbohydrates and lignin in the stems than susceptible varieties. A non-significant relationship was also observed between the starch content of the stem and lodging resistance (Zhang et al., 2009). Moreover, the accumulation of cellulose, hemicelluloses, and lignin improved the thickening and flexibility of the culm wall (Jones et al., 2001; Tanaka et al., 2003). Typically, stem strength depends on cellulose and lignin content; therefore, plant stems with lower lignin or cellulose levels are susceptible (Shah et al., 2017).

Lignin is one of the main components of and confers rigidity to cell walls; therefore, it is associated with the mechanical stability of plants (Loor et al., 2013). Low lignin content may result in weak mechanical strength of the cell wall and could easily cause stem lodging. Lignification of the cell wall could improve the stability of the cell wall and increase the physical strength of the stem (Chen et al., 2011). Therefore, lignin is an integral component of plant health and function (Chen et al., 2011). The application of silicon and nitrogen could considerably increase the lignin content in hardened cells and increase cellulose content, thereby reducing stem lodging index (Zhang et al., 2010; Chen et al., 2011).

Multiple reasons are involved in kernel abortion, which may be due to failure in pollination or defective ovary, and even abortion is noticed after successful fertilization (Gustin et al., 2018). Under high PD or under a lodging situation, when sources are deficient, plants are used to abort few of the ovaries as a survival tactic (Ruan, 2014; Tardieu et al., 2014). Reduction in the supply of sucrose and concentrations of assimilates causes kernel abortion (Shen et al., 2018). Many studies have discussed that under scarce sucrose conditions, apical kernels are more likely to abort as they have the weakest sink (Shen et al., 2018).

When it comes to the anatomy of female florescence, the kernel at the base of the ear is less likely to abort because even in the most competitive environment they have more photo assimilate supply as compared with the kernels at the terminal parts, which are more likely to abort. The same is the case under water deficit conditions, increasing PD under water deficit conditions increased the risk of kernel abortion for the kernels at the tip of ears (Setter et al., 2001; Setter and Parra, 2010). It is noted that the expression of the TPP gene (*trehalose phosphate phosphatase*) in the development stage of the ear reduces kernel abortion (Nuccio et al., 2015). Varieties with the TPP transgenes have comparatively lower kernel abortion in high planting density (Hannah et al., 2017). To date, little is known about the molecular mechanism that controls kernel abortion; therefore, there is a need for more studies that are focused on uncovering the exact mechanism of kernel abortion and its reasons. Based on previous studies, there is a hypothesis, on which current studies are relying, that inadequate supplies of carbohydrate and water in the development stage of kernel cause a reduction in kernel set (Gustin et al., 2018) under high PD. The other hypothesis is that reduction in supplies of water causes a reduction in cell expansion, which causes kernels to abort (Oury et al., 2016). The above-mentioned findings have important implications for future maize breeding.

In conclusion, plant characteristics play different roles in controlling the risk of lodging and kernel abortion in maize. These characteristics should be considered in future studies to develop lodging-resistant varieties and reduce kernel abortion.

ROLE OF STEM SUGARS IN LODGING RESISTANCE AND KERNEL ABORTION

Stem lodging is a significant constraint to the yield of maize at high PD; even high-yielding hybrids can be affected by it (Betra'n et al., 2003; Flint-Garcia et al., 2003). As stated above, stem lodging significantly depends on the distribution of structural chemical constituents of stems (Appenzeller et al., 2004; Chen et al., 2007). Additionally, the roles of structural and nonstructural carbohydrate and sugar contents in lodging resistance and kernel abortion are largely unknown. In this study, we attempted to summarize how sugar content in shoots relates to lodging resistance and kernel abortion, and the mechanisms involved in sugar biosynthesis, transport, and deposition. Sucrose phosphate synthase (*SPS*) and sucrose synthase genes (*SUS*) play a role in the accumulation of sugars in stems, and effectively minimize stem lodging and kernel abortion (Zinselmeier et al., 1999; Slewinski, 2012; Mizuno et al., 2018).

Two plausible possibilities are given here to elucidate the co-relationship of stem sugars and lodging. The first is that high sucrose content will require more water in parenchyma cells, thereby increasing cell turgor pressure and creating stiffer cells, thereby combating lodging. The second possibility is the presence of higher concentrations of sugar in the vicinity of the cell, which could facilitate carbohydrate mobilization and help the cell to maintain its integrity, which restricts necrosis that degrades dead cells and tissues and, consequently, compromises stem structure. However, sugar accumulation (controlled by *SUS* genes) delays senescence until the end of the season and increases resistance to stem lodging; thus, it minimizes kernel abortion and prolongs grain development (Thomas and Howarth, 2000).

Non-structural carbohydrates play a significant role in stem lodging tolerance and kernel abortion. When photosynthesis is at its peak, non-structural carbohydrates are stored in the parenchyma cells and vascular tissues, adding to the physical strength of the stem, thereby improving plant lodging tolerance. During later stages, when photosynthesis is compromised, stored non-structural carbohydrates act as secondary sources for grain filling, significantly reducing kernel abortion (Slewinski, 2012). Sustaining non-structural carbohydrates in the stem has been proposed as an effective way to control stem lodging (Shiferaw et al., 2011). It can be concluded that cultivars with high stem sugar deposits are more likely to resist lodging.

Kernel abortion is mainly caused by an insufficient carbohydrate supply (source-sink relationship; Shen et al., 2020). In the source-sink relationship, the stem buffer system is mainly dependent on sucrose (Daynard et al., 1969). Sugar transportation is regulated by invertase genes and is always noted from the source to sink organs. Initially, sugar is biosynthesized in the leaf by the action of *SPS* genes used for development, and any surplus is placed in the storage organs by the activity of the *SUS* genes. The stem in the vegetative growth stage acts as the storage organ (sink), but in later stages, during reproductive growth when sugar is needed for kernel development, the sugar content in the stem tissues begins to act as a source, and a flow from stem to kernel occurs (Slewinski, 2012). Therefore, stem sugars can maintain the supply of sugar for kernel development and can play a role as a limiting factor for kernel abortion. However, the role of stem sugars in important metabolic processes has been neglected in the past, and only recently has its roles in lodging resistance, kernel abortion, and other key metabolic processes been addressed (Saini and Westgate, 1999; McLaughlin and Boyer, 2004; Hiyane et al., 2010).

Sucrose in storage organs displays an apoplasmic movement, and its depletion can affect the total sugar content and total stem dry matter (Sayre et al., 1931; Setter and Flannigan, 1986; Slewinski, 2012). Thus, sugar partitioning is also important in relation to lodging resistance and kernel abortion. *ZmSUT1*, a member of the sucrose transporter family, is highly expressed in photosynthetic tissues and is responsible for the mobilization of carbohydrates from source to sink tissues (Carpaneto et al., 2005; Slewinski and Braun, 2010). Braun and Slewinski (2009) suggested that characterization of other family member genes could further clarify the roles of these genes in sugar mobilization, and is discussed in detail in later sections of this article. It will be interesting to discover the involvement of sucrose transporter *SUT* genes in regulating non-structural carbohydrate (NSC) reserves in maize.

In maize, the fertility of the ovaries has a more significant influence on kernel number than that of pollen, which is different from that of wheat, rice, and barley (Boyer and McLaughlin, 2007; Barnabás et al., 2008). During reproductive growth, ovaries require sugar in the form of sucrose for developing embryos, and a reduction in sugar supply results in the abortion of the embryos, resulting in aborted kernels (McLaughlin and Boyer, 2004; Hiyane et al., 2010). Therefore, sugar accumulation should be properly managed to ensure lodging resistance and minimal kernel abortion, because all these factors have an impact on the final yield. This must be understood for an in-depth elucidation of sugar metabolism and transport. A summary of stem sugar storage and translocation in overcoming stem lodging and kernel abortion is shown in **Figure 2**.

Sugar Biosynthesis and Metabolism

Sugar biosynthesis and metabolism are complex processes affected by different growth and environmental conditions. Briefly, as a result of photosynthesis, sugar is synthesized in the form of sucrose by the catalytic activity of sucrose-phosphate synthase (SPS) and converted into ADP-glucose or UDP-glucose, which is used in plant metabolism by the action of the SuSy enzyme (Hendriks et al., 2003; Kolbe et al., 2005). The SuSy enzyme is encoded by *SUS* genes that may be present in the cytoplasm, cell wall, and vacuoles (Stein and Granot, 2019). Plants with low activity of *SUS* genes exhibited stunted growth, whereas the overexpression of *SUS* genes produced significantly increased growth and strong cell structure with thickened cell walls (Stein and Granot, 2019; **Figure 3**). At the cellular level, the intercellular biosynthesis of sugar and starch is initiated by ADP-glucose pyrophosphosphorylase (AGPase), which provides ADP-glucose (Geigenberger, 2011). AGPases are primarily encoded in the cytosol and are minimally encoded in amyloplasts and plastids (Burton et al., 2002).

After biosynthesis, sugars reach the sink tissues *via* the phloem tubes either through the symplast or apoplast pathway. In the apoplasmic unloading pathway, sucrose is unloaded into the cell wall matrix from the sieve element/companion cell (SE/CC) complex mediated by sugars will eventually be exported transporter (SWEET) proteins, which are typically localized on the plasma membrane, to transport sucrose or hexoses down a concentration gradient in an energy-independent manner (Shen et al., 2019). Within the cell wall, sucrose is often hydrolyzed by cell wall invertase (CWIN; with an optimum pH of 5–6) into glucose and fructose (Ruan et al., 2010). Hexoses are then taken up by H^+-coupled hexose transporters (HXTs; Ruan, 2014). It is common for CWIN and HXTs to be co-expressed in the apoplasmic unloading region, indicating a synergistic functional relationship between CWIN and HXTs (Weber et al., 2005; Jin et al., 2009; McCurdy et al., 2010; Shen et al., 2019). The direct transfer of sucrose can also occur through plasmodesmata, where vacuolar invertase genes hydrolyze sucrose. SuSy and invertases cleave sucrose differently. SuSy proteins cleave sucrose into reversible UDP-glucose and ADP-glucose, whereas cell wall and vacuolar invertases catalyze an irreversible conversion of sucrose into glucose and fructose (Ma et al., 2019).

Additional sugars are transported to the sink tissues, which are the stem and roots in the case of maize, to be stored and added to the organic matter structural carbohydrates (Ruan, 2014; Stein and Granot, 2019). In maize, the involvement of *SUS* genes in starch synthesis was first studied by Chourey and Nelson (1976) who found that gene mutants exhibited a 90% reduction in SuSy and an overall 40% reduction in starch accumulation. However, in mutants with overexpressed *SUS* genes, a considerable increase in the accumulation of ADP-glucose was observed (Li et al., 2013). *SUS* genes appear to play a role in the development of young photosynthetic tissues and starch accumulation in the non-photosynthetic tissues of the plant (Hänggi and Fleming, 2001). Additionally, *SUS* genes were localized in the xylem tissue, and their overexpression resulted in increase in xylem cell wall thickness (Coleman et al.,

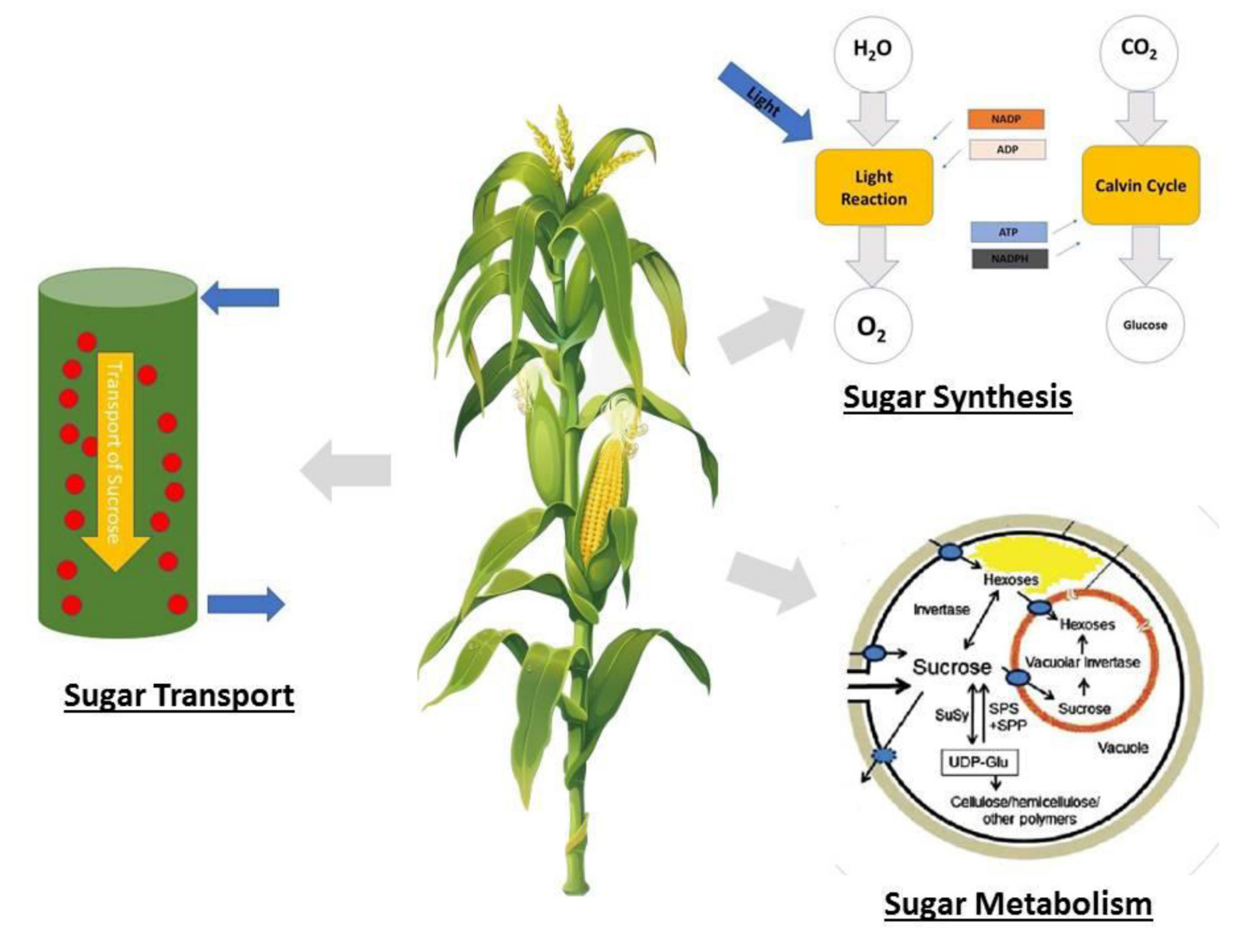

FIGURE 2 | Summary of stem sugar storage and translocation in maize.

2006; Bahaji et al., 2014; Wei et al., 2015). Hence, SuSy is responsible for directing carbon to cellulose synthesis, which is used in the development of xylem tissues (Barratt et al., 2009).

This raises the question regarding whether these genes protect plants from lodging and kernel abortion. Four cell wall invertase genes, two vacuolar invertase genes, and 21 invertase isogenes are identified in maize (Juarez-Colunga et al., 2018). Grain yield relies mainly on grain number, size, and starch content (Ngoune Tandzi and Mutengwa, 2020). Maize mutants with higher expression of cell wall invertase resulted in a 1.5-fold increase in production, a 20% increase in starch content, and a noticeable increase in the size of ears, grain size, and numbers, which relates invertase genes with resistance to lodging and kernel abortion. Higher activity of cell wall invertase results in increased shoot strength and sugar content, along with a significant increase in seed number and size (Li et al., 2013). It has also been documented that invertases regulate the production of other plant hormones, such as indole acetic acid (IAA), which directly affects kernel development (Chourey et al., 2010). The expression of genes involved in starch biosynthesis and accumulation is controlled by the number of transcription factors; *ZmNAC128* and *ZmNAC130* transcription factors are involved in sugar accumulation and *ZmEREB156* is involved in the regulation of key genes involved in sugar biosynthesis (Huang et al., 2016; Zhang et al., 2019). From these studies, it is inferred that invertase genes can improve lodging resistance in plants.

Genes Involved in Sugar Transport

Photosynthetic tissues are the powerhouses of plant sugars that are biosynthesized into different forms. Later, they are translocated to other organs, accumulated, and used in different development and reproduction processes. Processes involved in the translocation have been well-discussed, but little is known about the genes involved in sugar translocation and accumulation. The carbohydrate partitioning defective1 (*Cpd1*) gene plays a role in the early production of phloem and controls the distribution of carbohydrates throughout the plant (Julius et al., 2018). In the case of low expression of *cpd1*, plants are unable to transport sugars from the source to sink tissues, which

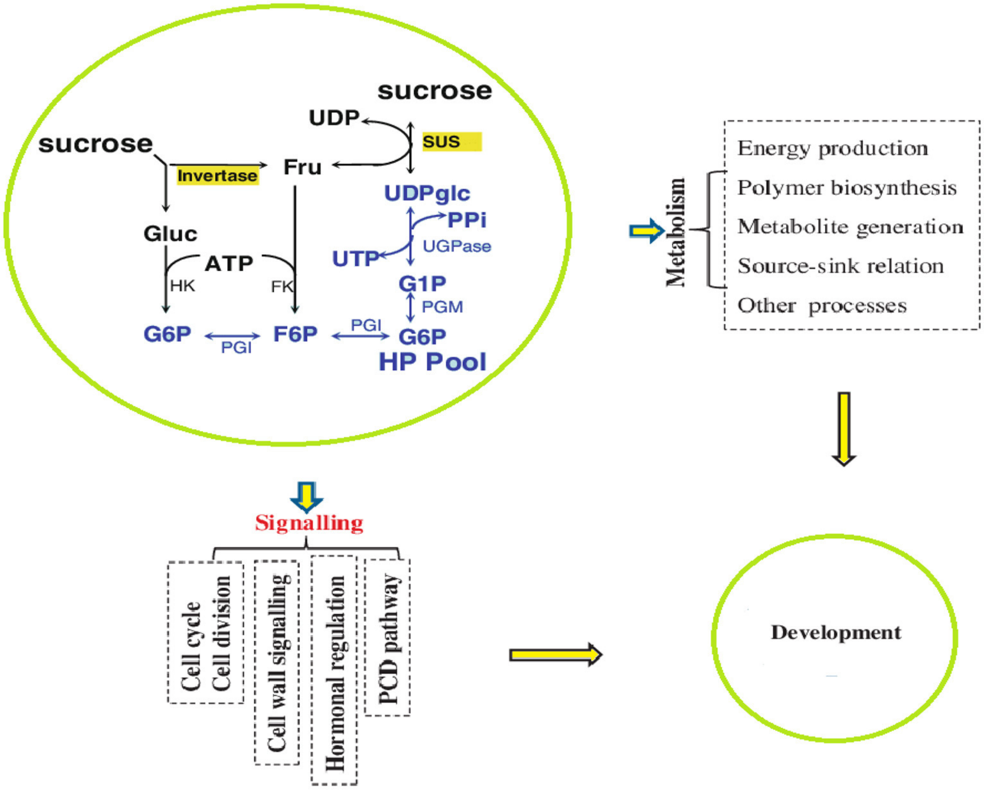

FIGURE 3 | Summary of sugar biosynthesis and metabolism.

results in the deposition of sugars in leaves and reduction in sink tissues (stems and roots) causing stunted growth, decreased sturdiness of the stem, and delayed silking and anthesis, which affect kernel size and weight (Julius et al., 2018). Agpsemzm and Agpllzm enzymes play roles in the regulation of starch levels in different tissues (Julius et al., 2018).

In addition to the phloem sieve tissues, other genetic factors are involved in the adequate distribution of carbohydrates throughout plants. Initially, sugars are uploaded to cell walls by the SWEET efflux proteins from source tissue cells, and then to sieve elements and companion cell complexes by sugar transporters (Julius et al., 2017). Sucrose moves passively from high-concentration tissues to low-concentration tissues; sucrose transferase (*Sut*) genes are responsible for the regulation of this symplasmic and apoplasmic movement of sucrose (Aoki et al., 1999, 2003; Zhang C. et al., 2014). Maize has apoplasmic sucrose movement null mutants for the *ZmSut1* gene, which identifies its function as a phloem loading with sucrose from source cell apoplastic regions. Retardation of the *ZmSut1* gene results in slow growth and deposition of sucrose in maize leaves (Slewinski et al., 2009; Slewinski and Braun, 2010).

For the identification of *ZmSut2* gene functions, null mutants were analyzed, and showed a reduction in growth and decreased ear length and kernel number, indicating that *ZmSut2* is responsible for resistance to kernel abortion and contributes to maintaining yields under high-density planting (Leach et al., 2017). Sucrose transporter genes also directly influence maize growth (Leach et al., 2017). Along with the *ZmSut* genes, *ZmSWEET13s* (a, b, and c) genes are involved in the translocation from the sink to source tissues, and knockout mutants showed low expression of genes involved in photosynthesis and carbohydrate metabolism (Bezrutczyk et al., 2018).

As the kernel is the main determinant of crop yield, the genetic factors involved in the transportation of sugars and their accumulation need to be elucidated. *ZmSWEET4c* has been identified in maize and is responsible for the transport and deposition of sugars across the basal endosperm transfer layer, which controls nutrient flow in and out of seeds (Sosso et al., 2015). In wild-type maize, the expression of *ZmSWEET4c* increases four times that of normal, along with the flow of maximum sugar to developing seeds, thereby supporting seed setting (Chourey et al., 2012). Along with *ZmSWEET4c*, another gene involved in sugar transport is *ZmMn1*. The expression of these genes induces the expression of *ZmMRP1*, which prepares the cell machinery for sugar transport (Sosso et al., 2015).

Linking Stem Lodging With Kernel Abortion Under High PD in Maize

A high PD can increase biomass and yield, but it also increases competition for nutrients and light interception among individual plants (Craine and Dybzinski, 2013). Moreover, plant morphogenesis, stem carbohydrate accumulation, cortex tissue, and mechanical strength are affected by high PD. Thus, maize plants under high PD are more vulnerable to stem lodging (Xue et al., 2016a; Jun et al., 2017). High PD promoted the abortion of kernels before the beginning of grain filling in maize (Sangoi, 2001; Ruffo et al., 2015). Abortion causes permanent losses in seed production and may result in decreased productivity (Salter and Goode, 1967; Hiyane et al., 2010). Floral abortion in maize disrupts carpel development (Hiyane et al., 2010). During the flowering stage, the first week of pollination is crucial for the seed set, during and after pollination. The continuous availability of sucrose for the development of seeds is necessary where an inadequate supply of sucrose results in the abortion of kernels in maize (McLaughlin and Boyer, 2004). Kernel abortion is associated with inhibited photosynthesis and reduced sugar supply to the stems under shade (McLaughlin and Boyer, 2004).

Additionally, under high PD, less interception of light decreases the synthesis of cellulose and lignin in maize, which may cause lodging (Li et al., 2008; Xue et al., 2016b). Stem lodging reduces photosynthesis (Setter et al., 1997; Ma et al., 2014) because of reduced photosynthetic activity, which ultimately reduces the sugar flow in plant storage tissues for metabolic processes. This results in a reduced supply of sucrose to developing kernels, which induces abortion (McLaughlin and Boyer, 2004; Hiyane et al., 2010). However, attack by corn borers increases the occurrence of lodging, reducing CO_2 assimilation, biomass accumulation, and carbohydrate partitioning, resulting in yield reduction from water disruption and nutrient translocation to the ear, and pre-harvest losses because of stem lodging and dropped ears (Riedell and Reese, 1999; Steffey and Gray, 2002; Rice, 2006).

Under high PD or lodging situations, reduction in the supply of sucrose and concentrations of assimilates causes kernel abortion (Shen et al., 2018). Many studies have discussed that under scarce sucrose conditions, apical kernels are more likely to abort, as they have the weakest sink (Shen et al., 2018). Kernel abortion can be minimized by adequate supplies of N, organic, and inorganic substances, and improving carbohydrate availability during the pollination stage (Paponov et al., 2020). At high PD, many kernels may not develop an event that occurs in some hybrids following poor pollination resulting from a silking period that is delayed relative to tassel emergence (Otegui, 1997) and/or owing to a limitation in assimilate supply that causes the grain and cob abortion in maize (1985). As stated above, there is no direct relationship between high PD and stem lodging and kernel abortion is mentioned in the literature. However, we attempted to draw a causal link between stem lodging and kernel abortion under high PD. This link needs to be validated in future studies.

MANAGEMENT OF STEM LODGING AND KERNEL ABORTION IN MAIZE

The Physiological and Genetic Approach

Genes Involved in Lodging Resistance and Kernel Abortion Reduction

Several studies have shown that various genes are involved in lodging resistance and kernel abortion reduction (**Table 2**). In cereals, stem diameter, rind penetrometer resistance, and stalk bending strength are determinants of stem lodging (Shah et al., 2017). In more than 250 inbred lines, a genome-wide association study was designed to identify the nucleotides involved in these quantitative traits. A total of 423 QTNs was identified in maize, in addition to 63 cyclin-dependent kinase coding and steroid-binding genes that were identified. Seven of these were classified as transcription factors. These genes are likely involved in cell elongation, stem girth improvement, and development, hence, adding up resistance to lodging; 17 genes for stem stalk bending, 19 for strength/diameter, and 30 for rind penetrometer resistance were identified (Hu et al., 2013; Dante et al., 2014; Zhang et al., 2018).

Other than these factors, lignin is also an evident factor that determines resistance to lodging (Frei, 2013). The maize miR528 family (miR528a and miR528b) regulates the expression of lignin synthesis genes *ZmLAC3* and *ZmLAC5* in an abundant nitrogen environment, and these genes are highly expressed in the internodes and are responsible for lignin deposition in the maize stem (Sun et al., 2018). Knocking down miR528 results in higher expression of ZmLAC3 and LAC5, which results in higher concentrations of lignin more resistant to lodging (Sun et al., 2018). Somssich (2020) identified the LAC10, PRX42, PRX72, PRX52, and PRX71 proteins as responsible for lignification in stems.

QTLs Involved in Lodging and Kernel Abortion Resistance

Marker-assisted quantitative trait loci (QTLs) are one of the approaches that can be used for the identification of genes and to interrelate qualitative, quantitative, chemical, physiological, and morphological traits. In maize, 22 QTLs for lignin, 2 for starch content, 7 for hemicellulose, and 11 for cellulose were identified, which increased lodging resistance and reduced kernel abortion (Santiago et al., 2016). One QTL for stem sugars was identified to match the *SWEET4-3* putative gene locus in maize (Mizuno et al., 2016). Under high PD, plants encounter 43 different QTLs for six different traits: plant height, ear height, reduced stem diameter, delayed days to tassel, delayed days to silk, and anthesis-silking interval (Yuan et al., 2012).

Different QTLs can be identified using a marker-assisted approach. QTLs for lodging resistance mostly overlap with QTLs for stem strength, which reveals factors responsible for stem strength directly related to lodging resistance (**Table 3**). QTLs were found on chromosomes 2, 4–8, 9, and 10, which are accountable for root lodging. Additionally, 28 QTLs were found in maize with respect to kernel size and shape (Farkhari et al., 2013). Considering QTLs, their heritability, and other genetic factors responsible for quantitative traits and resistance to lodging can be useful for transforming the food chain and ensuring food security.

Agronomic Approaches

Planting Time and Method

Planting time influences lodging intensity and kernel abortion in maize (Angel et al., 2019; Zhang et al., 2019). Delays in planting

TABLE 2 | Genes involved in lodging resistance and kernel abortion reduction.

S. No.	Genes name	Chromosomes numbers	Functions	References
1	GRMZM2G119357	1	Chromatin remodeling protein EBS	Pineiro et al., 2003
2	GRMZM5G856734	1	Membrane steroid-binding protein 1	Yang et al., 2008
3	GRMZM5G855808	2	Tetratricopeptide repeat (TPR)-like superfamily protein	Munoz-Martinez et al., 2012
4	GRMZM2G156016	3	Transcription factor VOZ1	Kumar et al., 2018
5	GRMZM2G093276	7	ZIP zinc/iron transport family protein	Fu et al., 2017
6	GRMZM2G073934	5	Tetratricopeptide repeat (TPR)-like superfamily protein	Munoz-Martinez et al., 2012
7	GRMZM2G155312	1	Leucine-rich repeat protein kinase family protein	Imkampe et al., 2017
8	GRMZM2G442523	2	Sugar transport protein 5	Han et al., 2017
9	GRMZM2G029692	7	Protein kinase superfamily protein	Lehti-Shiu and Shiu, 2012
10	GRMZM2G082586	7	DNA binding protein bHLH-transcription factor 105	Zheng et al., 2019
11	GRMZM2G156692	7	proline-rich family protein	Wong et al., 2019
12	GRMZM2G375975	8	Putative MAP kinase family protein	Kong et al., 2013
13	GRMZM2G324276	1	Acetylglucosaminyltransferase family protein	Guelette et al., 2012
14	GRMZM2G083504	2	Transcription factor bHLH62	Lehti-Shiu and Shiu, 2012
15	GRMZM2G160400	7	Spermidine hydroxycinnamoyl transferase	Peng et al., 2019

TABLE 3 | Number of quantitative trait loci identified for stem lodging resistance and kernel abortion reduction in maize.

Characteristics names	Quantitative trait locus numbers	Affects	References
Lignin contents	22	Resistance to lodging and kernel abortion	Santiago et al., 2016
Starch contents	2	Resistance to lodging and kernel abortion	Santiago et al., 2016
Cellulose	11	Resistance to lodging and kernel abortion	Santiago et al., 2016
Hemicellulose	7	Resistance to lodging and kernel abortion	Santiago et al., 2016

time accelerates plant growth between seedling emergence and silking, which minimizes crop exposure to cumulative incident radiation during the vegetative process. Therefore, lodging may be reduced if crops are sown at the optimum time (Dahiya et al., 2018). However, the optimum planting time differs with the area and environmental conditions (Andrade et al., 1996; Bruns and Abbas, 2006). Similarly, planting time affects kernel abortion (Zhang et al., 2019), and both early and late plantings can cause kernel abortion and reduce the cumulative intercepted photosynthetically active radiation because of delayed leaf area development and high temperature (Otegui et al., 1995; Zhang et al., 2019). During early planting, the effective grain-filling duration is shortened, owing to the maximum daily high temperature from the silking to the blister stage. Similarly, the grain-filling of apical kernels is restrained, which leads to an increase in kernel abortion (Zhang et al., 2019). Contrarily, delayed planting resulted in significant reductions in final kernel number per unit area, and the number of ears at harvest (Cirilo and Andrade, 1994). Moreover, it was suggested that with delayed planting date, radiation and thermal time during the grain-filling period decreased, leading finally to decreased maize kernel weight (Cirilo and Andrade, 1994; Zhou et al., 2017). Li Y. et al. (2003) reported that with delay in sowing date at Shihezi, Xinjiang Uygur Autonomous region, China, grain-filling rate and final kernel weight decreased. Kernel abortion under late planting time is highly associated with less pollen availability, delayed anthesis and silking of individual plants, anthesis-silking interval, decreased number of exposed silks per apical ear, number of pollen grains per square meter, and kernel number per ear (Uribelarrea et al., 2002). Therefore, optimum planting time can improve maize productivity under relatively appropriate climate conditions and with the avoidance of abiotic stresses during the critical period of kernel formation and growth (Arnold and Monteith, 1974; McMaster and Wilhelm, 1997).

In addition to planting time, planting methods also influence the susceptibility of maize plants to lodge and kernel abort (Bakht et al., 2011; Deng et al., 2019). Different planting methods are practiced worldwide at the time of sowing maize crops (Arif et al., 2001; Bakht et al., 2011; Deng et al., 2019). Inappropriate planting methods can result in sterile plants. Ears and plant size remain small, and crops become susceptible to lodging, diseases, and pests, resulting in lower yield per unit area (Liu and Yong, 2008; Bakht et al., 2011). Abdullah et al. (2008) reported that the ridge planting method was better compared with other planting methods examined (bed and flat methods). The ridge planting method reduces lodging and provides good soil conditions for root development and efficient use of irrigation water and nutrients for proper development (Bakht et al., 2006, 2011; Liu and Yong, 2008). Heat stress during fertilization and during flowering significantly reduces pollen viability and seed yield (Edreira et al., 2011; Wu et al., 2020), while adopting an appropriate planting method may alleviate such high temperature-induced negative effect on pollen development. Tao et al. (2013) reported that the ridge planting method enhances the ability of maize to resist heat stress during the grain filling stage, which may reduce kernel abortion. Likewise, the adoption of ridge-furrow with plastic film mulching resulted in reduced lodging index as compared with the well-watered planting method (Li et al., 2020; Li and Li, 2021).

Fertilization

Increasing the planting density and applying N fertilizer are very effective agronomic strategies for raising the yield of modern maize cultivars, but high plant density and excessive N application have led to thinner and taller stalks and increased lodging risk (Li H. et al., 2010; Ciampitti and Vyn, 2012; Shah et al., 2017). Higher nitrogen doses can increase the elongation rate and length of the basal internode and significantly reduce the cellulose content of maize stems (Rajkumara, 2008), thereby decreasing stem strength and increasing lodging rate. Wei et al. (2008) stated that N also increases the development of the upper plant canopy, which decreases basal internode length and, consequently, increases stem lodging. Because of dense canopy development, low light conditions occur, which tend to cause plants to grow vertically, resulting in the development of long internodes and stems with small diameters, and less lignification. This can be managed by the split application of N, rather than high concentration. N application timing should also be considered. During reproductive stages, N application is discouraged to avoid kernel abortion (Bian et al., 2017). Wang et al. (2020) reported that nitrogen application in splits can improve stem lodging resistance of maize under high PD.

Lack of kernel development and enhanced abortion is caused by insufficient supply of carbon and nitrogen assimilates in the ear (Hammad et al., 2020). Nitrogen deficiency may cause kernel abortion, resulting in infertility (Marahatta, 2020). Plants grown under high N levels and high PD have decreased kernel numbers because the floret set is established before silking (Gonzalez et al., 2019; Mueller et al., 2019; Paponov et al., 2020). Thus, an adequate supply of N during the lag phase of the grain-filling stage may reduce kernel abortion (Below et al., 2000; Mueller et al., 2019; Paponov et al., 2020). N deficiency decreases the number of kernels per cob by decreasing maize kernel growth and development (Savin et al., 2006). Optimum N fertilization may increase kernel quantity and weight, resulting in higher crop growth rates, while N deficiency decreases photosynthate production in plants (Worku et al., 2012), consequently reducing the kernel-filling period. Nitrogen deficiency in maize during vegetative growth can cause early maturity (Sharifi and Namvar, 2016) and consequently reduce the kernel-filling period (Mayer et al., 2012). Increasing N availability stimulated ear growth during the bracketing-silking period and during the fast grain-filling phase, consequently resulting in greater maize grain yield (Ning et al., 2018). Moreover, in developing ears, N fertilization likely enhanced the cleavage of sucrose to glucose and fructose in the cob prior to and at silking and the synthesis from glucose and fructose to sucrose in the kernels after silking, thus increasing kernel setting and filling (Ning et al., 2018). Therefore, proper N fertilization can assist in managing kernel abortion and stem lodging in maize under high PD. However, more focus is required to study N application timing and rate, as higher N application is also not beneficial under high PD.

The role of potassium (K) is less evident; but to some extent, it contributes to lodging resistance. An inadequacy of K leads to reduced culm length, diameter, and wall thickness, and plants with inadequate K fertilization exhibit weaker culms than those with proper K fertilization (Mulder, 1954; Shah et al., 2017). K plays an important role in the physical strength of the plant and considerably reduces the lodging index and kernel abortion (Zhang et al., 2010). K can promote lignification in thick-walled cells, thicken collenchyma cells, and increase cellulose content, which reduces the lodging index (Shah et al., 2017). In other studies, the application of K during early stalk development and flowering stages increased structural carbohydrate accumulation and stem strength (Liebhardt and Murdock, 1965; Sun et al., 1989; Li et al., 2012). K deficiency in plants often results in the accumulation of sucrose in source leaves because of insufficient loading of the phloem (Zhao et al., 2001; Cakmak, 2005; Shahzad et al., 2017). K is the major cation in the phloem, and deficiency can lead to poor functioning, including phloem-disrupted metabolism and transport of assimilates, which induces kernel abortion (Shahzad et al., 2017). Epron et al. (2016) and Shahzad et al. (2017) reported that K foliar application may reduce kernel abortion, probably by affecting the phloem transport of assimilates. In another study, it was found that K application increased fertilization by adjusting the period between tasseling and silking, which resulted in a greater number of grain rows, grain cob^{-1}, and produced higher grain weight cob^{-1} (Ur Rehman and Ishaque, 2011). Moreover, K, in combination with N, has a synergistic influence on the uptake, translocation, and utilization of nutrients, and it reduces the percentage of senescent stalks, lodging and increases crushing strength and rind thickness (Bukhsh et al., 2012).

Growth Regulator Application

Plant growth regulators are artificial chemical compounds used to decrease plant height and other lodging-related plant traits. Some regulators have recently been introduced to control maize lodging (Schluttenhofer et al., 2011). Plant growth regulators can optimize plant morphology and increase yield by regulating endogenous plant hormone signaling and metabolism (Naeem et al., 2012; Zeng et al., 2012). Different plant growth regulators have been applied to maize, such as ethephon (Shekoofa and Emam, 2008), mepiquat chloride (Kamran et al., 2018c), paclobutrazol (Kamran et al., 2020), and uniconazole (Schluttenhofer et al., 2011), in the context of lodging resistance and maize yield improvement; however, the effect on vegetative (and perhaps generative) plant growth is highly dependent on the time of application and dosage of plant growth regulator and probably varies with the maize cultivar used (Hütsch and Schubert, 2018).

Ethephon (2-chloroethyl phosphonic acid) is a plant growth regulator that inhibits stem elongation and promotes stem thickness, thereby improving plant morphological resistance to lodging (Li et al., 2019). Shekoofa and Emam (2008) reported that the application of ethephon is associated with reductions in plant height, leaf area index, and crop growth rate, decreasing lodging by 85–93%, resulting in better kernel filling but also slightly decreasing yield by 2–6% (Khosravi and Anderson, 1991). Yield reduction in maize increases with the application rate of ethephon (Earley and Slife, 1969). However, in another study, ethephon with diethyl aminoethyl hexanoate (DA-6) could offset the yield. Furthermore, the combination of ethephon and DA-6 shortened the length and increased the diameter of internodes below the ear position, improving lodging resistance and the yield of maize (Dong et al., 2006). Ethephon has been observed as a negative regulator of kernel development or grain yield maize because of its negative effects on leaf area development, crop growth rate, and photoassimilate reduction (Shekoofa and Emam, 2008; Gao et al., 2009). However, it has been shown that the application of low concentration of ethephon increases maize kernel yield under high PD (Gao et al., 2009). Given that, more physiological studies are required to explore the role of ethephon further in managing stem lodging and kernel abortion at the same time.

Mepiquat chloride is also a plant growth regulator that mainly reduces the length of internodes in dense plant populations and increases resistance to lodging (Kuai et al., 2015; Kamran et al., 2018b). Kamran et al. (2018b) stated that mepiquat chloride in dense maize populations reduces plant height and ear height and increases stem diameter and lignin content in basal nodes, which ultimately reduces kernel abortion and enhances lodging resistance. The ability of mepiquat chloride to reduce the percentage of lodging results in a more uniform canopy, which further improves grain yield at high density (Kamran et al., 2018b). Similarly, Zhang Q. et al. (2014), Zhang et al. (2017) reported that the application of growth regulators significantly increased the number of kernels per ear and maize yield by increasing the optimal plant density by reducing the lodging percentage.

Paclobutrazol is also a plant growth regulator that mainly reduces the length of the second internode, resulting in reduction in plant height and increased resistance to lodging (Peng et al., 2014). Furthermore, stem diameter, lodging resistance, lignin accumulation, and antioxidant activities were positively affected by its use. Additionally, several studies (Özmen et al., 2003; Dong et al., 2006; Cai et al., 2014) have shown that the canopy of plants is best established by its use, with a significant increase in photosynthetic activity and yield (Wang C. et al., 2015; Xu et al., 2017). Thus, the use of these plant growth regulators increases plant resistance to lodging (Peng et al., 2014; Wang C. et al., 2015).

The application of paclobutrazol increased the mechanical strength of basal internodes and decreased internode length and plant and ear height, thereby ultimately reducing lodging in maize (Kamran et al., 2018a). They also showed that paclobutrazol treatments significantly ($P < 0.05$) enhanced the ear characteristics (ear length and diameter, kernels ear^{-1}, and 1,000 kernel weight) and grain yield of summer maize when compared with control treatments (Kamran et al., 2018a). The increase in grain yield, in response to paclobutrazol, is attributed partly to decreased investment in above-ground parts, due to a relatively stouter canopy of paclobutrazol-treated plants, as well as enhanced grain filling, in the treated plants due to improved rooting system, which possibly increased the uptake of nutrients and water (Qi, 2012; Kamran et al., 2018b). Given the above findings, at anthesis, the start and duration of pollen production, the start of silking, and anthesis-silking interval were mostly unaffected by paclobutrazol application (Hütsch and Schubert, 2021). Nonetheless, maize grain yield improvements after paclobutrazol application have been attributed to better grain-filling due to broader canopy, delayed onset of senescence and, thus, the start of chlorophyll degradation and improved rooting system (Kamran et al., 2018b, 2020). Kamran et al. (2020) pointed out that higher photosynthetic rate and duration (longer duration of green leaf area) were thought to be mainly responsible for grain yield increases after paclobutrazol treatment.

Uniconazole, a plant growth regulator used mainly to retard plant growth, results in shorter internodes, thereby increasing stem diameter, strengthening the overall stem structure, and increasing lodging resistance (Sellmer et al., 2001). Particularly in maize, the use of uniconazole results in decrease in plant height because of decrease in gibberellins, which results in reduction in cell length but not a reduction in the number of nodes (Schluttenhofer et al., 2011). However, in buckwheat, the results are not much different; lodging resistance and the lodging index were significantly reduced because of reduced plant height and increased lignin content (Wang C. et al., 2015). Schluttenhofer et al. (2011) reported that uniconazole increases lignin content, mechanical strength of the culm, and rind penetration strength, and decreases plant and ear height, which reduces the risk of lodging stress in maize. The application of uniconazole improved maize grain yield by higher kernel number per cob and increased kernel weight due to enhanced seed filling (Ahmad et al., 2018a,b, 2019). A significant increase in cob size was observed when uniconazole was applied at early growth stages, pointing to the impact of application time on maize yield performance (Xu et al., 2004); thus, considering the time of application may result in increased stem lodging and reduced kernel abortion simultaneously under high PD.

CONCLUSION

Stem lodging and kernel abortion considerably reduce grain yield. This review provides an understanding of stem lodging and kernel abortion mechanisms in maize. Interestingly, we found that genes involved in starch biosynthesis and transportation metabolism are involved in stem lodging resistance and kernel abortion. However, targeting sugar metabolism and *via* agronomic management, stem lodging and kernel abortion can be reduced under high planting density. Although there is no direct relationship between all the discussed agronomic practices and kernel abortion; however, reducing stem lodging can reduce kernel abortion in maize under high planting density. Thus, further research should be designed to investigate the genes directly involved in stem lodging and, thereby, induce kernel abortion. This will improve our understanding of the molecular basis of maize resistance to lodging. More focused research is needed to elucidate how sugar synthesis, transport, and accumulation processes should be altered to maximize maize resistance to stressful stimuli under high planting density. Further research should be designed to investigate the genes directly involved in stem lodging, and, thereby, induce kernel abortion. This will improve our understanding of the molecular basis of maize resistance to lodging.

AUTHOR CONTRIBUTIONS

ANS, MT, AA, and YS conceived this review and drafted and finalized the paper. YS and MY helped to improve the draft by providing useful suggestions and information. AAS, MIA, ZW, and WS helped in finalizing the paper. All authors approved the work for publication.

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Optimum Planting Density Improves Resource Use Efficiency and Yield Stability of Rainfed Maize in Semiarid Climate

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Increasing planting density is an effective strategy for improving maize productivity, but grain yield does not increase linearly with the increase in plant density, especially in semiarid environments. However, how planting density regulates the integrated utilization of key input resources (i.e., radiation, water, and nutrients) to affect maize production is not clear. To evaluate the effects of planting density and cultivar on maize canopy structure, photosynthetic characteristics, yield, and resource use efficiency, we conducted a successive field experiment from 2013 to 2018 in Heyang County (Shaanxi Province, China) using three different cultivars [i.e., Yuyu22 (C1), Zhengdan958 (C2), and Xianyu335 (C3)] at four planting densities [i.e., 52,500 (D1), 67,500 (D2), 82,500 (D3), and 97,500 (D4) plants ha^{-1}]. Increasing planting density significantly increased the leaf area index (LAI) and the amount of intercepted photosynthetically active radiation (IPAR), thereby promoting plant growth and crop productivity. However, increased planting density reduced plant photosynthetic capacity [net photosynthetic rate (Pn)], stomatal conductance (Gc), and leaf chlorophyll content. These alterations constitute key mechanisms underlying the decline in crop productivity and yield stability at high planting density. Although improved planting density increased IPAR, it did not promote higher resource use efficiency. Compared with the D1 treatment, the grain yield, precipitation use efficiency (PUE), radiation use efficiency (RUE), and nitrogen use efficiency (NUE) increased by 5.6–12.5%, 2.8–7.1%, and –2.1 to 1.6% in D2, D3, and D4 treatments, respectively. These showed that pursuing too high planting density is not a desirable strategy in the rainfed farming system of semiarid environments. In addition, density-tolerant cultivars (C2 and C3) showed better canopy structure and photosynthetic capacity and recorded higher yield stability and resource use efficiency. Together, these results suggest that growing density-tolerant cultivars at moderate planting density could serve as a promising approach for stabilizing grain yield and realizing the sustainable development of agriculture in semiarid regions.

Keywords: density, resources use efficiency, photosynthetic characteristic, rainfed maize, grain yield

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INTRODUCTION

Rainfed farming is a main agricultural production system practiced on more than 70% of the arable land in the world and accounts for approximately 60–65% of the global grain production (Lin and Liu, 2016). Therefore, it is important to ensure food security and increasing the economic status of local populations in the face of climate change. The Loess Plateau region, a typical intensive agroecosystem that covers a total area of 630,000 km^2 in northwest China, has become an important cereal crop production belt (Zhang et al., 2014). This area has a long history of agricultural cultivation, and maize is one of the most important crops grown in this region. However, due to water scarcity, this area has always been dominated by dryland farming. Rainfall, which is the main resource for crop growth in this region, shows large inter- and intra-annual variability (Zhang et al., 2017), leading to low and unstable crop productivity. However, this region receives an ample amount of sunlight, which provides the energy required for obtaining a high yield (Teixeira et al., 2014). Therefore, to establish sustainable agriculture in this region, it is important to determine how the limited resources can be effectively utilized for improving crop yield and resource (i.e., radiation, water, and nutrient) use efficiency and for stabilizing crop productivity.

In maize (*Zea mays* L.), increasing planting density has proven to be an effective agronomic practice for improving grain yield and resource use efficiency worldwide (Testa et al., 2016; Jia et al., 2018; Fahad et al., 2020). However, only a few studies have explored how changes in the absorption and utilization of radiation, nutrients, and water caused by increasing planting density improve crop growth, development, and grain yield. Planting density affects the absorption and utilization of radiation, water, and nutrients in plants by changing the canopy and/or root system architecture (Hammer et al., 2009; Du et al., 2021). Increased planting density improves the intercepted photosynthetically active radiation (IPAR) by rapid canopy closure and increases the leaf area index (LAI) (Teixeira et al., 2014; Hernández et al., 2020). It is well-known that biomass yield is the production of IPAR, which ultimately converts into yield, and maize grain yield is determined by the product of total biomass (Du et al., 2021). Increasing planting density increases IPAR, but it also increases competition among plants for light, water, and nutrients (Ciampitti and Vyn, 2011; Rossini et al., 2011), causing abiotic stress in plants, which is often visually apparent in maize *via* the reduction in leaf area, leaf chlorophyll content, and grain biomass (Osakabe et al., 2014). Such phenomena decrease plant light interception and photoassimilate production, thereby decreasing crop productivity and resource use efficiency (Teixeira et al., 2014; Zhang et al., 2019b; Du et al., 2021). Under abiotic stress conditions, dry matter allocation to reproductive organs declines, leading to lower grain yield, yield components (i.e., kernel number and weight), and harvest index (HI) (Ciampitti and Vyn, 2011; Mylonas et al., 2020). Different cultivars also show different responses to planting density in terms of productivity and resource utilization efficiency (Balkcom et al., 2011; Tokatlidis et al., 2011; Tokatlidis, 2013). Therefore, it is important to understand how crop production and resource use efficiency respond to both planting density and plant genotype. In contrast, interactions within the above physiological indexes have also been recorded (Ciampitti and Vyn, 2012), and the enhanced knowledge of physiological relationships can be useful for developing maize management systems that improve resource use efficiency.

In this study, we conducted a 6-year successive field experiment on maize in the Loess Plateau region to (1) investigate the effects of planting density and cultivar on canopy structural characteristics, (2) explore the effects of planting density on plant growth and photosynthetic characteristics, and (3) evaluate the yield stability and resource (i.e., radiation, nitrogen, and water) use efficiency of dryland maize under different treatments.

MATERIALS AND METHODS

Field Management and Experimental Design

Successive field experiments were conducted from 2013 to 2018 at the experimental station of the Heyang Dryland Agricultural Research Station of Northwest A & F University, located in the Heyang County of Shaanxi Province (35°19’ N, 110°4’ E, and 877 m above sea level), in the southeast region of the Loess Plateau in northwest China. At the experimental site, the average annual precipitation is approximately 494 mm (2004–2017), with approximately 60% of the annual rainfall occurring in July–September. The soil type is dark loessial soil and is classified as middle loam soil, according to the FAO/UNESCO Soil Classification (1993).

The experiment was arranged in a split-plot design with three replications. Planting density was assigned to the main plots, and maize cultivar was assigned to subplots. Four planting densities were evaluated in the experiment as follows: 52,500 plants ha^{-1} (D1), 67,500 plants ha^{-1} (D2), 82,500 plants ha^{-1} (D3), and 97,500 plants ha^{-1} (D4), with a row-to-row spacing of 50 cm. Three cultivars with different levels of tolerance to planting density were used in the experiment as follows: Yuyu22 (C1), Zhengdan958 (C2), and Xianyu335 (C3) (Xue et al., 2010). Other field management practices followed in this study have been described previously (Zhang et al., 2019c).

Weather-Related Data

Daily weather datasets (i.e., solar radiation, air temperature, and rainfall) were obtained from the national meteorological database,[1] and the data from 2013 to 2018 are shown in **Figure 1**.

Leaf Area Index and Aboveground Biomass

Five plants were randomly selected at different stages to determine the green leaf area (leaf length × leaf width × 0.75) and LAI (total leaf area per ha) of each maize plant (Zhang et al., 2019b). After measuring leaf area, the same plants were used for measuring the aboveground biomass. To measure the

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[1] http://data.cma.cn/

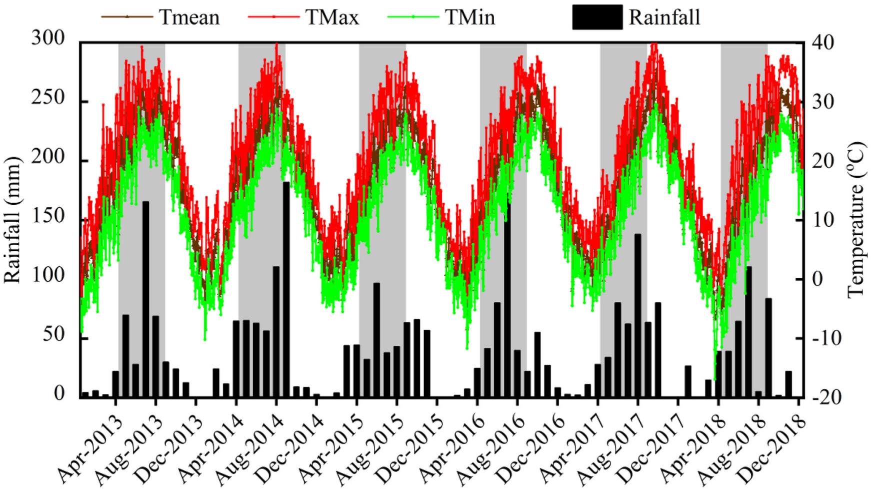

FIGURE 1 | Dynamics of temperature and rainfall during the experimental period. The gray areas represent the growing period of maize.

aboveground biomass, plants were fixated at 105°C for 0.5 h and then oven-dried at 85°C for a minimum of 48 h until a constant weight was achieved.

Leaf Photosynthetic Characteristics and Chlorophyll Content

Five plants were randomly selected from each plot at the jointing (V6), tasseling (VT), and filling (R3) stages, and the net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gc) of leaves were measured using a Li-6400 portable photosynthesis system (Li-COR Inc., Lincoln, NE, United States). These measurements were taken between 9:00 a.m. and 11:00 a.m. on a clear sunny day. The largest leaf was sampled at the V6 stage, while the maize ear leaf was sampled at the VT and R3 stages. Leaf chlorophyll content was determined using photometric methods, as described by Cui et al. (2019).

Intercepted Photosynthetically Active Radiation and Radiation Use Efficiency

The IPAR (MJ m^{-2}) per plant canopy and radiation use efficiency (RUE) (g MJ^{-1}) data were determined using the following equations (Zhang et al., 2019b):

$$IPAR = \sum 0.5R(1 - e^{-kLAI})$$

$$RUE = \frac{\text{Grain yield}}{\text{IPAR}}$$

where R is the daily solar radiation (MJ m^{-2} day^{-1}), k is the light extinction coefficient (0.65 for maize), and LAI is the LAI.

Grain Yield

In each treatment, three random quadrats covering a 9.0 m^2 area were selected to determine yield and yield components (kernel number per square meter and 100-kernel weight). Grain and biomass yield were determined at 14% moisture content. HI and precipitation use efficiency (PUE) were calculated using the following equations:

$$HI = \frac{Grain\ yield}{Biomass\ yield}$$

$$PUE = \frac{Grain\ yield}{P}$$

where P is the amount of precipitation (mm) during the growing season.

Crop yield stability, as affected by different treatments, was evaluated based on its variability by measuring the coefficient of variation (CV, %) using the following equation (Xu et al., 2019):

$$CV = \frac{STD(Yt)}{AVE(Yt)} \times 100$$

where *STD(Yt)* is the SD of grain yield of a particular treatment over the 6-year experiment period, and *AVE(Yt)* is the mean yield of that treatment over the same period.

The sustainable yield index (SYI) is a quantitative measure to assess the sustainability of any agricultural system (Sharma et al., 2013). The SYI was calculated using the following equation (Li et al., 2016):

$$SYI = \frac{AVE\,(Yt) - STD(Yt)}{Ymax}$$

where *Ymax* represents the maximum crop yield attained by any treatment during the study period, and *AVE(Yt)* is the mean yield of that treatment over the same period.

Nitrogen Uptake and Utilization

The sampled maize plants were separated into different organs. Samples were then oven-dried at 85°C to measure the dry matter weight. Nitrogen concentration in plant samples was analyzed based on the Kjeldahl method (Du et al., 2021). Nitrogen uptake, nitrogen harvest index (NHI), nitrogen use efficiency

(NUE), nitrogen productive efficiency (NPE), and nitrogen uptake efficiency (NUPE) were calculated as follows (Zhang et al., 2019b):

$$NUE = \frac{Grain\ yield}{Total\ nitrogen\ uptake}$$

$$NUPE = \frac{Total\ nitrogen\ uptake}{Nitrogen\ application\ rate}$$

$$NPE = \frac{Grain\ yield}{Nitrogen\ application\ rate}$$

$$NHI = \frac{Grain\ nitrogen\ uptake}{Total\ nitrogen\ uptake}$$

Statistical Analysis

The statistical significance of density, cultivar, and their interaction was assessed with two-way ANOVA. All data were analyzed using the IBM SPSS statistical software package (version 20.0, SPSS Inc., Chicago, IL, United States), followed by the least significant difference (LSD) test. Differences among treatments were considered statistically significant at $p < 0.05$, and figures were generated using Origin 2015 (v. Pro 2019; OriginLab Corp., Northampton, MA, United States).

RESULTS

Biomass and Grain Yield

Maize biomass yield varied significantly with planting density and cultivar over the six cropping seasons ($p < 0.05$) (**Table 1**). Aboveground biomass accumulation increased with the increase in planting density (**Figure 2**), with the highest value recorded in the D4 treatment. Biomass yield accumulation increased slowly from the V3 to V6 stage and rapidly from the V6 to VT stage, with the highest value recorded at physiological maturity (**Figure 2**). In contrast, HI decreased with the increase in planting density (**Table 1**).

Yield and its components were significantly affected by density and cultivar over the 6 years ($p < 0.05$) (**Table 1**). The average ear

TABLE 1 | Maize grain yield and its components in different treatments.

Factor		Kernel number per meter	Kernel weight (g 100 seed^{-1})	Grain yield (kg ha^{-1})	Biomass yield (kg ha^{-1})	HI (%)	PUE (kg ha^{-1} mm^{-1})
Density (D)	D1	2680c	28.1a	7592c	16592c	45.1a	24.8c
	D2	2993b	26.5b	8507a	18363b	46.1a	26.8a
	D3	3128a	25.7c	8126b	19785a	42.9b	25.5b
	D4	3150a	23.6d	7411c	20031a	36.1c	25.2bc
Cultivar (C)	C1	2827c	27.1a	7665b	18012b	42.3a	24.8b
	C2	3110b	25.9b	8095a	18556a	43.4a	26.2a
	C3	3186a	25.1b	8062a	18653a	42.8a	26.0a
Source of variation							
D		***	***	***	***	***	***
C		***	***	**	*	ns	***
D*C		*	*	*	**	**	***

D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335; HI, harvest index; PUE, precipitation use efficiency. Data represent the average values over the experimental period (2013–2018). Different letters within the same treatment represent significant differences at $p < 0.05$ [least significant difference (LSD) test]. Asterisks indicate the significance level of the correlation ($p < 0.05$, **$p < 0.01$, ***$p < 0.001$). ns, non-significant ($p > 0.05$).*

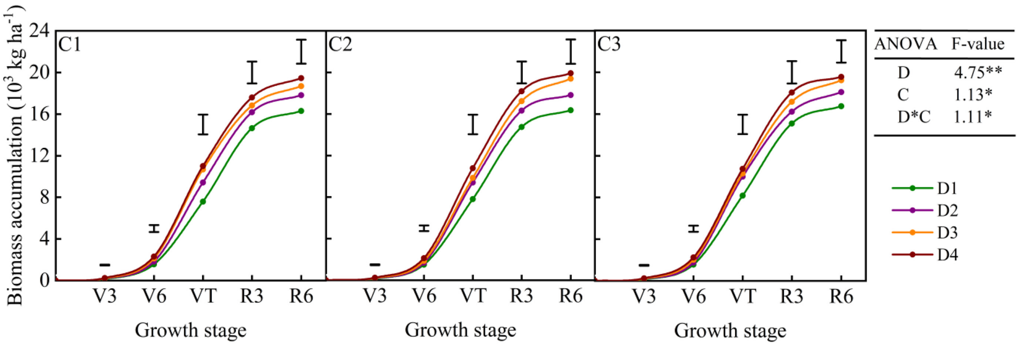

FIGURE 2 | Aboveground biomass accumulation of dryland maize under different planting densities and cultivar treatments. D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335; D, planting density; C, cultivar. Data represent the average values over the experimental period (2013–2018). Vertical bars represent the least significant difference (LSD) value at $p < 0.05$.

number per square meter increased, with the increase in planting density, whereas the 100-kernel weight decreased. Grain yield did not increase with the increase in planting density and showed the highest value in the D2 treatment (**Table 1**). The interaction between density and cultivar had significant effects on yield and its components ($p < 0.05$). The yield variation (CV) increased with the increase in planting density, but the SYI value decreased (**Table 2**). Differences in yield stability were detected among the three cultivars, and the C2 and C3 showed lower yield variation than the C1. These results indicate that high planting density raises the yield variability and decreases the yield sustainability of dryland maize, which was not conducive to the sustainable development of dryland farming.

Canopy Structural Characteristics

Dynamics of Leaf Area Development

The average value of LAI over the six cropping seasons increased with the increase in planting density (**Figure 3**), with the highest value recorded in the D4 treatment for all cultivars. The average LAI values for D2, D3, and D4 treatments were 19–27%, 38–44%, and 45–60%, respectively, higher than that in the D1 treatment. In all treatments, LAI increased slowly from the V3 to V6 stage before increasing rapidly from the V6 to VT stage, peaking at the VT stage, and then decreasing gradually. However, the amplitude of decline varied among the three cultivars, with the most rapid decline detected in the C1 (**Figure 3**).

TABLE 2 | Yield stability index (CV, %) and sustainable yield index (SYI) of dryland maize in different treatments.

Density	Cultivar	Mean (kg ha^{-1})	SD	CV (%)	SYI
D1	C1	7,544	1,983	26.3	0.59
	C2	7,539	2,021	26.8	0.57
	C3	7,291	1,809	24.8	0.61
D2	C1	7,776	2,127	27.4	0.55
	C2	8,458	2,209	26.1	0.58
	C3	8,333	2,149	25.8	0.58
D3	C1	7,396	2,620	35.4	0.51
	C2	8,314	2,754	33.1	0.52
	C3	8,204	2,726	33.2	0.51
D4	C1	6,212	2,563	41.3	0.40
	C2	6,778	2,620	38.6	0.42
	C3	7,855	2,803	35.7	0.50

D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335. SD, standard deviation; CV, coefficient of variation.

Intercepted Photosynthetically Active Radiation

The IPAR captured by maize canopy was significantly affected by planting density and cultivar over the six cropping seasons ($p < 0.05$) (**Table 3**). Compared with the D1 treatment, the IPAR values increased by 13.5, 18.6, and 23.7% in the D2, D3, and D4 treatments, respectively. The IPAR values of the C2 and C3 were 9.3 and 8.2%, respectively, lower than that of the C1.

Photosynthetic Characteristics and Chlorophyll Content

The photosynthetic characteristics of dryland maize were significantly affected by planting density over the 6 years ($p < 0.05$) (**Figure 4**). Compared with the D1 treatment, the Pn in D2, D3, and D4 treatments, respectively, decreased by an average of 1.2, 4.8, and 17.8% at the V6 stage, by 2.4, 8.4, and 24.1% at the VT stage, and by 7.3, 13.1, and 19.9% at the R3 stage. Similar trends were observed for Tr and Gc. The leaf chlorophyll content of D2, D3, and D4 treatments also decreased by 2.2–5.1%, 5.3–8.0%, and 9.1–12.5% (**Figure 4**), respectively, compared with the D1 treatment. However, no significant differences in photosynthetic characteristics and chlorophyll content were observed among the different maize cultivars in most years.

Resource Use Efficiency

Precipitation Use Efficiency

The PUE of maize was significantly affected by planting density and cultivar over the six cropping seasons ($p < 0.05$) (**Table 1**). Similar to the trend shown by grain yield, PUE decreased with the increase in planting density, reaching the highest level in the D2 treatment. Compared with the D2 treatment, the PUE of D3 and D4 treatments decreased by 4.9 and 6.0%, respectively.

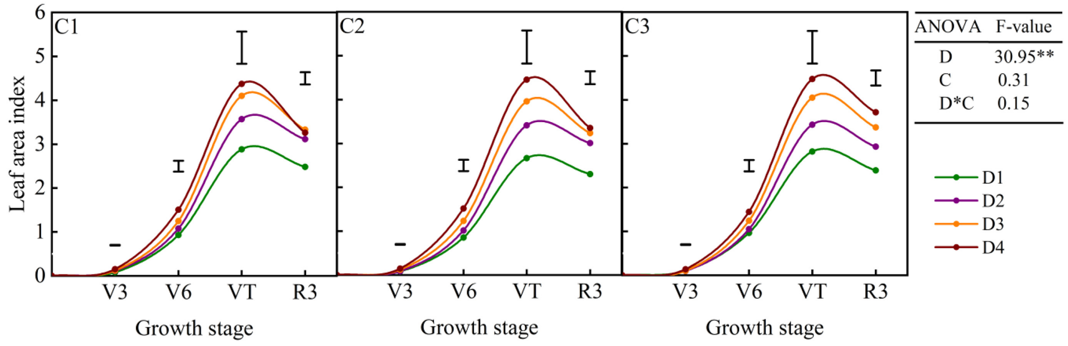

FIGURE 3 | Dynamics of the leaf area index (LAI) of dryland maize under different planting densities and cultivar treatments. D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335; D, planting density; C, cultivar. Data represent the average values over the experimental period (2013–2018). Vertical bars represent the LSD value at $p < 0.05$.

TABLE 3 | Intercepted photosynthetically active radiation (IPAR) and radiation use efficiency (RUE) of dryland maize in different treatments.

Factor		IPAR (MJ m^{-2})	RUE_{GY} (g MJ^{-1})	RUE_{BY} (g MJ^{-1})
Density (D)	D1	877d	0.86a	1.89a
	D2	995c	0.85a	1.84b
	D3	1040b	0.77b	1.81b
	D4	1085a	0.67c	1.80b
Cultivar (C)	C1	1001a	0.77b	1.80b
	C2	908b	0.82a	1.87a
	C3	919b	0.80a	1.87a
Source of variation				
D		***	***	*
C		*	**	**
D*C		ns	*	ns

D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335; RUE_{GY}, radiation use efficiency of grain yield; RUE_{BY}, radiation use efficiency of biomass yield. Data represent the average values over the experimental period (2013–2018). Different letters following means in different treatments represent significant differences at $p < 0.05$ (LSD test). Asterisks indicate the significance level of the correlation ($p < 0.05$, **$p < 0.01$, ***$p < 0.001$). ns, non-significant ($p > 0.05$).*

The interaction between planting density and cultivar had no significant effect on the PUE over the six cropping seasons.

Radiation Use Efficiency

The RUE of maize was significantly affected by planting density and cultivar among the six cropping seasons ($p < 0.05$) (**Table 3**). Although IPAR increased with the increase in planting density, the RUE showed the opposite trend (**Table 3**). Compared with the D2 treatment, the RUE of D3 and D4 treatments decreased by 9.4 and 21.2%, respectively, for grain yield and by 1.6 and 2.2%, respectively, for biomass yield. The interaction between planting density and cultivar had a significant effect on RUE for grain yield ($p < 0.05$).

Nitrogen Uptake and Utilization

The NUPE and NUE were significantly affected by planting density over the 6 years ($p < 0.05$) (**Table 4**). Total nitrogen uptake and nitrogen uptake for grain yield did not increase with the increasing planting density and reached the highest values in the D2 treatment. NHI decreased with the increase in planting density, indicating reduced translocation of nitrogen from vegetative organs to grains. Compared with the D1 treatment, the D2, D3, and D4 treatments showed an increase in NUE, NUPE, and NPE by −1.3 to 5.8%, −3.7 to 5.6%, and −2.2 to 12.2%, respectively. However, only NUPE and NPE showed significant differences among the three cultivars, and the C2 and C3 showed higher yields than the C1 (**Table 4**). The interaction between planting density and cultivar was significant for NPE ($p < 0.05$).

Relationships Among Yield, Harvest Index, Nitrogen Uptake Efficiency, Nitrogen Productive Efficiency, Precipitation Use Efficiency, and Radiation Use Efficiency

The relationships between maize grain yield and HI and those of NUPE with NUE, NPE, and RUE are shown in **Figure 5**. Grain yield was significantly positively correlated with HI, PUE, NUE, NPE, and RUE, but it showed no significant correlation with total nitrogen uptake and NHI. These correlations suggest that maize productivity under high planting density is limited by the relatively low translocation of assimilates from vegetative organs to grains, resulting in low resource use efficiency and relatively low productivity.

DISCUSSION

Canopy Structure and Photosynthetic Characteristics

Previous research has demonstrated that increasing planting density improves maize canopy closure, i.e., rapid canopy establishment and leaf area expansion, leading to greater IPAR, which contributes to greater radiation capture (Teixeira et al., 2014; Du et al., 2021). Similar results were obtained in this study. Compared with the D1 treatment, the average LAI values of the three cultivars increased by 19–27%, 38–44%, and 45–60% in D2, D3, and D4 treatments, respectively. After the VT stage, the LAI value decreased due to the shedding and senescence of plant leaves, but the amplitude of this decline was small in the low density, which is beneficial to the assimilating of photosynthetic products and resulting in higher partitioning of carbohydrates to the ear. This was mainly related to the lower interplant competition between plants, which has been reported in maize (Hammer et al., 2009; Rossini et al., 2011). Additionally, low-density crops maintain high green leaf area and leaf chlorophyll content and were accompanied by higher photosynthetic characteristics, such as Pn and Gc (**Figure 4**). The Gc affects the exchange of CO_2 and H_2O between leaves and the environment, as an adaptive mechanism to cope with drought stress (Hernández et al., 2020). Zhu et al. (2010) showed that photosynthetic efficiency is closely related to the regulation of stomatal opening and leaf chlorophyll content, and the increase in crop productivity relies on improved photosynthesis. Thus, optimizing canopy structure and maintaining photosynthetic capacity while increasing the resource use efficiency would be the key to improve the maize yield by optimizing planting density. One limitation of this study is that we monitored the photosynthetic characteristics and chlorophyll content of only the ear leaves, and the photosynthetic performance of the whole maize population remains unknown. Further investigation will help explain yield formation from the perspective of group light energy efficiency.

Grain Yield

In this study, biomass yield increased with the increase in plant density, whereas grain yield showed a parabolic relation with planting density (**Table 1**). The increasing of planting density results in lower light intensity in the canopy, but a certain grain yield needs more leaf area (to realize a high canopy photosynthesis rate) to support its grain filling and crop yield (Du et al., 2021). Thus, the HI decreased dramatically with increasing planting density. Increasing planting density significantly improved LAI and IPAR of the canopy, eventually resulting in a significant increase in aboveground dry matter

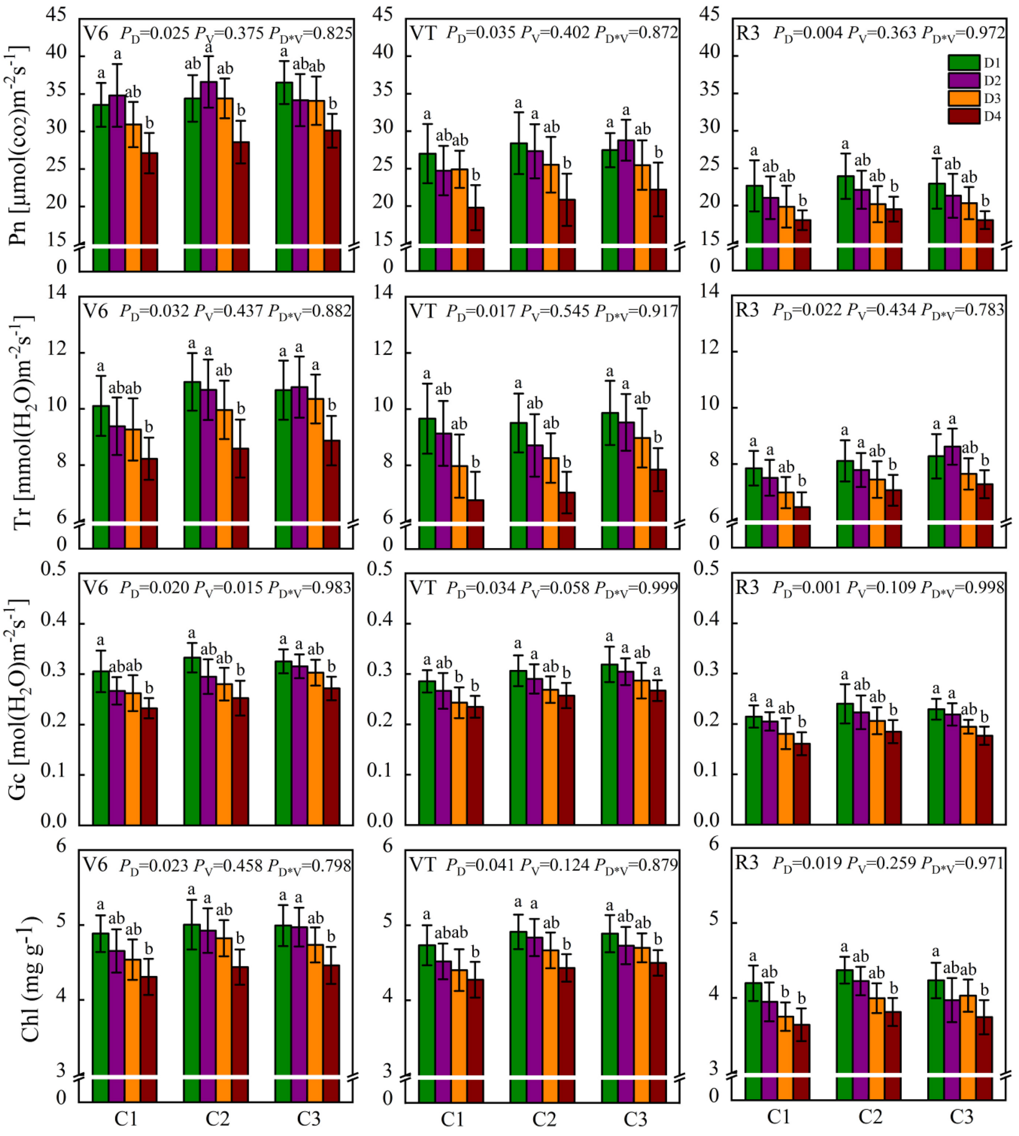

FIGURE 4 | Net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gc), and leaf chlorophyll content (Chl) of dryland maize under different treatments. D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335. Data represent mean ± SD over six cropping seasons. *P*-values of the ANOVA of density (P_D), cultivar (P_V), and their interaction (P_{D*V}) were also shown.

accumulation (Teixeira et al., 2014). However, as planting density increased, the photosynthetic characteristics of plants declined, resulting in lower crop photosynthetic assimilation and productivity per plant, which might explain the decrease in maize yield observed in this study at high planting density. These results indicate that dryland maize productivity at high planting density is limited by the relatively low translocation of assimilates to grains. Therefore, pursuing high planting density is not a desirable strategy in the rainfed farming system, while the relatively lower planting density may be more conducive to the effective use of limited resources of semiarid environments. Increasing planting density also increased the yield variability (CV, %) and decreased the yield sustainability of dryland maize (**Table 2**). Mylonas et al. (2020) also revealed that CV (%) values of plant yield increased when planting density increased, mainly due to increased competition for resources, especially for soil water in rainfall agroecosystems. While under lower planting density, the available water per plant increases, which can maintain the growth of crops and filling of grain. The cultivar is another factor affecting grain yield response to density and stability, as shown by previous studies (Berzsenyi and Tokatlidis, 2012; Chen et al., 2017; Solomon et al., 2017), as well as our current results. In this study, C2 and C3 showed higher yield and yield stability over the six cropping seasons than the C1 (**Table 2**). The lower yield of the C1 was associated with the rapid decline in LAI after the tasseling stage (**Figure 3**). This is consistent with previous

TABLE 4 | Nitrogen uptake and utilization by dryland maize in different treatments.

Factor		Nitrogen uptake (kg ha^{-1})		NHI (%)	NUE (kg kg^{-1})	NUPE (kg kg^{-1})	NPE (kg kg^{-1})
		Grain	Total				
Density (D)	D1	74.0ab	121.1ab	60.4ab	62.6b	0.54ab	33.7c
	D2	81.3a	128.6a	61.8a	66.1a	0.57a	37.8a
	D3	76.5ab	121.0ab	61.2a	66.4a	0.54ab	36.1b
	D4	71.5b	117.9b	58.8b	61.7b	0.52b	32.9c
Cultivar (C)	C1	71.6b	115.3b	60.9a	65.2a	0.51b	34.1b
	C2	77.6a	123.9a	61.2a	64.6a	0.55a	36.0a
	C3	79.5a	129.2a	60.7a	63.7a	0.57a	35.8a
Source of variation							
D		*	*	*	*	*	***
C		*	*	ns	ns	*	**
D*C		ns	ns	ns	ns	Ns	**

D1: 52,500 plants ha^{-1}; D2: 67,500 plants ha^{-1}; D3: 82,500 plants ha^{-1}; D4: 97,500 plants ha^{-1}; C1: Yuyu22; C2: Zhengdan958; C3: Xianyu335; NHI, nitrogen harvest index; NUE, nitrogen use efficiency; NUPE, nitrogen uptake efficiency; NPE, nitrogen productive efficiency. Data represent the average values over the experimental period (2013–2018). Different letters within the same treatment represent significant differences at $p < 0.05$ (LSD test). Asterisks indicate the significance level of the correlation ($p < 0.05$, **$p < 0.01$, ***$p < 0.001$). ns, non-significant ($p > 0.05$).*

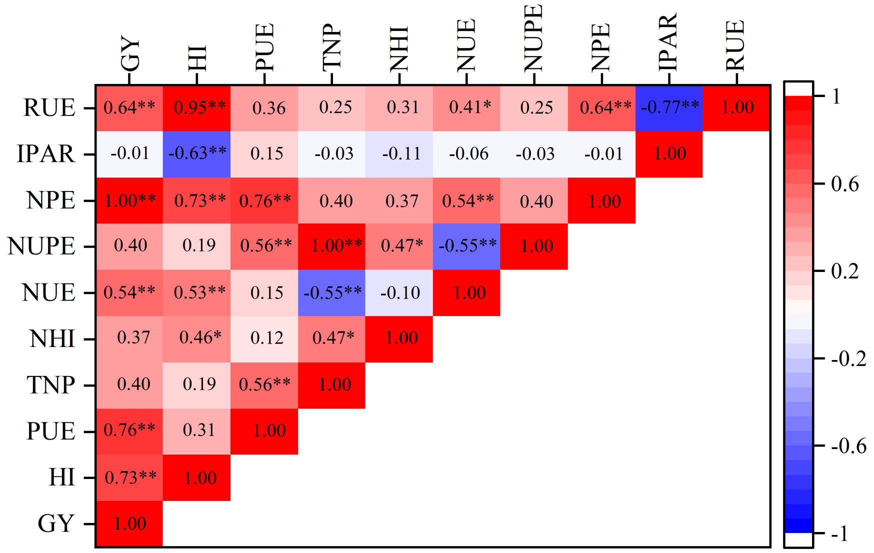

FIGURE 5 | Correlation coefficients of maize yield and resource use efficiency. GY, grain yield; HI, harvest index; PUE, precipitation use efficiency; TNP, total nitrogen uptake; NHI, nitrogen harvest index; NUE, nitrogen use efficiency; NUPE, nitrogen uptake efficiency; NPE, nitrogen productive efficiency; IPAR, intercepted photosynthetically active radiation; RUE, radiation use efficiency. Asterisks indicate the significance level of the correlation (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). ns, non-significant ($p > 0.05$).

findings reported that the reduction in green LAI results decreases the fraction of total radiation intercepted and leads to lower carbohydrate remobilization from leaves to the ear (Xue et al., 2010).

Resource Use Efficiency

Improving the resource use efficiency of crop plants is the main strategy to realize the sustainable development of agriculture. In this study, PUE was significantly affected by planting density and cultivar ($p < 0.05$) (**Table 1**). A similar trend was shown by grain yield, and PUE did not increase with the increase in planting density but showed a parabolic relation with planting density. Results by Tokatlidis et al. (2011) and Berzsenyi and Tokatlidis (2012) highlighted the importance of maize cultivars that are less dependent on high planting density to increase resource use efficiency in non-irrigated land. Although increments in IPAR were in accordance with increasing LAI, they did not promote higher RUE. This is partly because light attenuation within the canopy was increased under higher plant population due to shading, and relatively more light captured by the upper canopy has been suggested to reduce the whole plant photosynthetic efficiency, which in turn decreases the RUE (Du et al., 2021). In addition to water and radiation, crop productivity also depends on the absorption of nutrients and allocation of assimilates (Teixeira et al., 2014; Xu et al., 2017; Zhang et al., 2019a). In this study, increasing plant population did not increase the NUPE and NUE over 6 years (**Table 4**). This was mainly because increasing planting density decreases the capacity of the crop to accumulate nitrogen per unit green LAI (Ciampitti and Vyn, 2011), thus decreasing the NPE. Therefore, provided cultivars have high plant yield efficiency, and using lower planting density to enhance crop resilience to extremely fluctuating environments will be more meaningful for the long-term development of dryland agriculture. Differences in cultivar characteristics are one of the main reasons for the differences in resource use efficiency (i.e., radiation, water, and nutrients). Density-tolerant cultivars (C2 and C3) exhibited higher resource use efficiency than the C1 under the same climatic conditions. This was mainly related to the light distribution through the canopy, which was increased for density-tolerant cultivars due to their upright leaves and small leaf angles (Xue et al., 2010). This resulted in relatively more light being captured by the lower canopy of density-tolerant cultivars, thus improving their resource use efficiency.

CONCLUSION

This study evaluated the effects of maize planting density and cultivar on canopy structure, photosynthetic traits, yield, and resource use efficiency. The increase in planting density improved the LAI and canopy closure and consequently enhanced the capacity of maize plants to uptake nutrients, absorb soil water, and capture PAR, leading to higher crop productivity. However, increased planting density decreased the photosynthetic characteristics (Pn and Gc) and leaf chlorophyll content, which resulted in lower photosynthetic capacity. These alterations constitute the key mechanisms underlying the decline in yield and resource use efficiency at high planting density. These results suggest that high planting density reduces maize yields mainly through a decline in photosynthetic efficiency and conversion efficiency, which translates into a proportional reduction in resource use efficiency. Therefore, optimizing planting density *via* improved high plant yield efficiency and resource use efficiency to enhance yield stability will be more beneficial to the long-term development of dryland agriculture. Different cultivars also show different responses to planting density; C2 and C3 showed better canopy structure, yield stability, and resource use efficiency than C1. Different cultivars also show different responses to planting density regarding canopy structure, yield stability, and resource use efficiency. Provided of high plant yield efficiency, cultivation of density-tolerant cultivars with a reasonable decrease in planting density can increase maize yield stability and resource use efficiency in rainfed agroecosystems, thus facilitating the development of sustainable agriculture.

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

JL conceived and designed the experiments. YZ and ZX performed the experiments. YZ and RW analyzed the data and wrote the manuscript. YZ, JL, and RW reviewed and revised the manuscript and corrected the English language. All authors reviewed and approved the manuscript for publication.

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Managing Density Stress to Close the Maize Yield Gap

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Continued yield increases of maize (*Zea mays* L.) will require higher planting populations, and enhancement of other agronomic inputs could alleviate density-induced stress. Row spacing, plant population, P-S-Zn fertility, K-B fertility, N fertility, and foliar protection were evaluated for their individual and cumulative impacts on the productivity of maize in a maize-soybean [*Glycine max* (L.) Merr.] rotation. An incomplete factorial design with these agronomic factors in both 0.76 and 0.51 m row widths was implemented for 13 trials in Illinois, United States, from 2014 to 2018. The agronomic treatments were compared to two controls: enhanced and standard, comprising all the factors applied at the enhanced or standard level, respectively. The 0.51 m enhanced management control yielded 3.3 Mg ha^{-1} (1.8–4.6 Mg ha^{-1} across the environments) more grain (25%) than the 0.76 m standard management control, demonstrating the apparent yield gap between traditional farm practices and attainable yield through enhanced agronomic management. Narrow rows and the combination of P-S-Zn and K-B fertility were the factors that provided the most significant yield increases over the standard control. Increasing plant population from 79,000 to 109,000 plants ha^{-1} reduced the yield gap when all other inputs were applied at the enhanced level. However, increasing plant population alone did not increase yield when no other factors were enhanced. Some agronomic factors, such as narrow rows and availability of plant nutrition, become more critical with increasing plant population when density-induced stress is more significant. Changes in yield were dependent upon changes in kernel number. Kernel weight was the heaviest when all the management factors were applied at the enhanced level while only planting 79,000 plants ha^{-1}. Conversely, kernel weight was the lightest when increasing population to 109,000 plants ha^{-1} while all other factors were applied at the standard level. The yield contribution of each factor was generally greater when applied in combination with all other enhanced factors than when added individually to the standard input system. Additionally, the full value of high-input agronomic management was only realized when matched with greater plant density.

Keywords: maize, density, population, spacing, fertility, nitrogen, yield, kernel

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INTRODUCTION

Due to breeding advancements and improved crop management practices, substantial gains in maize (*Zea mays* L.) yield in the United States have been made to-date (Duvick, 2005; Lee and Tollenaar, 2007). However, on-farm maize yields are estimated to be only 65% of yield potential for the non-irrigated environments typical in the United States (Lobell et al., 2009). This yield gap (the difference between the realized and potential yield) can be lessened with an advanced understanding of the agronomic and genetic factors that influence yield (Dobermann et al., 2002; Ruffo et al., 2015).

Grain yield is the product function of the number of plants per unit area, the number of viable kernels on each plant, and the size of each kernel. Thus, from a physiological perspective, increasing maize yield requires either more kernels per plant or heavier kernels while keeping the plant population constant (i.e., greater yield potential) or the ability to maintain kernel number and weight while increasing the plant population (i.e., greater density tolerance) (Gonzalez et al., 2018). Contemporary maize hybrids have greater yield potential as a direct result of greater crowding-stress tolerance (Tokatlidis and Koutroubas, 2004; Lee and Tollenaar, 2007; Hammer et al., 2009; Gonzalez et al., 2018), which has led to greater within- and between-field variability in grain yield in the United States Corn Belt (Lobell and Azzari, 2017). Currently, maize hybrids are grown at an average population of about 79,000 plants ha^{-1} in the United States Corn Belt, which has increased by approximately 1% annually since the mid-1990s (USDA-NASS, 2021). As plant populations rise, intraspecific competition for limiting resources increases, leading to increased plant-to-plant variability (Boomsma et al., 2009) and reduced plant growth and survival (Casper and Jackson, 1997). Several physiological changes, such as decreased root biomass, occur due to increased plant populations, which can lessen the ability of the crop to obtain resources and potentially reduce grain yield (Jiang et al., 2013; Bernhard and Below, 2020). The future of maize yield improvement may need to focus on crop management strategies and hybrid selection that alleviate stresses at higher plant populations (Tollenaar and Lee, 2002).

Reducing row spacing (<0.76 m) increases plant-to-plant spacing within the row and potentially increases yield through better light interception and more efficient usage of available space and resources (Andrade et al., 2002; Sharratt and McWilliams, 2005; Barbieri et al., 2008). The root weight of individual maize plants decreases by 1.2% for every 1,000 plants ha^{-1} increase in population (Bernhard and Below, 2020). However, increasing plant-to-plant spacing within the row by decreasing row spacing from 0.76 to 0.51 m increased root weight by 22%, which improves the plant's ability to obtain limiting resourses (i.e., water and nutrients) at higher populations. Past research on narrow-row maize (row spacing less than the current average of 0.76 m in the United States) has shown mixed results (Nielson, 1988; Porter et al., 1997; Cox et al., 1998), suggesting geography, hybrid, and other factors may affect the yield response of maize to narrow row spacing.

Nutrient deficiency is the most common yield-limiting factor worldwide for maize (Mueller et al., 2012). Increased plant demand for soil nutrients at higher populations (Ciampitti and Vyn, 2012) and declining soil test levels in the United States Midwest (Fixen et al., 2010) necessitate improved fertilizer application methods to close the maize yield gap. Harvested grain removes more phosphorus (P) from the field than any other nutrient (Bender et al., 2013). However, P is the least soil-available of the major plant nutrients (Kovar and Claasen, 2005) and is the second most yield-limiting nutrient after nitrogen (N) (Andraski and Bundy, 2008). Additionally, since 2005, the median soil P test value of Illinois, United States, has declined (Fixen et al., 2010). Fertilization of immobile nutrients, such as P and potassium (K), is typically accomplished with broadcast applications, spreading fertilizer in an even distribution across the soil surface and incorporation through conventional tillage. An alternative to broadcast applications is the banding of P and K (i.e., concentrated band 10–15 cm below the soil surface) before planting, which can potentially reduce fixation, increase P and K soil test levels near the root zone, and increase nutrient uptake (Boomsma et al., 2007). Nitrogen, behind carbon, is the mineral nutrient required in the most significant quantities by plants (Hawkesford et al., 2012; Bender et al., 2013), explaining why N fertilizer demand for crop production in North America was approximately 14.5 million tons in 2019 (Food and Agriculture Organization [FAO], 2019). However, applied N that is in excess or unused by the crop is subject to loss and can result in environmental pollution (Dinnes et al., 2002). Practices, such as split applications of N fertilizer or the use of urease and nitrifications inhibitors, can synchronize N availability with crop need and limit losses to the environment (Dinnes et al., 2002; Fageria and Baligar, 2005). Sidedress N applications to maize can be especially practical at increasing grain yield at higher plant populations (Ciampitti and Vyn, 2011). Sulfur (S) deficiency is more frequent than any other secondary nutrient in the United States Corn Belt primarily due to the reduced atmospheric deposition resulting from more rigorous emission standards and rising removal rates by higher grain yields (Lynch et al., 2000; Camberato and Casteel, 2010; Sawyer et al., 2012). Sulfur is the secondary nutrient with the largest harvest index for maize and has season-long uptake (Bender et al., 2013). Zinc (Zn) is the micronutrient most commonly and severely limiting maize yield (Bell and Dell, 2008; Alloway, 2009). Furthermore, Zn is the micronutrient with the highest harvest index in maize (Bender et al., 2013).

A class of systemic fungicides called quinone-outside inhibitors, also referred to as strobilurin fungicides, can be effective against common fungal pathogens that hybrid maize is susceptible to Grossmann and Retzlaff (1997). However, research has shown that they can increase maize yields even when the fungal diseases are not detectable in the crop (Ruffo et al., 2015). These strobilurin fungicides can have a "greening effect," resulting in increased photosynthetic capacity and reduced respiration (Grossmann et al., 1999; Bartlett et al., 2002).

Further increasing maize yields necessitates greater planting populations. A clearer knowledge of which agronomic management practices have the most significant impact on maize yield and how these practices interact with increased density is needed. Therefore, this research aimed to demonstrate the potential for improved maize productivity *via* increased planting populations and enhanced crop management and to evaluate the individual and synergistic contributions of soil fertility, supplemental nitrogen, planting population, foliar protection, and row spacing on grain yield and yield components.

MATERIALS AND METHODS

In this research, 13 field trials were conducted in different environments during the 2014–2018 growing seasons at the Crop Sciences Research and Education Center in Champaign-Urbana (CU) (40°2′ N, 88°14′ W) in east-central Illinois and the Northern Illinois Agronomy Research Center near DeKalb (DK) (41°47′ N, 88°50′ W) in northern Illinois, United States. The fields used at each site were located within 1 km of each other and had similar soil types, fertility levels, and management histories. Soybean was the previous crop, and tillage practices were generally classified as conventional deep ripping followed by cultivation tillage at each field site. An average of two trials was established in each environment and differed in their maize hybrid and plant protection products. The number of trials in each environment, planting dates, and average soil properties are outlined in **Table 1**. A complete list of trials, hybrids and foliar protection products used, and soil properties are shown in **Supplementary Table 1**. All the hybrids planted in this study were commercially available and widely grown in Illinois, United States. Soil samples were taken from a depth of 0 to 15 cm from each trial area before planting, and the minerals were extracted and determined using Mehlich III solution (A&L Great Lakes Laboratories, Fort Wayne, IN, United States). The CU trials were located on soils classified as Flannagan silt loam (fine, smectitic, mesic Aquic Argiudolls) with 0–2% slope and had medium to high levels of P based on the spring soil tests. Research plots near DK were located on soils classified as Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls; 0–2% slope), with higher organic matter levels than the soils in CU.

The trials were planted in a randomized complete block design with six replications and two row widths (0.51 and 0.76 m) in a split-plot arrangement. The main-plot was row spacing, and the split-plot was agronomic treatment level. The experimental plots were four rows wide spaced 0.51 or 0.76 m apart and 11.4 m long. The plots were planted with a research plot planter (ALMACO, Nevada, IA, United States) with variable seeding rate capability. Planting dates ranged from late April to early June for all the trials and were reflective of typical planting dates for the region (**Table 1**). At planting, tefluthrin [(2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1α,3α)-(Z)-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] was applied in-furrow at a rate of 0.11 kg a.i. ha^{-1} for control of seedling insect pests. Weed control consisted of a pre-emergence application of S-metolachlor [acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-,(S)], atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), mesotrione {2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione}, and bicyclopyrone {bicyclo[3.2.1]oct-3-en-2-one, 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-(trifluoromethyl)-3-pyridinyl]carbonyl]-} and a post-emergence application of glyphosate [*N*-(phosphonomethyl)glycine].

The center two rows of each plot were mechanically harvested for determining crop grain weight and moisture. The grain yield was calculated based on 15.5% moisture content. The average individual kernel weight was estimated by randomly selecting 300 kernels from each plot and expressed at 0% moisture. Kernel number was estimated by dividing the total plot grain weight by the average individual kernel weight.

Agronomic Practices

Five management factors were implemented at two levels representing either the "Standard" or "Enhanced" system in 0.76 and 0.51 m row spacings for determining their individual and combined impacts on grain yield (**Table 2**). The five agronomic management factors considered were: (i) plant fertility to include P, S, and Zn; (ii) K and B fertility; (iii) N fertility; (iv) plant population; and (v) foliar protection.

The value of P-S-Zn and K-B containing fertilizers were tested separately and in combination. The treatment levels for P-S-Zn fertility were none or with added P, S, and Zn denoted as −P-S-Zn or +P-S-Zn, respectively. Immediately before planting, P, S, and Zn were applied as MicroEssentials SZ [12-40-0-10(S)-1(Zn)] (The Mosaic Company, Tampa, FL, United States) in a subsurface band 10–15 cm beneath the future crop row for 34 kg N, 112 kg P_2O_5, 28 kg S, and 2.6 kg Zn ha^{-1}. Similarly, the two levels for K-B fertility were none or with added K and B, denoted as −K-B or +K-B, respectively. K and B were applied as Aspire [0-0-58-0.5(B)] (the Mosaic Company, Tampa, FL, United States) broadcasted across the soil surface with light incorporation immediately before planting for 84 kg K_2O and 0.7 kg B ha^{-1} in the enhanced system. In addition, the first two factors were combined with the standard plots receiving no added fertility and the enhanced plots receiving added P-S-Zn and K-B fertility, denoted as −P-S-Zn and K-B or +P-S-Zn and K-B. The

TABLE 1 | Summary of trial information and soil properties for six environments at Champaign-Urbana (CU) or DeKalb (DK), IL from 2014–2018.

Environment	Total trials	Planting dates	CEC[†]	pH	OM	P	K	Ca	Mg	S	Zn	B
			meq $100g^{-1}$		%	ppm						
14CU	3	03–06 June 2014	17.9	5.4	3.4	42	133	1832	387	9	1.1	0.3
15CU	2	07–13 May 2015	23.1	5.6	4.0	12	112	2653	569	-	-	-
15DK	1	22 May 2015	27.3	6.7	6.5	42	172	3567	897	8	4.1	-
16CU	3	19–22 April 2016	18.7	6.0	3.3	34	127	2220	487	8	1.6	0.3
17CU	2	18 May 2017	20.7	5.5	3.9	15	100	2321	412	9	1.2	0.4
18CU	2	26 May 2018	19.8	6.4	3.5	38	128	2527	532	9	2.0	0.5

[†] *CEC, cation exchange capacity; OM, organic matter.*

TABLE 2 | Addition and omission treatment structure: the treatment exceptions are either added (+factor) to the standard system control or omitted (-factor) from the enhanced system control.

Treatment		Factor				
System	Exception	P-S-Zn	K-B	Nitrogen	Population	Protection
Standard	None[†]	None	None	Base	79,000	None
Standard	+P-S-Zn	P-S-Zn	None	Base	79,000	None
Standard	+K-B	None	K-B	Base	79,000	None
Standard	+P-S-Zn and K-B	P-S-Zn	K-B	Base	79,000	None
Standard	+N	None	None	Base + Sidedress	79,000	None
Standard	+Population	None	None	Base	109,000	None
Standard	+Protection	None	None	Base	79,000	Yes
Enhanced	None	P-S-Zn	K-B	Base + Sidedress	109,000	Yes
Enhanced	−P-S-Zn	None	K-B	Base + Sidedress	109,000	Yes
Enhanced	−K-B	P-S-Zn	None	Base + Sidedress	109,000	Yes
Enhanced	−P-S-Zn and K-B	None	None	Base + Sidedress	109,000	Yes
Enhanced	−N	P-S-Zn	K-B	Base	109,000	Yes
Enhanced	−Population	P-S-Zn	K-B	Base + Sidedress	79,000	Yes
Enhanced	−Protection	P-S-Zn	K-B	Base + Sidedress	109,000	None

[†] *"None" in the exception column indicates the control.*

−P-S-Zn and K-B would be the typical practice in most fields of this study since the soil test results for P and K were typically above the critical threshold (Culman et al., 2020).

The two levels for the N factor were application at the base rate or base application plus sidedressing, denoted as −N or +N, respectively. For the −N treatment, N was broadcast applied before planting in the spring as 28% urea-ammonium nitrate [UAN, $CO(NH_2)_2$ + NH_4NO_3 + H_2O; 28-0-0] for 180 kg N ha^{-1}. The +N treatment received an additional 90 kg N ha^{-1} sidedress at the V6 growth stage as urea with a urease inhibitor [CO(NH2)2 + N-(n-butyl) thiophosphoric triamide; 46-0-0] (BASF Corporation, Research Triangle Park, NC, United States).

Maize was planted for target populations of 79,000 or 109,000 plants ha^{-1}, representing a common and high population, denoted as −Pop and +Pop, respectively.

Foliar protection evaluation consisted primarily of a prophylactic fungicide application, but the source of fungicide and tank mixes varied depending on the trial. The applications were made once tassels emerged (plant growth stage VT/R1) using a pressurized CO_2 back-pack sprayer. The center two rows of each plot were treated with a spray volume of 140 L ha^{-1}. The trials received either the fungicide Headline AMP (13.64% Pyraclostrobin + 5.14% Metconazole; 1.05 L ha^{-1}; BASF Corporation, Research Triangle Park, NC, United States), the fungicide Quilt Xcel (13.5% Azoxystrobin + 11.7% Propiconazole; 1.05 L ha^{-1}; Syngenta Crop Protection, LLC, Greensboro, NC, United States), or the combination of the fungicide Trivapro (10.27% benzovindiflupyr + 10.5% azoxystrobin + 11.9% propiconazole; 1.07 L ha^{-1}; Syngenta Crop Protection, LLC, Greensboro, NC, United States) and insecticide Warrior II [22.8% Lambda-cyhalothrin (synthetic pyrethroid); 0.12 L ha^{-1}; Syngenta Crop Protection, LLC, Greensboro, NC, United States]. These applications were collectively named "foliar protection" and denoted as +Protection in the enhanced management system to simplify data analysis. In contrast, the standard system received no fungicide application, denoted as −Protection.

Addition Versus Omission Treatment Structure

The addition versus omission treatment structure used in this study assessed the individual and combined effects of different management factors, resulting in 14 treatments (**Table 2**). Six addition treatments (+P-S-Zn, +K-B, +P-S-Zn and K-B, +N, +Population, and +Protection) were established by individually substituting the enhanced level of each management factor while all the other management factors remained at the standard level. For example, the +Population treatment was created by substituting 109,000 plants ha^{-1} for 79,000 plants ha^{-1} while all the other management factors remained at the standard level. Similarly, six omission treatments (−P-S-Zn, −K-B, −P-S-Zn and K-B, −N, −Population, and −Protection) were individually substituted for the lower factor level while maintaining all the other factors at the enhanced level. Thus, the −Population treatment was created by substituting the lower plant population (79,000 plants ha^{-1}) for the higher plant population (109,000 plants ha^{-1}) while all the other management factors were maintained at the enhanced level. In this way, the value of each management factor was tested at the standard level of agronomic management and in an enhanced management system.

Statistical Analysis

Grain yield and yield components were analyzed with a linear mixed model using the MIXED procedure of SAS version 9.4 (SAS Institute, 2019). Environment ($n = 6$), row spacing ($n = 2$), agronomic management level ($n = 14$), and their interactions were considered to be fixed effects, while trial and replication nested within environment and trial were included in the model as random effects. The normality and homogeneity of the residuals was tested using the Shapiro–Wilks and Brown-Forsythe tests. T-tests were used to evaluate the significance of the differences of the least squared means estimates between specific treatments both within and across the row spacings at the 0.1 or 0.05 probability level. The comparisons were comprised of the difference between the enhanced and standard controls, between the six addition treatments (+P-S-Zn, +K-B, +P-S-Zn and K-B, +N, +Population, and +Protection) and the standard control, and between the six omission treatments (−P-S-Zn, −K-B, −P-S-Zn and K-B, −N, −Population, and −Protection) and the enhanced control. Lastly, 95% confidence intervals were estimated for the differences between the enhanced and standard controls across and within the row spacings.

RESULTS

Weather

The weather conditions at 14CU were characterized as below-average temperature and above-average precipitation throughout much of the growing season, including heavy rainfall through June and July (**Supplementary Table 2**). In 2015, Illinois experienced a warm April and May and cooler than average June, July, and August. The month of May had slightly above average rainfall recorded at both 15DK and 15CU. However, June brought extreme rainfalls, with 15DK and 15CU receiving 73 and 113 mm above normal, respectively. July and August were dry for 15DK and 15CU, with relatively favorable temperatures for pollination and grain-fill. The growing season at 16CU experienced near average temperatures and adequate rainfall throughout the growing season. Furthermore, 17CU and 18CU experienced weather that was conducive to high maize yields. The temperatures were near average except for above-average temperature in May at 18CU. Outside of a wet spring, the rain totals were below average for much of the growing season at 17CU. Minimal moisture stress occurred at 18CU, as precipitation did not drastically deviate from normal.

Row Spacing, Treatment, and Environment Effects on Grain Yield

Maize grain yield was affected by the environment, row spacing, agronomic treatment, and their interactions (**Table 3**). Across the six environments, narrowing row spacing from 0.76 to 0.51 m increased yield by 0.6 Mg ha^{-1} (4.5%) in the standard system and 1.2 Mg ha^{-1} (7.8%) in the enhanced system (**Table 4**), and grain yield was increased from narrowing row spacing at all the environments (**Figure 1A**). The enhanced management system resulted in a 2.1 and 2.7 Mg ha^{-1} (15.8 and 19.4%) yield increase over the standard control in the wide (0.76 m) and narrow (0.51 m) rows, respectively. Furthermore, the enhanced management system obtained the highest yield in all the environments (**Table 5**).

TABLE 3 | ANOVA for maize grain yield (Yield), kernel number (KN), and kernel weight (KW).

Source	Yield	KN	KW
		$P > F$	
Environment (E)	0.0008	0.0254	0.1513
Row Spacing (S)	< 0.0001	<0.0001	< 0.0001
E × S	0.0089	< 0.0001	0.0006
Treatment (T)	< 0.0001	< 0.0001	< 0.0001
E × T	< 0.0001	< 0.0001	< 0.0001
S × T	< 0.0001	< 0.0001	0.7271
E × S × T	0.7567	0.4185	0.4663

Fertility Effects on Grain Yield

Adding P, S, and Zn fertility to the standard control affected the yield at five of the six environments and, when averaged across all the environments, increased yield by 5% in both row arrangements (**Table 4**). Also, the omission of P-S-Zn fertility from the enhanced control reduced yield by 0.8 and 0.9 Mg ha^{-1} (5.2 and 5.4%) in wide and narrow rows, respectively. Notably, 15CU and 17CU, the environments with the lowest P soil test levels (**Table 1**), produced the highest yield responses to P-S-Zn fertility (**Table 5**). Nonetheless, positive yield responses to P-S-Zn fertilizer were observed in three environments (14CU, 16CU, and 18CU) where soil P levels would be considered adequate.

The potassium and boron fertilizer application did not affect the grain yield when added to the standard management system; however, omitting the K-B fertilizer from the enhanced system when in the wide rows resulted in a 0.5 Mg ha^{-1} (3.2%) yield loss (**Table 4**).

Removing the combined practices of banded P-S-Zn and broadcast K-B from the enhanced control reduced yield at all the environments (**Table 5**). Across environments, adding P-S-Zn and K-B fertility to the standard system increased yield by 0.8 Mg ha^{-1} (6.0%) in the wide rows and by 1.0 Mg ha^{-1} (7.2%) in the narrow rows, while their omission from the enhanced system decreased yield by 1.3 Mg ha^{-1} (8.4%) in the wide rows and by 1.2 Mg ha^{-1} (7.2%) in the narrow rows (**Table 4**). Notably, the yield increases from the individual P-S-Zn and K-B treatments were not additive to the yield response observed when the two treatments were added together, and the P-S-Zn treatment had the most significant contribution to yield response in each management system.

Sidedressing 90 kg N ha^{-1} in addition to the base rate of 180 kg N ha^{-1} in the standard control increased yield in four of the six environments and, on average, yielded an additional 0.7 Mg ha^{-1} (5.1%) over the standard control (**Table 5**). Additionally, the grain yield was reduced by 0.6 Mg ha^{-1} (3.8%) when the sidedress application was omitted from the enhanced management system.

Plant Population Effects on Grain Yield

Significant yield increases with the enhanced control over the standard control indicate that the environments tested in this study could support plant populations greater than 79,000 plants ha^{-1} (**Table 4**). However, increasing plant population from 79,000 to 109,000 plants ha^{-1} in the standard system only increased yield in two environments (14CU and 18CU) and led to yield decreases in two other environments (15CU

TABLE 4 | Maize grain yield (expressed at 15.5% moisture content) response to 14 management systems and the absolute and percentage-wise (in parentheses) difference in yield for the addition or omission treatments relative to the standard or enhanced system controls for two row spacings (0.51 and 0.76 m).

Treatment		0.51 m rows		0.76 m rows	
System	**Exception**	**Yield**	**Δ**	**Yield**	**Δ**
		Mg ha^{-1}			
Standard	None†	13.9		13.3	
Standard	+P-S-Zn	14.6	0.7 (5.3%)*	13.9	0.6 (4.5%)*
Standard	+K-B	14.1	0.2 (1.4%)	13.2	−0.1 (−0.8%)
Standard	+P-S-Zn-K-B	14.9	1.0 (7.2%)*	14.1	0.8 (6.0%)*
Standard	+N	14.6	0.7 (5.3%)*	13.9	0.6 (4.5%)*
Standard	+Population	13.8	−0.1 (−0.7%)	12.9	−0.4 (−3.0%)*
Standard	+Protection	14.0	0.1 (0.7%)	13.6	0.3 (2.3%)‡
Enhanced	None	16.6		15.4	
Enhanced	−P-S-Zn	15.7	−0.9 (−5.4%)*	14.6	−0.8 (−5.2%)*
Enhanced	−K-B	16.5	−0.1 (−0.6%)	14.9	−0.5 (−3.2%)*
Enhanced	−P-S-Zn-K-B	15.4	−1.2 (−7.2%)*	14.1	−1.3 (−8.4%)*
Enhanced	−N	16.0	−0.6 (−3.6%)*	14.7	−0.7 (−4.5%)*
Enhanced	−Population	15.9	−0.7 (−4.2%)*	15.2	−0.2 (−1.3%)
Enhanced	−Protection	16.3	−0.3 (−1.8%)‡	15.1	−0.3 (−1.9%)‡
Enhanced vs. Standard§			2.7 (19.4%)*		2.1 (15.8%)*

The values are the average of 13 trials from six environments in Illinois from 2014 to 2018.

† "None" in the exception column indicates the control.

‡ Significant at the 0.10 probability level compared to the respective control treatment.

**Significant at the 0.05 probability level compared to the respective control treatment.*

§ The percentage difference between the standard and enhanced system controls is expressed relative to the standard system control.

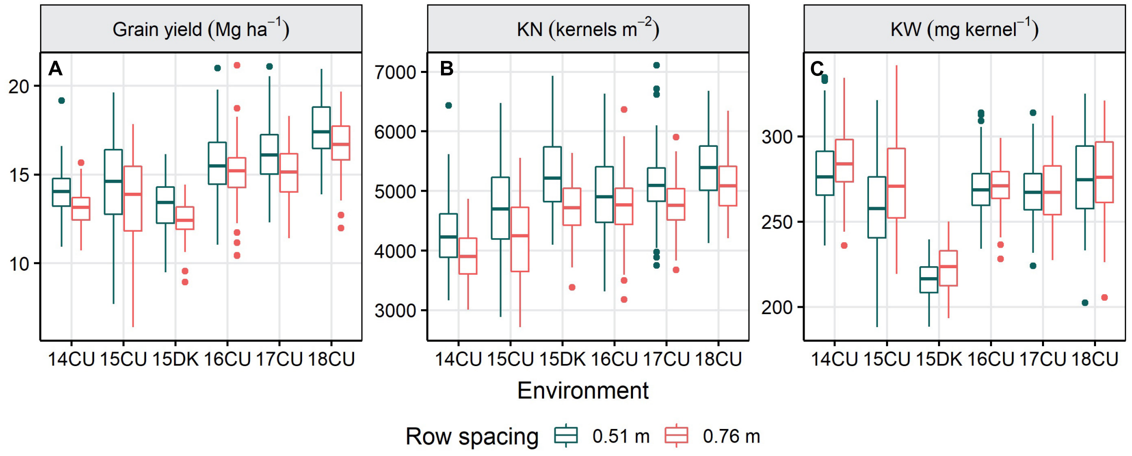

FIGURE 1 | Influence of environment on the grain yield **(A)**, kernel number [KN; **(B)**], and kernel weight [KW; **(C)**] of the 0.51 and 0.76 m row width plots for 14 agronomic treatments, six replications, and an average of two trials in each environment located at Champaign-Urbana (CU) and DeKalb (DK), Illinois from 2014 to 2018. The horizontal lines in the box plot indicate the median. Top and bottom edges of the box refer to the 75th and 25th percentiles, respectively, and whiskers extend to the 10th and 90th percentiles.

and 15DK), resulting in a slight average yield decrease (2.2%) (**Table 5**). The enhanced management system was better able to support the higher density as omitting the high plant population from the enhanced control reduced yield in four of the six environments and, on average, reduced grain yield by 0.4 Mg ha^{-1} (2.5%). The narrower rows were a better arrangement of the high plant population as reducing plant population from 109,000 to 79,000 plants ha^{-1} in the enhanced management system reduced yield by 0.7 Mg ha^{-1} (4.2%) in the 0.51 m spacing and did not affect the yield in the 0.76 m spacing (**Table 4**). Likewise, increasing the plant population from 79,000 to 109,000 plants ha^{-1} in the standard system only decreased yield in the 0.76 m row spacing while yield was unchanged in the 0.51 m row spacing.

TABLE 5 | Maize grain yield (expressed at 15.5% moisture content) response to 14 management systems for six environments in Illinois from 2014 to 2018 and the average of environments.

Treatment		Environment						Mean
System	**Exception**	**14CU**	**15CU**	**15DK**	**16CU**	**17CU**	**18CU**	
					Mg ha^{-1}			
Standard	None[†]	12.5	11.9	12.5	14.3	14.6	15.7	13.6
Standard	+P-S-Zn	13.1*	13.4*	12.7	14.8*	15.7*	16.1[‡]	14.3*
Standard	+K-B	12.9[‡]	11.5	12.6	14.1	14.6	16.0	13.6
Standard	+P-S-Zn-K-B	13.2*	14.0*	13.0	14.7[‡]	15.6*	16.5*	14.5*
Standard	+N	13.1*	13.3*	12.9	14.9*	14.9	16.5*	14.3*
Standard	+Population	12.9[‡]	10.9*	11.6*	14.2	14.4	16.1[‡]	13.3*
Standard	+Protection	13.3*	11.6	12.4	14.8*	14.4	16.0	13.8[‡]
Enhanced	None	14.6	15.5	13.5	16.7	17.0	18.6	16.0
Enhanced	−P-S-Zn	14.1*	13.7*	12.9	16.2*	15.6*	18.4	15.2*
Enhanced	−K-B	14.2[‡]	15.8	14.0	16.1*	16.0*	18.0*	15.7*
Enhanced	−P-S-Zn-K-B	14.0*	13.5*	12.6*	16.0*	14.8*	17.6*	14.7*
Enhanced	−N	14.3	14.4*	12.6*	16.2[‡]	16.4*	18.4	15.4*
Enhanced	−Population	13.8*	16.5*	13.9	15.3*	16.4*	17.4*	15.6*
Enhanced	−Protection	13.8*	16.1[‡]	13.4	16.1*	16.5[‡]	18.3	15.7*

The values are the average of two row spacings (0.76 and 0.51 m) and, on average, two trials within each environment.

[†] "None" in the exception column indicates the control.

[‡] Significant at the 0.10 probability level compared to the respective control treatment.

**Significant at the 0.05 probability level compared to the respective control treatment.*

Foliar Protection Effects on Grain Yield

Measurable fungal leaf infection was not observed in any of the six environments. However, the addition of foliar protection to the standard management control increased yield in two environments (**Table 5**). In comparison, the omission of foliar protection from the enhanced control affected yield at four environments and, on average, reduced the yield by 0.3 Mg ha^{-1} (1.9%).

Effects on Yield Components

Environment, row spacing, agronomic treatment, and their interactions strongly affected KN, while KW was affected by row spacing, agronomic treatment, and their interactions with environment (**Table 3**). Across the treatment levels, switching from 0.76 to 0.51 m row spacing increased KN in all the environments except 16CU and marginally decreased KW in three environments (14CU, 15CU, and 15DK; **Figures 1B,C**). The difference in KN between the enhanced and standard control treatments (19.3%), when averaged across environment and row spacing, was more significant ($P < 0.0001$) than the observed difference in KW (1.1%; $P = 0.0098$) (**Table 6**). Additionally, the grain yield was highly correlated with KN ($r = 0.81$, $P < 0.0001$) and less correlated with KW ($r = 0.22$, $P < 0.0001$), suggesting improving KN was more critical than KW for increasing grain yield.

Averaged across the environments and row spacings, P-S-Zn fertility, sidedress N, and plant population had the most prominent effects on KN with significant decreases when omitted from the enhanced control and increases when added to the standard control (**Table 6**). Plant population had the most significant impact on KW, which responded negatively to increased population and positively to decreased population. Additionally, KW decreased when K-B fertility, sidedress N, or foliar protection were removed from the enhanced control.

Indicated by a higher KN (6.3%), the preplant banded P-S-Zn application increased yield potential compared with the standard control (**Table 6**). Conversely, the yield responses to K-B fertilizer were generally associated with changes in KW. Positive yield responses to sidedressing N were associated with KN and KW, as both were increased when sidedress N was included in either the standard or enhanced system. The marginal plant population effect on grain yield resulted from contrasting changes in the yield components. Increasing plant population without increasing other crop inputs (i.e., standard system) resulted in a 5.8% increase in KN and a 6.8% reduction in KW. Decreasing plant population in the enhanced system resulted in an 8.5% decrease in KN and a 6.9% increase in KW. Kernel number response to increasing plant population was more significant in narrow rows than in wide rows for both management systems, suggesting that the plants had a heightened ability to maintain kernels per ear at the high plant population when in the narrow rows. Removing foliar protection from the enhanced system reduced KW by 1.5%.

System Effects

The maize yield gap was estimated as the difference between the standard management control with 0.76 m row spacing, representing typical farming practice, and the enhanced

TABLE 6 | Influence of 14 agronomic management treatments on yield components (kernel number and weight) for two row spacings (0.76 and 0.51 m).

Treatment		Kernel number			Kernel weight		
System	**Exception**	**0.51 m**	**0.76 m**	**Mean**	**0.51 m**	**0.76 m**	**Mean**
			kernels m^{-2}			mg $kernel^{-1}$	
Standard	None[†]	4,456	4,227	4,342	264	267	265
Standard	+P-S-Zn	4,728*	4,463*	4,595*	263	265	264
Standard	+K-B	4,540	4,146	4,343	263	270[‡]	267
Standard	+P-S-Zn-K-B	4,696*	4,389*	4,542*	269*	273*	271*
Standard	+N	4,641*	4,324[‡]	4,483*	268*	272*	270*
Standard	+Population	4,793*	4,385*	4,589*	245*	249*	247*
Standard	+Protection	4,509	4,267	4,388	263	269	266
Enhanced	None	5,398	4,961	5,180	261	263	262
Enhanced	−P-S-Zn	5,173*	4,693*	4,933*	259	265	262
Enhanced	−K-B	5,559*	4,896	5,228	253*	259*	256*
Enhanced	−P-S-Zn-K-B	5,170*	4,675*	4,923*	253*	257*	255*
Enhanced	−N	5,321	4,759*	5,040*	256*	259[‡]	258*
Enhanced	−Population	4,871*	4,606*	4,738*	277*	282*	280*
Enhanced	−Protection	5,388	4,922	5,155	257*	259*	258*

Values are the average of 13 trials from six environments in Illinois from 2014–2018.

[†] *"None" in the exception column indicates the control.*

[‡] *Significant at the 0.10 probability level compared to the respective control treatment.*

**Significant at the 0.05 probability level compared to the respective control treatment.*

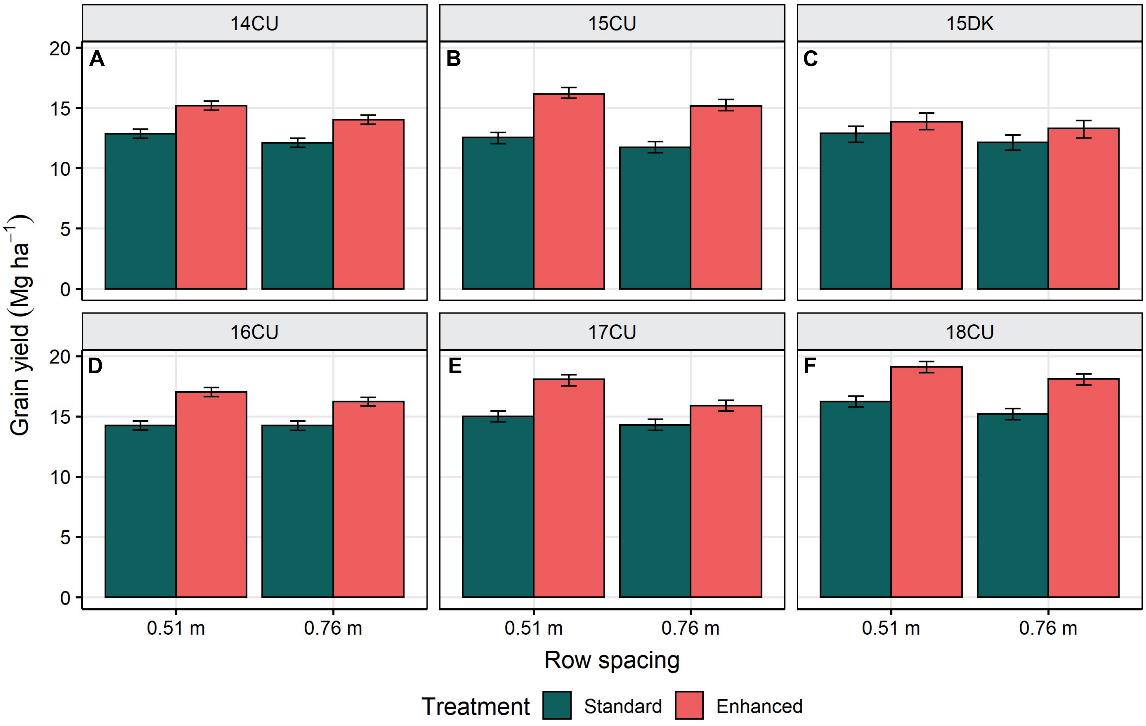

FIGURE 2 | Row spacing influence on grain yield for the standard and enhanced management control treatments at the environments 14CU **(A)**, 15CU **(B)**, 15DK **(C)**, 16CU **(D)**, 17CU **(E)**, and 18CU **(F)**. The bars represent ± 1 SE from the mean. All means are presented as the average of two trials and six replications.

management control with 0.51 m row spacing, representing attainable yield through the implementation of enhanced agronomic management technologies. The average yield gap across the six environments was 3.3 Mg ha^{-1} (25%) and ranged from 1.8 to 4.6 Mg ha^{-1} (15–40%) ($P < 0.0001$) (**Table 4** and **Figure 2**).

TABLE 7 | Comparisons between the overall yield difference between the enhanced (Enh) and standard (Std) control treatments (shown as the mean and 95% *CI*; $\mu_{Enh} - \mu_{Std}$) and the summation of the additional yield values provided by each added treatment to the Std control (i.e., Std + P-S-Zn, Std + P-K, Std + N, Std + Population, Std + Foliar protection).

	Row Spacing			
Treatment	**0.51 m**	**0.76 m**	**Average**	**0.51 Enh vs. 0.76 Std**
	Mg ha^{-1}			
$\mu_{Enh} - \mu_{Std}$	2.7 (2.3–3.0)	2.1 (1.8–3.0)	2.4 (2.2–2.6)	3.3 (3.0–3.7)
$\sum(Y_{+FACTOR} - Y_{Std})$‡	1.4	1.1	1.3	1.7

The additional yield value provided by each treatment was calculated as the difference between the + factor and the Std control yield when significant.

$^{‡}\sum\left[(Y_{+P-S-Zn} - Y_{Std}) + (Y_{+K-B} - Y_{Std}) + (Y_{+N} - Y_{Std}) + (Y_{+Pop} - Y_{Std}) + (Y_{+Foliar} - Y_{Std})\right]$,

$\sum\left[(Y_{+P-S-Zn} - Y_{Std}) + (Y_{+K-B} - Y_{Std}) + (Y_{+N} - Y_{Std}) + (Y_{+Pop} - Y_{Std}) + (Y_{+Foliar} - Y_{Std}) + (Y_{.51\ Std} - Y_{.76\ Std})\right]$.

The experimental design allows for assessing the additive and synergistic effects from combining the management factors, as portrayed by Ruffo et al. (2015). Estimating the individual yield value of any single management factor can be done with the difference between the standard addition and standard control treatments. Averaged across environments, individual factors that significantly changed yield when added to the standard control in 0.76 m row spacing were P-S-Zn fertility, sidedress N, plant population, and foliar protection, as well as narrowing row spacing to 0.51 m (**Table 4**). If combinations of factors acted additively in changing yield, summing the individual values for these significant factors gives an additive yield value of 1.7 Mg ha^{-1} (**Table 7**). However, the actual yield response from combining all the factors was 3.3 Mg ha^{-1} with a 95% *CI* of 3.0–3.7 Mg ha^{-1}, which was obtained by calculating the difference between the enhanced control in 0.51 m row spacing and the standard control in 0.76 m row spacing (i.e., the yield gap). Because the lower limit of 3.0 Mg ha^{-1} is markedly higher than the summation of all the individual factor contributions, 1.7 Mg ha^{-1}, these management factors are acting synergistically in their effects on grain yield when combined. A significant synergistic effect was also observed within either row spacing and when averaged across the row spacings.

DISCUSSION

This research estimates the yield gap present in the non-irrigated conditions of Illinois, United States, with contemporary maize hybrids. Across six environments, the combined factors of narrower row spacing, increased plant population, season-long crop nutrition, and foliar protection increased average yield by 25% (3.3 Mg ha^{-1}) compared with the standard management practices (**Table 4**). This data suggests that the maize yield gap can be significantly lessened with narrower row spacing and other enhanced agronomic management technologies. Because consistent yield responses to combining management factors were observed in all the environments of this study (**Figure 2**), it is expected that the apparent yield gap and management effects on yield would be similar in other highly-productive regions of the United States Corn Belt. However, the current maize yield may be relatively close to the potential yield in the water-limited regions of the Western United States Corn Belt with a higher dependency on irrigation than other management factors for achieving greater yields (Grassini et al., 2011; Balboa et al., 2019).

Notably, all the management factors were necessary for the higher maize yield achieved in the enhanced system, as demonstrated by the yield reductions when any one factor was removed from the system, and no single factor could account for the entirety of the observed yield gap (**Tables 4**, **5**). Narrow row spacing and the combination of P-S-Zn and K-B fertilizer applications resulted in the most significant yield increases of 7.8% and up to 8.4%, respectively, when combined with all other enhanced factors. The magnitude of yield response to the applied fertilizer was not necessarily indicative of the existing soil fertility levels. Banding P-S-Zn containing fertilizer was essential in determining yield potential through impacts on KN (**Table 6**). The nutrients P, S, and Zn are crucial to kernel development, considering their high harvest indices and remobilization to the grain after flowering (Bender et al., 2013). K-B fertilizer helped maintain KW, especially in high plant population and in wide rows when crowding was presumably higher. As the plant population increases, there is greater competition for nutrients, and K plays a vital role in stalk strength and harvestability (Bohling, 1975; Maria and Farina, 1984). The B supplied with K fertilizer may have aided increases in KW because of its significant translocations during pollination, especially in the presence of potassium fertilizer (Woodruf et al., 1987; Bender et al., 2013).

Sidedress N applications can increase N availability to the crop during pollination and grain-fill but do not always result in greater yield, especially when the initial N levels are adequate and N deficiency is not present before sidedress (Binder et al., 2000). Supplemental sidedress N could have been less impactful in the enhanced management system because of the additional 34 kg N ha^{-1} supplied with the banded P-S-Zn fertilizer. However, with a higher plant density, such as in the enhanced management system, a lower tolerance to low-N conditions and a higher response to sidedressed N applications is expected (Boomsma et al., 2009).

An inverse relationship was observed between KN and KW in response to increasing the plant population, resulting in marginal changes in the grain yield (**Tables 4**, **6**). This inverse relationship between yield components is called "yield component compensation" and is a vital developmental process of plants for maintaining yield when faced with stresses, such as intraspecific competition (Adams, 1967). When reducing plant population in the enhanced system, the reduction in KN per area was proportionally less than the reduction in plant population from 109,000 to 79,000 plants ha^{-1}, indicating more kernels developed per plant when at the lower population and all other enhanced factors remained in the system. Increasing plant population heightens intraspecific competition for limiting resources (Boomsma et al., 2009) and limits the ability of plants to obtain limiting resources due to reduced root biomass (Jiang et al., 2013; Bernhard and Below, 2020). Density-induced stress was likely partially alleviated with the applied fertilizer in the enhanced management system leading to a greater tolerance of the high plant population.

The narrow row spacing increased yield primarily through higher KN and was especially important at maintaining kernel set at the higher plant population (**Table 6**). Narrow rows are more commonly conducive to higher maize yields north of latitude 43°N, mainly because increased light interception from narrowing row spacing becomes more critical in shorter growing seasons (Lee, 2006). However, consistent yield increases from narrowing row spacing were observed across the environments of this study (**Figure 1**), all of which are south of latitude 43°N. Notably, the response to the narrower row spacing was most significant in the enhanced management system and lessened when reducing the plant population to 79,000 plant ha^{-1} (**Table 4**). While more favorable responses to narrowing row spacing would be expected in northern latitudes (Lee, 2006), reduced row spacing may be optimal in the central United States when yield potential or plant densities are higher. Reducing row spacing ($<$0.76 m) increases root biomass and the ability of plants to obtain limiting resources, allowing for greater optimal plant densities (Bernhard and Below, 2020). Increasing plant population beyond the United States average can increase grain yield with modern maize hybrids when other management factors are optimized to mitigate stresses (**Table 4**). However, other yield components, such as kernels per ear and kernel weight, cannot be maintained at higher densities when resources are limited. Greater planting densities necessitate enhanced management of other, potentially limiting, resources and are better suited for narrower row arrangements.

Foliar fungicides can be effective at increasing maize yield (Ruffo et al., 2015; Vitantonio-Mazzini et al., 2020), and growers more commonly utilize fungicides when the planting density and nutrient availability are higher (Vitantonio-Mazzini et al., 2020). In the environments where significant fungal leaf disease was absent, any observed yield response to strobilurin fungicide (**Table 5**) was likely due to their "greening effect," which can maximize grain-filling duration by extending photosynthetic capacity later in the season (Bartlett et al., 2002). Strobilurin fungicide was especially effective in the enhanced system, as the grain-filling rate and final kernel weight are typically depressed under high plant densities (Wei et al., 2019). Greater impacts of fungicide applications may have been observed if consistently more disease pressure was present across the trials, as foliar fungal diseases reduce the photosynthetic area and stalk strength of plants (Dodd, 1977; Wise and Mueller, 2011), resulting in reduced yields.

This work demonstrates that the yield reduction resulting from omitting an agronomic factor from the enhanced system was generally more significant than the yield increase from adding that factor to the standard control (**Table 4**). Additionally, the combination of enhanced management factors had a synergistic effect on the grain yield in this study. The yield increase from combining all factors in the enhanced system was more significant than the additive response from each management factor applied individually (**Table 7**). Therefore, when managing maize for greater yields, a comprehensive systems approach will often increase yield more than enhancing any one management factor alone. This research confirms that KN is the yield component most associated with changes in grain yield and is highly impacted by planting population and the availability of nutrients. Thus, closing the maize yield gap will require a systems approach to agronomic management, including better crop nutrition and optimization of spatial plant density.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

FB guided all facets of this research. TB and EW organized the design and implementation of experiments. EW analyzed the data and wrote the results. EW and FB prepared the manuscript for publication. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Yield and Quality in Main and Ratoon Crops of Grain Sorghum Under Different Nitrogen Rates and Planting Densities

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Ratooning is the cultivation practice of two harvests in one cropping season by producing a second crop from the original stubble, which could provide higher resource use efficiency and economic benefit compared with direct sown crops. Nitrogen (N) fertilizer and planting density (D) play a vital role in sorghum (*Sorghum bicolor* L.) production; however, limited information is available on the effects on yield and quality of the sorghum-ratoon system. To address this question, field experiments were conducted with three N treatments (120 kg N ha^{-1}, N1; 180 kg N ha^{-1}, N2; and 255 kg N ha^{-1}, N3) and three D treatments (82,500 plant ha^{-1}, D1; 105,000 plant ha^{-1}, D2; and 127,500 plant ha^{-1}, D3). The yield of the main crop was significantly higher than that of the ratoon crop. Increasing N could increase the yield and yield attributes of both main and ratoon crops, and the effect on the ratoon crop was greater than the main crop. With increasing D, the grain yield of both main and ratoon crops increased, though 1,000-grain weight and grain weight per ear decreased. The sorghum grain of the ratoon crop contained higher starch, protein, and tannin contents but lower fat content, indicating a better quality for liquor production. The quality traits were significantly affected by N and D, but the differences between treatments were smaller than that between the main and ratoon crop. Our results indicated that increasing the yield of ratoon crops could obtain a high yield and quality of the sorghum-ratoon system. It was recommended that 120 kg N ha^{-1} with 127,500 plant ha^{-1} for the main crop and a small amount of N be top-dressed in three new buds left on stubble in each hill for the ratoon crop.

Keywords: grain sorghum, ratooning, yield, quality, nitrogen fertilizer, planting density

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INTRODUCTION

Sorghum, the fifth most important cereal crop in the world with high-stress tolerance and wide adaptability, is grown for both human food and animal feed. In China, sorghum is planted in an area of about 751,793 hectares, produces about 3.60 million tons of grains[1]; this places China at 12th and 6th position in the world, respectively. Waxy sorghum is used as the main raw material for the production of liquor (Ni et al., 2015); about 80% of the domestic production in China is used for this purpose (Wang C. et al., 2016). In recent years, the demand for sorghum has increased substantially

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[1] http://www.fao.faostat/en/#data/QC

due to the rapid increase in the liquor industry in China. Due to the shortfall of domestic supply, a lot of the Chinese industries are producing liquor by using imported sorghum. Most of the imported sorghum is of forage quality; the use of this type reduces the quality of the liquor (Zou et al., 2020). The climate in southwest China is suitable for the production of waxy sorghum (Lu et al., 2009), and this can provide raw material to the liquor industries in China (Wang C. et al., 2017).

Ratooning of sorghum is often practiced in different parts of the world where a second crop is harvested in the same cropping season. In a ratoon crop, the basal buds of the stem grow shortly after cutting the main crop (Wilson, 2011). Normally, the duration of a ratoon crop is much shorter than the direct seeded crop (Al-Taweel et al., 2020), providing higher resource use efficiency per unit time and per unit land area (Santos et al., 2003). Therefore, ratooning of the sorghum crop is widely practiced in this region where photothermal resources exceed the demand of single-season sorghum but are insufficient for double-season sorghum (Yin et al., 2015). Ratooning has several advantages, such as there is no need for land preparation and new seed, and it also eliminates the risk of any seeding delay and other risks associated with crop establishment (Escalada and Plucknett, 1975; Gerik et al., 1988).

The yield of a ratoon crop can be increased through the identification and the use of appropriate management practices (Santos et al., 2003; Petroudi et al., 2011; Rogé et al., 2016). Of the different management practices, the application of nitrogen (N) fertilizer and planting density (D) play the most important roles for getting higher grain yield and better quality in the ratoon crops. N fertilizer enhances the formation of tillers and thus gives greater yield (Escalada and Plucknett, 1977). Often, a higher rate of N fertilization gives greater forage, dry matter, and grain yield (Mahfouz et al., 2015); however, grain yield does not increase linearly after a certain rate (Touchton and Martin, 1981). Some studies on N management have been carried out on both main and ratoon sorghum (Rao et al., 2011). In most cases, surplus N was found in soil after meeting the demand of the main crop (He et al., 2016). According to the study by Ceotto et al. (2014), it is not worthwhile to fertilize sorghum under good soil fertility conditions. It was widely confirmed that N fertilization affected the seed quality traits of both grain and sweet sorghum (Rashid et al., 2008; Tang et al., 2018). However, less information is available about the application of N fertilizers on the quality of the waxy sorghum, much less on the main and ratoon sorghum.

Plant density can affect the plant and canopy architecture of the crop and thus can influence the number of effective ear per unit area, leaf area index (LAI), and radiation interception (Westgate et al., 1997; Tabo et al., 2002). Plant density can also affect the growth of the crop, grain yield and its quality (Defoor et al., 2001; Carmi et al., 2006). Compared with the main crop, ratoon sorghum becomes taller but produces lower biomass yield (Vinutha et al., 2017); therefore, under the same D, ratoon crop may build a different population structure. To our knowledge, no literature is available on the effect of D on the main and ratoon crop of waxy sorghum. The objective of this study was to understand the effects of N fertilizer and D on the main and ratoon crop for yield and quality traits of waxy sorghum.

MATERIALS AND METHODS

Site Description

A 2-year field experiment was conducted in 2019 and 2020 at the Yuxi Crop Experimental Station of the Chongqing Academy of Agricultural Sciences, Yongchuan District (105.71°E longitude, 29.75°N latitude, 298 m altitude a.s.l.), Chongqing, China. Soil samples from the upper 20 cm were collected for soil analysis before seeding in 2019. The soil was in a clay loam texture with a pH of 4.5, organic matter content of 23.9 g kg^{-1}, total N of 0.76 g kg^{-1}, available N of 98.3 mg kg^{-1}, available P of 13.4 mg kg^{-1}, and available K of 102.7 mg kg^{-1}. The climate of this county is subtropical humid monsoon with an annual average temperature of 17.7°C, a maximum temperature of 42.1°C, and a minimum temperature of –2.9°C. The frost-free period is about 317 days, and the annual mean precipitation is about 1015.0 mm.

Experimental Design and Treatments

A waxy semidwarf sorghum hybrid cultivar "Jinyunuo 3" was used in this study. This cultivar is well adapted in southwest China, possessing high yield potential, good seed quality properties, and strong ratooning ability. The experiments were laid out in a split-plot design with N rates as the main plot and D as a subplot, and the number of replications was three.

The fertilizer urea containing 46.4% N was used as the source of N. The 3 N doses were as follows: 120 (N1), 180 (N2), and 255 (N3) kg ha^{-1} and were applied at two stages in the main crop at complete field emergence and at the jointing stage, in a ratio of 3:7; no N fertilizer was applied to the ratoon crop. The D treatments were 82,500 (D1), 105,000 (D2), and 127,500 (D3) plant ha^{-1}. The plot size was 6-m long × 5-m wide with 12 rows; row spacing was 50 cm. The hill seeding was performed, where the distance between the hills was 48.5 cm for D1, 38.1 cm for D2, and 31.4 cm for D3. The crop was seeded on March 21, 2019, and on April 4, 2020; seedlings were thinned at the five-leaf stage leaving two plants at each hill. The main crop was harvested by reaping ears manually on July 26, 2019, and August 6, 2020, followed by cutting the stem at 5–10 cm above the ground. After new tillers on stubble regenerated, one of them was left in each plant and allowed to grow to maturity. The ratoon sorghum was harvested on November 18, 2019, and November 19, 2020.

Growth Parameters and Dry Weight

Three plants from each plot were selected randomly and were used to measure LAI and chlorophyll content at flowering and mature stages in both seasons. The LAI was calculated as the total green leaf area divided by the harvest area of the plot. The green leaf area (cm^2) of the individual leaves was calculated using the formula as follows: Leaf length (cm) × maximum width of a leaf (cm) × 0.75. The chlorophyll content (soil-plant analysis and development, SPAD) was measured on the second leaf from the top of the plant using the SPAD-502 Plus device (Konica Minolta, Japan).

In both seasons, three representative plants were sampled from each plot at the mature stage to collect the data of both plant height (cm, PH) and stem diameter (cm, SD). PH was measured from the soil surface to the top of the panicle by a telemeter rod, and SD was measured at the second internode from the stem base by a vernier caliper. The plants were cut at the ground level, chopped, and dried in an oven at 80°C for at least 4 days to obtain aboveground total dry weight (TDW).

Yield and Yield Components

A random sample of 10 plants from each plot was harvested at maturity and was threshed manually to measure grain weight per ear (g, GWPE) and 1,000-grain weight (g, TGW); the remaining plants were harvested to estimate plot yield and data converted to kg per ha. Grain moisture content was determined immediately after threshing using a Riceter Grain Moisture Meter (Kett Electric Laboratory, Tokyo, Japan). The TGW, GWPE, and yield were reported at a moisture content of 130 g H_2O kg^{-1} fresh weight.

Grain Quality

Starch, protein, fat, and tannin contents of the seed samples were determined by using a DA 7250 near-IR grain quality analysis meter (Perten, Sweden). For this, three subsamples (small sample plate 44 cm^2) from each plot were used (Qu et al., 2019), and the mean values were used for statistical analysis.

Calculations and Data Analysis

The ANOVA was performed for each year on all traits using Statistical Product and Service Solutions (SPSS, version 19.0; IBM SPSS Inc. Chicago, IL, United States). The effects of the growing season (S; main and ratoon seasons), N, D, and their interactions on the traits were analyzed following the generalized linear model procedure. Tests for significant differences between the treatments were carried out using Duncan's multiple range tests at a 5% level of significance.

RESULTS

Weather Conditions

The precipitation and temperature at the experimental site during the crop growing periods were collected from a nearby meteorological station and are shown in **Figure 1**. Total precipitation during the growing season was 1,430.6 mm in 2019 and 1,097.6 mm in 2020. The accumulated precipitations during the main and ratoon seasons were higher in 2019 than in 2020, which was due to two heavy rainfalls in mid-April and late June in 2019.

The mean temperature was 21.4°C in 2019 and 20.0°C in 2020. The temperature increased across the main season and decreased over the ratoon season. The average temperatures during the main and ratoon seasons were both higher in 2019 than those in 2020.

Growth Parameters and Dry Weight

The SPAD was significantly affected by S, N, and D but not by their interactions except the S × N interaction (**Table 1**). The LAI was significantly affected by S, N, D, and their interactions in 2019, but in 2020, only LAI in the mature stage was affected by S × N, S × D, and N × D interactions (**Table 1**).

The SPAD and LAI of the main crop were significantly higher than those of the ratoon crop. Both SPAD and LAI decreased at the mature stage as compared to the flowering stage in both main and ratoon crops, and this decrease was more pronounced in the ratoon crop (**Table 1**). On average, the SPAD decreased by 5.21% at the mature stage compared with that at the flowering stage for the main crop but 11.81% for the ratoon crop. LAI at the mature stage was lower than that at the flowering stage by 9.87% for the main crop but 60.56% for the ratoon crop.

For both main and ratoon crops, the SPAD and LAI at flowering and mature stages increased with increasing N rate. With the increment of D, at flowering and mature stages, SPAD declined, while LAI increased, in both growing seasons.

The ANOVA results (**Table 2**) showed that the PH was significantly affected by S and N in both years and the interactions of S × N and S × D in 2020. The SD was significantly affected by S, N, and D but not by their interactions. The TDW was significantly affected by S, N, and D in both years and the interactions of S × D and N × D in 2019. The PH, SD, and TDW of the main crop were 17.18, 12.47, and 46.64% higher than the ratoon crop over 2 years. With the increment of N rates, the PH, SD, and TDW of the main and ratoon crops increased in 2019 and 2020. In case of the treatment D, no difference for PH was found in both main and ratoon crops in 2019; however, in 2020, the PH increased in the main crop but decreased in the ratoon crop with increasing D treatment. In both crops, the SD and TDW decreased with increasing D in 2019 and 2020.

Yield and Yield Components

The ANOVA results (**Table 3**) showed that S, N, and D had highly significant effects on the TGW, GWPE, and yield, and the interaction of S × N had a significant effect on yield in both years and the GWPE in 2020, and the interaction of S × D had a significant effect on yield in both years. The TGW, GWPE, and yield of the main crop were significantly higher than those of the ratoon crop by 26.39, 50.62, and 41.86% in 2019 and 34.51, 56.27, and 50.38% in 2020. It indicated that the yield of the main crop was significantly higher than that of the ratoon crop mainly due to more GWPE. The yield increased with increasing N, and the increment of the ratoon crop was greater than that of the main crop (**Table 3**). The yield of the main crop under N3 was higher than those under N1 and N2 by 7.92 and 3.60% in 2019 and by 8.83 and 2.41% in 2020. However, the yield of the ratoon crop under N3 was higher than those under N1 and N2 by 35.16 and 20.91% in 2019 and by 45.68 and 20.88% in 2020. The greatest total yield of main and ratoon sorghum was obtained under N3, which was 17.27 and 9.82% higher than those under N1 and N2 in 2019 and 19.73 and 6.75% in 2020. The TGW and GWPE of the main and ratoon crops increased with increasing N, and the increments of the ratoon crop were greater than those of the main crop. Averaged across years, the TGW under N3 was 5.63 and 2.15% higher than those under N1 and N2 in the main season, while those were 7.12 and 3.98% in the ratoon season. On average, the N3 treatment produced 14.02 and 7.47% higher GWPE than N1 and N2 in the main crop and 37.35 and 23.24% in the ratoon crop. It could be inferred that higher yield under a high N rate was mainly composed of increasing GWPE. Comparing the positive

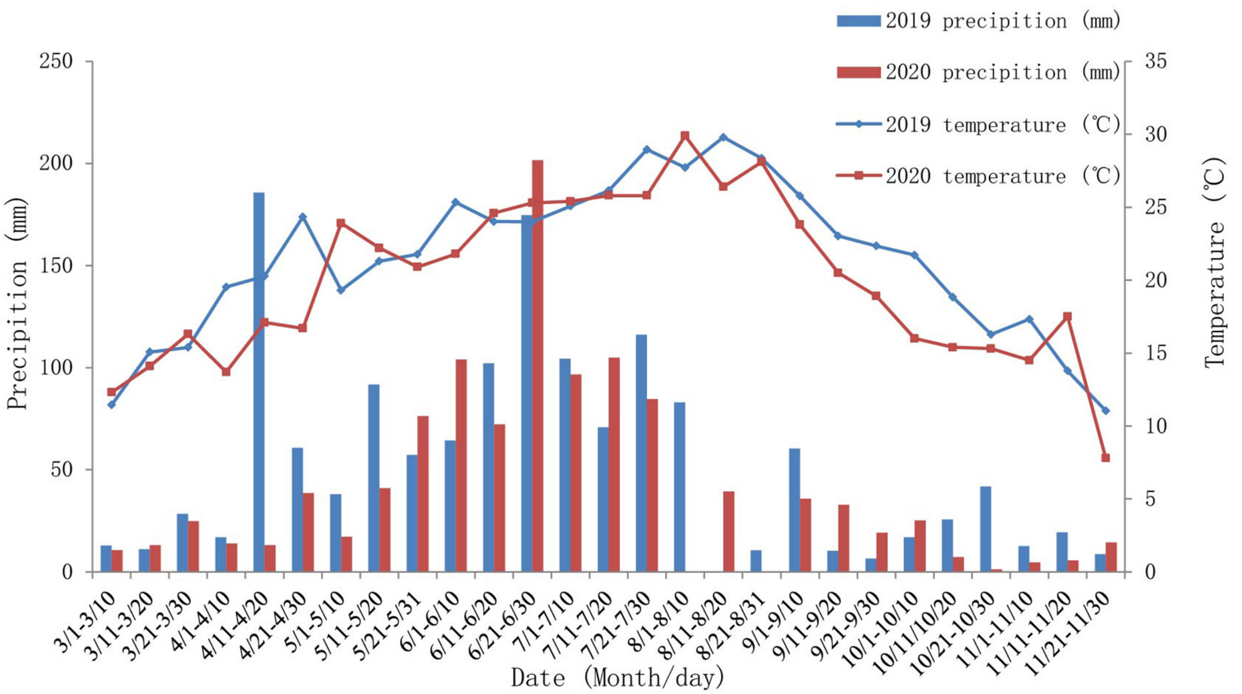

FIGURE 1 | Precipitation and mean temperature during the growth period (2019–2020) of the sorghum-ratoon system at the experimental site, Yongchuan County, Chongqing City, southwest China.

effects of N on productivity parameters of main and ratoon crops, N had a larger effect on the grain yield of ratoon crops.

With increasing D, the grain yield of both main and ratoon crops increased, while TGW and GWPE decreased (**Table 3**). D3 treatment produced more grain yield of the main crop than D1 and D2 by 25.51 and 11.62% in 2019 and 16.79 and 6.59% in 2020. However, the ratoon sorghum under D3 had 25.01 and 4.93% more yield than those under D1 and D2 in 2019 and 25.98 and 7.06% in 2020. D3 treatment produced more total yield than D1 and D2 by 25.01 and 4.93% in 2019 and 19.73 and 6.75% in 2020. Averaged across years, 5.39 and 8.75% more TGW were caused by D1 than those by D2 and D3 in the main crop, while 9.92 and 14.18% were in the ratoon crop. On average, the D1 treatment induced 7.78 and 15.13% higher GWPE than D2 and D3 in the main crop and 10.83 and 29.49% in the ratoon crop. The reductions of TGW and GWPE from D1 to D3 in the ratoon crop were greater than those in the main crop. Although the TGW and GWPE both decreased with increasing D, the yield still continued to increase, inferring that the main reason for higher yield under high density was the increase of effective panicle number per unit area.

Comparing the effects of N and D on the sorghum yield, it could be concluded that N was more efficient than D on the productivity parameters of the ratoon crop, while D was more efficient in the main season.

Grain Quality

The ANOVA results (**Table 4**) showed that the starch content was significantly affected by S in 2019 and S, N, and D in 2020 and by the interactions of S × N and S × D in both years. The protein content was significantly affected by S, N, D, and S × D interaction in both years. The fat content was significantly affected by S and N in both years and by S × D interaction in 2019 and D in 2020. The tannin content was significantly affected by S, N, D, and S × D interaction in both years and by the interactions of S × N and N × D in 2020. The grain starch, protein, and tannin contents of the ratoon crop were 3.02, 6.80, and 141.63% higher than those of the main crop in 2019 and 3.42, 8.87, and 151.48% in 2020. The ratoon crop contained a lower grain fat content than the main crop by 9.24% in 2019 and 7.64% in 2020.

With increasing N rate, the starch content decreased in the main crop, while increased in the ratoon crop with increasing N rate. The protein and tannin contents of both main and ratoon crops increased with increasing N rate, but the fat content declined.

In case of the treatment D, the grain quality traits varied by growing season and year. In 2019, no difference in the starch content was observed in both main and ratoon crops. The protein content increased in the main crop with increasing D treatment but decreased in the ratoon crop. The fat content decreased in the main crop with increasing D treatment but increased in the ratoon crop. The tannin content increased in the main crop with increasing D treatment, but there was no difference in the ratoon crop. In 2020, the highest starch content was observed under D1 treatment in the main crop but under D2 treatment in the ratoon crop. The protein content of both main and ratoon crops showed the highest value under D2 treatment. In the main crop,

TABLE 1 | Leaf area index (LAI) and soil-plant analysis and development (SPAD) at flowering and mature stage of the main and ratoon crops for different treatments in 2019 and 2020.

Treatment	2019				2020			
	SPAD		LAI		SPAD		LAI	
	Flowering	Mature	Flowering	Mature	Flowering	Mature	Flowering	Mature
Main crop								
N1	53.07 ± 2.47^{a}	49.39 ± 1.57^{b}	5.28 ± 0.57^{c}	4.93 ± 0.82^{c}	53.20 ± 1.74^{b}	50.53 ± 1.99^{c}	5.16 ± 0.89^{c}	4.89 ± 0.82^{c}
N2	54.23 ± 2.47^{a}	50.70 ± 1.25^{b}	5.67 ± 0.86^{b}	5.11 ± 0.88^{b}	54.89 ± 2.90^{a}	52.39 ± 1.94^{b}	5.81 ± 1.00^{b}	5.23 ± 0.93^{b}
N3	55.44 ± 2.25^{a}	52.74 ± 2.19^{a}	6.04 ± 0.90^{a}	5.23 ± 0.85^{a}	55.66 ± 2.71^{a}	53.72 ± 1.84^{a}	6.15 ± 0.93^{a}	5.35 ± 0.91^{a}
D1	56.50 ± 1.26^{a}	52.24 ± 2.00^{a}	4.94 ± 0.32^{c}	4.05 ± 0.12^{c}	57.27 ± 2.16^{a}	54.20 ± 1.70^{a}	4.65 ± 0.54^{c}	4.06 ± 0.19^{c}
D2	54.45 ± 1.56^{b}	50.30 ± 2.08^{a}	5.40 ± 0.22^{b}	5.23 ± 0.18^{b}	54.24 ± 1.41^{b}	51.94 ± 1.43^{b}	5.71 ± 0.29^{b}	5.35 ± 0.21^{b}
D3	51.80 ± 1.98^{b}	50.30 ± 2.03^{a}	6.65 ± 0.55^{a}	5.99 ± 0.15^{a}	52.24 ± 1.08^{c}	50.49 ± 2.01^{c}	6.77 ± 0.56^{a}	6.06 ± 0.32^{a}
Mean	54.25 ± 2.51	50.95 ± 2.17	5.66 ± 0.82	5.09 ± 0.83	54.58 ± 2.62	52.21 ± 2.28	5.71 ± 1.00	5.16 ± 0.88
Ratoon crop								
N1	48.30 ± 2.39^{a}	39.81 ± 2.65^{c}	4.59 ± 0.42^{c}	1.27 ± 0.25^{c}	47.59 ± 2.97^{c}	39.77 ± 1.95^{c}	4.42 ± 0.47^{c}	1.52 ± 0.09^{c}
N2	50.50 ± 2.12^{a}	44.26 ± 2.19^{b}	5.24 ± 0.55^{b}	2.17 ± 0.52^{b}	50.10 ± 1.73^{b}	43.13 ± 2.71^{b}	4.96 ± 0.69^{b}	2.01 ± 0.34^{b}
N3	51.73 ± 2.30^{a}	48.60 ± 2.99^{a}	5.57 ± 0.30^{a}	2.59 ± 0.32^{a}	52.60 ± 2.45^{a}	49.74 ± 2.53^{a}	5.39 ± 0.39^{a}	2.35 ± 0.23^{a}
D1	52.32 ± 1.86^{a}	44.87 ± 5.10^{a}	4.73 ± 0.46^{c}	1.66 ± 0.51^{b}	52.41 ± 2.16^{a}	45.62 ± 5.03^{a}	4.37 ± 0.42^{c}	1.73 ± 0.35^{c}
D2	49.82 ± 2.36^{b}	45.23 ± 3.20^{a}	5.07 ± 0.57^{b}	2.25 ± 0.58^{a}	50.05 ± 2.29^{b}	45.26 ± 4.81^{a}	4.92 ± 0.56^{b}	1.98 ± 0.36^{b}
D3	48.39 ± 2.08^{c}	42.57 ± 4.83^{b}	5.59 ± 0.42^{a}	2.11 ± 0.81^{a}	47.84 ± 3.21^{c}	41.77 ± 4.12^{b}	5.48 ± 0.44^{a}	2.16 ± 0.46^{a}
Mean	50.18 ± 2.62	44.22 ± 4.45	5.13 ± 0.59	2.01 ± 0.67	50.10 ± 3.14	44.22 ± 4.82	4.93 ± 0.65	1.96 ± 0.42
F-value								
S	107.85**	146.76**	166.49**	6108.66**	140.03**	381.77**	347.36**	10218.31**
N	9.92*	54.06**	196.32**	87.05**	51.17**	362.20**	108.09**	97.09**
D	40.48**	4.98*	336.47**	332.98**	53.75**	29.38**	496.05**	501.18**
S × N	0.80	8.05**	3.73*	59.31**	3.95*	25.25**	0.60	11.67**
S × D	0.82	2.26	41.25**	119.47**	0.27	2.59	48.36515506	213.21**
N × D	0.39	1.18	5.65**	7.17**	0.52	0.36	5.594269507	7.22**
S × N × D	0.32	0.38	7.60**	4.98**	1.79*	0.78	7.559578395	1.00

For each treatment (N or D), the lowercase letter in the same column indicates significant differences at 0.05 level.
**Significant at the 0.05 probability level. **Significant at the 0.01 probability level.*

the fat content decreased with increasing D treatment, but the tannin content increased. There were no differences in both fat and tannin contents in the ratoon crop.

DISCUSSION

The Yield of Main and Ratoon Crops

It is generally believed that the agronomic and reproductive characters of ratoon sorghum reduced compared to the main crop (Mourtzinis et al., 2016; Ardiyanti et al., 2019). In our study, the yield of the ratoon crop was significantly lower than that of the main crop, which might be due to smaller SPAD, LAI, TDW, and greater reductions of SPAD and LAI from flowering to mature stage in the main season. Similar results were reported in previous studies on forage sorghum, sweet sorghum (Rao et al., 2011; Vinutha et al., 2017). There are many factors leading to this difference, including greater insect, disease, and weed damages on the ratoon crop than on the main crop (Duncan and Gardner, 1984; Gerik et al., 1988). Under suitable environmental conditions and appropriate cultivations, the yield potential of ratoon sorghum could be fully exploited (Al-Taweel et al., 2020). Afzal et al. (2012) suggested that a higher level of N application was needed to prevent production differences between main and ratoon crops of sorghum. In this study, with increasing N, the yield gap between main and ratoon crops narrowed.

The N application could enhance biomass and grain yield of sorghum significantly (Wortmann et al., 2007; Kaizzi et al., 2012). Our study indicated that a high N rate increased the grain yield of both main and ratoon crops, and the effects of N on yield and yield attributes of ratoon crops were greater than that of the main crop. Previous experiments on single-crop sorghum revealed that 150–225 kg N ha^{-1} was recommended for a high yield of waxy sorghum variety (Liang et al., 2017; Wang J. S. et al., 2017). Nevertheless, in our previous study (Zhou et al., 2021), 120 kg N ha^{-1} application only in the main season, same as N1 in this study, was enough to promote buds in stubble emerging and ratoon sorghum yield formation. Therefore, we considered that the recommended N rate exceeded the need for single-crop sorghum, and the N surplus could promote the growth of ratoon sorghum. Also, in view of the fact that N fertilizer had a larger effect on the grain yield of the ratoon crop than that of the main crop, we suggest a postponed and reduced N application in the main season. The N rate of 120 kg ha^{-1} is enough for the main crop, and to improve ratoon sorghum yield, a small amount of N is recommended top-dressed 2 weeks after the harvesting of the main crop.

TABLE 2 | The plant height (PH), stem diameter (SD), and total dry weight (TDW) of the main and ratoon crops for different treatments in 2019 and 2020.

Treatment	2019			2020		
	PH (cm)	SD (mm)	TDW (g plant^{-1})	PH (cm)	SD (mm)	TDW (g plant^{-1})
Main crop						
N1	204.89 ± 4.01^{b}	18.53 ± 1.67^{b}	169.41 ± 8.53^{c}	204.67 ± 6.6^{b}	20.03 ± 1.41^{b}	178.88 ± 10.36^{c}
N2	209.78 ± 4.15^{a}	19.84 ± 1.54^{ab}	178.41 ± 6.08^{b}	214.56 ± 8.02^{a}	20.77 ± 1.31^{b}	193.70 ± 10.55^{b}
N3	209.22 ± 4.18^{a}	20.96 ± 1.86^{a}	185.77 ± 7.36^{a}	218.11 ± 5.35^{a}	22.55 ± 1.15^{a}	204.92 ± 12.73^{a}
D1	205.22 ± 5.04^{a}	21.03 ± 1.62^{a}	183.53 ± 7.74^{a}	208.33 ± 8.00^{b}	21.93 ± 1.43^{a}	202.55 ± 14.15^{a}
D2	208.67 ± 3.77^{a}	20.09 ± 1.42^{a}	177.91 ± 7.50^{b}	212.11 ± 7.69^{ab}	21.40 ± 1.47^{a}	194.26 ± 11.98^{b}
D3	210.00 ± 3.67^{a}	18.21 ± 1.62^{b}	172.14 ± 11.21^{c}	216.89 ± 9.01^{a}	20.02 ± 1.56^{b}	180.70 ± 12.12^{c}
Mean	207.96 ± 4.54	19.78 ± 1.92	177.86 ± 9.84	212.44 ± 8.69	21.12 ± 1.65	192.50 ± 15.34
Ratoon crop						
N1	173.33 ± 7.18^{a}	16.87 ± 0.96^{b}	89.08 ± 10.90^{c}	171.44 ± 4.03^{b}	17.23 ± 0.68^{b}	85.33 ± 9.99^{c}
N2	175.11 ± 4.70^{a}	17.52 ± 1.41^{b}	99.28 ± 13.80^{b}	172.89 ± 3.37^{ab}	17.84 ± 1.31^{b}	97.37 ± 9.98^{b}
N3	176.00 ± 7.31^{a}	18.57 ± 1.55^{a}	112.28 ± 13.15^{a}	175.78 ± 3.19^{a}	19.34 ± 0.86^{a}	109.57 ± 8.02^{a}
D1	174.67 ± 9.22^{a}	19.02 ± 1.30^{a}	114.21 ± 13.01^{a}	175.11 ± 2.98^{a}	18.78 ± 1.24^{a}	106.01 ± 9.78^{a}
D2	175.11 ± 4.96^{a}	17.18 ± 1.09^{b}	99.34 ± 7.75^{b}	174.56 ± 3.97^{a}	18.27 ± 1.10^{ab}	98.23 ± 12.91^{b}
D3	174.67 ± 4.64^{a}	16.76 ± 0.92^{b}	87.08 ± 11.91^{c}	170.44 ± 3.09^{b}	17.37 ± 1.30^{b}	88.04 ± 12.21^{c}
Mean	174.81 ± 6.36	17.66 ± 1.46	100.21 ± 15.55	173.37 ± 3.87	18.14 ± 1.31	97.43 ± 13.52
F-value						
S	355.44**	45.84**	4229.51**	842.43**	119.08**	3014.96**
N	30.07**	27.29**	84.55**	17.09**	89.37**	86.36**
D	0.70	22.02**	86.84**	0.79	12.84**	44.62**
S × N	0.26	0.550	3.120	4.74*	0.190	0.220
S × D	0.63	1.87	14.63**	8.36**	0.36	0.49
N × D	0.02	0.80	4.43**	0.63	0.31	0.07
S × N × D	0.19	0.17	2.01	0.63	0.29	0.86

For each treatment (N or D), the lowercase letter in the same column indicates significant differences at 0.05 level.
**Significant at the 0.05 probability level. **Significant at the 0.01 probability level.*

With the increment of D, the SPAD of both main and ratoon crops declined, while LAI increased. The higher D caused more leaf cover and an increase in LAI (Mahmood et al., 2013), leading to a higher light interception but less chlorophyll content (Xiao et al., 2018). The responses of yield, TGW, and GWPE to D were the same between the main and ratoon crops, in line with previous research on single-crop sorghum (Alderfasi et al., 2016; Sahu et al., 2018), whose results showed that increasing planting densities led to raising biomass production and seed yield per unit area but low number and grain weight per panicle. An increase in the spikes per unit area resulting from a high number of plants per unit area compensated for the reduction in TGW and GWPE and produced a higher yield in higher density. However, beyond a certain density range, the grain yield of sorghum would decline (Yang et al., 2013; Zhou et al., 2016). In this study, the yield increased continuously with increasing D, indicating a higher density should be adopted for a higher yield, especially for ratoon crops with less dry biomass (Vinutha et al., 2017), LAI, and PH (Wang Y. C. et al., 2019). The density of over 127,500 plant ha^{-1} was recommended for the main crop, and a higher density for the ratoon crop could be reached by leaving three new buds on stubble in each hill.

Grain Quality of Main and Ratoon Crops

Waxy sorghum is the best raw material for brewing liquor in China, especially famous liquor including Maotai, Wuliangye, Fenjiu, and so on (Liu et al., 2012). Starch is the main material to produce alcohol, and theoretically, the higher the starch content, the higher the liquor yield is. The protein hydrolyzes into amino acids, which can promote the growth of fermentation microorganisms and generate flavor substances to enhance the liquor taste (Yan et al., 2017). However, overmuch amino acids are harmful to liquor quality due to excess fusel oil; hence, an 8–10% protein content of sorghum grain was recommended for brewing (Tang, 2000). Fat is unfavorable to liquor-making, causing a fast and abundant acid generation, low liquor yield, and poor taste (Li, 1990). Tannin, suitable with a range of 0.5–2.0%, can inhibit harmful microorganisms and produce phenols in the fermentation process (Chen et al., 2012).

In this study, the ratoon crop recorded higher starch and tannin content but lower fat content compared with the main crop, indicating a better quality of ratoon crop. The quality of forage and sweet sorghum also showed differences between the main and ratoon crops (Rao et al., 2011; Vinutha et al., 2017). In terms of rice ratooning, extensive research has confirmed a higher rice quality of ratoon crop (Liu et al., 2002; Wu et al., 2019), due to a lower chalkiness and white vitreous and a higher milled rice recovery than that of the main crop (Cai et al., 2019). Duan et al. (2018) considered that the lower temperature and larger diurnal temperature range during the filling stage might be the reasons for the better quality of grain in the ratoon crop. Li et al. (2009) reported that 19.5–20.5℃ was a suitable daily mean temperature

TABLE 3 | Grain yield and yield components of the main and ratoon crops for different treatments in 2019 and 2020.

Treatment	**2019**			**2020**		
	TGW (g)	**GWPE (g)**	**Yield (kg ha^{-1})**	**TGW (g)**	**GWPE (g)**	**Yield (kg ha^{-1})**
Main crop						
N1	25.22 ± 1.45^{b}	56.33 ± 4.90^{c}	5038.95 ± 588.42^{c}	26.67 ± 1.23^{b}	59.28 ± 3.26^{c}	5369.04 ± 437.58^{c}
N2	25.75 ± 1.08^{ab}	59.92 ± 4.58^{b}	5249.28 ± 488.69^{b}	27.91 ± 1.13^{a}	62.75 ± 3.34^{b}	5705.27 ± 383.62^{b}
N3	26.51 ± 0.96^{a}	65.03 ± 5.06^{a}	5438.05 ± 514.23^{a}	28.30 ± 1.18^{a}	66.79 ± 3.38^{a}	5843.01 ± 348.32^{a}
D1	27.04 ± 0.91^{a}	65.69 ± 4.07^{a}	4653.28 ± 237.87^{c}	28.86 ± 0.96^{a}	66.65 ± 3.59^{a}	5183.84 ± 263.11^{c}
D2	25.63 ± 0.96^{b}	59.99 ± 4.56^{b}	5232.57 ± 220.89^{b}	27.41 ± 0.75^{b}	62.80 ± 3.01^{b}	5679.49 ± 245.90^{b}
D3	24.80 ± 0.73^{c}	55.60 ± 4.32^{c}	5840.43 ± 202.22^{a}	26.61 ± 1.18^{b}	59.36 ± 3.62^{c}	6053.99 ± 188.06^{a}
Mean	25.82 ± 1.26	60.43 ± 5.91	5242.09 ± 537.56	27.63 ± 1.34	62.94 ± 4.47	5639.11 ± 427.41
Ratoon crop						
N1	18.46 ± 1.43^{b}	25.96 ± 3.14^{c}	2634.72 ± 273.84^{c}	17.43 ± 1.25^{a}	23.38 ± 2.58^{c}	2292.39 ± 264.61^{c}
N2	19.11 ± 1.01^{a}	28.53 ± 4.50^{b}	2945.12 ± 287.71^{b}	17.86 ± 1.50^{a}	26.46 ± 3.51^{b}	2762.82 ± 318.82^{b}
N3	19.45 ± 1.31^{a}	35.03 ± 4.04^{a}	3561.01 ± 406.19^{a}	18.99 ± 1.14^{a}	32.73 ± 3.04^{a}	3339.58 ± 319.53^{a}
D1	20.52 ± 0.67^{a}	33.85 ± 4.64^{a}	2656.10 ± 323.19^{c}	19.43 ± 0.84^{a}	30.49 ± 4.43^{a}	2442.83 ± 457.91^{c}
D2	18.54 ± 0.56^{b}	30.09 ± 4.40^{b}	3164.39 ± 532.76^{b}	17.81 ± 1.05^{b}	27.97 ± 4.57^{b}	2874.51 ± 454.90^{b}
D3	17.95 ± 0.72^{c}	25.58 ± 4.02^{c}	3320.35 ± 403.90^{a}	17.04 ± 1.18^{c}	24.11 ± 3.96^{c}	3077.45 ± 489.66^{a}
Mean	19.01 ± 1.28	29.84 ± 5.43	3046.95 ± 503.19	18.09 ± 1.42	27.52 ± 4.94	2798.26 ± 524.11
F-value						
S	1576.77**	3057.18**	3698.19**	1988.50**	14863.10**	8626.65**
N	52.04**	69.46**	96.27**	34.48**	102.33**	132.21**
D	69.04**	91.88**	221.45**	40.84**	184.94**	205.34**
S × N	0.53	0.56	20.06**	1.47	5.63**	32.03**
S × D	0.94	1.29	20.58**	0.06	1.82	5.29*
N × D	1.72	0.45	1.41	0.70	1.51	0.12
S × N × D	0.68	0.22	1.57	0.62	1.30	0.92

For each treatment (N or D), the lowercase letter in the same column indicates significant differences at 0.05 level.
**Significant at the 0.05 probability level. **Significant at the 0.01 probability level.*

for starch accumulation. The effect of environment on fat content was greater than those of genotype and genotype × environment (Zhang G. X. et al., 2010), and the fat content increased with the increase of daily temperature (Qu et al., 2019). Both high and low temperature stresses can promote the synthesis of plant tannin (Zhuang and Pan, 2008). The abovementioned research explained that the part of the reason for higher starch and tannin contents and lower fat content in ratoon sorghum may be lower daily temperature. As for the effects of cutting the main crop, depletion of nutrient levels in the soil and other meteorological factors for ratoon crop on quality differences in the main and ratoon crops, it is needed to conduct further experiments for confirmation.

The N fertilizer treatment is the key determinant among the three main fertilizers in effectively enhancing the quality of waxy sorghum (Wang C. et al., 2017). Appropriate N level can improve the capacity of carbon assimilation and N assimilation in the source organs, promote the translocation of assimilates from the vegetative organs to grains, and increase the activities of enzymes related to starch synthesis and N assimilation enzymes in grains, leading to the simultaneous increase of starch and protein content (Wang et al., 2003; Liu et al., 2017). In the process of grain filling, the synthesis pathways of starch and protein are synchronized, which are interdependent and competitive (Huppe and Turpin, 1994). It is generally believed that N fertilization increased grain protein content (Miao et al., 2006; Rashid et al., 2008), but high levels of N application might reduce starch content due to the reduced activities of enzymes involved in carbon assimilation and starch synthesis (Shen et al., 2006; Zhang X. L. et al., 2010). In this study, with increasing N rate, the starch content of the main crop decreased but that of the ratoon crop increased, while the protein content of both main and ratoon crops increased. The results indicated that after the consumption of N by the main crop, the residual N in the soil promoted the synthesis of starch and protein of the ratoon crop synchronously. Based on the abovementioned results, the yield improvement by N was mainly attributed to increased GWPE, and the increment of the ratoon crop was greater than that of the main crop. Starch is the main component of grain, whose content directly determines the grain weight. In the main crop, the starch content decreased with increasing N rate. It could be inferred that higher N input significantly enhanced spikelets per panicle, seed number per spike, and grain filling rate.

The D affected the photosynthetic rate and carbon assimilation ability of leaves by influencing the plant nutritional status and light distribution in the population (Sun et al., 2015). Most efforts to elucidate the effect of D on quality were focused on forage and sweet sorghum (Cavalaris et al., 2017; Sher et al., 2017), with less attention paid to grain sorghum. Available research results about the effect of D on grain quality of single-crop sorghum have not been consistent. Wang H. X. et al. (2016)

TABLE 4 | Grain quality traits of the main and ratoon crops for different treatments in 2019 and 2020.

Treatment	2019				2020			
	Starch (%)	**Protein (%)**	**Fat (%)**	**Tannin (%)**	**Starch (%)**	**Protein (%)**	**Fat (%)**	**Tannin (%)**
Main crop								
N1	73.56 ± 0.98^{a}	9.12 ± 0.28^{c}	3.87 ± 0.12^{a}	0.36 ± 0.02^{b}	73.32 ± 1.13^{a}	8.67 ± 0.19^{c}	3.90 ± 0.26^{a}	0.37 ± 0.02^{b}
N2	72.88 ± 0.65^{a}	9.28 ± 0.32^{b}	3.69 ± 0.11^{b}	0.36 ± 0.03^{b}	72.99 ± 0.54^{a}	9.02 ± 0.25^{b}	3.77 ± 0.18^{a}	0.38 ± 0.06^{ab}
N3	71.60 ± 0.89^{b}	9.60 ± 0.48^{a}	3.54 ± 0.11^{c}	0.40 ± 0.04^{a}	71.09 ± 0.68^{b}	9.26 ± 0.24^{a}	3.55 ± 0.19^{b}	0.40 ± 0.03^{a}
D1	72.13 ± 1.21^{a}	8.87 ± 0.15^{b}	3.78 ± 0.19^{a}	0.33 ± 0.01^{c}	73.02 ± 1.68^{a}	8.78 ± 0.29^{c}	3.88 ± 0.17^{a}	0.36 ± 0.02^{b}
D2	72.79 ± 1.21^{a}	9.58 ± 0.30^{a}	3.65 ± 0.20^{b}	0.38 ± 0.02^{b}	72.41 ± 1.02^{ab}	9.20 ± 0.35^{a}	3.72 ± 0.32^{ab}	0.37 ± 0.01^{b}
D3	73.12 ± 0.95^{a}	9.55 ± 0.27^{a}	3.67 ± 0.14^{b}	0.40 ± 0.03^{a}	71.97 ± 0.90^{b}	8.98 ± 0.21^{b}	3.63 ± 0.18^{b}	0.42 ± 0.04^{a}
Mean	72.68 ± 1.16	9.34 ± 0.41	3.70 ± 0.18	0.37 ± 0.04	72.47 ± 1.28	8.99 ± 0.33	3.74 ± 0.25	0.38 ± 0.04
Ratoon crop								
N1	74.61 ± 0.99^{b}	9.80 ± 0.22^{c}	3.49 ± 0.16^{a}	0.86 ± 0.04^{a}	73.89 ± 0.60^{c}	9.55 ± 0.33^{c}	3.53 ± 0.15^{a}	0.91 ± 0.02^{b}
N2	74.01 ± 0.42^{b}	9.95 ± 0.13^{b}	3.37 ± 0.15^{a}	0.90 ± 0.03^{a}	75.04 ± 0.86^{b}	9.80 ± 0.42^{b}	3.47 ± 0.14^{a}	0.94 ± 0.02^{b}
N3	76.00 ± 0.85^{a}	10.16 ± 0.16^{a}	3.22 ± 0.18^{b}	0.92 ± 0.02^{a}	75.90 ± 0.79^{a}	10.01 ± 0.33^{a}	3.37 ± 0.10^{a}	1.03 ± 0.07^{a}
D1	75.17 ± 1.28^{a}	10.11 ± 0.17^{a}	3.20 ± 0.20^{b}	0.91 ± 0.04^{a}	74.80 ± 0.88^{b}	9.90 ± 0.19^{b}	3.46 ± 0.05^{a}	0.98 ± 0.10^{a}
D2	75.11 ± 1.04^{a}	10.01 ± 0.13^{b}	3.45 ± 0.12^{a}	0.89 ± 0.04^{a}	75.50 ± 1.26^{a}	10.11 ± 0.25^{a}	3.52 ± 0.19^{a}	0.95 ± 0.04^{a}
D3	74.35 ± 1.03^{a}	9.78 ± 0.23^{c}	3.42 ± 0.16^{a}	0.90 ± 0.03^{a}	74.53 ± 1.05^{b}	9.34 ± 0.24^{c}	3.39 ± 0.15^{a}	0.95 ± 0.04^{a}
Mean	74.87 ± 1.14	9.97 ± 0.23	3.36 ± 0.19	0.90 ± 0.04	74.94 ± 1.11	9.78 ± 0.40	3.46 ± 0.14	0.96 ± 0.07
F-value								
S	119.99**	525.29**	120.64**	6349.11**	167.49**	610.00**	47.41**	5734.05**
N	2.65	79.89**	31.15**	17.60**	6.99*	73.47**	23.20**	14.24*
D	0.76	39.89**	1.61	6.92**	5.66**	81.13**	5.07*	4.28*
S × N	30.29**	2.12	0.51	1.960	42.34**	1.460	1.790	12.33**
S × D	6.83**	124.04**	14.07**	13.29**	3.98*	49.94**	2.48	12.47**
N × D	1.64	2.53	0.65	0.40	1.92	1.85	2.66	3.61*
S × N × D	0.25	4.46**	0.96	1.47	0.49	1.40	0.14	5.30**

For each treatment (N or D), the lowercase letter in the same column indicates significant differences at 0.05 level.
**Significant at the 0.05 probability level. **Significant at the 0.01 probability level.*

reported that the starch content of sorghum grain decreased with increasing D, similarly with the result of our study on the main crop in 2020, while Wang C. et al. (2019) reported that the starch content first increased and then decreased. The protein content was not significantly influenced by D (Soleymani et al., 2011) or first increased and then decreased with the increase of density (Wang C. et al., 2019), which was consistent with our results on both main and ratoon crops. Although the protein content varied with N and D variation, it all met the demand of liquor production, so more attention should be paid to the effect of treatment on starch content and yield.

Previous studies showed that the effect of N application on fat content varies among the varieties (Yang et al., 2015), but the effect of D on fat content has not been reported. In our study, the variations of fat content according to N and D treatment were remarkably less and all lower 4%, meeting the criteria of raw materials for liquor production. Some reports showed that the effect of N on tannin content was not significant (Yin et al., 1990; Liu et al., 2017). Wang H. X. et al. (2016) reported that tannin content increased with increasing D, while Wang C. et al. (2019) demonstrated that tannin content first decreased and then increased. In this study, the tannin content increased with increasing N in both main and ratoon crops but increased with increasing D only in the main crop.

Compared with N and D treatments, growing season had a larger effect on sorghum grain quality. Therefore, it must be of great significance to further study the influence of growing season on quality parameters and why the influence occurred.

CONCLUSION

The yield of the main crop was significantly higher than that of the ratoon crop. Increasing N fertilizer could increase the TGW, GWPE, and yield of both main and ratoon sorghum and could narrow the yield gap between the main and ratoon crop. N fertilizer had a larger effect on the grain yield of the ratoon crop than that of the main crop. Increasing D led to lower TGW and GWPE but higher grain yield. The ratoon crop recorded higher starch and tannin content but lower fat content compared with the main crop, indicating a better quality of ratoon crop. Compared with the growing season, N and D treatments had a smaller effect on sorghum grain quality. The simultaneous improvement of yield and quality can be obtained by increasing the yield of ratoon crops. Our results suggest that 120 kg N ha^{-1} in the main crop and a small amount of N top-dressed 2 weeks after main crop harvesting could obtain a high ratoon yield. A density of over 127,500 plant ha^{-1} is recommended for the main crop, and a higher density is suitable for the ratoon crop.

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 author/s.

AUTHOR CONTRIBUTIONS

YZho and JH performed the experiments, analyzed data, and wrote the manuscript. ZL and YW carried out the experiment. JZ and YZha designed the experiments and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Differential Maize Yield Hybrid Responses to Stand Density Are Correlated to Their Response to Radiation Reductions Around Flowering

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Altered stand density affects maize yields by producing changes in both numerical yield components, kernel number per plant (KNP), and kernel weight (KW). Kernel number is determined by the accumulation of ear biomass during the flowering period, whereas KW is determined by the sink potential established during flowering and the capacity of the plant to fulfill this potential during effective grain filling. Here, we tested if different short shading treatments during different stages around flowering can help discriminate genotypic differences in eco-physiological parameters relevant for maize stand density yield response and associated yield components. Our specific objectives were to: (i) identify hybrids with differential shading stress response, (ii) explore shading effects over eco-physiological parameters mechanistically related to KNP and KW, and (iii) test if shading stress can be used for detecting differential genotypic yield responses to stand density. The objectives were tested using four commercial maize hybrids. Results indicated that KNP was the yield component most related to yield changes across the different shading treatments, and that the specific shading imposed soon after anthesis generated the highest yield reductions. Hybrids less sensitive to shading stress were those that reduced their plant growth rate the least and the ones that accumulated more ear biomass during flowering. Genotype susceptibility to shading stress around flowering was correlated to stand density responses. This indicated that specific shading stress treatments are a useful tool to phenotype for differential stand density responses of commercial hybrids.

Keywords: corn, stand density, shading treatments, plant population (densities), shading stress

INTRODUCTION

Commercial maize breeding programs have been successful in making continuous genetic improvements in maize grain yields (Duvick et al., 2004; Echarte et al., 2004; Luque et al., 2006; Di Matteo et al., 2016; Borrás and Vitantonio-Mazzini, 2018). Hybrid selections are done using multienvironmental trials, where a group of hybrids are grown across several experiments during the years to provide information covering the performance of genotypes in a target population of environments (Delacy et al., 1996). This methodology responds to the requirement of exploring a range of possible environments, with contrasting yield potential and stress conditions. This testing represents a large cost for the production of commercial hybrids. Public and private breeding programs are commonly interested in finding alternative selection methods that allow reducing the number of trials/years during the selection process. Manipulating environmental factors within field experiments can help breeding programs improve genotype selection and agronomic management recommendations, reducing testing costs (Blum and Pnuel, 1990) or increasing their efficiency (Campos et al., 2004).

Environmental conditions, like water (Andrade et al., 2002), nutrient (Caviglia et al., 2014), and radiation levels (Andrade et al., 1999, 2002; Cerrudo et al., 2013) affect maize crop growth and grain yield. Physiological and numeric yield components can help predict crop yield variability associated with different environmental conditions (Rotundo et al., 2012; Di Mauro et al., 2019). Environmental conditions affect maize yields due to changes on kernel number per plant (KNP) or individual kernel weight (KW; Claassen and Shaw, 1970b; Hall et al., 1981). KNP is commonly associated with ear biomass (EB) accumulation around flowering (Echarte et al., 2004; Severini et al., 2011; Borrás and Vitantonio-Mazzini, 2018), and is also associated with plant growth rate (PGR) during this period (Andrade et al., 1999).

Changes in KNP are associated with maize yield variability, and this variability is closely related to changes in PGR around flowering (Fischer and Palmer, 1984; Andrade et al., 1999). Previous studies to determine maize yield susceptibility across the flowering period showed mixed results. Some studies indicated that the close postanthesis period is the most sensitive period for kernel setting and yield (Kiniry and Ritchie, 1985). Other authors, however, indicated that maize yield is most sensitive to changes in canopy growth during 2 weeks bracketing flowering (Hawkins and Cooper, 1981; Cirilo and Andrade, 1994). Otegui and Bonhomme (1998) described the most sensitive period starts −227 Cd (growing degree days) before flowering and ends 100 Cd after flowering, and a recent study found that this period is from −300 to 780 Cd around flowering (Cerrudo et al., 2013).

Several treatments have been used to generate crop stress and reduce canopy growth to test for the crop responses. Yield reductions can be managed using water deficit conditions (Claassen and Shaw, 1970a,b; Hall et al., 1981; Kiniry and Ritchie, 1985; Andrade et al., 2002; Campos et al., 2004), inadequate nutrition (Uhart and Andrade, 1995; Andrade et al., 2002; D'Andrea et al., 2008; Caviglia et al., 2014), increased plant density (Otegui, 1997; Andrade et al., 1999, 2002; Echarte et al., 2000; Sarlangue et al., 2007; Tokatlidis et al., 2011; Hernández et al., 2014), or reductions in radiation levels (Fischer and Palmer, 1984; Kiniry and Ritchie, 1985; Reed et al., 1988; Andrade et al., 1999, 2002). Among all these manipulative stress treatments, artificial shading has practical advantages. The most important is associated with its flexibility for regulating stress timing, intensity, and duration, as shown in the recent study by Cerrudo et al. (2013). Additionally, because canopy growth reductions can be achieved through a number of these treatments, the responses are not specific to the type of stress (Knight and Knight, 2001). In the present manuscript, we tested if genotype differential responses to manipulative shading treatments can be extrapolated to other conditions that reduce canopy growth, like stand density.

Our specific objectives were to: (i) identify tolerant hybrids to shading stress, (ii) explore shading responses using yield numerical and physiological components approaches, and (iii) test if shading stress can be used for detecting differential genotypic yield responses to stand density. We hypothesized that the genotypes with more shading tolerance are the ones with higher optimum stand densities (they tolerate higher stand densities). To test this hypothesis, four maize hybrids were evaluated across different shading and stand density treatments.

MATERIALS AND METHODS

Genotypes

We tested four maize hybrids (H1–H4) with relative maturity between 118 and 125. Genotypes are the result of a single cross between a common female of one heterotic pool and four males with different backgrounds of a second heterotic pool of KWS Group breeding program. Genotype H1 is commercially known as KM3800 (relative maturity 118), H2 is KM4200 (relative maturity 122), H3 is KM4321 (relative maturity 123), and H4 is KM4500 (relative maturity 125).

Field Experiments With Shading Treatments

Field experiments were conducted in the year 2014 in Zavalla, Santa Fe, Argentina (33° 2′ 24.75″ S, 60° 53′ 11.76″ W). Plots were eight rows with 6 m long and 0.52 m row spacing. Plots were kept free of weeds, insects, and diseases. Weeds were controlled using standard agronomic practices and manually removed whenever necessary. Soil was Vertic Argiudoll, Roldan series. One shading experiment was conducted at Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario (named Env 1). Sowing date was September 27 and was conducted under no till and rainfed conditions. All plots were oversown and hand-thinned at V2 (Ritchie and Hanway, 1982) to 8 plants m^{-2}. A second shading experiment was conducted at KWS Experimental Station (named Env 2). Sowing date was December 20 and was managed under tillage and rainfed conditions. Plots were hand-planted at three seeds per hill and hand-thinned to one plant per hill at V2 (Ritchie and Hanway, 1982), resulting in a final stand density of 5.5 plants m^{-2}.

In both experiments, all the hybrids were shaded during periods of 7 days with 80% reduction of incident photosynthetic active radiation. Five shading treatments were centered around

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Abbreviations: EB, ear biomass; PGR, plant growth rate; KNP, kernel number per plant; KW, kernel weight; RY, relative yield; CV_{PGR}, coefficient of variation of plant growth rate; BA, barrenness; PEF, partition efficiency; SSEF, seed set efficiency; IS_{EB}, initial slope for ear biomass accumulation; PGR_b, base plant growth rate for ear biomass accumulation; C_{EB}, curvilinearity of the relationship between ear biomass accumulation and plant growth rate; IS_{KN}, initial slope for kernel number per plant; EB_b, base ear biomass for kernel set; C_{KN}, curvilinearity of the relationship between kernel number per plant and accumulated ear biomass; Env, environment; H, hybrids; S, shading; SD, stand density.

the flowering period. Shading treatments went from 14 to 7 days previous to anthesis (named S−7), from 7 days preanthesis to anthesis (named S0), from anthesis to 7 days after anthesis (named S + 7), and from 7 days after anthesis to 14 after anthesis (named S + 14). A control treatment without shading (named T0) was also evaluated, and in Env 1 an additional treatment starting 21 days before anthesis and ending 14 days before anthesis was also tested (named S−14). The experimental design of both shading experiments was a randomized complete block with three replicates. Shading cloth blocked 80% natural light intensity and was suspended above canopy.

Field Experiments With Stand Density Treatments

Three additional field experiments testing hybrid response to stand density were conducted at KWS Experimental Station. The three experiments were sown on September 23, October 20, and November 20, 2014, and named Env 3, Env 4, and Env 5, respectively. They included the same four hybrids used in Env 1 and Env 2. Experiments were managed under tillage and rainfed conditions. Plots were four rows with 6 m long and 0.52 m row spacing. Hybrids H1–H4 were tested at four stand densities (5, 7, 9, and 11 plants m^{-2}). Plots were oversown and hand-thinned to the desired stand density at V2 (Ritchie and Hanway, 1982). Experiments were fertilized with 40 kg N ha^{-1} before sowing plus an additional 120 kg N ha^{-1} at V6. Plots were kept free of weeds, insects, and diseases. Each stand density experiment had a randomized complete block design of hybrids and stand densities with three replicates.

Phenotypic Measurements in Shading Experiments

In both shading experiments, yield was calculated from harvesting all ears of two central rows per plot at harvest maturity. Individual kernel weight (KW) was determined after weighting 400 kernels per plot, and KNP was calculated using yield and stand density. Yield and individual KW are reported with 145 g kg^{-1} moisture. Relative yield (RY) was calculated as the ratio between the yield of any shade treatment and the control plot from the same genotype and block.

In the shading experiment of Campo Experimental Villarino, 15 consecutive plants per plot were tagged at V8 in center rows. These plants were used for describing plant growth and kernel number differences across treatments.

At the pre- and postflowering stages, non-destructive allometric models were used to estimate shoot biomass and partitioning at the individual plant level (Vega et al., 2000; Echarte et al., 2004; Gambín et al., 2008). The preflowering model was based on the linear regression between shoot biomass and stem volume (Vega et al., 2001; Gambín et al., 2008). Stem volume was calculated from plant height (ground level up to the uppermost leaf collar) and stem diameter at the base of the stalk. The preflowering biomass sample was done 15 days before 50% anthesis, and the postflowering one was done 15 days after anthesis. In each plot, two plants from border rows were used to develop the allometric preflowering and postflowering models (Vega et al., 2001). All plant samples were determined after cutting plants and drying them in a forced-air oven at 65°C for at least 7 days. The r^2 values for the preflowering model ranged from 0.79 to 0.91 ($p < 0.01$; $n = 27$) across hybrids.

The postflowering biomass sample was done using a multiple linear regression model with stem volume and maximum ear diameter from all ears having extruded visible silks (Vega et al., 2001; Gambín et al., 2008). The r^2 values for this model ranged from 0.81 to 0.98 ($p < 0.01$; $n = 27$) across hybrids. In the postflowering biomass sample, we also estimated the ear biomass 15 days after anthesis by fitting a linear regression between ear biomass and the square of ear diameter (similar to Hernández et al., 2014).

Plant growth rate around flowering (mg $plant^{-1}$°C d^{-1}) was calculated as the ratio between the plant biomass (mg $plant^{-1}$) difference and the thermal time accumulated from pre- to postflowering samples in each specific plot. Daily thermal time values were calculated using a base temperature of 8°C. PGR was determined for each tagged plant and the values were presented as an average individual PGR, and its plant-to-plant variability was expressed as the coefficient of variation of PGR (CVPGR) for each genotype × treatment combination.

Barrenness was calculated as the percentage of barren plants per plot. Plants with less than 10 kernels at harvest maturity were considered barren (Tollenaar et al., 1992). For each individual plot, we also calculated the partition efficiency (PEF) as the ratio between EB and the total plant biomass 15 days after anthesis, and the seed set efficiency (SSEF) as the ratio between KNP and the accumulated EB.

For comparing hybrids, we fitted the relationship between KNP and EB 15 days after anthesis, and between EB and PGR around flowering, similar to Hernández et al. (2014). Both relationships were described by a hyperbolic function with their specific parameters [**Figure 1**; Eqs. (1–4)]. Descriptive parameters of the models are PGR_b, IS_{EB}, C_{EB}, EB_b, IS_{KN}, and C_{KN} (**Figure 1**). Models were fit to each genotype × replicate combination and included in the same curve the five shading treatments utilized within each replicate; so the parameters were estimated for genotype × replicate combinations. Replicates were used for an ANOVA test, and r^2 values ranged from 0.41 to 0.92 ($p < 0.01$).

$$EB = 0 \qquad \text{if } PGR \le PGR_b \tag{1}$$

$$EB = [IS_{EB} \times (PGR - PGR_b)]/[1 + C_{EB} \times (PGR - PGR_b)] \qquad \text{if } PGR > PGR_b \tag{2}$$

$$KNP = 0 \qquad \text{if } EB \le EB_b \tag{3}$$

$$KNP = [IS_{KN} \times (EB - EB_b)]/[1 + C_{KN} \times (EB - EB_b)] \qquad \text{if } EB > EB_b \tag{4}$$

where in Eq. (2) IS_{EB} is the initial slope of the relationship between EB and PGR, PGR_b is the base PGR for ear growth, and C_{EB} is the curvilinearity of the hyperbolic function (curvature)

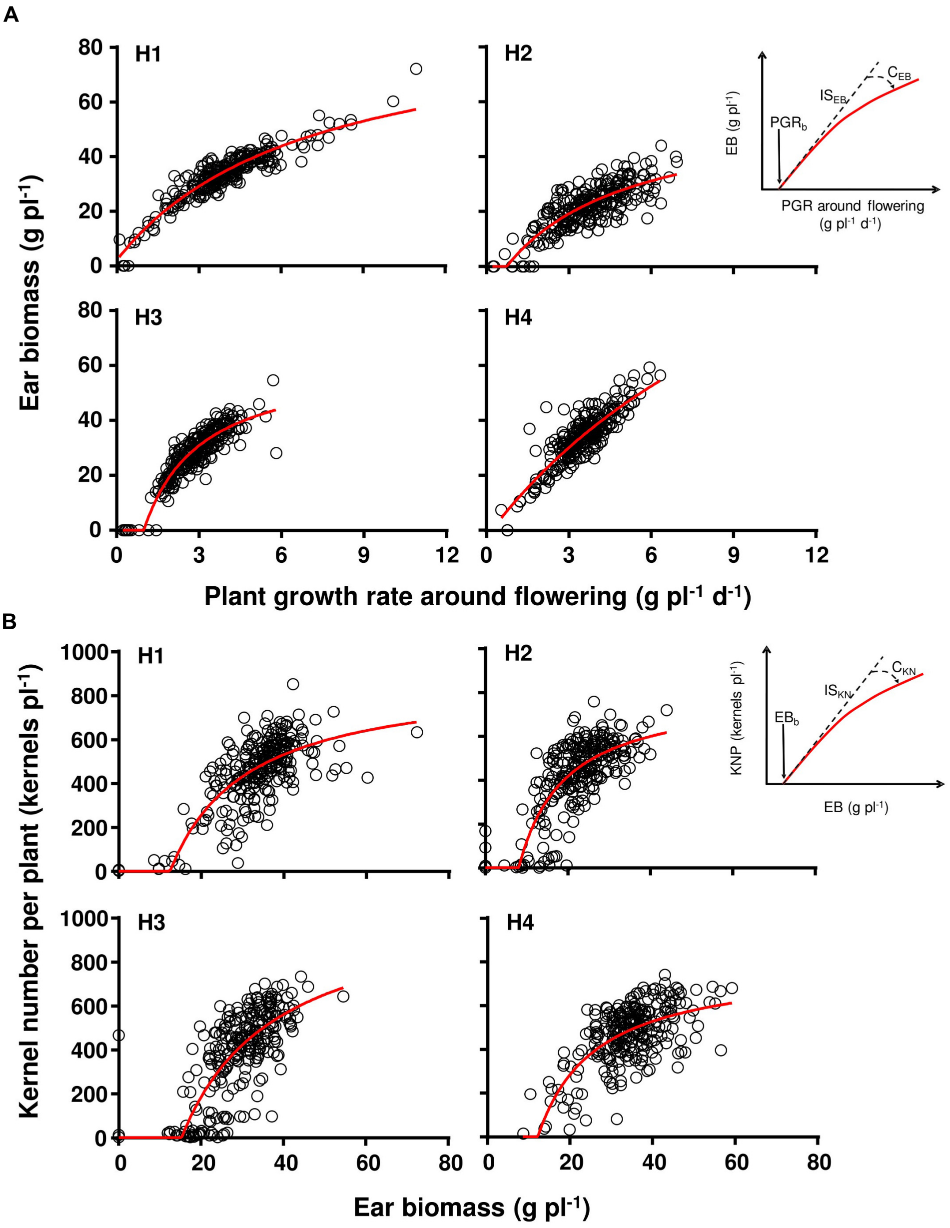

FIGURE 1 | Ear biomass as a function of plant growth rate **(A)**, and kernel number as a function of ear biomass **(B)** for the four evaluated genotypes (H1, H2, H3, and H4). The left inset in **(A,B)** describe the parameters for each relationship, fully described in **Table 3**. All correlations were significant [**(A)** H1 had $n = 263$, $r^2 = 0.54$; H2 had $n = 261$, $r^2 = 0.59$; H3 had $n = 252$, $r^2 = 0.55$; H4 had $n = 259$, $r^2 = 0.45$; in **(B)** H1 had $n = 265$, $r^2 = 0.88$; H2 had $n = 270$, $r^2 = 0.65$; H3 had $n = 269$, $r^2 = 0.84$; H4 had $n = 268$, $r^2 = 0.75$]. Red lines describe the fitted curves.

of the relationship between EB and PGR. In Eq. (4) IS_{KN} is the initial slope of the relationship between KNP and EB, EB_b is the base ear biomass for initial kernel set, and C_{KN} is the curvilinearity of the hyperbolic function (curvature) of the relationship between KNP and EB. All these parameters are considered genotypic coefficients. Applying these coefficients uniformly to all the plants is supported by several studies that show a consistent relationship between ear growth and PGR around flowering across environments (Andrade et al., 1999; Vega et al., 2001; Borrás et al., 2007). All curves were fitted using the GraphPad Prism version 5.0 (Raduschev, 2007) iterative optimization technique.

Phenotypic Measurements in Stand Density Experiments

Yield was calculated after harvesting all ears from 3 m^2 in central rows at harvest maturity in all genotype × stand density × environment combinations. Yield is reported with 145 g kg^{-1} moisture.

Statistical Analysis

Data were analyzed separately for each experiment (shading or stand density) in R software (R Core Team, 2020). We used a randomized complete block design with three replications in all the trials. Sources of variation were environment (sowing date), hybrids, treatment (shading or stand density), and blocks. Main or interaction effects were tested with ANOVA. Treatment marginal means were estimated with "emmeans" function from EMMEANS R package (Russell, 2021). Tukey test was done for pairwise comparisons of estimated means.

RESULTS

Shading Stress Effects on Maize Yields and Critical Period

Environments, hybrids, and shading treatments all showed significant yield differences ($p < 0.001$; **Table 1**). A hybrid × shading × environment treatment interaction ($p < 0.001$; **Table 1**) was also significant, showing that shading stress responses were different among hybrids and environments ($p < 0.001$; **Table 1**). In both environments, the effects of shading treatments S−14, S + 7, and S + 14 on yield did not differ across hybrids, but large hybrid yield differences were observed in the treatments closer to anthesis (S−7 and S0 treatments; **Supplementary Table 1**). In Env 1 the S−7 treatment hybrids H1, H2, H3, and H4 yielded 13.0, 11.9, 8.7, and 14.2 Mg ha^{-1}, respectively (**Supplementary Table 1**), and in the S0 treatment genotypes H1, H2, H3, and H4 yielded 11.6, 4.6, 8.2, and 12.7 Mg ha^{-1}, respectively (**Supplementary Table 1**). These genotype differential yield responses were even more evident in Env 2.

When analyzed in relative terms (here called relative yield), the interaction environment × hybrid × shading treatment was significant ($p < 0.05$; **Table 1**), illustrating that yield reductions associated with shading treatments were different depending on the particular hybrid and environment. For example, the relative yield of H1 and H4 in Env 1 did not differ among shading treatments, but the shading treatment finishing at anthesis (S0) applied to H1 had a 26% relative yield, whereas it did not reduce H4 relative yields in Env 2 (**Supplementary Table 1**, contrast $p < 0.001$). Likewise, hybrids H2 and H3 showed significant relative yield reductions when the shading treatment S0 was applied to both the environments (**Supplementary Table 1**).

Shading Effects on Yield Physiological Components

Kernel Number per Plant

Kernel number per plant was affected by environment ($p < 0.05$), hybrids ($p < 0.001$), shading ($p < 0.001$), and hybrid × shading treatment interactions (**Table 1**). This showed that KNP was different according to hybrid and shading treatments (**Supplementary Table 1**). Hybrid H4 presented the highest KNP values ($p < 0.001$), and when comparing shading treatments S−7, S0, S + 7, and S + 14 significantly lower KNP values were generated in comparison to T0 ($p < 0.001$). Averaged across hybrids, the treatment S0 had the lowest KNP value (322 kernels plant^{-1}), in agreement with the described yield response and the known relevance of KNP for yield determination.

Changes in KNP can be described as a function of changes in EB accumulated 15 days after flowering. Significant differences for accumulated EB among hybrids ($p < 0.001$) and shading treatment ($p < 0.001$) were observed. Genotype maximum and minimum EB were 33.7 and 22.1 g ear^{-1}, corresponding to H4 and H2, respectively (**Table 2**). Shading treatments showed maximum values for T0 (32.9 g ear^{-1}), and minimum values for S0 (25 g ear^{-1}, **Table 2**).

The proportion of barren plants within the canopy was well correlated to changes in accumulated EB 15 days after anthesis across hybrids and shading treatments, with the S0 treatment being the one with higher barrenness values (**Table 2**). This was particularly evident in the genotypes H2 and H3 that showed the largest yield and KNP decline in this specific shading treatment, S0 (**Supplementary Table 2**).

Plant growth rate also showed significant differences for hybrids ($p < 0.01$) and shading treatments ($p < 0.05$; **Table 2**), and the non-significant hybrid × shading interaction ($p > 0.05$) showed that all the genotypes reduced their growth to a similar extent across shading treatments. Pant growth rate of H1 and H4 was significantly different from H3 (3.93 and 3.48 vs. 2.88 g plant^{-1} day^{-1}, respectively; **Table 2**; contrast $p < 0.001$ and $p < 0.05$, respectively). PGR was significantly reduced for S−14, S−7, and S0 regarding to T0 (**Table 2**).

Variations in CV_{PGR} only showed significant differences among hybrids ($p < 0.05$, **Table 2**). H2 was the most uniform genotype, whereas H1 was the most variable one in terms of plant-to-plant growth variability.

Plant biomass partitioning to the ear during the flowering period (called partitioning efficiency, PEF) showed a significant hybrid × shading treatment interaction ($p < 0.01$, **Supplementary Table 2**). Lowest PEF values were observed for H2, and were especially lower in the shading treatment S0, the one that reduced yield, KNP, and ear biomass accumulation the most.

Also, hybrids ($p < 0.001$) and shading treatments ($p < 0.01$) showed significant differences for their seed set efficiency per unit of accumulated ear biomass (SSEF; **Table 2**), but no differential hybrid responses were evident for this trait (no significant hybrid × shading interaction, $p > 0.05$; **Table 2**). The lowest efficiency was observed in the treatment having the highest yield detrimental effect (S0; **Table 2**).

Figure 1A describes the relationship between ear biomass accumulation and PGR around flowering for each hybrid, and **Figure 1B** shows the relationship between KNP and ear biomass accumulation for each hybrid. **Table 3** describes the parameters of the adjusted models describing the differential response patterns shown by each hybrid. In brief, hybrids showed different response patterns. Parameter PGR_b was significantly lower for H1 than H3 ($p < 0.05$, **Table 4**). When compared with IS_{EB}, H3 showed the highest magnitude (31.6 g EB g plant^{-1} d^{-1}; $p < 0.01$; **Table 4**) but was also the genotype with the highest curvature value (C_{EB} for H3 was 0.52 g plant^{-1} d^{-1}; **Table 3**). EB_b presented significant differences between hybrids ($p < 0.05$), ranging from 9.2 to 16.5 g (**Table 3**), and the lowest value was observed in H2. As such,

TABLE 1 | Yield, relative yield (yield relative to the T0 control treatment), kernel number per plant (KNP), and individual kernel weight (KW) for four genotypes tested at six reduced radiation treatments (S−14, S−7, S0, S + 7, S + 14, and T0) in two different environments (Env 1 and Env 2).

Environment	Hybrid	Shading	Yield	Relative yield	KNP	Kernel weight
			Mg ha^{-1}	%	kernels plant^{-1}	mg kernel^{-1}
Env 1			12.6	80	433	323
Env 2			8.0	71	401	340
	H1		11.0	80	436	317
	H2		10.0	70	404	329
	H3		10.2	72	386	320
	H4		12.6	86	461	342
		S−14	13.8	91	490	335
		S−7	10.2	77	432	317
		S0	7.1	51	322	343
		S + 7	11.2	85	413	335
		S + 14	11.3	86	401	332
		To	13.2	100	499	316
Env (E)			***(0.5)¥	ns	*(23)	***(6)
Hybrid (H)			***(0.9)	***(8)	***(41)	***(12)
Shading (S)			***(1.3)	***(10)	***(57)	***(16)
E × H			ns	*(10)	ns	***(20)
E × S			ns	***(16)	ns	***(24)
H × S			***(3.3)	***(28)	**(150)	*(42)
E × H × S			**(4.8)	*(41)	ns	***(62)

The S−14 was only tested in Env 1. See section "Materials and Methods" for a description of shading treatments. Treatment mean for interactions are available in ***Supplementary Table 1****.*
*¥Significance of ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, and ns is not significant ($p > 0.05$). Values in parenthesis are Tukey values ($p < 0.05$).*

TABLE 2 | Kernel number per plant (KNP), accumulated ear biomass 15 days after anthesis (EB), individual plant growth rate around flowering (PGR) and their coefficient of variation (CVPGR), barrenness (BA), partition efficiency (PEF), and seed set efficiency (SSEF) for the four genotypes (H1, H2, H3, and H4) tested at six shading treatments (S−14, S−7, S0, S + 7, S + 14, and T0).

Hybrid	Shading	KNP	EB	PGR	CV_{PGR}	BA	PEF	SSEF
		kernels plant^{-1}	g ear^{-1}	g plant^{-1} d^{-1}	%	%	g g^{-1}	KNPEB^{-1}
H1		456	33.2	3.93	36	3.3	0.21	13.6
H2		425	22.1	3.78	24	7.0	0.13	18.7
H3		383	28.2	2.88	31	8.9	0.20	12.4
H4		468	33.7	3.48	27	3.0	0.21	14.0
	S−14	490	31.2	3.44	25	1.1	0.19	15.7
	S−7	434	26.9	3.31	32	8.3	0.18	15.6
	S0	309	25.0	3.00	30	13.9	0.18	11.6
	S + 7	432	27.9	3.58	32	5.0	0.18	16.4
	S + 14	408	32.0	3.82	27	1.7	0.20	13.0
	T0	525	32.9	3.94	31	3.3	0.19	15.9
Hybrid (H)		¥***(50)	***(2.8)	**(0.47)	*(11)	*(5.5)	***(0.01)	***(2.2)
Shading (S)		***(69)	***(3.8)	*(0.64)	ns	***(7.5)	**(0.02)	***(3.0)
H × S		**(178)	ns	Ns	ns	ns	**(0.05)	ns

See section "Materials and Methods" for a description of the treatments. Treatment mean for interactions are available in ***Supplementary Table 2****.*
*¥Significance of ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, and ns is not significant ($p > 0.05$). Values in parenthesis are Tukey values ($p < 0.05$).*

genotypes differed in the parameters that described how much of the total plant biomass is partitioned to the growing ear around flowering, and in how is accumulated ear biomass turned into kernels per plant. The poor plant biomass partitioning described in H2 in **Table 2** is also evident in **Figure 1** and is coincident with the hybrid susceptibility to shading.

Hybrid Differential Yield Response to Stand Density

In a second round of experiments, we tested how these same hybrids responded to stand density changes, and a stand density × hybrid experiment was repeated across three environments.

TABLE 3 | Descriptive parameters of model relating kernel number per plant (KNP) with ear biomass accumulated 15 days after anthesis (EB), and EB as a function of plant growth rate (PGR) around flowering.

Hybrid	PGR_b	IS_{EB}	C_{EB}	EB_b	IS_{KN}	C_{KN}
	g plant^{-1} d^{-1}	g EB g plant^{-1} d^{-1}	g plant^{-1} d^{-1}	g	KNP g EB^{-1}	g EB
H1	0.13	14.5	0.17	11.2	40.8	0.04
H2	0.52	11.9	0.20	9.2	105.0	0.15
H3	1.00	31.6	0.52	16.5	58.0	0.06
H4	0.24	13.3	0.08	12.2	56.1	0.07
Hybrid	*(0.85)	**(14.5)$^{¥}$	*(0.40)	*(6.6)	ns	ns

PGR_b is the minimum base plant growth rate around flowering for ear biomass accumulation, IS_{EB} is the initial slope of the relationship between plant growth rate and ear biomass accumulated at 15 days after anthesis, C_{EB} is the curvature of the relationship between ear biomass and plant growth rate, EB_b is the base ear biomass around flowering for kernel number per plant, IS_{KN} is the initial slope of the ear biomass vs. kernel number per plant relationship, and C_{KN} is the curvature of the relationship between ear biomass and kernel number per plant relationship. This is described for four genotypes (H1, H2, H3, and H4). Additional data available in the section "Materials and Methods."

$^{¥}$*Significance of ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, and ns is not significant ($p > 0.05$). Values in parenthesis are Tukey values ($p < 0.05$).*

TABLE 4 | Yield of four genotypes (H1, H2, H3, and H4) tested at three environments (Env 3, Env 4, and Env 5), and four stands density treatments (D1, D2, D3, and D4 were 5, 7, 9, and 11 plants m^{-2}).

Environment	Hybrid	Stand density	Yield
			Mg ha^{-1}
Env 3			14.1
Env 4			13.9
Env 5			12.3
	H1	D1	10.9
		D2	13.8
		D3	14.1
		D4	14.0
	H2	D1	11.7
		D2	13.6
		D3	14.0
		D4	12.9
	H3	D1	11.9
		D2	15.0
		D3	13.8
		D4	13.0
	H4	D1	12.4
		D2	15.0
		D3	14.9
		D4	14.2
Environment (E)			***(0.5)$^{¥}$
Hybrid (H)			***(0.6)
Stand density (SD)			***(0.6)
H × SD			*(1.6)
E × H			*(1.3)
E × SD			ns
E × H × SD			ns

*Treatment mean for interactions are available in **Supplementary Table 3**.*

$^{¥}$*Significance of ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, and ns is not significant ($p > 0.05$). Values in parenthesis are Tukey values ($p < 0.05$).*

Yield results showed that all three main effects (hybrids, stand densities, and environments) were highly significant ($p < 0.001$), and that the interactions hybrid × stand density and hybrid × environment were also statistically significant for yield ($p < 0.05$; **Table 4**). The significant interaction hybrid × stand density showed that hybrids responded differently to changes in stand density.

Analyzing hybrids across densities, H4 produced highest yields in all densities, yielding 12.4, 15.0, 14.9, and 14.2 Mg ha^{-1} for stand densities 5, 7, 9, and 11 plants m^{-2}, respectively. Hybrid H1 also presented its highest yields in the highest stand densities, with 14.1 and 14.0 Mg ha^{-1}, at 9 and 11 plants m^{-2}, respectively (**Table 4**). Contrary to this response, hybrids H2 and H3 did not maximize their yields at the highest densities and showed a significant decline in their yields at the highest stand density of 11 plants m^{-2}. Hybrids H2 and H3 showed that the maximum yields were achieved at the lower stand densities of 9 and 5 plants m^{-2}, respectively (**Table 4**). This differential yield response to stand the density of hybrids H2 and H3 compared to H1 and H4 was more evident in the lowest yield environment Env 5 (**Supplementary Table 3**).

DISCUSSION

Differences among commercial maize hybrids in their yield response to water availability (Campos et al., 2004; Messina et al., 2019), stand density (Sarlangue et al., 2007; Tokatlidis et al., 2011; Hernández et al., 2014; Mylonas et al., 2020), and *N* availability (Gambin et al., 2016) are known. Crop managers are seeking information about hybrid × stand density interactions, and many seed companies are currently providing hybrid-specific recommendations for stand density management. The generation of this information comes with a large effort, in which commercial and precommercial hybrids are tested at a range of stand densities and environments to provide accurate recommendations (Lacasa et al., 2020).

In the present manuscript, we tested the hypothesis that hybrid response to shading treatments around flowering can help predict hybrid differential responses to stand density. This hypothesis is based on the concept that all these stressful environmental scenarios (lack of water, of radiation, of N) have common responses affecting kernel set though changes in PGR around the flowering period (Andrade et al., 1999). We do realize that our study tested a limited number of hybrids, locations, and stand densities, but results have large implications for phenotyping hybrid responses to management changes. Our results testing a number of commercial genotypes support the use of specific shading treatments to predict hybrid stand density performance.

We tested five different short shading moments to identify if there was any specific timing that helped discriminate hybrids in their response to shading. Results indicated that the shading ending at anthesis (S0) was the most powerful one to discriminate differential hybrid responses to shading stress. The treatments that were more distanced in time from anthesis (starting 14 days before or after anthesis, treatments S−14 and S + 14, respectively) were the ones showing minor yield effects. This is coincident with the early articles about the maize yield critical period around flowering (Fischer and Palmer, 1984; Otegui and Bonhomme, 1998), and contradicts the results from Cerrudo et al. (2013) that predicted a similar effect for a large period around flowering. We hypothesize that differences with this later study might be a consequence of testing a single genotype, our hybrid × shading significant interaction for yield (**Table 1**) shows that not all genotypes have similar yield responses.

Maize grain yield response to stand density changes is usually dissected into two components, potential yield per plant, and tolerance to crowding stress. Although evidence is available that both components have changed with breeding for yield, the latter component has been more successfully increased by breeding and is responsible for the most yield improvements (Tollenaar and Wu, 1999; Duvick et al., 2004; Tokatlidis and Koutroubas, 2004; Egli, 2015; Assefa et al., 2018). In the present manuscript, we described that a direct specific shading treatment around flowering can help predict hybrid performance to higher stand densities, as shown by the differential response of the commercial evaluated hybrids.

Amelong et al. (2017) reported that hybrid yield response to stand density can be predicted from parental inbred line information. In the present study, we used four genotypes that only differed in one parental line. This will allow us to track the genotypic basis for the differential shading, and stand density described yield responses.

CONCLUSION

Evaluated hybrids differ in their yield and relative yield response to changes in shading stress. The treatments that exposed hybrid differences the most were those specifically centered around the flowering period.

Yield responses to shading stress were related to known physiological determinants of kernel set, namely plant growth and biomass partitioning to the ear during flowering. These physiological traits helped understand commercial hybrid differences in their yield sensitivity to shading stress.

Hybrid differences in their yield response to high stand density were correlated to their yield response to shading stress, indicating that shading treatments can be used to effectively test hybrid yield performance to crowing tolerance.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

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

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Single-Plant Selection at Ultra-Low Density Enhances Buffering Capacity of Barley Varieties and Landraces to Unpredictable Environments and Improves Their Agronomic Performance

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Rainfall and temperature are unpredictable factors in Mediterranean environments that result in irregular environmental conditions for crop growth, thus being a critical source of uncertainty for farmers. This study applied divergent single-plant selection for high and low yield within five barley varieties and two Tunisian landraces under semi-arid conditions at an ultra-low density of 1.2 plants/m^2 for two consecutive years. Progeny evaluation under dense stands following farmers' practices was conducted in two semi-arid locations in Tunisia during one cropping season and in one location during a second season, totalling three environments. The results revealed significant genotypic effects for all recorded agronomic and physiological traits. No genotype × environment interaction was shown for biological yield, implying a biomass buffering capacity for selected lines under different environmental conditions. However, genotype × environment interaction was present in terms of grain yield since plasticity for biomass production under drought stress conditions was not translated directly to yield compensation for some of the lines. Nevertheless, several lines selected for high yield were identified to surpass their source material and best checks in each environment, while one line (IH4-4) outperformed consistently by 62.99% on average, in terms of grain yield, the best check across all environments. In addition, improved agronomic performance under drought conditions induced an indirect effect on some grain quality traits. Most of the lines selected for high yield maintained or even improved their grain protein content in comparison to their source material (average increase by 2.33%). On the other hand, most of the lines selected for low yield indicated a poor agronomic performance, further confirming the coherence between selection under ultra-low density and performance under dense stand.

Keywords: barley, buffering capacity, drought conditions, single-plant selection, ultra-low density, yield compensation

INTRODUCTION

On a global scale, barley (*Hordeum vulgare* L. subsp. *vulgare*) ranks fourth among cereals in terms of production quantity, after wheat, maize, and rice, providing nutrient benefits for both livestock and humans (Newton et al., 2011; FAOSTAT, 2021). Barley is a member of the grass family (Poaceae). It is a versatile crop with the ability to adapt to unfavourable conditions that distinguish it as one of the best models and most suited crops for studying adaptation to climate change (Dawson et al., 2015). Despite its resilient nature to climate disruptions, high relative yield gap rates have been estimated for barley crop, ranging between 12 and 75% for the rainfed systems in Europe (Schils et al., 2018) and up to 25% for the rainfed barley fields in Alberta, Canada (Chapagain and Good, 2015). Volatile climate conditions, management practices, genetic factors, as well as social restrictions on the use of inputs and economic disincentives to intensify crop production are amongst the main causes of considerable variation and stagnated or even declined yields for barley and other major crops (Peltonen-Sainio et al., 2009; Lin and Huybers, 2012; Mueller et al., 2012; Tokatlidis, 2014; Ray et al., 2015; Hochman and Horan, 2018).

In this regard, climate change seems to induce severe yield losses for barley crops mainly due to an increase in maximum temperature during the grain filling period causing heat stress, as well as due to an increased frequency of drought events during the stem elongation period (Brisson et al., 2010; Bento et al., 2021). Climate change impacts and, hence, their consequences do not follow an evenly distributed spatial pattern with their magnitude varying from region to region (Trenberth, 2011). Among the most fragile areas, the Mediterranean region has been well recognised as a prominent climate change hot spot (Diffenbaugh and Giorgi, 2012; Alessandri et al., 2014). Mediterranean environments are characterised by high inter-annual variability of temperature and rainfall patterns, increasing the uncertainty of maintaining production at higher levels (Cammarano et al., 2019). This unforeseen variation is likely to affect yield and yield quality directly, due to impact on crop physiology and indirectly, due to alterations in nutrient mineralisation and availability for crops (Henson, 2011; Cammarano et al., 2019).

However, the resource-limited regime in crop stands results in plant-to-plant competition due to the concurrent demand of individual plants for the available resources (Weiner and Damgaard, 2006). As highlighted by Farrior et al. (2013), resource limitation creates incentives for plants to over-invest in resource capture at levels that are suboptimal for the productivity of a plant in isolation but pay off for the plant interference with the others, such as investment in height growth for light capture or in fine roots for belowground resources. Competition between individuals within a crop may lead to developmental dissimilarities and intra-crop inequality (Weiner and Damgaard, 2006; Tokatlidis, 2017). This intra-crop inequality, in turn, further aggravates the unequal share of limited resources, thus intensifying inter-plant competition, functioning as a vicious circle between plant asymmetry and competition that perpetuates all along the crop cycle. Evidently, this condition affects plant growth detrimentally to crop yield performance (Pan et al., 2003; Pagano and Maddonni, 2007). Complexity is exacerbated under high stand densities since plants are more prone to early established inequalities leading to pronounced morphological and physiological differences which in turn affect resource use efficiency during critical developmental stages of the crop (Tollenaar et al., 2006; Rossini et al., 2011; Yan et al., 2017; Sher et al., 2018).

Planting density is one of the key factors in achieving crop uniformity by minimising interplant competition and ensuring an equal share of resources, eventually attaining maximum profitability. Recommending an optimum planting density is not an easy task, since the relationship between planting density and grain yield is governed by several parameters that fall under the genotype, environment, and crop management effect (Assefa et al., 2016, 2018; Carciochi et al., 2019; Bastos et al., 2020). In wheat, for example, Bastos et al. (2020) concluded that for high yielding environments and less limited resources the number of plants required to maximise yields was very low and below any commercially recommended number of plants for this crop, while for low yielding environments a higher density was needed to sustain maximum yields. Likewise, according to Matsuyama and Ookawa (2020), a lower seeding rate than the one commonly practiced in Japan was more suitable in achieving high yields and improved lodging resistance for those wheat cultivars that were characterised by a high number of grains per spike when these cultivars were planted in soils with abundant resources. In barley, most of the research concludes that a seeding rate which establishes between 300 and 360 plants m^{-2} is usually the optimal one (Thomason et al., 2009; O'Donovan et al., 2012; Perrott et al., 2018). However, the recommended density can vary considerably depending on the field properties and climate conditions or even on the interaction with the genotype (Jedel and Helm, 1995; O'Donovan et al., 2012; Bekele et al., 2020). Undoubtedly, the main constraint to define optimum plant density lies on the large environmental variability that occurs in a particular zone across seasons, as well as on the unpredictability of the inter-annual variation in terms of weather conditions, and predominantly in the amount and distribution of atmospheric precipitations in the rain-fed cropping systems (Tokatlidis, 2014). Therefore, Tokatlidis (2017), highlights the importance of breeding to target varieties that are characterised by homeostasis, that is the ability to withstand external forces that induce acquired plant-to-plant inequality and concomitant intra-crop competition, as well as by density-independence, to perform satisfactorily at relatively low densities.

Since intra-crop inequality and inter-plant competition are related to high densities, a condition for selection under ultra-low density that excludes plant-to-plant interference for resources (i.e., nil-competition) is a prerequisite. Such a condition exploits the honeycomb breeding model (Fasoulas, 1988, 1993). Owing to their systematic entry arrangement, locating each plant in the centre of a circular replicate/ring to ensure increased local control and allocating the plants of each entry in a moving triangular grid spread across the whole field for an effective sampling of soil heterogeneity, honeycomb designs objectively evaluate sister-lines and apply single-plant selection under a pattern of ultra-low planting density (Fasoulas and Fasoula, 1995). The nil-competition regime maximises the phenotypic expression of genetic differences among individuals, facilitating, further, the detection of desirable genotypes (Kyriakou and Fasoulas, 1985; Fasoula and Fasoula, 2002; Tokatlidis et al., 2010). Moreover, the selection under ultra-low density erases the confounding effects of competition on the identification of high yielding genotypes, induced by the negative relationship between yielding and competitive ability (Kyriakou and Fasoulas, 1985; Chatzoglou and Tokatlidis, 2012; Ninou et al., 2014), while attaining greater heritability by minimising the acquired variance arising from non-genetic sources (Fasoula and Fasoula, 2002; Tokatlidis, 2015). The computation of mathematical parameters that account for relative plant yield efficiency and stability of performance is easily performed and can be applied from the early stages of selection for selecting superior plants, thus reducing the time frame required for the release of improved varieties.

Considering the challenges imposed by climate variation and volatility and the need to expand the range of optimum planting density in field crops, the development of barley cultivars with an innate buffering capacity to perform well enough under varying and unpredictable climate conditions and making optimum use of the available resources, sound as a prudent approach to reduce the gap between actual and attainable yield in barley crop. Hence, the objective of the present study was to investigate the performance and buffering capacity of barley lines under favourable and drought stress conditions in Tunisia. These lines were derived from three commercially released cultivars and two Tunisian landraces, using single-plant selection at ultra-low density. Furthermore, the potential to exploit latent or *de novo* variation within barley cultivars for the development of high-yielding lines with elevated homeostasis and competent qualitative traits is discussed.

MATERIALS AND METHODS

Plant Material

To obtain the barley lines evaluated in this study, selection started in 2014 cropping season among five commercially released cultivars in Tunisia (Imen, Kounouz, Lemsi, Manel, and Rihane) and two Tunisian landraces (Ardhaoui and Djebali) planted under the ultra-low density of 1.2 plants/m^2 according to an R-7 honeycomb field layout (Fasoulas and Fasoula, 1995). The selection between entries was based on the computation for each of the entries of the three parameters described by Fasoula and Fasoula (2000), that is (i) the entry's mean ($\bar{x}$), (ii) the entry's standardised mean ($\bar{x}/_{s}$), and (iii) the entry's standardised selection differential ($\frac{\bar{x}_{sel}-\bar{x}}{s}$). Then, divergent single-plant selection for high and low yield within the top entries was applied by the moving-circle procedure (Fasoulas and Fasoula, 1995) to form the first cycle selected lines. These lines along with the best commercial checks of the region were further subjected to selection in the following cropping season, by applying the same principles of single-plant selection for high and low yield under an ultra-low-density regime of 1.2 plants/m^2 according to an R-21 honeycomb field layout (Fasoulas and Fasoula, 1995). In both years, the selected high-yielding plants were the ones that showed the highest grain weight compared with the mean of the 36 surrounding plants (i.e., 0.027 selection pressure). Low-yielding individuals were identified using the same selection pressure, but in this case, selected plants should weigh at least 10 g of grains and then bulked according to the source material, to get enough seeds for the next selection cycle and for further evaluation. The whole procedure resulted in 12 first cycle lines (8 high yielding and 4 low yielding) and 38 second cycle lines (30 high yielding and 8 low yielding) to be assessed in the next seasons' dense stand trials. The honeycomb experimental field layouts and the selection procedure applied for two consecutive years are described in detail by Ben Ghanem et al. (2018). A summary of the selection history of the progeny lines is given in **Table 1**.

Field Evaluation Trials

In the 2016 growing season, the 50 first and second cycle selected lines along with five checks (source seed lots of Imen, Ardhaoui, Djebali, Manel, and Rihane) were planted as dense stand field trials at the National Agricultural Research Institute of Tunisia (INRAT) experimental stations in El Kef (36° 14′ N; 8° 27′ E; 518 m) and Mornag (36° 37′ N; 10° 17′ E; 54 m) in Tunisia. These materials were also planted as dense stand trial the following growing season at Mornag experimental station. The two research stations represent two distinct production environments for Tunisia. Mornag is characterised by clay soil and average annual precipitation of 450 mm. El Kef is

TABLE 1 | Selection history of the single-plant progeny lines derived through divergent selection at ultra-low density and evaluated at the dense stand trials (modified by Ben Ghanem et al., 2018).

Source material	First cycle HY lines	First cycle LY lines	Second cycle HY lines	Second cycle LY lines
Ardhaoui	AH9, AH10	AL0	AH9-H1, AH9-H2, AH9-H3, AH10-H1, AH10-H2, AH10-H3	AH9-L0, AH10-L0
Imen	IH4, IH16, IH17	IL0	IH4-H1, IH4-H2, IH4-H3, IH4-H4, IH16-H1, IH16-H2, IH16-H3, IH17-H1, IH17-H2, IH17-H3, IH5-VS	IH4-L0, IH16-L0, IH17-L0
Djebali	DH2, DH12	DL0	DH2-H1, DH2-H2, DH2-H3, DH2-H4, DH2-H5, DH12-H1, DH12-H2, DH12-H3, DH14-VS	DH2-L0, DH12-L0
Manel	MH18	ML0	MH18-H1, MH18-H2, MH18-H3	MH18-L0
Rihane	–	–	RH8-VS	–

The coding of lines is based on two letters and the number of the selected plant. In the case of the bulk sample, this is indicated with 0. The first letter indicates the source material from which the line has been selected (A stands for Ardhaoui, I for Imen, D for Djebali, M for Manel, and R for Rihane). The second letter indicates whether the selection is based on high yield (H) or low yield (L). Cases indicated with VS, stand for visual selection.

TABLE 2 | Monthly precipitation at the two experimental sites for the growing seasons of selection and evaluation trials.

Site	Growing season	Trial type	September	October	November	December	January	February	March	April	May	June	July	August	Total
El Kef	13/14	Selection	52	25	103	52	52	35	81	8	53	17	0	1	479
El Kef	14/15	Selection	20	35	43	74	70	66	66	0	22	2	5	46	449
El Kef	15/16	Evaluation	18	26	48	5	55	13	89	30	32	5	0	2	325
Mornag	15/16	Evaluation	6	57	44	23	18	40	63	14	25	0	0	5	295
Mornag	16/17	Evaluation	80	33	77	140	53	36	4	16	0	19	0	0	458

characterised by clay loam soil and average annual precipitation of 452 mm with barley being the most common rainfed crop of the region. The monthly precipitation at the two experimental sites for the growing seasons during which the selection and evaluation trials were held is given in **Table 2**.

A non-replicated augmented design field trial was established in all cases, with five incomplete blocks and 15 entries per block. Plots were composed of four rows of 2.5 m long, each with 0.25 m spacing between rows, occupying a plot area of 2.5 m^2. Plot by plot distance within the same alleyway was 0.75 m and between alleyways 1.5 m. All trials were planted under a uniform seed rate of 360 seeds/m^2. To ensure the robust establishment of field plots, seeds were treated before planting with Celest top [Diféconazole (25 g/L) + Fludioxonil (25 g/L) + Thiamethoxam (262.5 g/L)] at a rate of 200 ml/hl of seeds. Basic fertiliser in the form of diammonium phosphate (18-46-0) was applied before planting at a rate of 100 kg/ha. Complete weed control was attained by chemical applications (Axial: pinoxaden (100 g/L) + cloquintocet-methyl (25 g/L) at a dose of 1 L/ha for the narrow leaf weeds and Zoom: dicamba (66%) + Triasulfuron (4%) at a dose of 180 g/ha for the broadleaf weeds) and hand weeding. Two spring foliar spray applications of Ogam [Kresoxim-methyl (125 g/L) + Epoxiconazole (125 g/L)] at a rate of 0.7 L/ha were applied as a preventive measure to minimise yield reductions due to fungal diseases. The harvest took place beginning of June, and all four rows per plot were harvested.

Data Records for Agronomic and Physiological Traits

Several agronomic and physiological traits were recorded across the three environments. Regarding agronomic traits, biological yield (BY: t/ha) and grain yield (GY: t/ha) per plot were measured at maturity and, harvest index (HI) was derived as the quotient between grain and biological yield. Plant height (PH) was measured at maturity from five randomly selected plants within each plot and recorded as the distance in centimetres from soil level to the tip of spikes excluding the awns. Spike length (SL) was recorded as the average of ten representative spikes of each plot from the base up to the tip of the spike. Each of these spikes was then threshed individually and the average grain weight per spike (SGW) expressed in g for each of the entries was also recorded. Powdery mildew (PM) reaction was scored based on the prevalence of the disease at the seedling stage at El Kef and Mornag stations during the 2016 cropping season based on a disease severity scale from 1 to 5, with 1 as no symptoms and 5 as highly susceptible.

For physiological parameters, measurements were performed only in the 2016 growing season in the two locations where the trials had been planted. Soil Plant Analysis Development (SPAD) values at the heading stage SPAD were measured on fully expanded flag leaves of three representative plants of each plot using a MINOLTA SPAD 502 Plus chlorophyll meter. Leaf canopy temperature (LCT) was recorded as the average of five representative positions within each plot using an infrared scantemp 440 thermometer. Chlorophyll fluorescence F_0, F_m, and F_v parameters were measured at heading time at the fully expanded flag leaves of the three representative plants within each plot, for which the SPAD values were also taken, using an OPTI-SCIENCE 0530 + handheld portable fluorometer. These measurements were then used to calculate the ratios F_v/F_m and F_v/F_o and thus, test for differences in the activity of photosystem II (PSII).

Grain Quality Parameters

Representative grain samples from all field plots of the two locations planted in the 2016 cropping season were transferred and evaluated in International Center for Agricultural Research in the Dry Areas (ICARDA) Quality Laboratory. In particular, grain colour, morphology, physiochemical parameters, and β-Glucans content were assessed.

Grain Morphology and Grain Colour

Random samples of 70 grains were received from all seed lots representing each plot at the field and scanned using a flatbed scanner (CanoScan LiDE 220; Canon). The images collected were analysed using Grainscan software (Whan et al., 2014), which generated the morphological and colour profile for every single grain. Grain morphology traits, such as perimeter in mm (PRM), grain length in mm (LNG), and width in mm (WDT) were calculated for each sample as means of the 70 seeds. In addition, a colour channel intensity output similar to the standardised CIELAB colour space produced by the software (Whan et al., 2014). The GrainScan colours (ColCha1, ColCha2, and ColCha3) were therefore considered proxies for L, a, and b, respectively, representing the lightness of the colour, green or magenta, and blue or yellow.

Physiochemical Parameters

Barley protein content (PRT) and starch (STRCH) were determined using near-infrared spectroscopy (NIR, Infratec 1241, Foss). To determine the β-glucan content (β-GLC) the calcofluor-fluorimetric method using a flow analyser (SKALAR san^{++}) was employed. Before this determination,

TABLE 3 | Genotypic and environmental effects and their interaction on the agronomic traits of barley lines selected under ultra-low density when evaluated under dense stand trials in different environments in Tunisia.

A. Traits recorded in three environments

Source of variation	DF	BY	GY	HI	PH
Entry	54	36.9***	46.2***	36.3***	52.6***
Environment	2	4.8***	36.5***	3.3	5.6***
Entry × Environment	108	9.4	12.5*	12.2**	12.0

B. Traits recorded in two environments

Source of variation	DF	SL	SGW	TKW	PM
Entry	54	12.5***	35.4***	15.9***	32.5***
Environment	1	2.1	35.4**	31.9	3.6
Entry × Environment	54	17.9	35.4*	31.5	33.6

**Significant at α = 0.05; **Significant at α = 0.01; ***Significant at α = 0.001.*

an acid extraction was carried out according to the method recommended by the European Brewery Convention (Manzanares and Sendra, 1996). Briefly, 100 mg of barley flour was weighed. A volume of 10 mL distilled water was added jointly with 100 μL of alpha-amylase and dispersed with a vortex mixer. Then, the tube was boiled for 1 h and after cooling, 10 ml of sulphuric acid was added. The mixture was homogenised, boiled for 10 min, cooled to room temperature, and finally centrifuged and the aliquot filtered prior to being loaded into the sampler of the flow analyser.

Data Analysis

Raw data values for agronomic, physiological, and grain quality traits were analysed by employing the analysis of variance using linear mixed models. For this purpose, locations and years were combined into a single factor (environment). Genotypes (entries), environments, and entry × environment interaction were considered as fixed effects, while the block effect and the plot effect nested in each block as random. Based on this model, the best linear unbiased estimations (BLUEs) were computed for all recorded traits. To identify the best performing lines across and within each environment, entries were analysed in relation to their source material by performing a GGE biplot analysis based on the grain yield BLUEs values of the entries in each distinct environment. In addition, Pearson correlation coefficients between all recorded traits were computed and a heat map was generated based on correlations. Statistical analysis was performed with JMP statistical package ver. 14.0.0.

RESULTS

Agronomic Performance Traits

The combined ANOVA revealed a significant effect of the environment for BY, GY, PH, and SGW traits, while there was no effect for HI, SL, TKW, and PM (**Table 3**). The three environments differed considerably in terms of annual precipitation with Mornag_16 being the driest one with 295 mm of rainfall, followed by El Kef_16 receiving 325 mm of rain. A very different annual precipitation pattern was observed the following year when the Mornag_17 environment recorded a total of 458 mm of rain (**Table 2**). Hence, in terms of BY the lowest values were recorded at the driest environment Mornag_16 with a mean value of 3.75 t/ha reduced by 46 and 48% compared to the respective BY values in El Kef_16 and Mornag_17 (**Table 4**). The same trend was also revealed for GY with the driest environment Mornag_16 to indicate a mean value of 1.46 t/ha, being significantly lower from the mean GY in El Kef_16 and Mornag_17 with the difference exceeding 1 t/ha (**Table 4**). Regarding the PH, distinct values were recorded among the three environments, with Mornag_17 demonstrating the tallest stands with an average value of 81.63 cm, followed by a 13 and 33% reduction at El Kef_16 and Mornag_16 environments, respectively (**Table 4**). Furthermore, the driest environment Mornag_16 revealed the lowest values for the SGW, with the mean value of 2.13 g being by 7% reduced by the respective value in the El Kef_16 environment (**Table 4**).

Significant entry effects were revealed for all the recorded agronomic traits (**Table 3**). Almost for all traits the effect of selection status, i.e., first- and second-year HY and LY lines and source materials, as well as the effect of the source variety/landrace of the derived lines was significant (**Figure 1**). More specifically, the first- and second-year HY lines recorded the highest BY values at El Kef_16 with a total biomass of 7.65 and 7.46 t/ha, respectively, surpassing the original genotypes

TABLE 4 | Agronomic traits means and confidence intervals of barley entries evaluated in different environments in Tunisia.

	El Kef_16			Mornag_16			Mornag_17		
Trait	**Mean**	**Lower 95%**	**Upper 95%**	**Mean**	**Lower 95%**	**Upper 95%**	**Mean**	**Lower 95%**	**Upper 95%**
BY (t/ha)	6.90	6.522	7.276	3.75	3.495	4.007	7.18	6.672	7.697
GY (t/ha)	2.49	2.275	2.713	1.46	1.325	1.596	2.78	2.516	3.039
HI	0.36	0.339	0.384	0.38	0.3567	0.400	0.37	0.358	0.392
PH (cm)	71.04	68.717	73.362	54.73	53.209	56.258	81.63	79.749	83.505
SL (cm)	7.23	7.059	7.398	7.09	6.950	7.228	NA	NA	NA
SGW (g)	2.29	2.218	2.366	2.13	2.037	2.216	NA	NA	NA
TKW (g)	34.63	32.767	36.484	33.53	31.195	35.868	NA	NA	NA
PM	2.67	2.545	2.788	3.19	3.037	3.337	NA	NA	NA

NA, Not applicable, measurements not made.

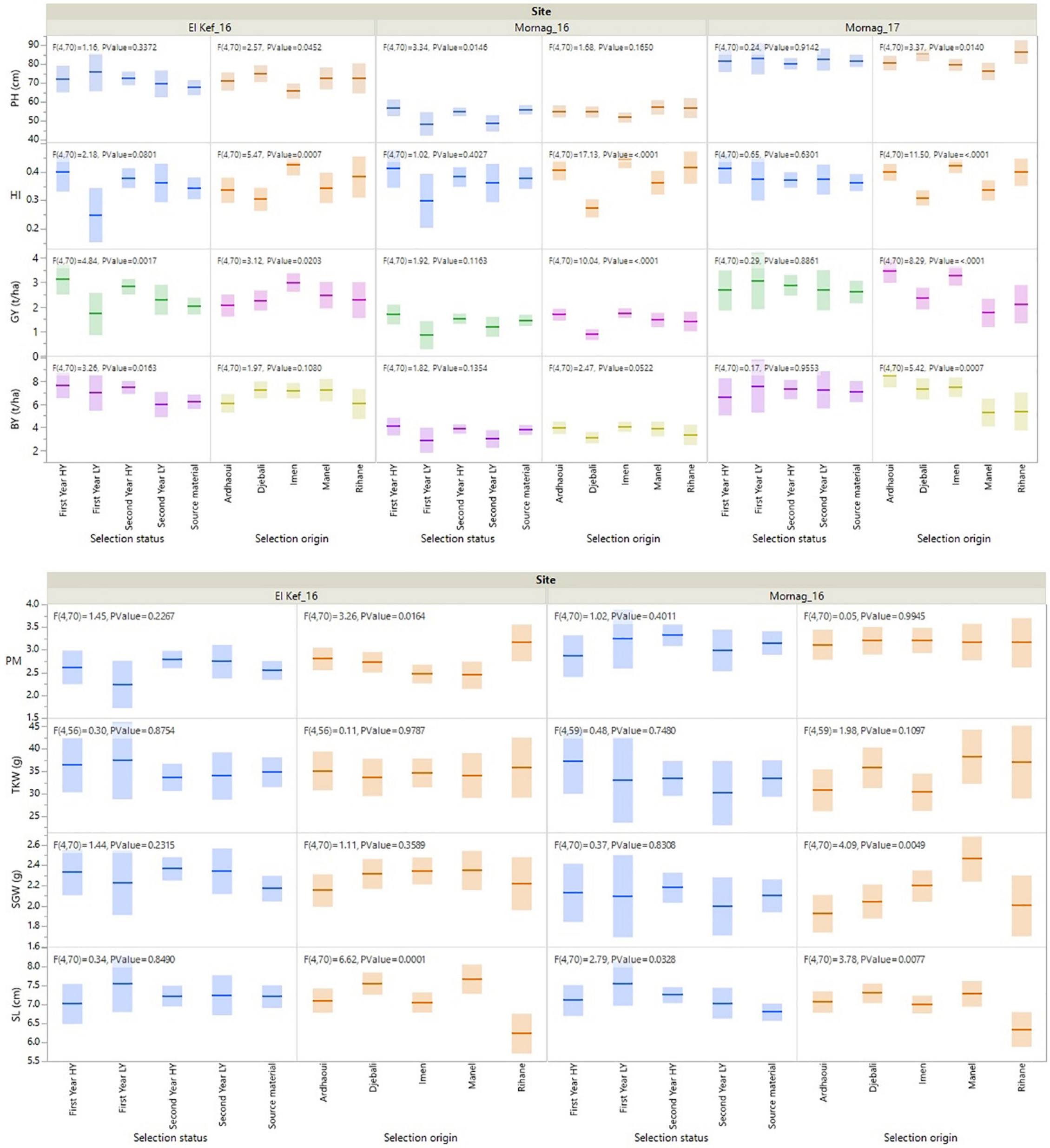

FIGURE 1 | Fit of means for the agronomic traits of barley lines selected under ultra-low density when evaluated under dense stand trials in distinct environments in Tunisia.

by 20%. Moreover, for the same environment, the first- and second-year HY lines showed the highest GY with 3.14 and 2.84 t/ha outperforming on average the source materials by 36 and 23%, respectively (**Figure 1**). No significant effects were found for the HI based on the selection status of the lines, even though a clear trend for high HI values was revealed for the first year HY lines that recorded a mean HI value of 0.41 across the three environments compared to the 0.36 HI value of the source materials. Concerning PH, the first- and second-year LY lines showed the lowest values at the Mornag_16 environment, with significantly shorter stands by a minimum of 6 cm compared to all other lines (**Figure 1**).

For the agronomic traits recorded in two environments, the first-year LY lines revealed the longest spikes in the Mornag_16 environment with an average of 7.56 cm, longer by 11% in comparison to the average length of the source materials. The same trend for the first year LY lines was revealed also at the Kef_16 environment, however, in this case, the differences did not reach the significance level (**Figure 1**). Despite the differences in terms of SL, no significant effects were found for the SGW based on the selection status of the lines in both environments. The same was also true for the TKW and PM, traits for which the selection status of the lines did not reveal any significant difference (**Figure 1**).

When the effect of the source variety/landrace of the derived lines was assessed, the lines derived from variety Imen appeared consistent high values across the three environments with a mean BY of 6.26 t/ha producing on average 12 and 21% more biomass than the lines acquired from Manel and Rihane varieties, respectively (**Figure 1**). For the lines originated from the two landraces, the ones from Ardhaoui produced high biomass with a mean BY value of 6.20 t/ha across the three environments, while the lines from Djebali showed contrasting results being among the high biomass producing lines for the favourable environment of Mornag_17 but ranked amongst the least producing lines for the dry environment of Mornag_16 (**Figure 1**). In all three environments, lines derived from variety Imen were among the high yielders with a mean GY value of 2.69 t/ha outperforming significantly the lines acquired from Rihane, Manel, and Djebali by 26, 29, and 32%, respectively (**Figure 1**). Only lines from Ardhaoui showed similar high GY values to Imen derived lines, even though in one of the environments, El Kef_16, these lines indicated also a significantly lower GY value by 31% (**Figure 1**). The same pattern for GY was also depicted for the HI trait, for which lines originated from Imen showed a mean value of 0.43 across the three environments, being significantly higher from the mean HI values of Manel and Djebali derived lines by 19 and 30%, respectively (**Figure 1**). Concerning PH, lines originated from Djebali and Rihane were those that demonstrated the tallest stands with the differences being more profound in the Mornag_17 environment, where these lines showed mean PH values of 86.86 and 85.47 cm, respectively, surpassing the lines derived from Imen and Manel (**Figure 1**). Three distinct groups based on the source of the derived lines were shaped for SL. Lines originated from Manel and Djebali recorded the longest spikes with 7.49 and 7.44 cm, respectively, significantly higher from the group of Ardhaoui and Imen lines with 7.09 and 7.03 cm, as well as from the lines derived from variety Imen, which showed the shortest spikes with a mean value of 6.29 cm across the two environments that the measurement recorded (**Figure 1**). For the SGW, differentiation was found only in Mornag_17, where the lines acquired from Manel recorded a mean SGW value of 2.47 g being higher by 17, 19, and 22% from the respective values of the lines originated from Djebali, Rihane, and Ardhaoui (**Figure 1**). For PM, the lines acquired from Rihane showed higher susceptibility in the environment Kef_16 compared to all other lines recording average symptoms higher than the value of 3 in the disease scale (**Figure 1**). No differentiation was revealed for the TKW based on the source of the variety/landrace of the derived lines (**Figure 1**).

Among all agronomic traits, a significant genotype × environment interaction (G × E) was observed for GY, HI, and SGW. No significant G × E effects were detected for BY, PH, SL, TKW, and PM with the lines demonstrating a consistent performance across all the environments for these traits (**Table 3**). To a large extent, the significant G × E effects for GY were due to the contrasting performance of first-year LY lines, which were found to be the less productive lines in El Kef_16 and Mornag_16 environments showing a significant gap in GY compared to the first- and second-year HY lines by 47

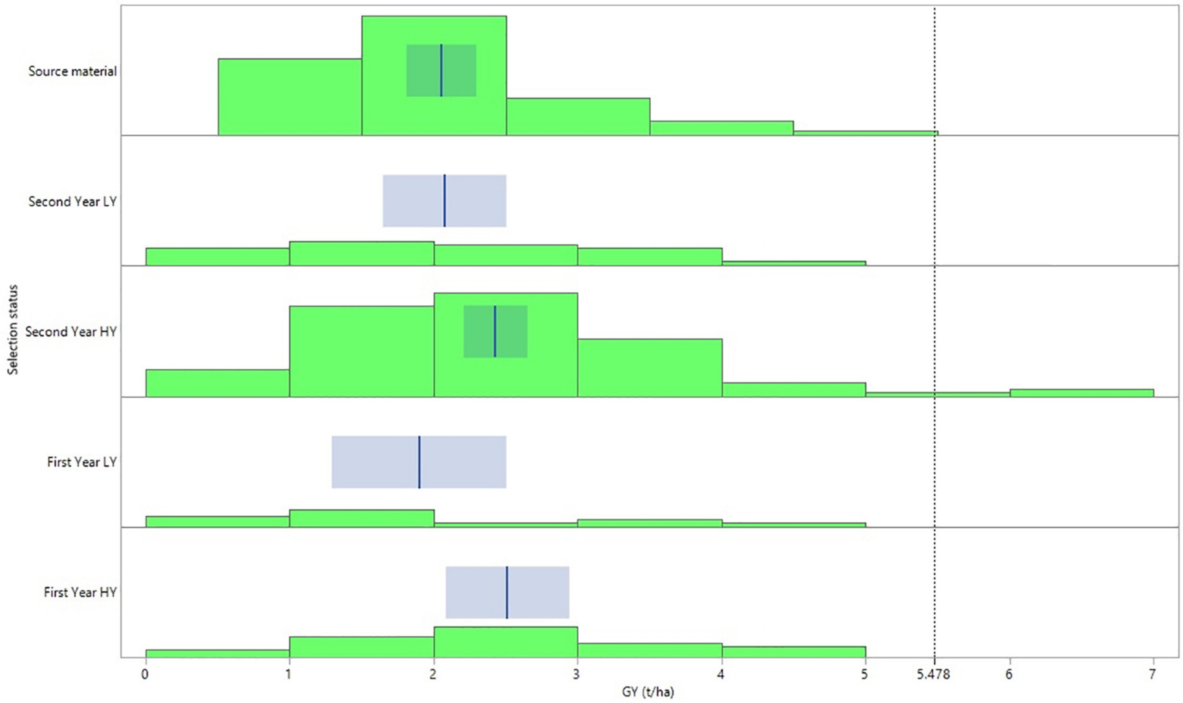

FIGURE 2 | Distribution graph and fit of means for grain yield performance according to the selection status of the barley lines evaluated under dense stand trials in three environments in Tunisia.

and 41%, respectively, while ranked at the top as an average GY performance in the environment of Mornag_17, even though the differences with the other lines did not reach significance levels (**Figure 1**). An increase in HI at Mornag_17 environment was also apparent in the first year LY lines, since this index increased for these lines from 0.28 in El Kef_16 and Mornag_16 to 0.38 in Mornag_17, while all other lines maintained the same value of HI across all environments (**Figure 1**).

Despite the significant G × E effect for GY, the general pattern across the three environments reflected with high consistency the selection status of the lines (**Figure 2**). Thus, the first- and second-year HY lines demonstrated the higher mean values for grain yield with 2.51 and 2.43 t/ha, significantly outperforming the source materials. The source materials in turn revealed the same GY mean value with the second year LY lines reaching at 2.05 and 2.07 t/ha, respectively. The least performing lines in terms of grain yield were the first-year LY lines with a mean GY of 1.90 t/ha across the three environments (**Figure 2**). Furthermore, the group of the second year HY lines was the only one in which some of the lines demonstrated a mean GY across the three environments that exceeded the right cutting-edge threshold value of the curve (5.478 t/ha), defined by the overall mean GY value plus three standard deviations (**Figure 2**).

To avoid biased assumptions from a joint analysis due to the significant effects of the source material of the derived lines, GGE biplot analysis was performed separately for each of the different source varieties/landraces and their respective derived lines (**Figure 3**). Based on the analysis, the three environments were very contrasting for the lines derived from Ardhaoui and none of the lines recorded high grain yield in all three environments. Combining environments by two showed that most of the first- and second-year HY lines demonstrated high grain yield, while the first- and second- year LY lines, as well as the original population of Ardhaoui, either performed well only in one environment each time or their performance was poor for all the three environments (**Figure 3**). Four second-year HY lines from Djebali (DH2-3, DH2-4, DH2-5, and DH12-1) revealed high grain yield across all environments compared to their original population, with their scores to be plotted among the vectors that defined the three evaluation environments (**Figure 3**). Contrary, the second-year LY lines (DH2-L0 and DH12-L0) were the ones with the lowest grain yield among all

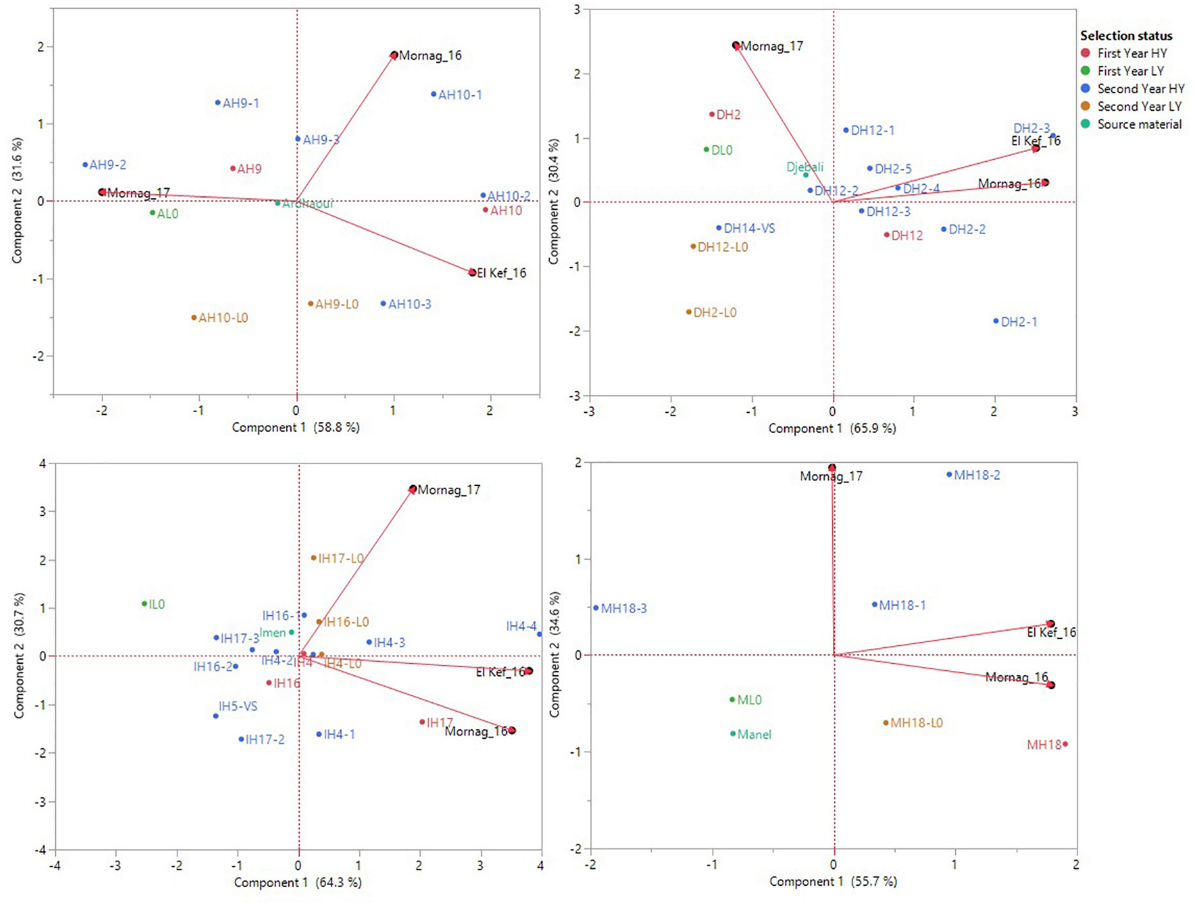

FIGURE 3 | Source material-based GGE biplot analysis for grain yield performance of the barley lines evaluated under dense stand trials in three environments in Tunisia. Upper left: landraces Ardhaoui and derived lines, upper right: landrace Djebali and derived lines, bottom left: variety Imen and derived lines, bottom right: variety Manel and derived lines. Due to the limited number of lines derived from variety Rihane, GGE biplot analysis was not performed.

Djebali lines (**Figure 3**). A more diverse pattern of selection status revealed high grain yield across the three environments for the lines derived from the variety Imen. Two second-year HY lines (IH4-4 and IH4-3) were predominantly the ones showing the highest grain yield for all three environments. Furthermore, line IH4-4 was the one that revealed consistently the highest grain yield for the three environments amongst all the entries tested, ranking first in El Kef_16 and third in Mornag_16 and Mornag_17 with a mean grain yield of 4.44 t/ha (**Figure 3**). Another one second-year HY line (IH17-1) and one second-year LY line (IH4-L0) derived from variety Imen showed good performance for grain yield for all three environments, while surprisingly three second-year HY lines (IH16-2, IH17-2, and IH5-VS) and one first-year HY line (IH16) were those with poor performance in all three environments (**Figure 3**). Having Manel as source material, two second-year HY lines (MH18-2 and MH18-1) showed consistency in terms of high grain yield in all the three environments, contrary to one first-year LY line and the source material of variety Manel that demonstrated low grain yield in each environment that the evaluation took place (**Figure 3**).

Physiological Parameters

Regarding the physiological parameters, combined ANOVA revealed significant environmental effects for SPAD and LCT. Mornag_16 environment-induced higher values for the barley lines in comparison to El Kef_16 (**Tables 5**, **6**). However, no significant G × E effects were revealed for none of the recorded physiological parameters in the trials (**Table 5**).

Significant entry effects were revealed for these physiological traits (**Table 5**). Across all environments, the second year HY line IH4-4, which showed a consistent elite performance in terms of grain yield, was the one that exhibited the highest values for the ratios related to the photosynthetic activity with 0.76 for F_v/F_m and 3.3 for F_v/F_0, significantly higher than the respective ratios of almost all other lines (**Figure 4**). Meanwhile, its source variety Imen was ranked among the entries that showed the lowest ratios for the two parameters of PSII (**Figure 4**). No other specific pattern, however, was observed, by means of selection status or source materials from which the lines were derived, regarding the F_v/F_m and F_v/F_0 ratios (**Figure 4**). For LCT the second year HY line IH4-4 was again the one indicating the highest value among all other lines with a mean leaf canopy temperature of 25.7°C across all environments (**Figure 4**). Even though there was no specific pattern for LCT in terms of selection status or source material from which the lines were derived, a trend for high LCT values was observed for all the original varieties/landraces that were ranked among the top entries indicating high mean temperature values (**Figure 4**). As for the SPAD parameter, a trend for low SPAD values appeared for the lines derived by Djebali with a mean of 45.66. However, this trend did not reach a significant level when lines from Djebali were compared to the lines of other source materials (**Figure 4**).

TABLE 5 | Genotypic and environmental effects and their interaction on the physiological parameters of barley lines selected under ultra-low density when evaluated under dense stand trials in different environments in Tunisia.

Source of variation	DF	F_v/F_m	F_v/F_0	DF	SPAD	LCT
Entry	54	17.7***	34.0***	54	15.2***	26.3***
Environment	2	1.2	1.5	1	2.3***	1.5*
Entry × Environment	108	6.5	6.0	54	19.7	12.1

**Significant at α = 0.05; ***Significant at α = 0.001.*

Grain Quality Parameters

Significant environmental effects on grain quality were detected for seed colour parameters as well as for the starch content (**Table 7**). The environment of Mornag_16 favoured the colour lightness and colour intensity of the grains with the three-colour parameters recording mean values of 152.55 for CLR_a, 119.47 for CLR_b, and 174.68 for CLR_L, significantly higher than the ones in El Kef_16, where the mean values for CLR_a, CLR_b, and CLR_L were 148.90, 114.05, and 172.29, respectively (**Table 8**). The starch grain content appeared to be higher in El Kef_16 with a mean value of 51.67% surpassing the respective mean starch content value of 50.43% in the Mornag_16 environment (**Table 8**). Regarding the G × E interaction, only a few of the considered grain quality parameters showed a significant effect. Thus, significant G × E interactions were limited to CLR_b and β-GLC, while all other grain quality parameters did not reveal any interaction between the barley lines and the environment (**Table 7**).

Highly significant entry effects were indicated for all the grain quality parameters, from the grain shape and size (PRM, LNG, WDT) up to the colouration (CLR_a, CLR_b, CLR_L) and seed nutrient content (PRT, STRCH, β-GLC) (**Table 7**). A clear trend based on the source materials that the lines derived was observed for the grain shape and size traits. Lines originating from Djebali showed significantly longer grains than all other lines with a mean LNG value of 11.24 mm. On the contrary, lines derived from variety Imen were the ones with the shortest grain length with a mean value of 9.93 mm (**Figure 5**). Djebali lines also showed a high value for grain width ranked second after the lines acquired from Manel for the specific trait. Thus, the mean WDT values for lines acquired from Manel was 3.02 mm, significantly higher than the value of 2.97, which was the mean value of lines derived from Djebali (**Figure 5**). The high LNG and WDT values from Djebali lines had a direct impact on the grain perimeter for which these lines were top-ranked with a mean value of 33.14 mm with a difference of a minimum of 3 mm in terms of perimeter compared to all other lines (**Figure 5**).

Regarding the lightness of the grain colour, no specific trend was indicated for barley lines. Some of the lines originated from Manel and Djebali, such as DH12-3, DH2-3, DH12-L0, MH18, MH18-2 appeared to be the ones with the lighter grain colour indicating significantly higher values for CLR_L compared to most of the other lines (**Figure 5**). However, these differences were more profound and source material specific for colour intensity. In this case, lines originated from Djebali showed, on average, higher values for CLR_a and CLR_b with a mean of 152.13 and 119.75, respectively, while lines derived from Imen were the ones that had lower values among all entries with CLR_a mean value 148.99 and CLR_b mean value 113.54 (**Figure 5**).

TABLE 6 | Physiological parameters means and confidence intervals of barley entries evaluated in different environments in Tunisia.

	El Kef_16			Mornag_16			Mornag_17		
Trait	**Mean**	**Lower 95%**	**Upper 95%**	**Mean**	**Lower 95%**	**Upper 95%**	**Mean**	**Lower 95%**	**Upper 95%**
F_v/F_m	0.66	0.651	0.663	0.69	0.684	0.702	0.70	0.689	0.711
F_v/F_0	1.97	1.921	2.028	2.36	2.268	2.455	2.41	2.302	2.515
SPAD	42.48	41.760	43.195	51.66	51.061	52.256	NA	NA	NA
LCT	20.11	19.380	20.839	23.77	23.473	24.073	NA	NA	NA

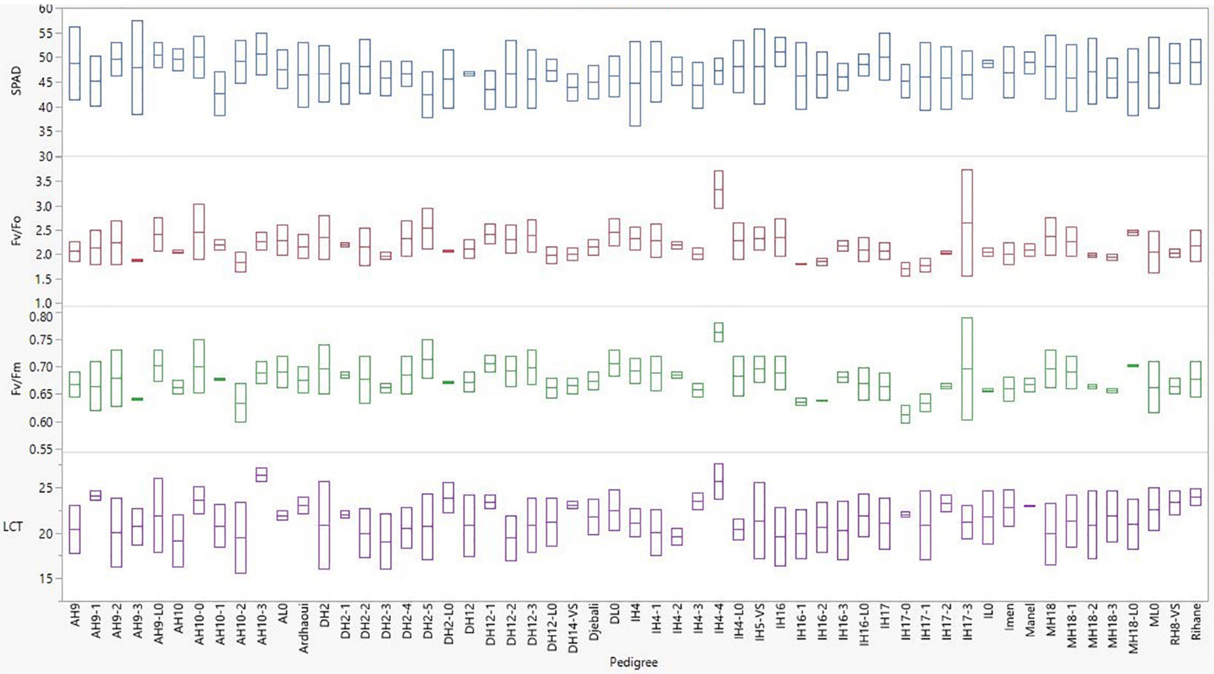

FIGURE 4 | Entry box plots for the physiological parameters measured during the evaluation of the barley lines in the distinct environments in Tunisia.

TABLE 7 | Genotypic and environmental effects and their interaction on the grain quality parameters of barley lines selected under ultra-low density when evaluated under dense stand trials in different environments in Tunisia.

Source of variation	DF	PRM	LNG	WDT	CLR_a	CLR_b	CLR_L	PRT	STRCH	β-GLC
Entry	54	15.8***	16.5***	5.5***	8.4***	7.6***	7.6***	12.1***	11.4***	16.5***
Environment	1	28.5	31.6	2.7	3.2*	2.8**	2.8**	2.2	2.7*	30.8
Entry × Environment	54	28.4	31.4	13.9	14.5	15.1*	12.8	20.0	15.9	30.6*

**Significant at α = 0.05; **Significant at α = 0.01; ***Significant at α = 0.001.*

An increase in grain protein content by 2.34% on average was also revealed for the second-year HY lines in comparison to their respective source materials (**Figure 5**). This increase was consistent among all different source varieties/landraces and was more profound in the case of Ardhaoui, for which the second year HY lines significantly surpassed the source material of Ardhaoui by 7.34% for grain protein content (**Figure 5**). At the same time, β-glucan content appeared to be reduced among second-year HY lines by 7.24% in comparison to their respective source materials (**Figure 5**). The trend was specific to the source material, since the second year HY lines derived from Ardhaoui showed significantly lower β-glucan content by 9.95% from their source material, while on the other side the second year HY lines from Manel found by 17.2% on average higher than original variety Manel in terms of β-glucan content (**Figure 5**). Regarding grain starch content, selected HY lines did not reveal, as a general trend, any difference from the source material. However, among all lines, some second-year HY lines derived from Imen (IH4-3, IH16-3, IH4-1, IH17-2, IH16-1, IH4-4) were identified to show significantly higher starch content values among tested entries (**Figure 5**). On the opposite side, the lines derived from Djebali independently their selection status, along with their original population were those with the lower values for grain starch content (**Figure 5**).

TABLE 8 | Grain quality parameters means and confidence intervals of barley entries evaluated in different environments in Tunisia.

Trait	El Kef_16			Mornag_16		
	Mean	Lower 95%	Upper 95%	Mean	Lower 95%	Upper 95%
PRM (mm)	31.05	30.545	31.555	30.59	30.133	31.041
LNG (mm)	10.50	10.314	10.684	10.31	10.145	10.473
WDT (mm)	2.92	2.902	2.949	2.95	2.929	2.977
CLR_a	148.90	148.231	149.559	152.45	151.577	153.318
CLR_b	114.05	113.065	115.035	119.57	118.516	120.620
CLR_L	172.29	171.707	172.880	174.68	173.952	175.404
PRT (%)	10.47	10.279	10.665	10.59	10.378	10.806
STRCH (%)	51.67	51.371	51.964	50.43	50.200	50.669
β-GLC (%)	4.46	4.147	4.768	4.63	4.328	4.922

Correlations Among Traits

Based on barley lines' general performance, some distinct clusters of intercorrelated traits were revealed (**Figure 6**). In particular, GY was positively correlated with the agronomic performance traits of BY ($r = 0.88$), HI ($r = 0.57$), and PH ($r = 0.47$) (**Figure 6**). Surprisingly, no significant correlation was revealed between GY and TKW, as well as between GY and SL (**Figure 6**). Regarding the correlation to the physiological traits, GY was negatively correlated to SPAD ($r = -0.42$) and LCT ($r = -0.46$), even though for LCT the line that revealed consistently the higher grain yield across all environments was the one revealing the higher leaf canopy temperature (**Figures 4**, **6**). On the other hand, the PSII related physiological traits, i.e., F_v/F_m and F_v/F_0 did not show any correlation with GY (**Figure 6**). As far as the grain quality parameters, a significant correlation was found between GY and STRCH ($r = 0.68$), while significant negative correlations were found between GY and CLR_b colour intensity ($r = -0.50$) (**Figure 6**). Another interesting cluster for intercorrelated traits was the one shaped among the four measured physiological parameters (F_v/F_m, F_v/F_0, SPAD, LCT) for which the paired correlations were in all cases significant ranging from $r = 0.41$ (between SPAD and F_v/F_m) up to $r = 0.98$ (between F_v/F_m and F_v/F_0) (**Figure 6**).

DISCUSSION

In this study, 50 barley lines selected by applying divergent single plant selection at ultra-low density within three commercial cultivars and two Tunisian landraces were evaluated in comparison to their source material in an open field under highly contrasting environmental conditions in Tunisia, ranging from dry (Mornag_16; 295 mm annual rainfall) to moderately dry (El Kef_16; 325 mm annual rainfall) up to favourable ones (Mornag_17; 458 mm annual rainfall). The results of this study revealed that the selection applied under ultra-low density reflected with high consistency the grain yield patterns under dense stands with the first- and second-year HY lines to outperform the source material, and the first year LY lines to rank under all entries in terms of grain yield. These lines were derived after applying intra-cultivar selection within source materials of different genetic backgrounds regarding their genetic constitution. That is, while some genetic diversity was expected to be exploitable within the two landraces, no genetic variation was expected theoretically to occur within the improved barley varieties. However, the present study revealed that even within improved varieties, individual plant selection under ultra-low density was efficient to select for HY lines that outperformed their respective source material across all environments.

Although intra-cultivar variation has long been recognised in crop species (Sprague et al., 1960; Russell et al., 1963; Byth and Weber, 1968), it is oftentimes ignored due to the common belief that elite cultivars are highly homogeneous (Fasoula and Boerma, 2007; Haun et al., 2011). Nevertheless, even within homogeneous gene pools, an intrinsic amount of latent genetic variation may still occur, whereas mechanisms that generate *de novo* variation may also be present. Residual heterozygosity, due to segregation of parental loci during the breeding process is presumably one source of genetic variation (Haun et al., 2011; Tokatlidis, 2015). On the other hand, additional heterogeneity might stem from *de novo* generated variation, resulting from spontaneous mutations (Shaw et al., 2000; Ossowski et al., 2010) or *via* genetic and epigenetic mechanisms, such as intragenic recombination, unequal crossing over, gene duplications, or deletions, DNA methylation, excision or insertion of transposable elements, chromatin alterations, and others (Rasmusson and Phillips, 1997; Sani et al., 2013; Cavrak et al., 2014; Kim and Zilberman, 2014).

Despite the wide variability in terms of annual precipitations among the three testing environments, no significant G × E interactions were found for most of the recorded traits. Thus, the barley lines selected under ultra-low density revealed a high buffering capacity for biological yield, demonstrating similar patterns for biomass production across all environments, regardless of the unpredictable precipitation rates. The plasticity of the selected lines as a response to environmental conditions was also maintained for other agronomic traits, such as plant height, spike length, thousand kernel weight, and powdery mildew resistance. However, a significant G × E effect was indicated for the grain yield mainly as a response to the strong G × E interactions for the harvest index and the grain weight per spike. Yet, as the high correlation to the biological yield entails the G × E effect for grain yield was marginally significant, implying a good buffering capacity of the selected lines for this particular trait, too. Furthermore, some selected lines were found to outperform their source material and the best checks across all environments consistently.

Buffering capacity is a crucial feature for the development of modern varieties, to tackle the unpredictable environmental conditions by making optimum use of available resources in both marginal and favourable environments. Defining the optimum planting density under these variable and fragile conditions to accomplish the attainable yield depends on many crop parameters, as well as on several factors related to the genotype itself and the applied cultivation practices. In maize, abiotic adversities show a more pronounced effect under dense stands (Berzsenyi and Tokatlidis, 2012;

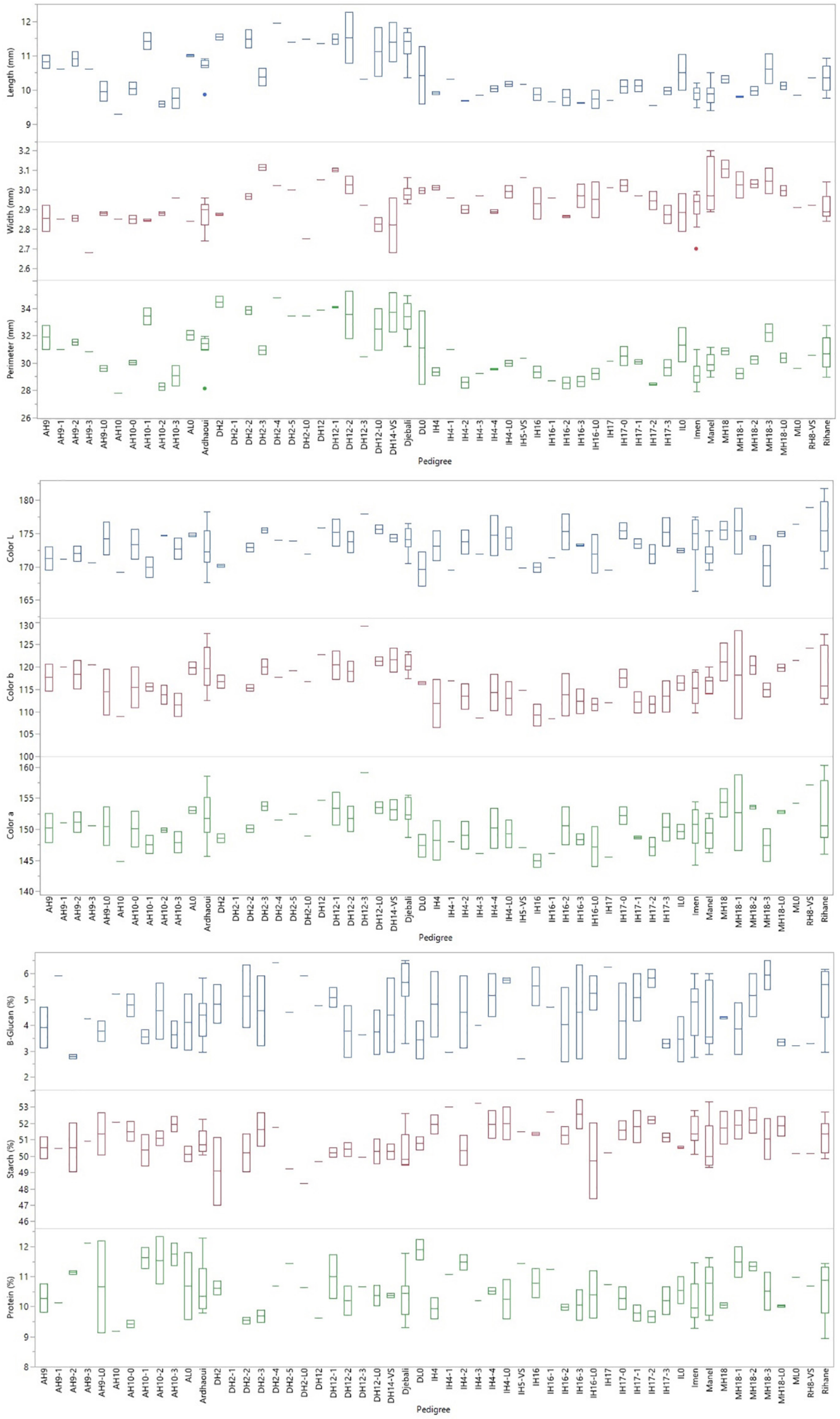

FIGURE 5 | Entry box plots for the grain quality parameters measured during the evaluation of the barley lines in the distinct environments in Tunisia.

Solomon et al., 2017; Mylonas et al., 2020). On the other hand, Bastos et al. (2020), mentioned for wheat that under high-yielding and less limited resources environments the number of plants required to maximise yields was very low, below any commercial recommended number of plants for this crop. However, a higher planting density was needed for the low-yielding environments to sustain maximum yields (Bastos et al., 2020).

To this end, Tokatlidis et al. (2001) indicated the need to extend the lower and upper limits of optimum crop plant density. The authors highlight the concept of developing

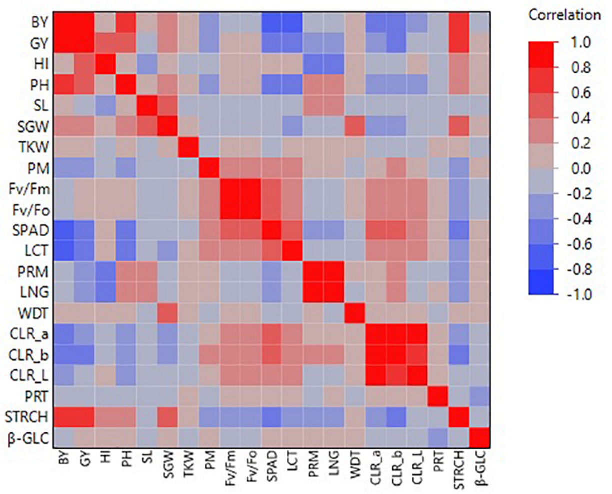

FIGURE 6 | Colour map on pairwise correlations for the different traits recorded during the evaluation of the barley lines in the distinct environments in Tunisia.

density-independent varieties that offer flexibility and plasticity to environmental diversity and secure over-season stability (Tokatlidis et al., 2001; Tokatlidis, 2017). Lower and upper limits of the optimum density are determined by individual plant yield efficiency and tolerance to high densities, respectively (Tokatlidis et al., 2001). Extending the lower limits of the optimum crop density has been proven more challenging than expected. Hence, evidence from research on maize suggests that plant yield potential of maize hybrids remained practically without significant change along the years of maize hybrid development, and it is the hybrid performance as a response to a steadily increasing density rate that is improved (Tollenaar and Lee, 2002; Tokatlidis and Koutroubas, 2004; Duvick, 2005; Gonzalez et al., 2018) or in the best-case scenario, a positive impact on yield components as other sources for yield gain was also identified (Assefa et al., 2018). Given the inverse relationship between the yield of a genotype and its competitive ability (Sedgley, 1991; Fasoula and Fasoula, 1997; Pan et al., 2003; Chatzoglou and Tokatlidis, 2012; Ninou et al., 2014), Tokatlidis (2017) introduced the idea of exploiting in plant breeding the "weak competitor" ideotype. In other words, since under dense stand conditions, the superiority of a plant that stands out could stem from being a strong competitor, while a weak neighbour devoid of genetic competitive ability might be the one with the higher yield potential, Tokatlidis (2017) recommended evaluation and selection of individual plants adequately spaced under a regime that simulates conditions of nil-competition. Evaluation of genotypes under ultra-low density in a regime that practically resembles nil-competition has been successfully also applied as a predictive tool for plant yield efficiency and stability (Sinapidou et al., 2020). Our findings confirm the above remarks, since selection under ultra-low density for high plant yield efficiency, resulted in the selection of superior barley lines with enhanced buffering capacity, revealing high stability in unpredictable environments that ranged from dry (Mornag_2016) up to favourable (Mornag_2017) ones.

Correlation between physiological parameters and agronomic performance traits for the evaluated barley lines showed variable results. According to Fang and Xiong (2015), to overcome drought stress at the physiological level, plants adjust their rates of photosynthesis by modifying photosystem II, inducing the stomatal closure, and lowering the carbohydrate and nitrogen metabolism, as well as the nucleic acid, and protein activity. The effect of drought stress on PSII in plants has been found controversial. Hence, while in some studies chlorophyll fluorescence was found to be useful to evaluate yield performance under rainfed Mediterranean conditions in durum wheat (Araus et al., 1998) and barley (Li et al., 2006), in some others it has been considered as of limited use (Aberkane et al., 2021). On the other hand, leaf canopy temperature has been reported as a useful criterion to select for water-stressed environments and a high correlation has been found between lower canopy temperature and grain yield in wheat (Amani et al., 1996; Reynolds et al., 1998). A significant negative correlation between grain yield and leaf canopy temperature was also revealed from our study, implying that higher grain yield was associated with lower canopy temperature. However, it is worth mentioning that the line which outperformed consistently all other lines across all environments was the one that showed the higher leaf canopy temperature among all the evaluated entries, meaning that other factors are also crucial to determine efficient response to drought conditions. Regarding chlorophyll content, a significant negative correlation was observed between grain yield and SPAD values, which was not expected based on some research evidence that drought and heat stress affect the photosynthetic activity by reducing chlorophyll content (Feng et al., 2014; Sangwan et al., 2018). However, other researchers have reported limited or no association between chlorophyll content and grain yield under heat and drought stress conditions (Pinto et al., 2010; Aberkane et al., 2021).

Good plasticity of barley lines was also indicated for the grain quality parameters since no G × E effects were revealed for most of the recorded quality traits. Furthermore, the improved agronomic performance of the barley selected lines, induced an indirect positive effect on grain protein content with most of the selected high yielding lines to maintain or even improve their protein content in comparison to their source material. Such results are very promising, particularly under the view of a global trend that has been reported toward the lowering of grain quality in high yielding agronomic conditions and among modern cultivars, because breeders are selecting for grain yield but not for quality (Fan et al., 2008; Laidig et al., 2017; Marcos-Barbero et al., 2021). Nevertheless, as Simmonds (1996) highlighted, despite the consensus for strongly negative correlations between grain yield and protein content in cereals a positive expected relationship also holds by making, however, some compromises between attainable high yield or high protein content. The results of our study indicated that small progress in grain protein content has been achieved, while selecting for high grain yield, in accordance with Simmonds's (1996) remark. Working with lentil crops, following a 2-year selection cycle for individual plant yield under ultra-low density, Ninou et al. (2019) ensured that the selection of high yielding lines maintained or even improved their seed quality characteristics.

Overall, the development of varieties with enhanced buffering capacity, characterised by density independence and resource use efficiency is of utmost importance for the farmers to sustain the yield under the unpredictability and inter-annual variation of agricultural environments. Toward this direction, selection for plant yield efficiency at ultra-low-density conditions sounds like a prudent tool to narrow the gap between the actual and the attainable yield and to meet future challenges in agriculture.

CONCLUSION

Considering the challenges imposed by climate variation and volatility of agricultural environments, the development of modern cultivars with high and stable performance across a wide range of environments is an imperative need. The results of our study revealed that selection for high plant yield efficiency under ultra-low density resulted in the development of high yielding lines with an innate buffering capacity, outperforming their source materials and the best checks consistently under contrasting environments. In addition, the potential at the nil-competition regime for efficient selection within narrow gene pools has been well demonstrated. Furthermore, results suggest that single-plant selection under ultra-low density could serve as an effective strategy for developing high-yielding barley varieties maintaining concurrently a high grain quality profile.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

AT drafted the manuscript. AT and HBG conceptualised the study and designed the field trials. HBG coordinated the field trials and the collection of agronomic and physiological traits. AE-B performed the grain quality analysis and collected and curated the quality data. ZK and AT coordinated data curation and performed the statistical analysis. All authors contributed to the development of the entire manuscript and reviewed and edited the final version.

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Modulation of Wheat Yield Components in Response to Management Intensification to Reduce Yield Gaps

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Appropriate genotype selection and management can impact wheat (*Triticum aestivum* L.) yield in dryland environments, but their impact on yield components and their role in yield modulation are not well understood. Our objectives were to evaluate the yield response of commercial winter wheat genotypes to different management practices reflecting a stepwise increase in management intensity (including a reduction in crop density under high input), and to quantify how the different yield components modulate wheat yield. A factorial experiment evaluated six management (M) intensities ["farmer practice" (FP), "enhanced fertility" (EF), "ecological intensification" (EI), "increased foliar protection" (IFP), "water-limited yield" (Yw), and "increased plant productivity" (IPP)] and four winter wheat genotypes (G) in four Kansas environments (E). Average grain yield was 4.9 Mg ha^{-1} and ranged from 2.0 to 7.4 Mg ha^{-1}, with significant two-way interactions (E × M and E × G). The EF usually maximized yields in dry environments, while EI, which consisted of EF plus one fungicide application, maximized yields in environments with greater water availability. Across all sources of variation, kernels m^{-2} and aboveground biomass were the strongest modulators of yield as compared to kernel weight and harvest index, while spikes m^{-2} and kernels $spike^{-1}$ modulated yields at a similar magnitude. Foliar fungicides improved yield through increased green canopy cover duration and greater radiation intercepted during grain filling. When crop density was reduced from 2.7 to 1.1 million plants per hectare in an otherwise high-input system, plants produced more productive tillers (with genotype-specific response); however, reduced green canopy cover at anthesis and reduced cumulative solar radiation intercepted during grain filling limited wheat yield—although large differences in canopy cover or intercepted radiation were needed to cause modest changes in yield. Treatments more intensive than EI were not warranted as EF or EI maximized yields at all environments, and practices that promote biomass and kernels m^{-2} are to be targeted for future increases in wheat yield.

Keywords: intensive management, *Triticum aestivum* L., crop density, fungicide, fertility, biomass, kernels m^{-2}, kernel weight

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is cultivated in more than 200 million ha across the world, being an essential component of the human diet and the primary source of calories for the world's population (Reynolds et al., 2012). Thus, increases in wheat production are crucial for global food security (Shiferaw et al., 2013), especially as yield gains fail to sustain historical rates (Grassini et al., 2013). Within this context, increasing crop yield in current cropland can help to meet future food demand and minimize the expansion of agricultural lands (Cassman, 1999).

The majority of global wheat production occurs under rainfed conditions. These non-irrigated cropping systems are subject to droughts due to insufficient and/or poorly distributed precipitation (Sadras, 2002; Sadras and Angus, 2006; Torres et al., 2013; Lollato et al., 2017, 2020a). This leads to a more conservative approach from producers in terms of adoption of management practices with the objective of increasing yield. The underlying rationale is that water availability is the most yield-limiting factor and reduces the return on added inputs (Jaenisch et al., 2019; de Oliveira Silva et al., 2020b), following Liebig's law of the minimum, which states that the growth of a plant is proportional to the scarcest of the essential nutrients available. However, empirical and theoretical evidence supports that crop yield might not be limited by a single factor but rather determined by interactions between two or more factors (Sadras, 2004; Cossani and Sadras, 2018; Carciochi et al., 2020). Thus, it can be hypothesized that improvements in crop management could increase grain yield despite water limitation (de Oliveira Silva et al., 2020b).

The state of Kansas (United States) provides a great case study for testing the management and genotype opportunities for future yield increases in dryland wheat-growing regions. With 3–4 million ha of winter wheat sown annually and a production of ∼8 million metric tons, Kansas is the largest winter wheat-producing state in the country (USDA-NASS, 2017). The crop is grown predominantly under dryland conditions (∼94%, USDA-NASS, 2018), with a 10-year average yield of 2.8 Mg ha^{-1}, which corresponds to only 50–55% of the dryland yield potential (∼5.2 Mg ha^{-1}; Patrignani et al., 2014; Lollato et al., 2017). A range of genotypic traits and agronomic management practices is proposed to modulate wheat yield in this region (Lollato et al., 2020b; Munaro et al., 2020; Jaenisch et al., 2021). For instance, improved fertility management, including the adoption of in-furrow starter fertilizer (McConnell et al., 1986; Lollato et al., 2013; Maeoka et al., 2020), increased nitrogen rates (Thomason et al., 2002; Walsh et al., 2018; Lollato et al., 2019a, 2021), and use micronutrients (Zain et al., 2015), has been associated positively with yields. Likewise, genetic resistance to major diseases and its interaction with foliar fungicides are management variables of interest (Lollato et al., 2019b; de Oliveira Silva et al., 2020b; Cruppe et al., 2021). The role of crop density seems a variable and dependent resource availability (Fischer et al., 2019; Lollato et al., 2019b; Bastos et al., 2020); thus, its potential to interact with other practices (e.g., Jaenisch et al., 2019) deserves further exploration.

The studies above provided insights into individual management practices to improve wheat grain yield. Others attempted to quantify wheat yield response to intensified management, combining the prophylactic use of a number of inputs to minimize yield gaps (Mohamed et al., 1990; Jaenisch et al., 2019; Quinn and Steinke, 2019; de Oliveira Silva et al., 2020b; Herrera et al., 2020; Roth et al., 2021; Steinke et al., 2021). However, with few exceptions (de Oliveira Silva et al., 2020b, 2021), these efforts mostly overlooked the mechanisms behind the yield responses and simply quantified the magnitude of yield improvements. Because organogenesis is linked to crop developmental stages (Slafer et al., 2021), we argue that it is relevant to maximize yield within the time frame of yield component determination.

The relationships between wheat yield and its components [i.e., biomass, harvest index (HI), spikes m^{-2}, kernels spike^{-1}, kernels m^{-2}, and kernel weight] have been researched for decades across a wide range of environments (Austin et al., 1980, 1989; Calderini et al., 1999; Acreche et al., 2008; Slafer et al., 2014). The majority of the literature suggests that wheat is mostly sink limited, with kernels m^{-2} explaining a larger variation of yield than kernel weight, and with changes in assimilate supply only offering modest changes in yield (Slafer and Savin, 1994; Borrás et al., 2004; Slafer et al., 2014; and citations therein). Thus, management practices that affect kernels m^{-2} would expectedly have a greater impact on yield. Still, some management practices that mostly modulate kernel weight might also relate positively to yield in some environments (Cruppe et al., 2021). To our knowledge, there have been no attempts to explicitly manipulate management practices that match important stages of crop development when different organs are produced and quantify their relationship to yield within a context of management intensification, which is crucial for food security (Cassman and Grassini, 2020).

Organs that eventually become a source and a sink are initiated during different times in the vegetative and reproductive stages in wheat (Slafer and Rawson, 1994; Ochagavía et al., 2021). Crop density is determined during the vegetative stage as seedlings emerge and establish; tillers m^{-2} (and thus potential spikes m^{-2}) are determined between seedling emergence and the terminal spikelet stage (although less productive tillers can be produced later); potential spikelets spike^{-1} is determined prior to the first visible node; and kernels spikelet^{-1} is determined between the onset of stem elongation until harvest maturity through the process of floret development (which ends by anthesis) and grain filling (Ochagavía et al., 2021). Grain weight is determined between booting and maturity, with different sensitivities to weather conditions between the heading and grain-setting stages (Calderini et al., 2001) as compared to and the grain filling stage (Bergkamp et al., 2018). Meanwhile, the source capacity (e.g., leaf area index) is usually maximized prior to anthesis and decreases with maturity (Lollato and Edwards, 2015). Disentangling the effects of genotype (G), environment (E), management (M), and their interactions—with the specific goal of modulating different yield components and tradeoffs—can provide a physiological basis for future yield increases in wheat.

While genotypic and management factors associated with wheat yield gaps in Kansas and other dryland regions have been explored individually in different studies, their role to improve crop yield and its components within an integrated management perspective having a goal to optimize yield components has not been explored. Thus, our objectives were to (i) evaluate the yield and yield components response of commercial winter wheat genotypes to different management practices reflecting a stepwise increase in management intensity using as baseline the current technology level followed by an average producer in the region and investigating levels of yield gaps; and (ii) quantify how different yield components modulate wheat yield in this dryland region. Because wheat response to crop density seems to depend on resource availability (Fischer et al., 2019; Bastos et al., 2020), we also tested whether reducing seeding rates from the most intensive treatment would be a promising strategy to reduce yield gaps. We hypothesize that a more intensive management will increase grain yield, and that yield increases will be genotype and environment specific. Additionally, we hypothesize that fertilizer-based practices will affect yield components that are coarse regulators of yield (i.e., spikes m^{-2} and kernels m^{-2}), while fungicide-based practices will affect fine regulators of yield (i.e., kernel weight, kernels $spike^{-1}$) (Slafer et al., 2014).

MATERIALS AND METHODS

Experimental Locations and Agronomic Management

Rainfed field experiments were conducted in Kansas, United States, near Belleville (39.81°N, 97.67°W; 471 m; moderately well-drained Crete silt loam) and near Hutchinson (37.93°N, 98.03°W; 468 m; well-drained Ost loam) during the winter wheat seasons of 2017–2018 and 2018–2019. Each environment will be referred to as Bel18, Bel19, Hut18, and Hut19. Winter wheat was sown under conventional tillage after a summer fallow using a Great Plains 606 no-till drill (7 rows spaced at 19 cm) with plot dimensions of 1.3 m × 9.1 m. Seeds were treated with 6.9-g a.i. ha^{-1} thiamethoxam, 1.4-g a.i. ha^{-1} mefenoxam, and 8.9-g a.i. ha^{-1} difenoconazole to avoid early-season diseases and insects. Composite soil samples (i.e., 15 individual soil cores) were collected at sowing from the 0–15- and 15–60-cm depth to quantify initial soil nutrient status (**Supplementary Table 1**). Weeds were controlled and insect pressure was not observed across the study.

Treatment Structure and Experimental Design

Treatments were arranged in a complete factorial structure established in a split-plot design with four replications. Whole plots were assigned to six management intensities, and sub-plots were assigned to four winter wheat genotypes. Treatment combinations represented stepwise increases in management intensity from a baseline reflecting the level of technology adoption of an average producer in the region and will, hereafter, be referred to as “farmer practice” (FP), “enhanced fertility” (EF), “ecological intensification” (EI), “increased foliar protection” (IFP), “water-limited yield” (Yw), and “increased plant productivity” (IPP) (**Table 1**).

The FP consisted of a seeding rate of 2.7 million seeds ha^{-1} plus an N application at Zadoks GS23-25 with a rate reflecting a yield goal of the 10-year wheat grain yield average in the county where the experiment was located (~2.4–2.8 Mg ha^{-1}). The first increase in intensity was the enhanced fertility (EF) treatment, which included 112 kg ha^{-1} micro essentials (MESZ; 13-kg N ha^{-1}, 45-kg P ha^{-1}, 11-kg S ha^{-1}, and 1-kg Zn ha^{-1}) placed in a furrow with the seed, and an increased N rate for a 6.7 Mg ha^{-1} yield goal applied at Zadoks GS23–25 in the spring (**Table 1**). The fertilizer treatments aimed at increasing tiller and biomass production. The N rate in this treatment was selected so that N was not a limiting factor based on the long-term wheat yield potential of ~5.2 Mg ha^{-1} (Lollato et al., 2017). The next step was ecological intensification (EI), which consisted of EF plus one fungicide application (fluxapyroxad-26 g ha^{-1}, pyraclostrobin-171 g ha^{-1}, propiconazole-107 g ha^{-1}) at Zadoks GS55. Increased foliar protection (IFP) was the next step, consisting of EI plus the same fungicide product and the rate applied at Zadoks GS31. The aim of these fungicide applications was to protect the green canopy cover of the crop (i.e., source) during the different stages of development. The water-limited yield potential (Yw) treatment consisted of IFP plus micronutrients (81-g S ha^{-1}, 90-g Zn ha^{-1}, 67-g Mn ha^{-1}, and 2-g B ha^{-1}) applied at Zadoks GS31. Finally, the increased plant

TABLE 1 | Description of the six management intensities and four winter wheat genotypes evaluated in the current study.

	Management intensity							Genotype			
Input	FP	EF	EI	IFP	Yw	IPP	Trait	WB4303	WB4458	WB-Grainfield	Zenda
N Rate for Yield Goal (Mg ha^{-1})	2.4	6.7	6.7	6.7	6.7	6.7	YOR	2017	2013	2012	2017
In-furrow starter N, P, S, and Zn	No	Yes	Yes	Yes	Yes	Yes	Maturity	ME	M to ME	M	M
Foliar Fungicide Feekes GS10.5	No	No	Yes	Yes	Yes	Yes	Straw strength	E	G	A	E
Foliar Fungicide Feekes GS6	No	No	No	Yes	Yes	Yes	Drought tolerance	BA	AA	AA	BA
Foliar S, Zn, Mg, and B	No	No	No	No	Yes	Yes	Stripe rust	MS	S	MR	MR
Seeding rate (million seeds ha^{-1})	2.7	2.7	2.7	2.7	2.7	1.1	Leaf rust	MS	S	MR	MR

Farmer practice (FP) was followed by stepwise additions of five inputs: enhanced fertility (EF), ecological intensification (EI), increased foliar protection (IFP), water-limited yield potential (Yw), increased plant productivity (IPP). Abbreviations: YOR, year of release; M, medium maturity for heading date; ME, medium-early maturity for heading date; E, excellent straw strength; G, good straw strength; A, average straw strength; and for disease-resistant ratings, S, susceptible; MS, moderately susceptible; MR, moderately resistant. We note that these resistance ratings reflected the study period, but some cultivars have lost their resistance since the study was conducted.

productivity (IPP) treatment was designed to explore whether a high resource availability scenario allowed for reduced crop density; thus, the seeding rate was 1.1 million seeds ha^{-1}, reflecting the low seeding rates used by progressive growers in the region (Lollato et al., 2019b).

Wheat genotypes were selected based on their adoption by growers, adaptation to the region, and contrasting traits of interest for intensive management as well as performances in regional trials. The genotypes tested and their percent of the seeded area in central Kansas during 2020–2021 were WB4303 (<1%), WB4458 (2.2%), WB-Grainfield (5.5%), and Zenda (7.8%) (USDA-NASS, 2020). Information about traits of interest of each genotype within the context of management intensification is provided in **Table 1**.

The nitrogen rate was determined considering the soil NO_3-N measured at sowing, potential N released from the organic matter, and a 40 kg ha^{-1}-applied N per a Mg ha^{-1} grain yield goal (Leikam et al., 2003). Due to the residual soil NO_3-N carry over from the previous growing season and estimated N release from organic matter, the N rate varied across environments (**Supplementary Table 2**). A pressurized CO_2 backpack sprayer with a three-nozzle boom was used to apply the N as urea ammonium nitrate (UAN, 28-0-0) with a streamer nozzle (SJ3-03-VP), and foliar fungicide and micronutrients using a flat-fan nozzle (XR11002) with a constant volume of 140 L ha^{-1}. Treatment application dates are provided in **Supplementary Table 2**.

Measurements

Crop density was recorded in two linear meters per plot, 3–4 weeks after sowing. Percent green canopy cover was measured approximately at bi-weekly intervals from heading (Zadoks GS55) until maturity (Zadoks GS 95) from downward-facing digital photographs from an area of about 1 m^2 processed using Canopeo (Patrignani and Ochsner, 2015). Aboveground biomass was sampled from a one-linear row-meter area (~0.19 m^2) from one of the center rows of each plot the same day of wheat harvest. Samples were dried at 65°C until constant weight and dry aboveground biomass were measured. The spikes were counted and separated from the stover prior to threshing to remove the chaff from the kernels. Grain weight was measured after threshing. The grain weight divided by the total aboveground biomass weight (including stover, chaff, and grain) determined the harvest index (HI). A 1,000-kernel weight was determined from a random kernel sub-sample. The ratio between total grain weight and 1,000 kernel weight determined kernels m^{-2}; and the ratio between kernels m^{-2} by spikes m^{-2} determined kernels per spike. The number of productive tillers per plant was calculated as the ratio of spikes m^{-2} and plants m^{-2}. Plots were trimmed prior to harvest to avoid edge effects, and wheat was harvested from a ~13-m^2 area using a small-plot Massey Ferguson 8XP combine. Grain moisture was measured at harvest, and grain yield was corrected for 135-g kg^{-1} water content. Grain protein concentration was measured using near-infrared spectroscopy.

Weather data, including precipitation, reference evapotranspiration (ETo), and maximum and minimum temperatures, were collected from a station pertaining to the Kansas Mesonet (Patrignani et al., 2020) located ~50 m from the experiments. Plant available water at sowing was estimated using non-growing season precipitation and the soil's available water-holding capacity (Lollato et al., 2016). At each environment, the weather variables were averaged (Tmax, Tmin) or accumulated (precipitation, ETo) for the entire growing season, as well as separated into four distinct phases: fall (the period between sowing and December 31); winter (January 1 to March 31), critical period [20 days prior to anthesis through 10 days afterward (Fischer, 1985)], and grain filling (10 days after anthesis through harvest). This sub-division intended to reflect (i) the conditions surrounding sowing that affect crop establishment and fall tiller initiation; (ii) the dormant period that can affect tillering and winterkill; and (iii) the yield determination period in the spring, similar to previous reports in the region (e.g., Lollato and Edwards, 2015).

Statistical Analyses

Pearson's correlation analysis was performed in R using the "corrplot" package (Wei et al., 2017) to determine the degree of linear association between the weather variables at the different periods and the measured crop variables. Because the data only derived from four environments, we relaxed the assumptions of *p*-values for this specific analysis to 0.15, while, for all other analyses, effects were significant at $\alpha = 0.05$. ANOVA was performed using "lmerTest" in R software version 3.4.0 (Kuznetsova et al., 2017). Management, genotype, environment, and their interactions were fixed effects, while block nested within environment and management intensity nested within the block were random effects (the latter accounted for the split-plot design).

We used the stability method (Eberhart and Russell, 1966) to further understand the genotypic and management effects on grain yield and on productive tillers per plant (the latter to quantify the impact of crop density). This method consists of a linear regression of trait expression of each genotype (or management) versus an environmental index calculated as the mean trait expression of all genotypes at each environment minus the overall mean trait expression across all environments. Each management-by-environment combination was considered an environment ($n = 24$) for the genotype analyses (e.g., Ferrante et al., 2017; Lollato et al., 2021), and each genotype-by-environment combination was considered an environment ($n = 16$) for the management analyses (e.g., Raun et al., 1993). The slope (α) indicates whether the genotype has broad adaptability ($\alpha = 1$) or adaptability specific to low ($\alpha < 1$) or high- ($\alpha > 1$) trait-expression environments, and is associated with phenotypic plasticity (Sadras and Richards, 2014). The intercept (β) is an estimate of the trait expression across environments; and a model goodness of fit index (i.e., R^2) quantifies stability.

The modulators of yield in response to management were quantified as the relationships between yield components and yield using linear regression (e.g., de Oliveira Silva et al., 2020b). Differences in grain yield between the FP and each management for each genotype were calculated and regressed for: (i) all environment and management practices by wheat genotype combinations (n = 96), (ii) on average of each management intensity (n = 24; 6 managements × 4 environments), and (iii) on average for each genotype (n = 24; 6 managements × 4 genotypes). To understand the drivers of yield improvements in response to each step within the management intensification practices evaluated, we explored the relationships between the responsiveness of yield and the responsiveness of each yield component using linear regression (Slafer et al., 2014). Responsiveness was calculated as the ratio of each trait in a given management intensity over the same trait measured in the preceding management intensity so that we could quantify the effects of each management addition (e.g., responsiveness calculated as EF over FP associated with changes resulting from improved fertility).

Finally, we evaluated the green canopy cover data and the cumulative radiation intercepted during grain filling to better interpret the effects of fungicide and of crop density on grain yield in terms of source limitation. First, we calculated the linear slope of canopy cover dynamics between heading and maturity to detect whether the presence of foliar fungicides delayed canopy senescence, which would be indicated by a less-negative slope. This comparison was made between treatments EF and EI to isolate the effect of a single fungicide application at Zadoks GS55. Second, green canopy cover values at anthesis and their association with grain yield were compared for the Yw and IPP treatments to detect whether grain yield limitation from lower crop density could be explained by reduced green canopy cover. Finally, for the selected treatments above, cumulative radiation intercepted between anthesis and harvest maturity was calculated as the product between daily solar radiation and percent green canopy cover (Purcell, 2000). Daily values of green canopy cover were estimated for days between measurements using linear interpolation between consecutive measurements (Lollato and Edwards, 2015).

RESULTS

Weather Conditions and Associations With Yield Components

Growing season total precipitation ranged from 297 to 823 mm, and seasonal ETo ranged from 637 to 801 mm (**Figure 1**). Environments in 2017–2018 had a cold and dry fall, winter, and early spring, and a hot and dry late spring and early summer. Environments in 2018–2019 had warm and moist fall and cool and moist late spring and early summer, increasing disease pressure (Hollandbeck et al., 2019). Above-normal May and June temperatures in 2017–2018 (average temperatures between 23 and 27°C vs. 15–23°C in 2018–2019) accelerated and shortened the reproductive crop development (duration of grain fill, ranging from 27 to 29 days in 2017–2018 and from 33 to 52 days in 2018–2019; **Figure 1**), consequently decreasing the yield potential of the crop. The contrasting environments resulted in growing season length ranging from 239 to 288 days.

Table 2 shows the correlations between weather variables during specific crop developmental stages and yield components. Productive tillers plant^{-1} related negatively with fall Tmin and positively with Tmin during the critical period. Harvest index related positively to winter Tmin. Spikes m^{-2} related negatively to Tmin and precipitation during the winter. The negative relation between winter Tmin and spikes m^{-2} or productive tillers plant^{-1} reflects a delayed incorporation of the N fertilizer into the root zone until late spring in these environments, reducing the formation of spring tillers. Kernels spike^{-1} related

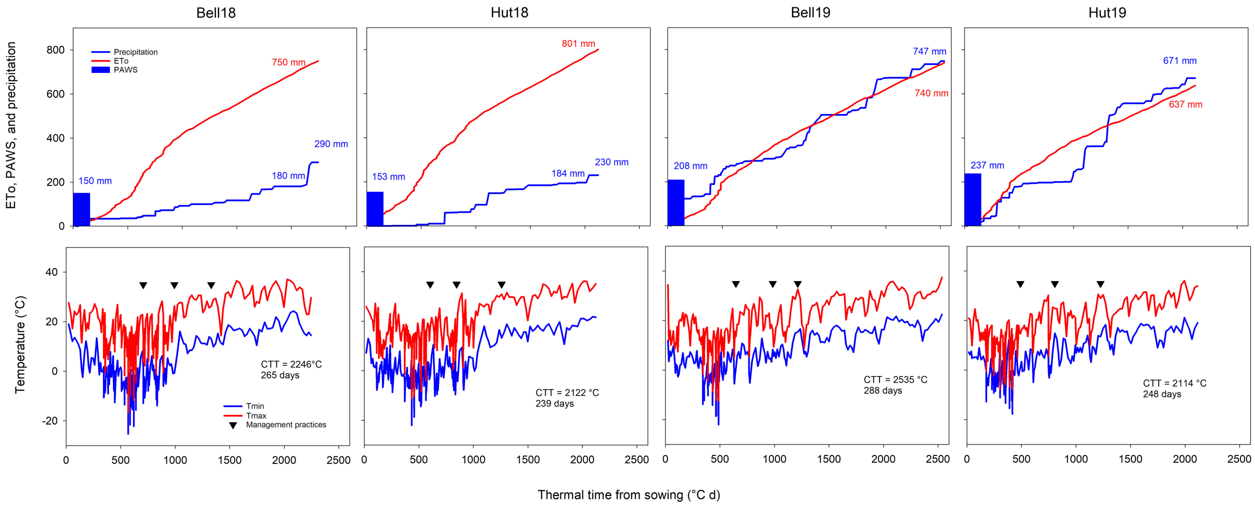

FIGURE 1 | Weather conditions experienced during the winter wheat-growing season at the four Kansas environments resulting from two locations (Bell, Belleville; Hut, Hutchinson) and two growing seasons (18, 2017–2018 season; 19, 2018–2019 season). The upper row shows plant available water at sowing (PAWS), cumulative reference evapotranspiration (ETo) and precipitation, the bottom row shows maximum and minimum temperatures. Downward facing triangles show, respectively dates for N application at Zadoks GS25, fungicide and micronutrient application at GS32, and fungicide application at GS55. Inset values show cumulative ETo, precipitation, PAWS, cumulative thermal time between sowing and harvest (CTT), and season duration in days. Two cumulative precipitation values are shown for 2018 environments as considerable rainfall occurred after the crop was mature.

TABLE 2 | Correlations between yield components, averaged across four varieties and six management intensities, and daily average or cumulative values of environmental factors during specific crop development periods.

Trait	Environmental factor	Period	*r*
Productive tillers plant^{-1}	Tmin	Fall	−0.99
	Tmin	Critical period	0.89
Harvest index	Tmin	Winter	0.96
Spikesm^{-2}	Tmin	Winter	−0.88
	Precipitation	Winter	−0.87
Kernels spike^{-1}	Tmax	Growing season	−0.99
	Precipitation	Growing season	0.97
	Water supply	Growing season	0.96
	Tmax	Fall	−0.89
	Precipitation	Fall	0.96
	Tmax	Winter	−0.91
	Tmax	Critical period	−0.86
	Tmax	Grain filling	−0.87
	Tmin	Grain filling	−0.9
	Precipitation	Grain filling	0.91
	Duration	Grain filling	0.86
Kernels m^{-2}	Tmax	Winter	−0.88
	Precipitation	Grain filling	0.9
Kernel weight	Tmin	Winter	0.9
	Precipitation	Winter	0.93
	Precipitation	Critical period	0.89

Weather variables included in the analysis were minimum (Tmin,°C) and maximum (Tmax,°C) temperatures, cumulative precipitation (mm), plant available water at sowing (PAWS, mm), water supply (growing-season precipitation plus PAWS, mm), and photothermal quotient (MJ m^{-2} C^{-1}). Developmental periods evaluated were the fall (from the sowing date until December 31), the winter (from January 1 until March 31), the critical period (20 days prior to until 10 days after anthesis), and the grain-filling period (from 10 days after anthesis until harvest).

positively to precipitation and water supply during the season, fall and grain-filling precipitation, and duration of the grain-filling period; and negatively to Tmax (growing season, and at each stage evaluated), and Tmin during grain filling. Kernel weight associated positively with winter Tmin and precipitation, as well as critical period precipitation.

Management and Genotype Effects on Grain Yield and Yield Components

Across all sources of variation, mean grain yield ranged from 2.3 to 7.2 Mg ha^{-1} (**Figure 2**). Environmental mean yield (across management and genotypes) ranged from 3.3 Mg ha^{-1} in Hut18 to 5.6 Mg ha^{-1} in Bel19, with overall greater yields in 2019 (5.43 Mg ha^{-1}) as compared to 2018 (4.28 Mg ha^{-1}). Mean grain yield for the genotypes was highest for WB4303 (5.11 Mg ha^{-1}), followed by Zenda (4.96 Mg ha^{-1}), WB-Grainfield (4.72 Mg ha^{-1}), and WB4458 (4.58 Mg ha^{-1}) (**Figure 2A**). Mean yield for the different management intensities was 3.96, 4.46, 5.34, 5.11, 5.34, and 4.82 for FP, EF, EI, IFP, Yw, and IPP, respectively (**Figure 2B**).

There were significant G × E and M × E interactions for grain yield, but no three-way interaction (**Supplementary Table 3**). General trends as related to the G × E interaction were: (i) WB4303 was in the highest yielding group at all environments; (ii) Zenda was in the highest yielding group in three out of four environments; and (iii) WB4458 yielded well in dryer conditions (i.e., Hut18) but yielded poorly at the higher-yielding environments (Bel19) (**Table 3**). General trends as related to M × E interaction were: (i) the FP yielded similarly to other treatments only in one environment (Bel18); (ii) EF yielded higher from FP in three environments; (iii) increases in grain yield from foliar protection (i.e., EI) only occurred in environments with greater rainfall (i.e., Bel19 and Hut19); (iv) the addition of the early fungicide (i.e., IFP) did not increase yields compared to a single fungicide application later in the season; (v) wheat grain yield benefited from all the management practices combined (i.e., Yw) only in one environment (i.e., Hut19); and (vi) reducing crop density under an otherwise highly managed system had no effect on grain yield except in one environment (i.e., Hut19) (**Table 3**).

Further exploration of the significant interactions through the adaptability and stability indices suggested that wheat genotypes varied in stability and adaptability across the different yield environments (**Figure 2A**). The wheat genotype WB4458 had the lowest α (0.78 ± 0.11), suggesting that this genotype was the least adapted to high-yielding environments and was unstable with a high variation about the fitted line (R^2 = 0.67). Due to their α equal to one (1.12 ± 0.09, and 0.96 ± 0.09), the wheat genotypes WB4303 and WB-Grainfield showed broad adaptability and greater stability (R^2 > 0.83), while Zenda was adapted to high-yielding environments (α = 1.18 ± 0.09). Management practices also showed environmental-specific adaptability, with EF showing greater yields in low-yielding environments (α = 0.72 ± 0.14), Yw showing adaptability to high-yielding environments (α = 1.45 ± 0.15), and the remaining management intensities showing broad adaptability (**Figure 2B**). Yield stability improved from the FP to the Yw treatments (R^2 ranging from 0.67 to 0.87, **Figure 2B**).

With the exception of 1,000 kernel weight and grain protein, the yield components followed the yield analysis and were not affected by the three-way interaction, mostly reflecting G × E and M × E interactions (**Supplementary Table 3**). Briefly, in terms of crop density, the IPP treatment had fewer plants m^{-2} (149–163) as compared to other treatments (223–266 plants m^{-2}) as expected (**Table 4**), which resulted in more productive tillers per plant (3.18–4.97 vs. 2.16–4.22) (**Table 5**). Management intensification tended to increase aboveground biomass as compared to the FP (magnitude: 18–100%), while the latter usually resulted in the greatest HI—with exception of Bel19 –although the magnitude of change was not large (16–46%) (**Supplementary Table 4**). The magnitude in the differences in spikes m^{-2} due to management and genotype was similar (38–72%) as those compared to changes in kernels spike^{-1} (39–64%) (**Supplementary Table 5**). The results of kernels m^{-2} reflected those for grain yield (**Supplementary Table 6**), while 1,000 kernel weight and grain protein were impacted by a G × E × M interaction (**Supplementary Table 7**).

Different genotypes had different tillering abilities and adaptation to tillering environments, which were mostly modulated by reduced crop density (**Figure 2C**). Zenda had the highest tillering ability across environments (mean: 3.81

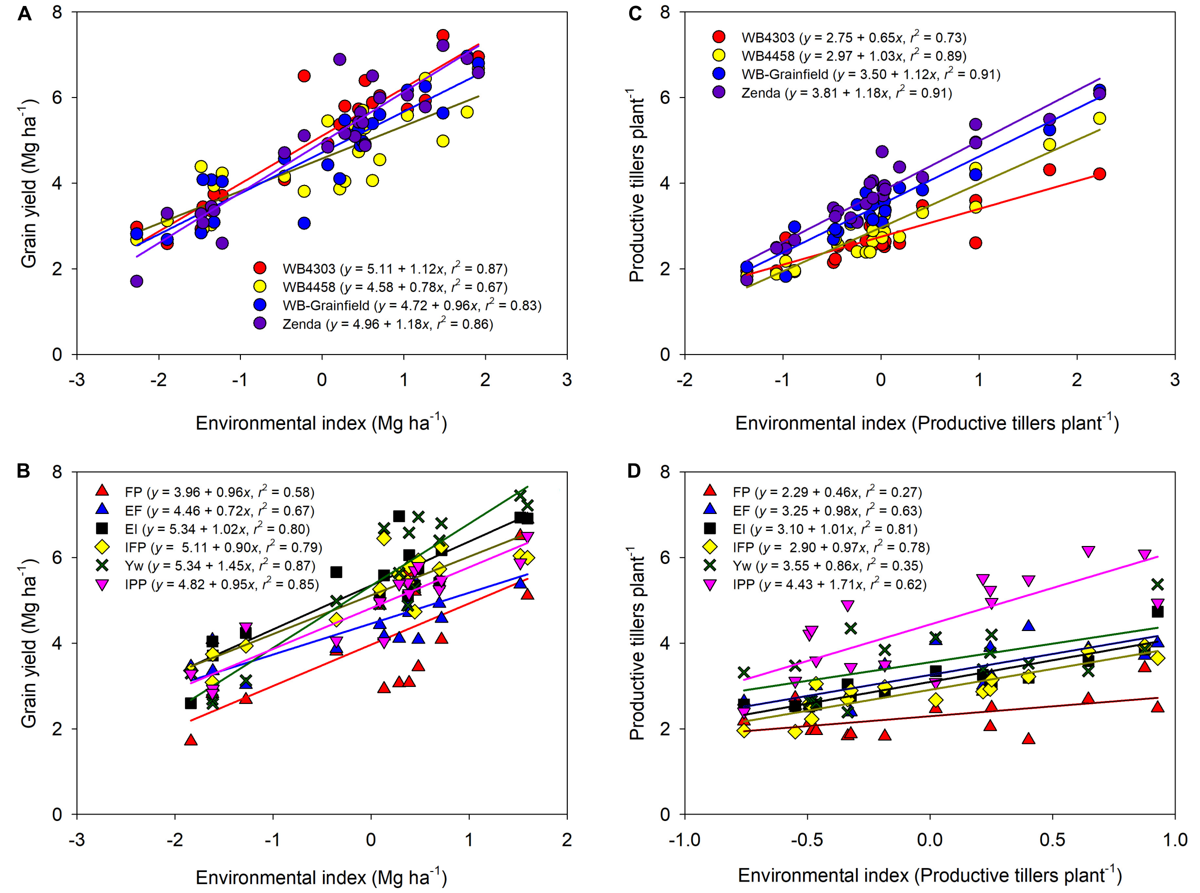

FIGURE 2 | Wheat grain yield **(A,B)** and productive tillers per plant **(C,D)** as affected by the environment index for each wheat genotype (WB4303, WB4458, WB-Grainfield, and Zenda) **(A,C)** and management intensity (FP, farmer practice; EF, enhanced fertility; EI, ecological intensification; IFP, increased foliar protection, Yw, water-limited yield potential; and IPP, increased plant productivity) **(B,D)**. Environmental indices were calculated as the combination of environment (Bel18, Hut18, Bel19, and Hut19) and **(A,C)** management practices or **(B,D)** genotypes.

productive tillers per plant) with even greater tillering expression in high-tillering environments (α = 1.18 ± 0.19), which was followed by WB-Grainfield, WB4458, and WB4303 (3.50, 2.97, and 2.75 productive tillers per plant) (**Figure 2C**). While WB-Grainfield and WB4458 had wide adaptability of productive tillers per plant, the ability of WB4303 to produce tillers decreased in reference to the other genotypes as tillering environment increased (α = 0.66 ± 0.13). Reduced crop density (IPP) allowed for the greatest expression and maintenance of tillers (mean of 4.43 productive tillers plant^{-1}), which increased at α = 1.71 ± 0.36 with the environmental index for tillering production (**Figure 2D**). The lowest tillering production and response to tillering environment occurred at the FP (mean of 2.29 productive tillers plant^{-1}, α = 0.46 ± 0.20).

Yield Component Modulation of Wheat Grain Yield

Across E, M, and G, aboveground biomass at maturity explained 77% of the variation in yield, showing a positive relationship (**Figure 3A**). Although significant, a negative relationship of HI only explained 8% of the variation in yield (**Figure 3D**). Across environments, differences in grain yield were dependent on differences in biomass accumulation (**Figure 3B**) and independent of differences in HI (**Figure 3E**). Following the same trend, differences in biomass accumulation among the different wheat genotypes under different managements were also strongly related to differences in grain yield (**Figure 3C**) as compared to HI (**Figure 3F**). Increasing management intensity (the difference of each management practice to FP) significantly increased biomass accumulation, which increased yield across environments (**Figure 3B**, insert). Likewise, increased management intensity increased the responsiveness of biomass accumulation for wheat genotypes, which increased grain yield (**Figure 3C**, insert). Meanwhile, increased management intensity had limited effect on HI across environments or across genotypes (**Figures 3E,F**, inserts).

Kernels m^{-2} had greater importance in increasing grain yield as compared to kernel weight (**Figure 4**). Across E, M, and G, a positive relationship of kernels m^{-2} explained 78% of the variation in grain yield (**Figure 4A**), while no relationship between kernel weight and yield occurred (**Figure 4D**). Averaged

TABLE 3 | Least square mean winter wheat grain yield as affected by management practices (FP, EF, EI, IFP, Yw, and IPP), wheat genotypes (WB4303, WB4458, WB-Grainfield, and Zenda), and environments (Bel18, Hut18, Bel19, and Hut19).

	Environment				
	Bel18	**Hut18**	**Bel19**	**Hut19**	**Mean**
Genotype	**Grain yield (Mg ha^{-1})**				
WB4303	5.54Aa	3.17Bab	6.36Aa	5.32Aab	4.10
WB4458	5.29Aab	3.56Ba	4.49ABc	4.97Ab	4.58
WB-Grainfield	4.93Ab	3.22Bab	5.13Ab	5.56Aa	4.71
Zenda	5.22Bab	2.93Cb	6.44Aa	5.23Bab	4.96
Mean	5.25	3.22	5.61	5.27	
Management					
FP	5.31Aa	2.53Bb	4.62Ad	3.39Be	3.96
EF	4.91ABa	3.42Ba	5.06Acd	4.38ABd	4.44
EI	5.24Ba	3.60Ca	6.62Aa	5.89ABbc	5.34
IFP	5.29Aa	3.56Ba	5.55Abc	6.10Aab	5.13
Yw	5.37Ba	2.87Cab	6.32ABab	6.75Aa	5.33
IPP	5.34Aa	3.36Ba	5.46Abc	5.12Acd	4.82
Mean	5.24	3.22	5.61	5.27	

Least square means followed by a common uppercase letter (comparisons across environments) or a lowercase letter (comparisons across management practices or genotypes) are not significantly different by the Tukey test at the 5% level of significance.

across genotypes, increasing management intensity increased grain yield through differences in kernels m^{-2} (**Figure 4B**), and yield responses to management practices were associated with increases in kernels m^{-2} (**Figure 4B**, insert). Similarly, averaged across management practices, wheat genotypes that had greater kernels m^{-2} also had greater grain yield (**Figure 4C**), and yield responses were dependent on the genotype's kernels m^{-2} responsiveness (**Figure 4C**, insert). Following a different trend, increases in grain yield were independent of kernel weight for both management practices and wheat genotypes (**Figures 4D–F**); however, increases in kernel weight due to management were associated with increased grain yield within environment (**Figure 4E**, insert). Differences in kernel weight within each genotype were not associated with increases in grain yield (**Figure 4F**, insert).

Spikes m^{-2} and kernels spike^{-1} both had a positive effect on grain yield (**Figure 5**). Across G, E, and M, a positive relationship of spikes m^{-2} and of kernels spike^{-1} explained 19 and 39% of the variation in yield, respectively (**Figure 5A**). Averaged across either management practices or wheat genotypes, grain yield differences were dependent on differences in spikes m^{-2} (**Figures 5B,C**). Likewise, wheat genotype responsiveness to spikes m^{-2} resulted in positive differences in grain yield (**Figure 5C**, insert). Interestingly, management practices resulting in greater number of kernels spike^{-1} also significantly affected yield (**Figure 5E**), but there were no differences across genotypes (**Figure 5F**). Likewise, the responsiveness of kernels spike^{-1} to management practices affected grain yield, with no differences among genotypes (**Figure 5F**, inserts).

Each stepwise increase in management intensity modulated different yield components (**Figure 6**). In the first step (i.e., addition of enhanced fertility to the FP), the responsiveness of yield ranged from 0.85 to 2.22 (mean: 1.23 ± 0.03) and was positively linked to the responsiveness of the productive tillers plant^{-1} (range: 0.48–4.28, mean: 1.49 ± 0.06), biomass (range: 0.50–4.26, mean: 1.40 ± 0.06), spikes m^{-2} (range: 0.53–2.75, mean: 1.40 ± 0.04), and kernels m^{-2} (range: 0.39–4.12, mean: 1.44 ± 0.06) (**Figure 6**, first row). We also note that yield responsiveness was positively associated with grain protein responsiveness (range: 0.93–1.52, mean: 1.11 ± 0.01) when fertility drove yield increase. When one fungicide application was added to the EF, yield responsiveness ranged from 0.77 to 1.82 (mean: 1.20 ± 0.02) and associated positively with responsiveness of biomass (range: 0.61–1.86, mean: 1.14 ± 0.03), spikes m^{-2} (range: 0.55–1.68, mean: 1.06 ± 0.02), and kernel weight (range: 0.79–1.58, mean: 1.11 ± 0.02) (**Figure 6**, second row). The addition of an early fungicide application to the EI had very weak relationships of yield responsiveness (range: 0.65–1.36, mean: 1.02 ± 0.01) to the responsiveness of biomass (range: 0.58–1.44, mean:0.97 ± 0.02) and HI (range: 0.70–1.84, mean: 1.08 ± 0.03) (**Figure 6**, third row). Likewise, the addition of micronutrients to the IFP treatment suggested that responsiveness of biomass (range: 0.77–1.67, mean: 1.12 ± 0.06) and of HI (range: 0.62–1.43, mean: 0.96 ± 0.03) associated with responsiveness of yield (range: 0.81–1.53, mean: 1.06 ± 0.01) (**Figure 6**, fourth row). Finally, when crop density was reduced from the Yw, responsiveness in yield (range: 0.61–1.21, mean: 0.91 ± 0.01) was positively related to responsiveness of biomass (range: 0.45–1.53, mean: 0.89 ± 0.03), of HI (range: 0.54–2.20, mean: 1.08 ± 0.03), and of kernel weight (range: 0.75–1.47, mean: 1.05 ± 0.02), and negatively related to responsiveness of plants m^{-2} (range: 0.32–2.75, mean: 0.80 ± 0.06) and protein (range: 0.72–1.10, mean: 1.00 ± 0.01) (**Figure 6**, fifth row).

The slope of green canopy cover dynamics following fungicide application, as well as the cumulative radiation intercepted during the grain filling period, was positively associated with grain yield for the selected treatments that allowed for a direct comparison between fungicide and non-fungicide application (EF versus EI) (**Figures 7A,B**). Likewise, the difference between slopes of these treatments or intercepted radiation was positively related to grain yield difference (**Figures 7A,B**, insert). For each individual slope, intercept, and regression fit, please refer to **Supplementary Table 8**. Following a similar trend, green canopy cover values measured at anthesis, and the cumulative radiation intercepted after anthesis for the Yw and IPP treatments, related positively with grain yield (**Figures 7C,D**), as did their differences (**Figures 7C,D**, insert), providing empirical evidence for the reason behind decreased yields from reduced crop density in an otherwise well high-input system.

DISCUSSION

We aimed to expand on the knowledge of the interactions G × E × M to identify opportunities for future yield increases for dryland winter wheat through yield component manipulation

TABLE 4 | Least square mean winter wheat plants m^{-2} as affected by management practices (FP, EF, EI, IFP, Yw, and IPP), environments (Bel18, Hut18, Bel19, and Hut19) and genotypes.

	Environment					**Genotype**				
	Bel18	**Hut18**	**Bel19**	**Hut19**	**Mean**	**WB4303**	**WB4458**	**WB-Grainfield**	**Zenda**	**Mean**
Management	**Plants m^{-2}**					**Plants m^{-2}**				
FP	275.3Aa	231.3Aab	275.7Aa	196.0Ab	244.6	242.8Aa	245.0ABa	249.6ABa	240.9Aa	244.6
EF	284.1Aa	211.5Ab	264.9ABa	202.6Ab	240.8	236.9Ab	258.5ABa	226.1Bb	241.6Aab	240.8
EI	293.1Aa	224.2Ab	281.9Aa	207.0Ab	251.6	256.0Aa	254.2ABa	257.4Aa	238.6Aa	251.6
IFP	273.6Aa	233.1Aab	280.3Aa	203.7Ab	247.7	248.4Aab	266.0Aa	238.6ABb	237.6Ab	247.7
Yw	291.7Aa	219.2Ab	219.3Bb	195.0Ab	231.3	247.7Aa	228.1Bab	223.0Bb	226.5Ab	231.3
IPP	144.2Bb	100.7Bb	242.3ABa	138.5Bb	156.4	156.1Ba	157.6Ca	149.2Ca	162.8Ba	156.4
Mean	260.3	203.3	260.7	190.5		231.3	234.9	224.0	224.7	

Least square means followed by a common uppercase letter (comparisons across management) or a lowercase letter (comparisons across environments or genotypes) are not significantly different by the Tukey test at the 5% level of significance.

TABLE 5 | Least square mean winter wheat productive tillers plants^{-1} affected by wheat genotypes (WB4303, WB4458, WB-Grainfield, and Zenda), environments (Bel18, Hut18, Bel19, and Hut19).

	Environment					**Management**						
	Bel18	**Hut18**	**Bel19**	**Hut19**	**Mean**	**FP**	**EF**	**EI**	**IFP**	**Yw**	**IPP**	**Mean**
Genotype	**Productive tillers plant^{-1}**					**Productive tillers plant^{-1}**						
WB4303	2.78Ca	2.73Ba	2.72Ba	2.99Ca	2.81	2.16Ac	2.66Bbc	2.54CBCc	2.50Bbc	3.16Cab	3.81Ba	2.81
WB4458	3.48Ba	2.93Bab	2.51Bb	2.94Cab	2.97	2.19Ac	2.70Bbc	2.90BCbc	2.60Bc	3.36Cab	4.06Ba	2.97
WB-Grainfield	3.91Aa	3.47Aab	3.08Ab	3.52Bab	3.50	2.26Ac	3.62Ab	3.11Bb	3.22Ab	3.79Bb	4.97Aa	3.50
Zenda	4.14Aa	3.62Aab	3.29Ab	4.20Aa	3.81	2.57Ac	4.0Abc	3.78Abc	3.37Ac	4.22Aab	4.90Aa	3.81
Mean	3.5775	3.1875	2.9	3.4125		2.295	3.2525	3.0825	2.9225	3.6325	4.435	

Least square means followed by a common uppercase letter (comparisons across genotypes) or a lowercase letter (comparisons across environments or genotypes) are not significantly different by the Tukey test at the 5% level of significance.

using Kansas, United States, as a case study. The average grain yield in the FP was 4.01 Mg ha^{-1}, which resulted in a yield gap of 1.37 Mg ha^{-1} when compared to the highest yielding treatment (Yw). Similar yield levels and yield gaps have been reported for the area under intensified management (Jaenisch et al., 2019, 2021; de Oliveira Silva et al., 2020b), confirming the opportunity to increase current yields through management intensification.

The management comprised of enhanced fertility plus one foliar fungicide application around heading (i.e., EI) resulted in average yield of 5.36 Mg ha^{-1}, which was similar to the Yw treatment, although the latter received an additional fungicide application and micronutrients. Thus, these additional practices might not be necessary to fill the bulk of the yield gap, although this was environment-dependent (i.e., Hut19). Additionally, in environments where water deficit limited the yield potential of the crop, EF was sufficient to maximize grain yield, precluding application of foliar fungicides. Furthermore, in one dry environment with high NO_3-N carryover (Bell18), the FP was enough to maximize grain yield. These findings support the idea that managing with the goal of reaching the yield potential might not be economical (Lobell et al., 2009).

Wheat genotypes responded differently to increased yielding conditions but similarly to management (**Figure 2** and **Table 3**), suggesting that selecting wheat genotypes either with performance specific to the most reoccurring environment in a given region or with broad adaptability seems more promising than genotype-specific management. We note, however, that the lack of significant G × M interaction in this research might be due to a small sample size, as previous research with larger sample size showed significant G × M (Thompson et al., 2014; Cruppe et al., 2021).

Management Practices and Their Effects on Wheat Yield Components

Our results align well with previous literature reporting that, across all sources of variation, wheat grain yield relates closely to aboveground biomass and kernels m^{-2}, and is relatively independent of harvest index and kernel weight (Slafer et al., 2014; Ferrante et al., 2017; de Oliveira Silva et al., 2020b). However, an original contribution of our research is the detailed yield responsiveness analysis and its relation to yield component responsiveness for each individual step in management intensification (**Figure 6**). To our knowledge, this has not been previously attempted in the existing literature of wheat response to management intensification. From this analysis, it was clear that the yield responsiveness was greater for added fertility (EF) and one fungicide application (EI) (mean

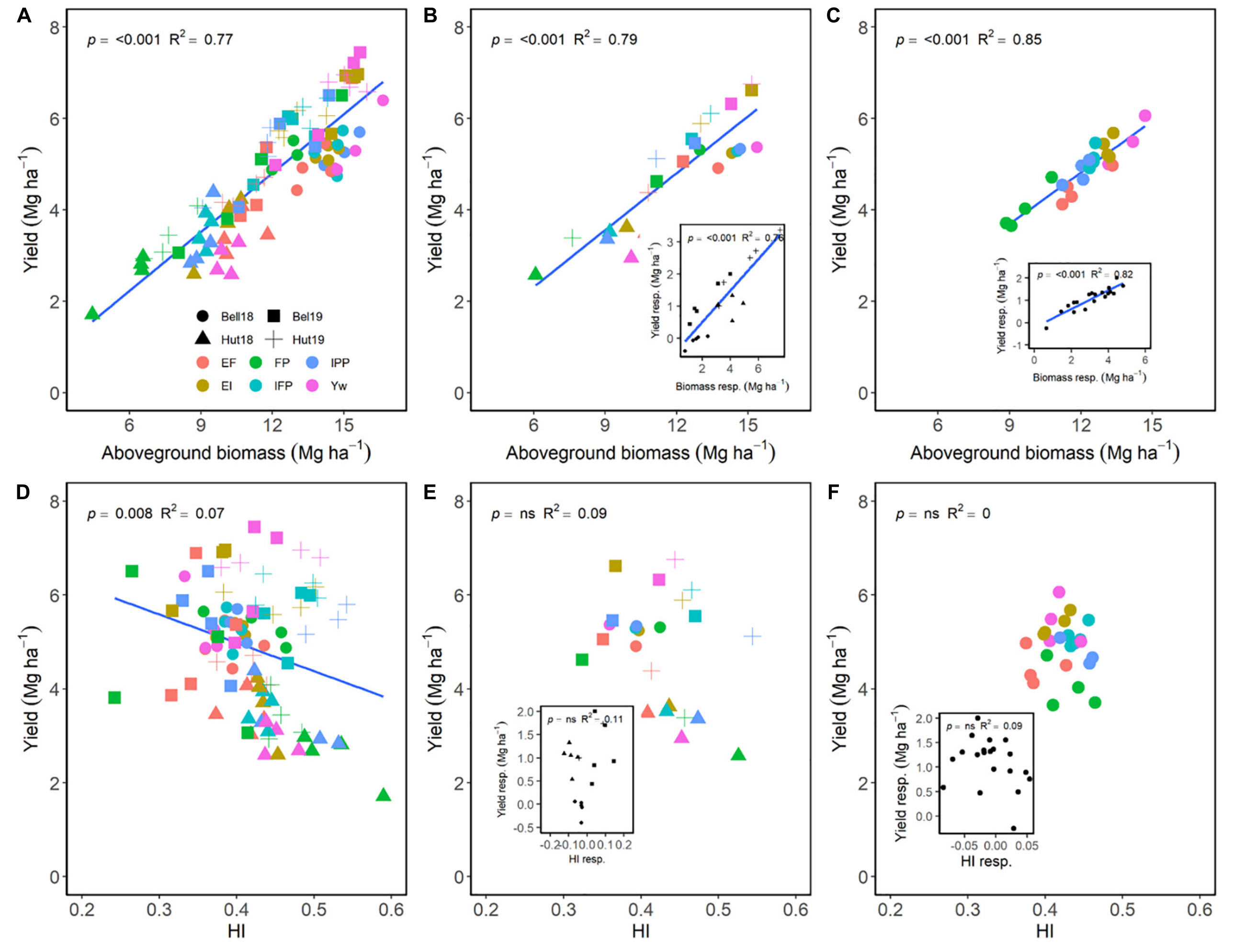

FIGURE 3 | Relationship between yield and aboveground biomass **(A–C)** or harvest index **(D–F)** at maturity across environments, wheat genotypes, and management systems (n = 96) **(A,D)**, on average of each management for each environment (n = 24; 6 management practices × 4 environments) **(B,E)**, on average of each genotype for each environment (n = 24; 6 management practices × 4 genotypes) **(C,F)**. Inset graphs are the relationships between the responses of the variables to each management practices (difference between each management practice from the FP) averaged across either genotype for each management practice (n = 20) or management for each environment (n = 20) **(C,F)**.

responsiveness of 1.20–1.23) as compared to the remaining practices (mean responsiveness of 0.91–1.06). The added fertility drove improvements in yield mostly through greater number of productive tillers plant^{-1}, biomass, spikes m^{-2}, and kernels m^{-2}, while the added fungicide modulated yield through biomass, spikes m^{-2}, and kernel weight (**Figure 6**). Interestingly, the reduced crop density mostly decreased yield (responsiveness: 0.91) through reductions in biomass (responsiveness: 0.89), although there was some compensation through increased in harvest index (responsiveness: 1.08). The remaining practices only slightly modulated biomass and harvest index, having little effect on yield.

The modulation of yield through kernels m^{-2} driven by the added fertilizer (EF) is justified as both in-furrow P fertilizer, and N fertilizer increases tiller initiation (Spiertz and De Vos, 1983; Rodríguez et al., 1999), and N fertilizer can reduce floret abortion (Ferrante et al., 2010; González et al., 2011). Tiller production determines the potential spikes m^{-2}, and floret development determines the potential kernels spike^{-1}. Both yield components interact with environmental conditions to determine kernels m^{-2}, which were highly positively related to yield (**Figure 4**). Thus, N availability has to meet the requirements for both of these processes during the growing season as untimely N deficiency can result in floret abortion and reduce kernels m^{-2}, potentially reducing yield. Nitrogen rates offer an opportunity for increased yields (Lollato et al., 2021), especially in favorable seasons where the crop can capitalize on a greater yield potential (Cruppe et al., 2017; Lollato et al., 2019a). Expected N uptake based on yield potential can serve as a guide for managing N rates in the season (Leikam et al., 2003); and, for wheat, a recent synthesis of global literature has suggested that N uptake ranges from ∼20 to 400 kg N ha^{-1} (de Oliveira Silva et al., 2020a). Thus, matching N availability with the time when the potential number of kernels m^{-2} is determined (i.e., early stem elongation) results

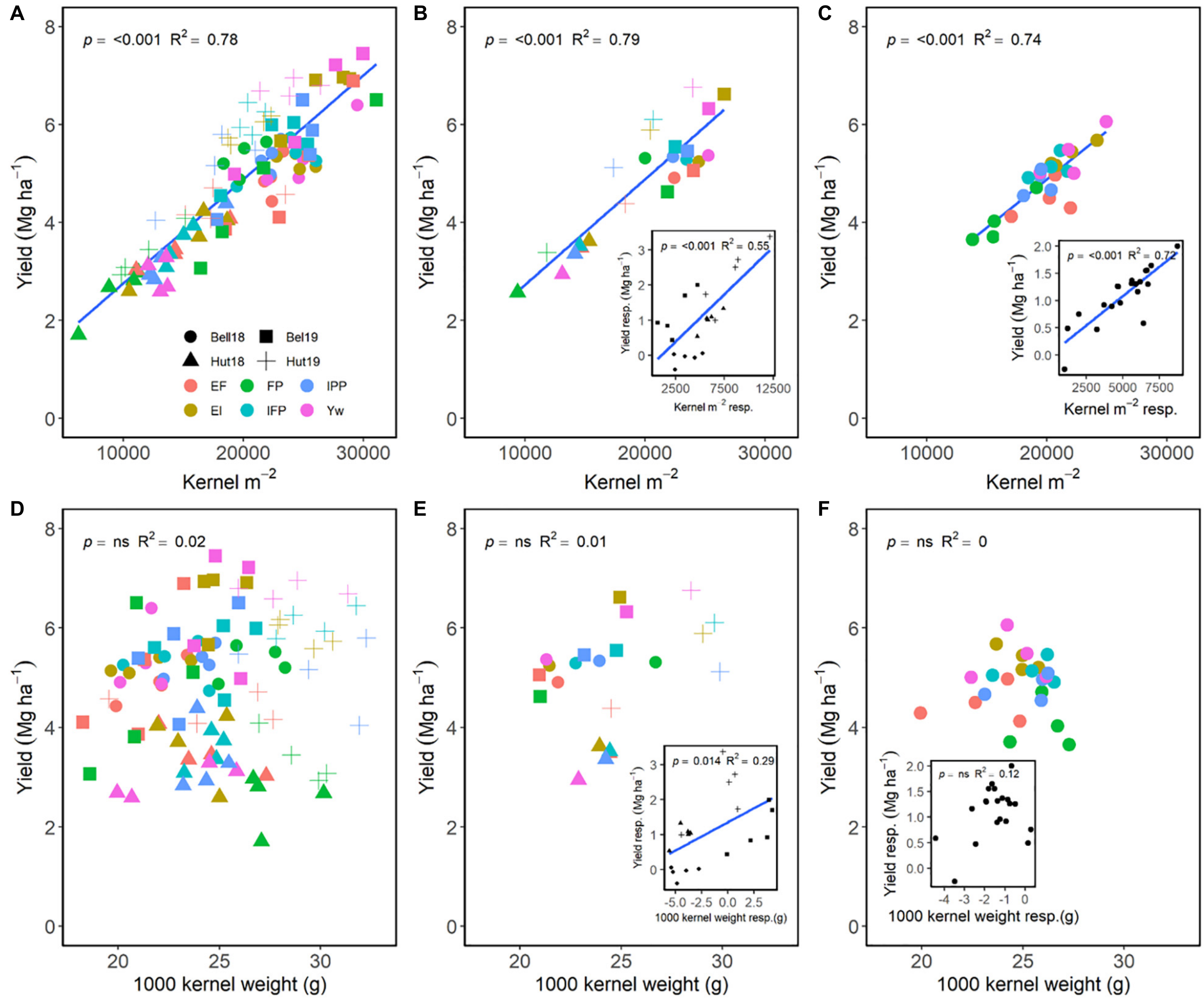

FIGURE 4 | Relationship between yield and kernels m^{-2} **(A–C)** or 1,000 kernel weight **(D–F)** across environments, wheat genotypes, and management systems (n = 96) **(A,D)**, on average, each management for each environment (n = 24; 6 management practices × 4 environments) **(B,E)**, on each genotype for each environment (n = 24; 6 management practices × 4 genotypes) **(C,F)**. Inset graphs are the relationships between the responses of the variables to each management practices (difference between each management practice from the FP) averaged across either genotype for each management practice (n = 20) or management for each environment (n = 20) **(C,F)**.

in yield increases as grain number is the dominant driver of yield (Borrás et al., 2004; Slafer et al., 2014). We also note that this developmental stage coincides with the greatest N uptake rate by the crop, which increases under intensive management (de Oliveira Silva et al., 2021).

Kernels m^{-2} and kernel weight are affected by complex interactions among many environmental factors in the late reproductive stages. Our results support available literature that suggests that kernels m^{-2} is a coarse regulator of wheat yield as compared to kernel weight (Borrás et al., 2004; Slafer et al., 2014), which is justified as each individual kernel has a narrow range in size (Sadras, 2007); thus, greater increases in grain yield come from filling more kernels (Borrás et al., 2004). We note, however, that increases in kernel weight through management associated positively with increases in yield (insert, **Figure 4E**), in particular through the application of foliar fungicides (**Figure 6**). These findings agree with previous reports of highly managed wheat in the U.S. Great Plains (Lollato and Edwards, 2015; Jaenisch et al., 2019; Cruppe et al., 2021) suggesting that kernel weight might, in some conditions, partially explain increases in yield for wheat.

Foliar diseases can occur prior to anthesis and last throughout the grain-filling period, coinciding with a period of significant demand for photosynthesized resources by the developing grain (i.e., a very strong sink; Fischer, 1985). These foliar diseases decrease the green leaf area of the plant (Schierenbeck et al., 2019), reducing radiation interception and radiation use efficiency (Schierenbeck et al., 2016), and ultimately decreasing the source of assimilates to the developing sink. This mismatch between a reduced assimilate supply (i.e., source) during a period with large demand can cause kernel abortion and reduce yield (Ferrante et al., 2010; González et al., 2011). Foliar fungicides

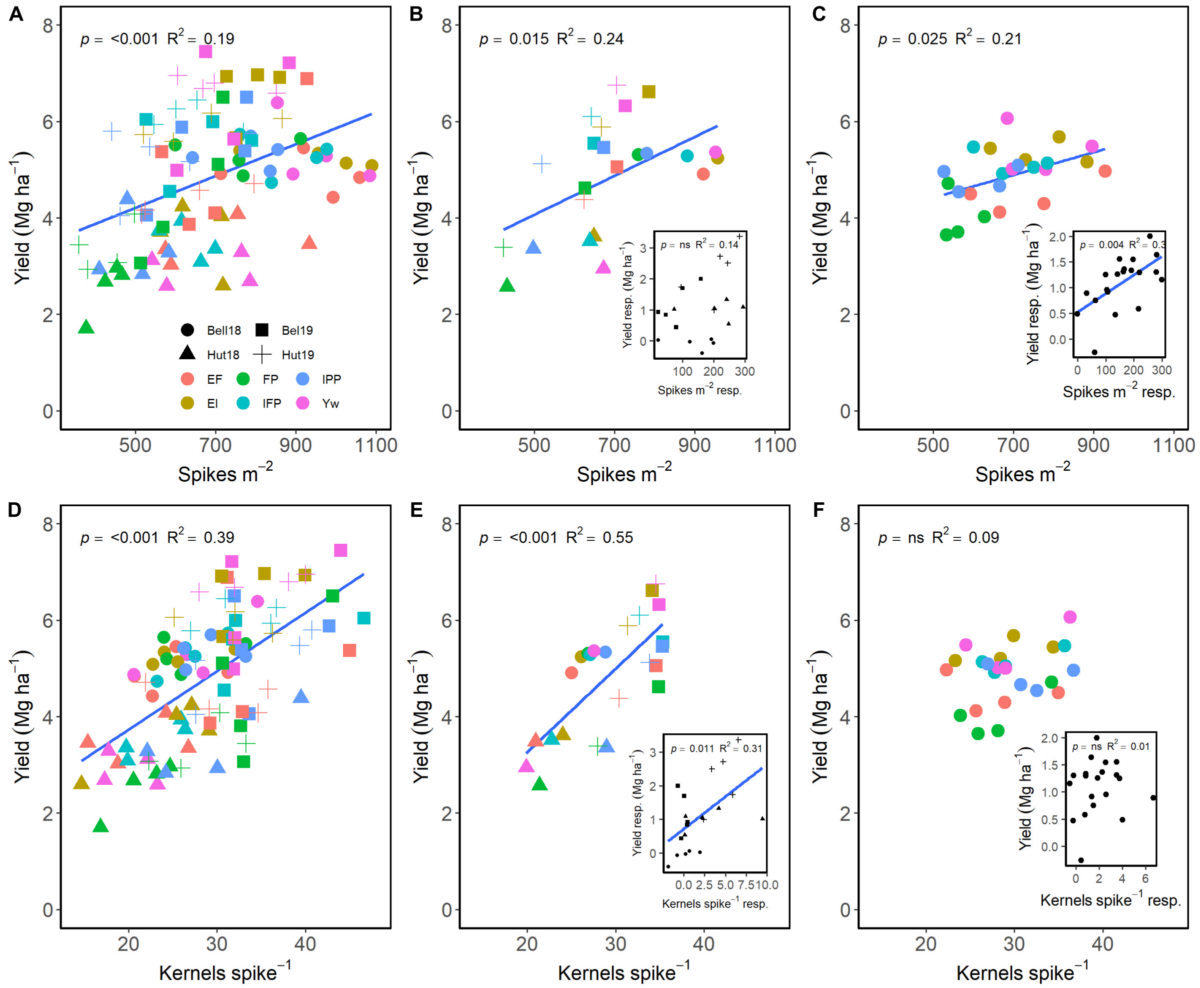

FIGURE 5 | Relationship between yield and spikes m^{-2} **(A–C)** and kernels spike^{-1} **(D–F)** across environments, wheat genotypes, and management systems ($n = 96$) **(A,D)**, on average, each management for each environment ($n = 24$; 6 management practices × 4 environments) **(B,E)**, on each genotype for each environment ($n = 24$; 6 management practices × 4 genotypes) **(C,F)**. Inset graphs are the relationships between the responses of the variables to each management practice (difference between each management practice from the FP) averaged across either genotype for each management practice ($n = 20$) or management for each environment ($n = 20$) **(C,F)**.

can also increase kernel weight under severe disease infestations, which can reflect increases in grain yield (Cruppe et al., 2021), although this increase is environment specific (Lynch et al., 2017). Wheat kernel weight is sensitive to environmental stresses (e.g., heat or drought) between booting to anthesis when carpel (which will turn into the external grain structures) growth increases rapidly (Calderini et al., 2001), and from anthesis to maturity during kernel weight determination (Bergkamp et al., 2018). Foliar diseases during these developmental stages can reduce kernel weight, which could reduce yield (**Figure 4E**, insert; **Figure 6**). Similarly, increases in kernel weight associate with kernel-filling rate, and foliar diseases can reduce the rate of fill due competition for assimilates (Simmons et al., 1982).

Foliar fungicides maintain the yield potential at time of application by protecting the upper canopy and spikes, which supply a large portion of the carbohydrates that determine yield (Rawson et al., 1983) and can increase kernels m^{-2} (Brinkman et al., 2014). The prolonged green leaf area maintained through fungicides also allows for longer duration of active photosynthesis, ultimately increasing N uptake (de Oliveira Silva et al., 2021) and grain yield (Joshi et al., 2019; Nehe et al., 2020). This was shown in the current research as a more negative slope of the green canopy cover dynamics and a lower cumulative radiation interception after anthesis in the treatments not receiving foliar fungicides (**Figure 7**). The positive relationship between the slope of canopy cover and grain yield also suggests that treatments not receiving foliar fungicides were, at least, to some extent, source limited, which was also evidenced by the greater grain protein concentration of treatments receiving foliar fungicides (data not shown). Further evidence for this source limitation is shown in the inset of **Figures 4E**, **6**, in which increases in kernel weight through management associated positively with yield increases. However, we note that large reductions in the green leaf area or radiation

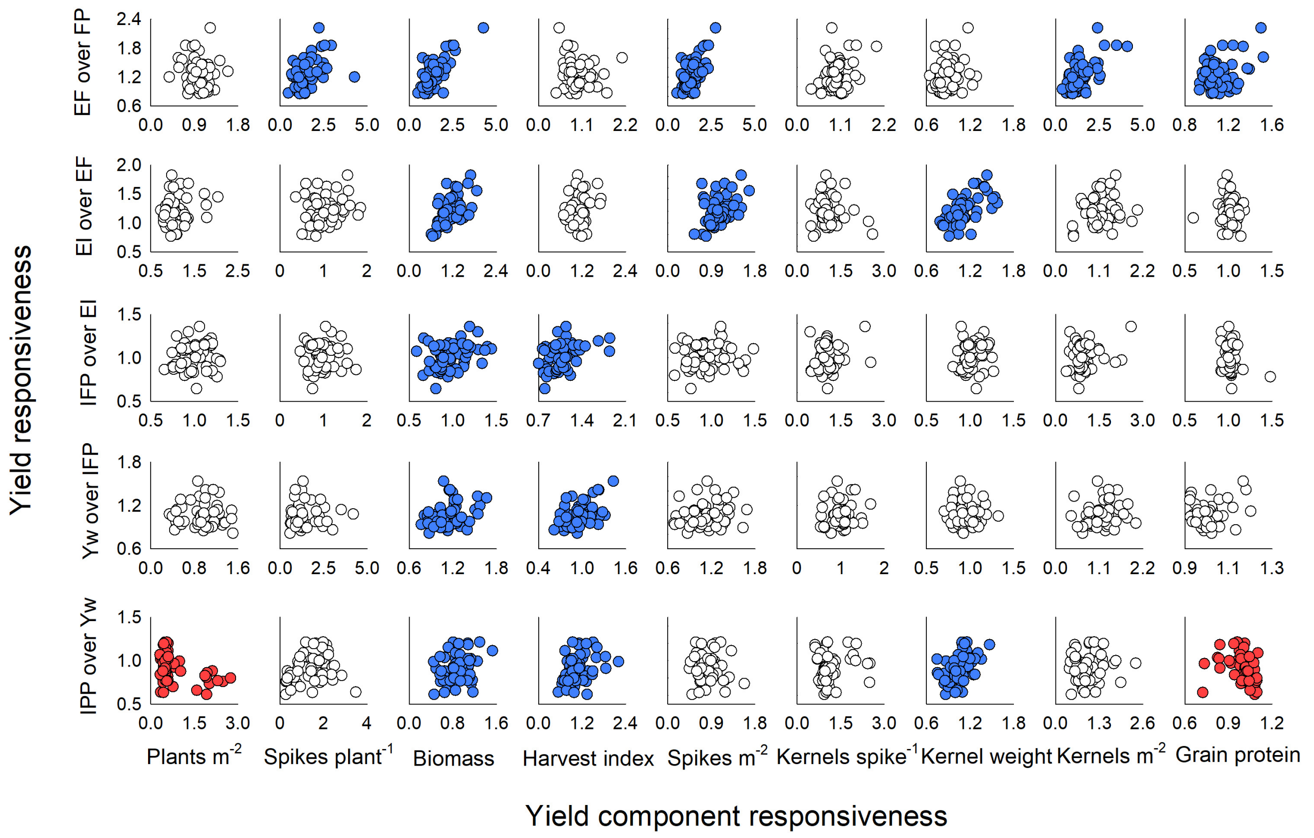

FIGURE 6 | Winter wheat yield responsiveness and its relationship with responsiveness of yield components (plants m^{-2}, biomass, harvest index, spikes m^{-2}, kernels $spike^{-1}$, and kernel weight) and grain protein concentration for each step of management intensification evaluated in the current study. Responsiveness values were calculated as enhanced fertility (EF) over farmer's practice (FP) (first row); ecological intensification (EF) adding a fungicide application at Zadoks GS55 over EF (second row); increased foliar protection (IFP), adding a fungicide application at Zadoks GS31 to EI (third row); rainfed yield potential (Yw), adding micronutrients at Zadoks GS31 to the IFP (fourth row); and increased plant productivity (IPP), reducing the seeding rate from Yw (fifth row). Circles in blue denote a significant positive and circles in red a significant negative relationship between variables at $p < 0.05$.

intercepted were needed to cause modest reductions in yield (**Figure 7**), likely because wheat is mostly sink-limited and very efficient in translocating stem reserves to the developing kernels (Borrás et al., 2004). Even though foliar fungicides applied around anthesis have increased wheat yield and reduced the yield gap in the region (Thompson et al., 2014; Jaenisch et al., 2019), producers may be reluctant to apply it consistently due to high environmental unpredictability (Couëdel et al., 2021) and inconsistencies in yield response (Cruppe et al., 2021).

The evaluation of a reduced crop density under an otherwise highly managed system (IPP) suggested that yield responsiveness was negatively related to responsiveness in plants m^{-2} (**Figure 6**), reflected on the overall yield reduction of IPP as compared to Yw (4.82 vs. 5.39 Mg ha^{-1}; **Supplementary Table 3**). This aligns with findings suggesting that crop density is an important determinant of the yield gap in rainfed wheat (Tokatlidis, 2014). Furthermore, it seems like the opportunity to reduce crop density in dryland conditions for winter wheat might not be as evident as that for irrigated spring wheat in low latitudes (Fischer et al., 2019), likely due to the unpredictability of conditions for tillering in the fall, which is dependent on many environmental variables (Tokatlidis, 2014). Nonetheless, we showed that there was a large genotypic component of tillering plasticity (**Figure 2C**) that might be further explored in this region. Tillering allows wheat plants to compensate for a low crop density, with greater opportunities in higher-yielding environments (Bastos et al., 2020), which was shown in this study with the IPP producing more tillers than other treatments. Tillering plasticity regulates the ability of a given genotype to tiller in different environments, which also interacts with crop density. Thus, a wheat variety with high-tillering potential and tillering plasticity (e.g., Zenda, **Figure 2**) has the ability to produce more productive tillers at reduced density (**Figure 2C**) and modulate yield through harvest index and kernel weight (**Figure 6**). On the other hand, a variety with low-tillering potential and plasticity (e.g., WB4303, **Figure 2**) is reliant on higher crop densities to attain desirable yields because individual plants are inefficient in using available resources (Tokatlidis, 2017). Evidence for other cereals suggests that high phenotypic plasticity of tillering can result in increased panicle weight under low-seeding rates (Kikuchi et al., 2017). Thus, selecting wheat genotypes for increased tillering capacity through conventional breeding could help reduce the risk associated with low-crop density (Fischer et al., 2019), which aligns with the

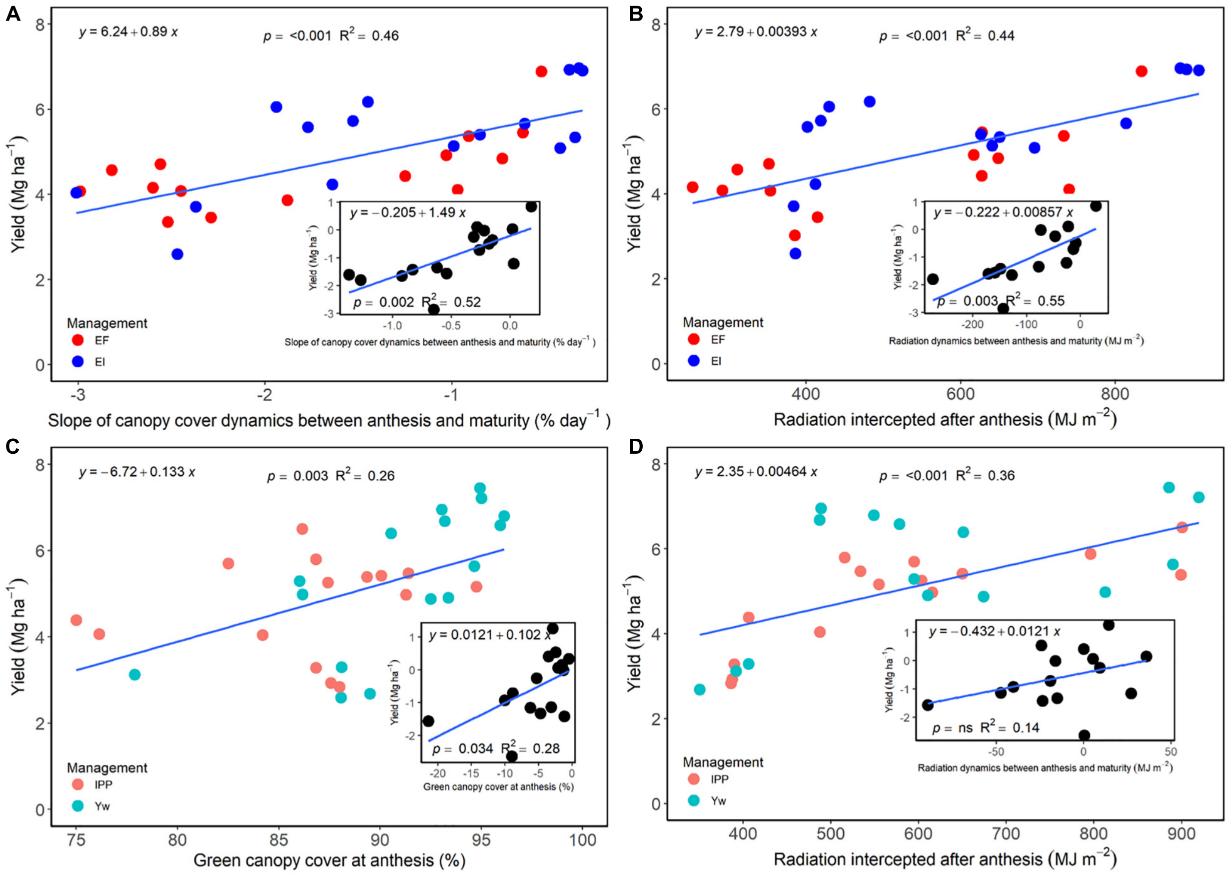

FIGURE 7 | (A) Relationship between wheat grain yield and a slope of the green canopy cover dynamics between anthesis and maturity for the enhanced fertility (EF) and ecological intensification (EI) treatments across genotypes and environments. The inset panel in **(A)** shows the relationship between the difference in both grain yield and a canopy cover dynamics slope between the two treatments. **(B)** Relationship between wheat grain yield and percent green canopy cover values measured at anthesis for the "yield potential" (Yw) and "increased plant productivity" (IPP) treatments across genotypes and environments. The inset panel **(B)** shows the relationship between the difference between IPP and Yw for grain yield and percent green canopy cover. **(C)** Relationship between wheat grain yield and radiation dynamics between anthesis and maturity for the EF and EI treatments across genotypes and environments. The inset panel in **(C)** shows the relationship between the difference in both grain yield and radiation dynamics between the two treatments. **(D)** Relationship between wheat grain yield and radiation values measured at anthesis for the Yw and IPP treatments across genotypes and environments. The inset panel **(D)** shows the relationship between the difference between IPP and Yw for grain yield and radiation.

early concept (Fasoulas, 1973) and more recent developments (Tokatlidis et al., 2006; Fasoulas, 2013) of selecting per-plant yield under nil competition.

Genotypic Characteristics to Increase Grain Yield

Wheat genotypes responded to the environment differently but not to management practices or to the interaction of management and environment. Thus, our findings suggest that wheat genotypes have to be adapted to specific reoccurring environmental conditions or broadly adaptable and have other desirable agronomic traits, such as high-yield potential (Ferrante et al., 2017), disease resistance (Serrago et al., 2011), heat or drought stress tolerance (Bergkamp et al., 2018), to match those commonly experienced in the environment where the genotype is grown. While the lack of G × E × M in our data might result from the limited number of observations (i.e., four environments), previous research in the region also only found weak evidence for G × E × M in response to management intensification ($p = 0.14$; de Oliveira Silva et al., 2020b).

The wheat genotype WB4303 was better adapted to higher-yielding environments and responded to increased environmental index by producing more kernels m^{-2}, which was highly correlated with increases in grain yield (**Figure 4**). These findings agree with those for other growing regions where modern genotypes were more adapted to higher-yielding environments and led to the hypothesis that the growers use older genotypes in their lowest-yielding soils and modern genotypes in their highest-yielding soils (Ferrante et al., 2017). While we did not test this hypothesis in Kansas, our findings suggest that this could be a promising strategy as the older genotype WB4458 was more adapted to lower-yielding environments, although further research is needed on this topic. For producers, selecting newer released genotypes might offer opportunities to capitalize on their ability to capture greater yields in higher-yielding environments (Slafer and Andrade, 1993; Perronne et al., 2017; de Oliveira Silva et al., 2020b) despite the challenge of finding information on new genotypes coupled with their limited life span (Perronne et al., 2017).

CONCLUSION

The results from this research confirmed a large yield gap that can be fulfilled through management, while highlighting the opportunity to modulate different yield components through specific management practices in a stepwise increase in management intensification. Overall, the results reinforced the need for an integrated wheat management based on crop scouting, as environmental conditions determined which management practices resulted in the greatest grain yields; in higher-yielding, high-moisture environments, increased fertility and one application of foliar fungicide at anthesis seemed to maximize grain yields; while in lower-yielding, dry environments, increased fertility alone was sufficient to maximize grain yields—and the increased fertility was only warranted over farmer's practice when the soil did not have enough fertility at sowing.

This research also confirmed the important role of aboveground biomass and kernels m^{-2} in maximizing grain yield at the expense of harvest index and kernel weight. Likewise, management of fertility led to yield modulation through improved biomass and kernels m^{-2}. We note, however, that independent steps in management intensification impacted different yield components, and a fungicide application around Zadoks GS55 had an important impact on grain yield partially through biomass, kernel weight, and maintenance of green canopy cover longer into the grain-filling period. While the positive relation between green canopy cover (or radiation interception) during grain filling and yield suggests some potential for source limitation, large changes in green canopy cover were needed to cause modest changes in yield. The reduction of crop density in an otherwise highly managed system provided varying results and seems to limit yield through decreased green canopy cover at anthesis, decreased radiation interception during the grain-filling period, harvest index, and kernel weight. Thus, future research could focus on optimizing seeding rates and identifying genotypes with increased phenotypic plasticity of tillering to maximize winter wheat yields within a highly managed system.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

BJ: conceptualization, data curation, formal analysis, data acquisition, investigation, methodology, and writing—review and editing. LM: statistical analysis and writing—review and editing. KJ: writing—review and editing. RL: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, visualization, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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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.2022.772232/full#supplementary-material

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Plant Population and Row Spacing Affects Growth and Yield of Rainfed Maize in Semi-arid Environments

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Increased tolerance to competition for soil resources of modern maize (*Zea mays* L.) hybrids increases soil resource use efficiency and yield. Yet little information is available on the relationship between maize population density and yield under no-tillage in semi-arid environments. A 2-year field trial was conducted in South Africa during the 2017/2018 (Season 1) and 2018/2019 (Season 2) production seasons to evaluate growth and water use productivity of rainfed maize established at seven diverse plant population (20,000–60,000 plants ha^{-1}) and row spacing (0.52 and 0.76 m) configurations. In Season 1, light interception was 6.8% greater at 0.76 m row spacing compared to 0.52 m row spacing ($p < 0.05$). In Season 2, despite dry and hot growing conditions, a well-developed leaf canopy cover was present at 0.52 m row spacing indicating a 10.4% greater intercepted photosynthetically active radiation (IPAR) compared to 0.76 m row spacing. In Season 1, with more uniform rainfall distribution, no biomass or yield benefits were found with increased plant population, except at 50,000 plants ha^{-1} at 0.76 m row spacing. In Season 2, plant populations at 0.76 m row spacing out-yielded any given plant population at 0.52 m row spacing. The optimal plant population and row spacing will ultimately be a compromise between obtaining high maize grain yield and minimizing the potential for crop failure in semi-arid environments.

Keywords: leaf area index, conservation agricultural practices, soil water, dryland agriculture, regenerative agriculture, corn, row width, plant density

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INTRODUCTION

Maize (*Zea mays* L.) produced under rainfed conditions is among the most important crops in semi-arid environments in various regions in the world, including parts of the United States, Northeast China, and South Africa (Clay et al., 2014; Qin et al., 2016; Haarhoff and Swanepoel, 2020). Semi-arid environments are characterized by high summer day temperatures and low or inconsistent rainfall where lengthy dry spells commonly occur during the growing season (Zuma-Netshiukhwi et al., 2013). As a result, the evapotranspiration greatly exceeds rainfall in semi-arid environments. For example, across the semi-arid maize production region of South Africa, evapotranspiration may exceed 2,500 mm *per annum* (Walker and Schulze, 2008), while long-term annual rainfall ranges between 400 and 550 mm. This disparity between rainfall and evapotranspiration highlights the importance of utilizing the available soil resources, particularly plant-available water, effectively (Haarhoff et al., 2020).

Improved agronomic practices often lead to improved efficiency of maize production (Swanepoel, 2021). Modern weed and pest management practices (Teasdale, 1998), crop residue retention (Sindelar et al., 2013), and soil tillage management strategies (Perez-Bidegain et al., 2007), provide pathways to reduce the effect of drought conditions on yield. Genetic advances coupled with increased plant population were major factors explaining recent maize grain yield improvements (Duvick, 2005). Modern hybrids are more stress-resilient and can withstand greater interplant competition enabling producers to increase maize grain yields through increasing the number of plants per unit area in more humid environments (Ma et al., 2014). Yield benefits of narrow row spacing depend on increased radiation interception (Andrade et al., 1999), which is generally accompanied by a reduction in evaporation from the soil surface. Additional benefits include a more uniform crop root distribution (Hammer et al., 2009) and improved weed control strategies (Sardana et al., 2017). Previous studies indicated that weed growth and nutrient uptake by weeds were significantly reduced when increased maize plant populations coupled with narrower row spacing was established (Arvadiya et al., 2012; Jha et al., 2017). Early leaf canopy closure and the greater shading of weeds results in an increase in the competitive ability of the growing crop (Singh et al., 2013). Promoting more efficient uptake and use of available soil water and nutrients (Sandler et al., 2015) using greater plant densities is critical in semi-arid environments for achieving sustainable yields under rainfed conditions (Haarhoff and Swanepoel, 2018). Despite these benefits, producers in semi-arid environments still opt for low plant populations (<30,000 plants ha^{-1}) established at a wide row spacing (> 0.91 m) to minimize risk of crop failure. Therefore, there exists a need to re-evaluate plant population and row spacing configurations under newly introduced agronomic practices to improve crop performance in these drier environments while still preserving the farmers' needs to minimize risk (Haarhoff et al., 2020).

Functional processes may depend on site and season characteristics (environment), such as soil water availability (Nielsen et al., 2010), soil water content at planting, rainfall amount and distribution, and the interaction between these characteristics with management practices. For example, plant population and row spacing determine the onset of competition between plants for resources and different biomass production (Tetio-Kagho and Gardner, 1988). Ample soil water during early vegetative growth stages may promote leaf expansion (thereby increasing radiation interception) and lead to excessive biomass production. When a prolonged dry spell occurs later in the growing season, a high leaf area index (LAI) promotes soil water extraction, resulting in a dry soil during the critical period for kernel set, hence severely affecting grain production.

The success of increased plant population and/or narrow row spacing is well-known in wet and humid environments such as in the United States Corn Belt (Duvick, 2005), Southwestern China (Qin et al., 2016), and the Argentine Pampas (Echarte et al., 2000). A comprehensive systematic review revealed that despite the increasing number of studies performed globally on plant population and row spacing, less than 5% were performed under no-tillage in semi-arid environments (Haarhoff and Swanepoel, 2018). Therefore, to fill this gap in information for these environments, rainfed field trials were conducted in the semi-arid maize production region of South Africa to evaluate the effects of maize plant population and row spacing on (i) aboveground growth and development; (ii) soil water-use productivity; and (iii) grain yield and yield components under no-tillage.

MATERIALS AND METHODS

Site Description

Field trials were conducted near Ottosdal (26°47′ S, 25°56′ E; altitude 1,490 m), North-West Province, South Africa, during the 2017/2018 (Season 1) and 2018/2019 (Season 2) production seasons. The region has a semi-arid climate (BSk) with a mean annual rainfall of 447 mm (Kottek et al., 2006). Approximately 90% of the annual rainfall occurs in the summer growing season (October to April). Rainfall patterns are highly inconsistent between seasons and dry spells during the growing season are common phenomena.

Soil type is a hard-xanthic Plinthic Haplustox (Soil Survey Staff, 2003). Soil bulk density in the 0–60 cm soil depth was 1.6 g cm^{-3} at the onset of the trial in Season 1. Soil texture was sandy loam with organic matter content of 0.9%. The experimental site has been under no-tillage since 2011. Maize monoculture practices were followed in the field trial and soil cover was approximately 95% in the 2 months following harvest. Strong winds during winter removed a large portion of the crop residues resulting in a soil cover of 35%–40% on the day of planting in each season. Maize monoculture is a common practice across the summer grain production region of South Africa due to favorable markets and livestock feed needs during the winter months (Haarhoff et al., 2020).

Cumulative growing degree days (GDD) were calculated according to Gilmore and Rogers (1958) using daily air temperature data provided by the South African Weather Service. The GDD base temperature was set as 10°C. Air temperature was measured at a weather station approximately 10 km from the trial site. Rainfall was recorded at the trial site using a manual rain gage.

Field Trial Design and Treatments

The experimental design was a randomized split-plot design with four blocked replicates. Whole-plots were row spacing (0.52 and 0.76 m), while plant population formed sub-plots, randomly nested within whole-plots (**Table 1**). These plant population configurations were chosen to achieve similar intra-row spacings in each row spacing as practiced by local producers. Plant populations of between 15,000 and 28,000 plants ha^{-1} are currently established by rainfed producers under conventional tillage conditions across the local region.

Plot lengths were 20 m and plot width depended on row spacing. Plots with 0.52 m row spacing had 12 rows leading to plot widths of 6.2 m, while the 0.76 m row spacing plots had 10 rows leading to 7.6 m widths. Plots were overplanted

TABLE 1 | Plant population and row spacing configurations and resultant intra-row spacings between plants as treatments.

Row spacing (m)			
0.52		0.76	
Plant population (plants ha^{-1})	Intra-row spacing (cm)	Plant population (plants ha^{-1})	Intra-row spacing (cm)
25,000	76	20,000	66
38,000	48	30,000	44
50,000	38	40,000	33
60,000	32	50,000	26

at 65, 000 plants ha^{-1} to ensure a high stand, and hand-thinned to the target plant populations at the fifth-leaf collar (V5) development stage (Ritchie et al., 1993), leaving a stand with uniform intra-row spacing in each treatment. The plots used in Season 1 were also used in Season 2 to include compounding effects of root biomass accumulation in the soil from the use of different plant densities. Maize plant density prior to Season 1 was 25, 000 plants ha^{-1}.

Trial Management and Calculations

Representative soil samples were taken prior to planting to establish baseline chemical properties. In both seasons, nitrogen was broadcasted prior to planting as urea at 75 kg N ha^{-1}, while 14 kg N ha^{-1} was band-placed as monoammonium phosphate at planting. Maize was planted by means of direct-drilling, using a 10-row John Deere 2117 no-tillage planter (John Deere Pty (Ltd.), Iowa, United States) and a six-row Jumil 2670-EX POP no-tillage planter [Jumil, Pty (Ltd.), Castelo, Espírito Santo, Brazil] in the 0.76 and 0.52 m row spacing plots, respectively.

The trials were established on 14 December 2017 and 4 January 2019 in Season 1 and 2, respectively. The optimal planting window for achieving maximum maize grain yield potential in the North-West province ranges between mid-November to mid-December. Early-autumn frost may occur at the end of April during kernel filling resulting in complete crop loss. Due to very hot conditions and low rainfall at the onset of Season 2, as recommended to farmers, planting was delayed beyond these dates. The 120-day Pioneer maize hybrid P2864WBR was used in both seasons (DuPont Pioneer Hi-Bred International). This hybrid was selected because it is one of the highest yielding cultivars in the region and commonly planted by local rainfed maize producers. Weeds were chemically controlled with pre-emergence herbicides after planting. Although weed pressure was low, hand-weeding was done throughout the growing seasons if necessary to keep plots weed free.

Total biomass was evaluated after emergence by randomly selecting five plants in each plot at 30, 60, 90, and 120 days after emergence (DAE). At least, 75% of plants reached the sixth-leaf collar (V6) stage at 30 DAE, tasseling (VT) at 60 DAE, the linear development phase of kernel filling (R3–R4) at 90 DAE, and physiological maturity (R5–R6) development stage at 120 DAE. Biomass samples were oven-dried at 60°C for 72 h to remove all moisture.

Intercepted photosynthetically active radiation (IPAR) and LAI were measured at VT, when maximum LAI was achieved, using an LP-80 AccuPAR ceptometer (Decagon Devices Inc., 2017). The 84 cm long probe was placed diagonally across two crop rows, with the two ends of the probe located in adjacent crop rows. This measuring regime is advised for row crops, as it provides a representative sample of the entire PAR environment below and between crop rows. The AccuPAR ceptometer calculates LAI based on the above and below-canopy measurements along with additional variables that relate to the canopy architecture and position of the sun. The IPAR and LAI measurements were done at five random spots within each plot above the leaf canopy (reference measurement, Qa) and at ground level (below-canopy measurement, Qb) between 12:00 and 14:00 on clear and windless days. The IPAR is reported as a percentage and was calculated using Equation 1:

$$IPAR\left(\%\right)=\left[1-\frac{Qb}{Qa}\right]x\,100 \qquad (1)$$

Soil water content was monitored at 2- to 3-week intervals in Seasons 1 and 2 from planting until R5-R6. One galvanized access tube (length 120 cm, diameter 4 cm) was in-stalled per plot using a hand auger (diameter 4 cm) immediately after planting in the middle of two crop rows. A neutron probe (503DR Elite Hydroprobe Model, CPN Inc., Concord, CA, United States) was used to record soil water content at 30, 60, 90, and 120 cm soil depths. To calibrate soil water data, gravimetric soil samples were taken approximately 100 cm from the access tubes at planting (at the same time as the neutron probe readings) using a hand auger (diameter 7 cm) at soil layers 0–30, 30–60, 60–90, and 90–120 cm to determine gravimetric soil water content using the standard gravimetric method (Schmugge et al., 1980). The soil samples were oven-dried for 72 h at 105°C to remove all water. The gravimetric soil water content of each soil sample was converted to volumetric water content by multiplying by the soil bulk density. A linear regression of calibration readings against volumetric water values was calculated and used to calculate volumetric water content from the growing season soil water readings. Volumetric soil water content (cm^{-3} cm^{-3}) was then converted to soil water content (mm) per layer by multiplying the volumetric soil water content by the soil layer depth (mm). Crop evapotranspiration (crop ET) was calculated as rainfall minus the change in soil water content (accumulated 0–120 cm soil depth) between subsequent measurements, minus drainage. Runoff was considered negligible as the experimental site is flat (<0.5% slope) and well drained. Water productivity for grain (WPg) and biomass production (WPb) were estimated by dividing maize grain yield and total biomass at R5–R6 by the seasonal crop ET (Hatfield and Dold, 2019).

Maize grain yield was determined by hand harvesting the full length of the center eight and six rows of the 0.52 and 0.76 m plots, respectively. Yield components were determined by randomly selecting 10 plants per plot at harvest. Grain samples were oven-dried at 60°C until constant weight and kernel weight was calculated by weighing a sample of 1,000 kernels. Harvest index was calculated by dividing maize grain yield by biomass as determined at R5–R6. All grain yield data were standardized to a moisture level of 12.5%.

Statistical Analyses

Statistical analyses were performed by using Statistica version 13.5.0.17 (TIBCO Software, 2018). The Restricted Maximum Likelihood (REML) procedure was used to analyze according to the split-plot design. Three treatment factors were specified as fixed effects, i.e., plant population, row spacing and season, as well as the interaction between all three factors. Block, the interaction between block and plant population and block and row spacing were specified as random terms. The REML procedure was followed because the random factors of the dependent variables are also estimated, which allowed the evaluation of the effects of both row spacing and plant population as well as the interactions, despite dissimilar plant population treatments between the 0.52 and 0.76 m row spacings. Fisher's least significant differences (LSD) test were conducted at a 5% significance level to determine whether interactions among the three factors of interest were significant. The Bonferroni correction test was used as validation of the Fisher's LSD test to reduce the chances of obtaining false-positive results (type I errors), since multiple pairwise tests were performed on a single set of data. Normality of residuals and homogeneity of variances were tested and fulfilled the assumptions of the statistical model.

Growing Conditions

The total amount of rainfall during the growing period of Seasons 1 and 2 were 263 and 310 mm, respectively. The amount of rainfall for the 8 weeks prior to planting of trials in Seasons 1 and 2 were 83 and 62 mm, respectively. The distribution of rainfall during the cropping seasons was variable and dry spells occurred in both seasons (**Figures 1A,B**). Despite the late planting date in Season 2, average air temperature was comparable between seasons with cumulative growing degree days (GDD) totaling 1,404 and 1,386 from seedling emergence (VE) to R5–R6 in Season 1 and 2, respectively.

In Season 1, the total amount of rainfall from VE to 14-leaf collar (V14) was 149 mm, corresponding to a deficit of 70 mm compared to the 30-year average. In spite of the low rainfall during this period, soil water status was adequate and early vegetative growth was not affected by the prevailing growing conditions. A dry spell occurred from 57 to 88 DAE when plants were in the early reproductive development stages (VT to R3–R4). Maize plants across all treatments were under severe water stress, thereby negatively affecting kernel development. From 88 DAE onward, wet conditions prevailed with 102 mm received between R3–R4 and R5–R6 allowing satisfactory kernel filling.

Season 2 was characterized by challenging growing conditions from the onset of the season. Between VE and V14, a total of 138 mm of rainfall was received, with only two rainfall events recording more than 15 mm. Between V10 and R3–R4, a prolonged dry spell combined with high air temperatures occurred. Only 15 mm of rainfall was received between the V10 and R3–R4. At this point in the growing season, rainfall received was 130 mm below the 30-year average. Water-stress conditions negatively affected final vegetative growth, pollination, and ear growth across all treatments. Wet conditions and cool air temperatures characterized the period between R3–R4 and R5–R6, allowing maize plants to conclude the latter stages of kernel filling under stress-free growing conditions.

RESULTS

IPAR and LAI

Both IPAR and LAI were affected by the interaction between row spacing and season ($p < 0.05$). In Season 1, IPAR was 6.8% greater at the 0.76 m row spacing compared to the 0.52 m row spacing (**Table 2**; $p < 0.05$). In Season 2, despite challenging growing conditions, a well-developed leaf canopy cover was present at 0.52 m row spacing indicating a 10.4% greater IPAR compared to the 0.76 m row spacing. No differences in LAI were observed between row spacings in Season 1 ($p > 0.05$), however, LAI was 21.8% greater at the 0.52 m row spacing compared to 0.76 m row spacing in Season 2 ($p < 0.05$).

Soil Water Content and Crop ET

The water content of the soil profile varied over the seasons as a result of variable crop uptake, evaporation rate, and rainfall occurrence (**Supplementary Table S1**). The soil water content varied with time due to variable plant uptake, evaporation rate, and seasonal rainfall occurrence. Soil water contents were similar at the start and end of the growing season between plant population treatments, irrespective of the row spacing. Also, the timing of water loss from the soil was similar between treatments throughout the growing season. This indicates evaporation at low plant population (< 38,000 plants ha^{-1}) due to poor leaf canopy was similar to the evapotranspiration rate at higher plant populations. Crop ET was only affected by the main effect of season and was 255 and 333 mm in Season 1 and 2, respectively (results not shown; $p < 0.05$).

Crop ET as a function of IPAR at the 0.52 and 0.76 m row spacings is illustrated in **Figure 2**. At the 0.52 m row spacing, there was a strong positive response of crop ET to IPAR in both seasons ($r^2 > 0.8$; $p < 0.05$). At the 0.76 m row spacing, there was a positive response of crop ET to IPAR in both seasons, however, this response was weak ($r^2 > 0.3$) in both seasons. Crop ET was greater for a given IPAR in the 0.76 m row spacing than in the 0.52 m row spacing, but both showed similar responses to increasing IPAR.

Biomass Production

Total biomass at V6 was affected by the interaction of plant population and row spacing, without a season effect ($p < 0.05$). Total biomass at ≥50,000 plants ha^{-1} was greater than at lower plant populations at a similar row spacing due to lower interplant competition combined with adequate soil water levels during the first 4 weeks following planting (**Table 3**; $p < 0.05$). At 0.76 m

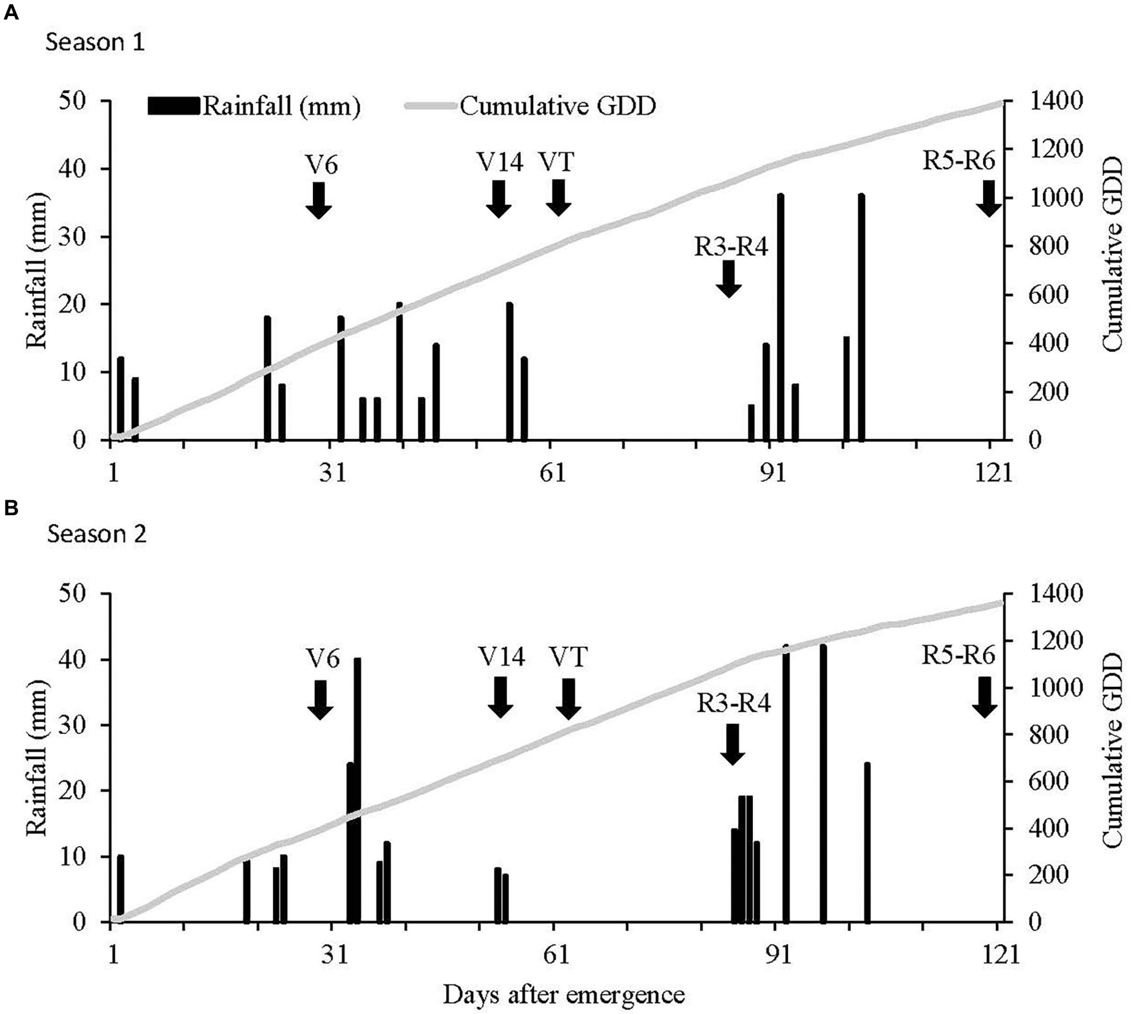

FIGURE 1 | Rainfall events and cumulative growing degree days (GDD) from 0 to 120 days after emergence (DAE) during **(A)** Season 1 and **(B)** Season 2 at the trial site near Ottosdal, South Africa. V6 = sixth-leaf collar, V14 = fourteenth-leaf collar, R3–R4 = the linear development phase of kernel filling, and R5–R6 = physiological maturity.

TABLE 2 | Effect of row spacing on IPAR and leaf area index (LAI) at tasseling (VT) across all plant populations in Seasons 1 and 2.

Season	Row spacing (m)	IPAR (%)	LAI
Season 1	0.52	74.88^{b}	3.75^{bc}
	0.76	80.31^{a}	4.01^{ab}
Season 2	0.52	82.46^{a}	4.36^{a}
	0.76	73.92^{b}	3.41^{c}

No common letter indicates a significant difference at level $p < 0.05$ in the ANOVA.

row spacing, total biomass at 50,000 plants ha^{-1} was less than at 40,000 and 50,000 plants ha^{-1} ($p < 0.05$).

Total biomass at the VT, R3–R4, and R5–R6 development stages was affected by the interaction between row spacing and season ($p < 0.05$). There was, however, no response of total biomass at R5–R6 to plant population indicating the trade-off associated with increased plant population in semi-arid environments when available soil resources are insufficient to address the greater demand at higher densities. At VT in Season 1, total biomass was 14% greater at 0.52 m than at 0.76 m row spacing ($p < 0.05$), while no differences were observed in total biomass between row spacings at R3–R4 and R5–R6 development stages (**Table 4**; $p > 0.05$). In Season 2, total biomass was lower at VT, R3–R4, and R5–R6 development stages with both row spacings compared to Season 1 ($p < 0.05$). Total biomass at 0.76 m row spacing was 32, 30, and 33% more than at the 0.52 m row spacing at the VT, R3–R4, and R5–R6 development stages, respectively.

Total biomass as a function of IPAR at 0.52 and 0.76 m row spacings is illustrated in **Figure 3**. In Season 1, there was a strong response of total biomass to IPAR at 0.76 m row spacing with increases of 468, 716, and 1,403 kg ha^{-1} for each additional 10% of IPAR at the VT, R3–R4, and R5–R6 stages, respectively. However, at the 0.52 m row spacing, the response of total biomass was positive at the VT stage (452 kg ha^{-1} per 10% increase in IPAR) but negative at the later growth stages.

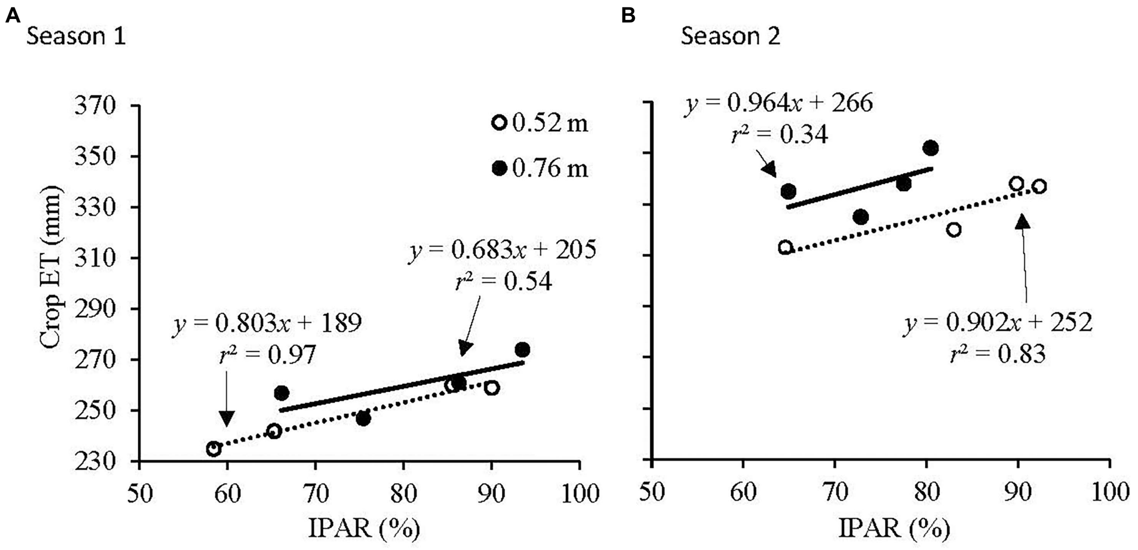

FIGURE 2 | Relationship of seasonal crop evapotranspiration (crop ET) to intercepted photosynthetically active radiation (IPAR) at 0.52 and 0.76 m row spacings in **(A)** Season 1 and **(B)** Season 2.

TABLE 3 | Effect of row spacing and plant population on total biomass at the sixth-leaf collar (V6) development stage across season.

Row spacing (m)	Plant population (ha^{-1})	Total biomass (kg ha^{-1})
0.52	25,000	976de
	38,000	1,255^{bcd}
	50,000	1,963^{a}
	60,000	2,029^{a}
0.76	20,000	868^{d}
	30,000	1,065^{cde}
	40,000	1,381^{bc}
	50,000	1,465^{b}

No common letter indicates a significant difference at level p < 0.05 in the ANOVA.

TABLE 4 | Effect of season and row spacing on total biomass at the tasseling (VT) stage, the linear development phase of kernel filling (R3–R4), and physiological maturity (R5–R6) across all plant populations.

Season	Row spacing (m)	Total biomass (kg ha^{-1})		
		VT	R3–R4	R5–R6
Season 1	0.52	9,483^{a}	10,476^{a}	12,796^{a}
	0.76	8,175^{b}	9,887^{a}	13,425^{a}
Season 2	0.52	4,170^{d}	5,290^{c}	6,591^{c}
	0.76	6,112^{c}	7,501^{b}	9,752^{b}

No common letter indicates a significant difference at level p < 0.05 in the ANOVA.

Contrasting responses of total biomass to IPAR were observed in the drier Season 2. Total biomass at 0.52 m row spacing had a negative response to IPAR at VT, R3–R4, and R5–R6 (**Figure 3**). Although total biomass at 0.76 m row spacing had a positive response to IPAR at VT, the response was weak. At R3–R4 and R5–R6, a weak negative response of total biomass to IPAR was observed at 0.76 m.

Grain Yield and Yield Components

Mean grain yield was considerably greater in Season 1 (8,119 kg ha^{-1}) than in Season 2 (7,162 kg ha^{-1}; **Table 5**). In Season 1, there were no yield differences between plant populations at the 0.52 m spacing or at populations less than 40,000 plants ha^{-1} at the 0.76 m spacing. However, the crop at 0.76 m spacing and 50,000 plants ha^{-1} yielded significantly more than all the other treatments. In Season 2 yield declined as population increased at the 0.52 m spacing, although the difference between yields at 50,000 and 60,000 plants ha^{-1} was not significant ($p > 0.05$). Yield was greater at the 0.76 m spacing and there were no yield differences between plant populations ($p > 0.05$).

Kernel weight was similar across all treatments is Season 1 (**Table 5**; $p > 0.05$). In Season 2, kernel weight decreased with increasing plant population at 0.52 m row spacing ($p < 0.05$). Kernels per plant and grain yield per plant decreased with increasing plant population at both spacings and in both years although differences were not always significant ($p < 0.05$). Grain yield per plant decreased with increasing plant population at 0.52 and 0.76 m row spacing (**Table 5**; $p < 0.05$). In Season 1, grain yield per plant was higher at 0.76 m row spacing compared 0.52 m, with the opposite effect observed in Season 2 ($p < 0.05$). Harvest index remained constant across all treatments in Season 1, however, at 0.52 m row spacing in Season 2, harvest index decreased with increasing plant population ($p < 0.05$).

Neither crop ET nor WPb was affected by the plant population and row spacing treatments and were 24.58 and 51.85 kg mm^{-1} in Season 1 and 2 across treatments, respectively (data not shown). The response of WPg to the treatments was similar to the response of grain yield. In Season 1, WPg ranged from 24.8 to 31.3 kg mm^{-1}, with differences between 25,000 and 60,000 plants ha^{-1} at 0.52 m row spacing and between 50,000 and 20,000 and 40,000 plants ha^{-1} at 0.76 m row spacing (**Table 5**; $p < 0.05$). In Season 2, WPg

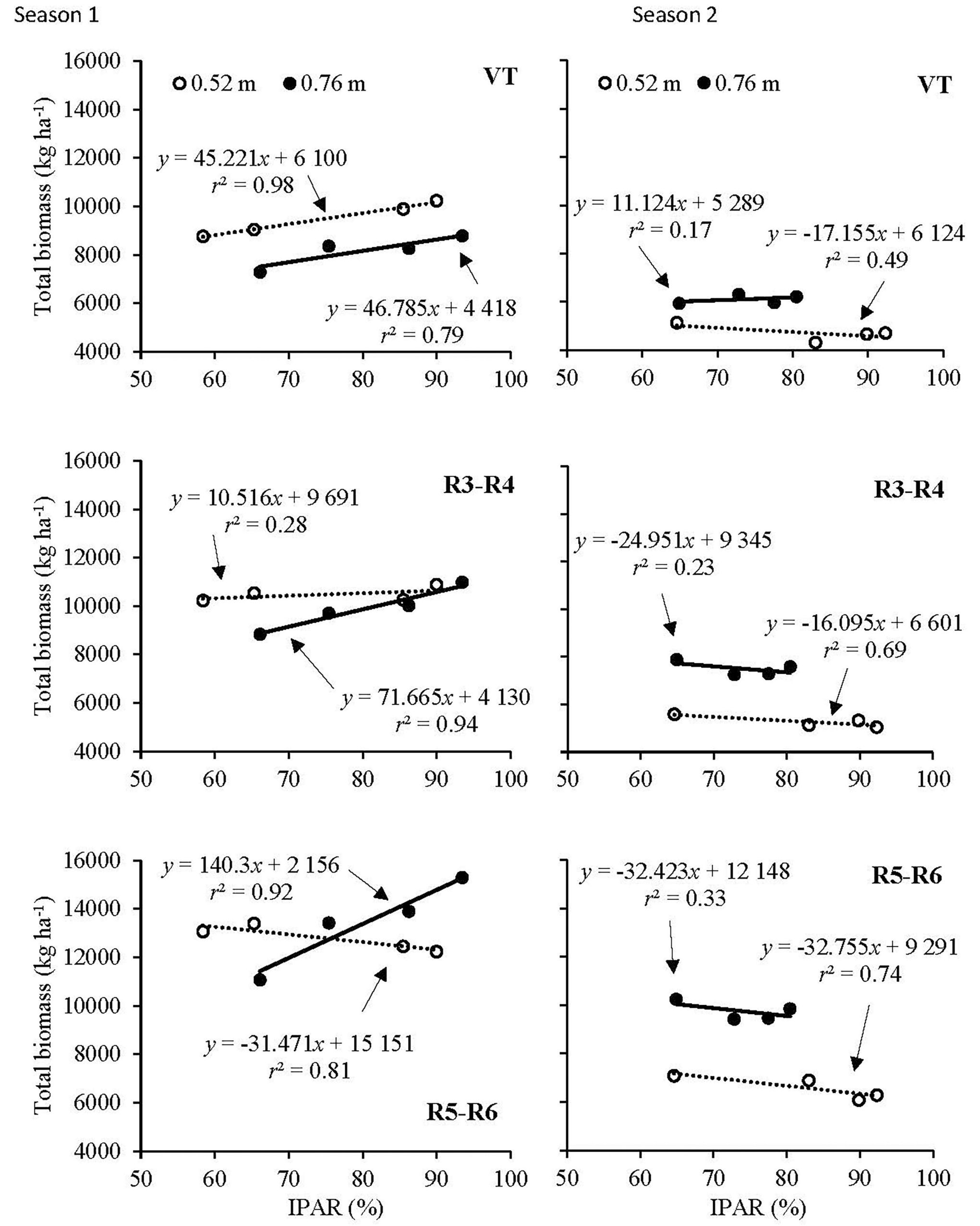

FIGURE 3 | Relationship of total biomass to IPAR at the tasseling (VT) development stage, the linear development phase of kernel filling (R3–R4) and physiological maturity (R5–R6) development stage at the 0.52 and 0.76 m row spacings in Season 1 (left) and Season 2 (right).

decreased ($p < 0.05$) with increasing plant population at 0.52 m row spacing, while WPg remained constant ($p > 0.05$) across plant population at 0.76 m row spacing. Treatment and seasonal effects on crop ET, WPb, and WPg during 2-week periods throughout the growing season were explored, however, no differences ($p > 0.05$) were found between treatments.

Grain yield as a function of crop ET at the 0.52 and 0.76 m row spacings is illustrated in **Figure 4**. In Season 1 at 0.52 m row spacing, a weak negative response of grain yield to crop ET was found, while a positive response in grain yield to crop ET was found at 0.76 m row spacing (**Figure 4A**). At 0.76 m row spacing, for each additional 10 mm of crop ET, maize grain yield increased by 651 kg ha^{-1}. In Season 2, a strong negative response of grain yield to crop ET was found at 0.52 m row spacing, while a weak positive response of grain yield to crop ET was found at 0.76 m row spacing (**Figure 4B**). At 0.52 m row spacing, for each additional 10 mm of crop ET, grain yield decreased by 945 kg ha^{-1}.

Grain yield as a function of IPAR at 0.52 and 0.76 m row spacings is illustrated in **Figure 5**. In Season 1, a negative response

TABLE 5 | Effect of row spacing and plant population on grain yield, kernel weight, kernels per plant, grain yield per plant, and harvest index in Seasons 1 and 2.

Season	Row spacing (m)	Plant population (ha^{-1})	Grain yield ($kg\,ha^{-1}$)	Kernel weight (g)	Kernels $plant^{-1}$	Grain yield $plant^{-1}$ (g)	Harvest index	WP_g($kg\,mm^{-1}$)
Season 1	0.52	25,000	$6,745^{bc}$	0.41^{bc}	656^{b}	270^{b}	0.52^{abc}	28.81^{ab}
		38,000	$6,804^{bc}$	0.41^{bc}	439^{de}	179^{c}	0.51^{abc}	28.08^{abc}
		50,000	$6,739^{bc}$	0.38^{bc}	357^{fgh}	135^{d}	0.54^{abc}	25.98^{bc}
		60,000	$6,366^{c}$	0.39^{bc}	274^{i}	106^{de}	0.52^{abc}	24.78^{c}
	0.76	20,000	$6,850^{bc}$	0.45^{bc}	759^{a}	342^{a}	0.61^{a}	26.68^{bc}
		30,000	$7,185^{b}$	0.44^{bc}	535^{c}	240^{b}	0.53^{ab}	29.11^{ab}
		40,000	$6,970^{bc}$	0.41^{bc}	422^{de}	174^{c}	0.50^{abc}	26.96^{bc}
		50,000	$8,580^{a}$	0.38^{bc}	450^{d}	172^{c}	0.56^{ab}	31.26^{a}
Season 2	0.52	25,000	$4,120^{e}$	0.41^{bc}	404^{def}	165^{c}	0.58^{ab}	13.20^{de}
		38,000	$3,001^{f}$	0.21^{e}	379^{efg}	76^{f}	0.45^{c}	9.39^{ef}
		50,000	$1,952^{g}$	0.12^{f}	329^{ghi}	41^{g}	0.33^{d}	5.79^{fg}
		60,000	$1,318^{g}$	0.09^{g}	327^{ghi}	22^{h}	0.21^{e}	3.97^{g}
	0.76	20,000	$5,280^{d}$	0.56^{a}	418^{def}	261^{b}	0.52^{abc}	15.79^{d}
		30,000	$4,685^{de}$	0.39^{bc}	401^{def}	155^{c}	0.50^{bc}	14.40^{d}
		40,000	$4,855^{de}$	0.34^{cd}	356^{efghi}	121^{d}	0.51^{abc}	14.35^{d}
		50,000	$5,100^{d}$	0.34^{cd}	292^{hi}	101^{de}	0.51^{abc}	14.47^{d}

No common letter within the same column indicates a significant difference at level $p<0.05$ in the ANOVA.

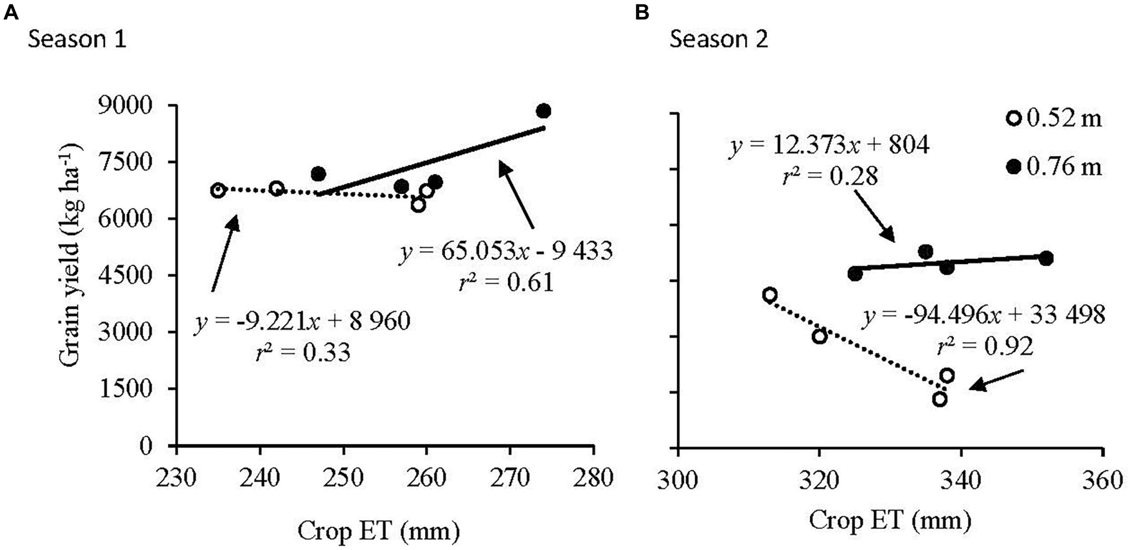

FIGURE 4 | Relationship of grain yield to accumulated crop evapotranspiration (crop ET) at the 0.52 and 0.76 m row spacings in **(A)** Season 1 and **(B)** Season 2.

of maize grain yield to IPAR was observed at 0.52 m row spacing, while the opposite was true at 0.76 m row spacing (**Figure 5A**). In Season 2, a strong negative response in grain yield to IPAR was observed at 0.52 m row spacing, while grain yield could not be explained by IPAR at 0.76 m row spacing (**Figure 5B**).

DISCUSSION

Intercepted photosynthetically active radiation is directly related to incident leaf canopy size and architecture (Flénet et al., 1996). Increased IPAR with increasing LAI is associated with higher plant populations (Fromme et al., 2019). Newly released maize hybrids underwent changes in aboveground morphology traits contributing to the success of greater plant populations (Duvick, 2005). Breeding efforts resulted in more vertical leaf growth above ears allowing more efficient sunlight interception and distribution throughout the leaf canopy (Mantilla-Perez and Salas Fernandez, 2017). Conserving soil moisture early in the season by developing less leaf area alongside improved root development may be beneficial in semi-arid environments with terminal droughts (Milander, 2015). In this study, when water-stress conditions occurred in Season 2, higher LAI and IPAR values were found at the narrower row spacing (0.52 m) compared to the 0.76 m row spacing when plant population were greater than 30,000 plants ha^{-1} (**Table 2**). The lower LAI and IPAR at the wider row spacing were advantageous later in the growing season when plants were in the reproductive stages, especially when soil water was limiting. Less vigorous

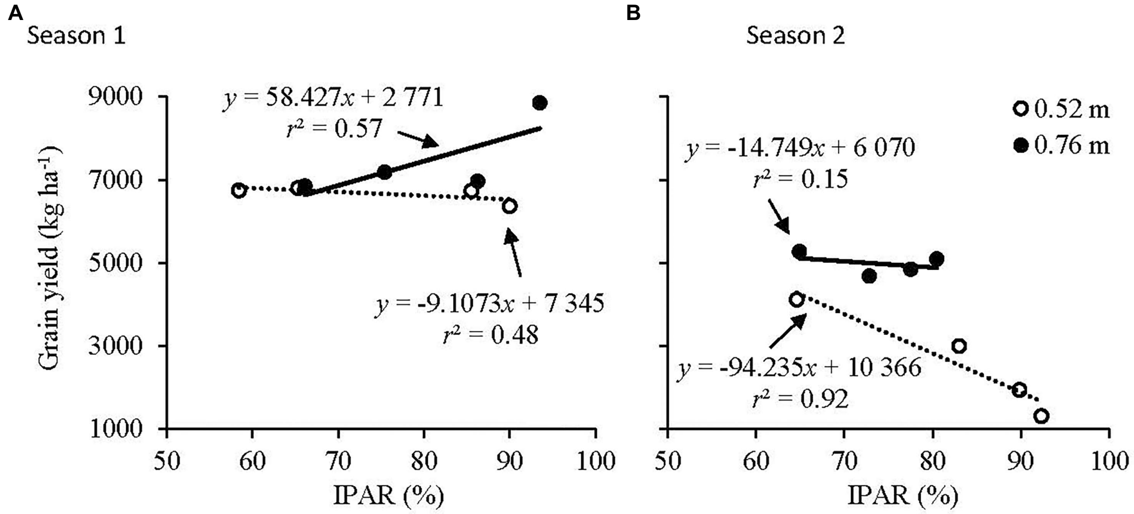

FIGURE 5 | Relationship of grain yield to IPAR at the 0.52 and 0.76 m row spacings in **(A)** Season 1 and **(B)** Season 2.

vegetative growth and investment in biomass production early in the growing season (**Table 3**) may have resulted in a lower transpiration demand. This enabled the crop to utilize available soil water more effectively for grain production when rainfall arrived later in the season. At the higher plant populations, soil water levels became depleted as dry conditions persisted as the growing season advanced, resulting in greater competitiveness between developing plants. As a result, biomass production was similar between plant populations at physiological maturity (**Table 4**). Despite greater sunlight interception at the higher plant populations, greater biomass was not observed. Closed stomata may have inhibited photosynthesis when stressed conditions occurred, while senesced leaf area may have been included in the IPAR measurements. Allen (2012) found increased biomass and grain yield at lower plant populations when less than 300 mm of rainfall was received during the growing season, however, the plant populations investigated was much lower compared to the plant populations in our study.

Timing of water-stress influences the relationship between grain yield and yield components (Blumenthal et al., 2003; Milander, 2015). High rainfall at R3–R4 in Season 1 provided favorable conditions for kernel growth onward and may have reduced the competition for carbon-assimilates (Uribelarrea et al., 2008). The 41% decrease in kernel number per plant alongside no significant decrease in kernel weight from the lowest to highest plant population at 0.52 and 0.76 m row spacings counterbalanced the increase in the number of plants per ha. This led to no grain yield response to plant population in Season 1, except for the 50,000 plants ha^{-1} established at 0.76 m treatment. A similar decrease in kernel number per plant and ear length with increasing plant population was reported in below-average rainfall seasons (Cox and Cherney, 2012; Zhang et al., 2014).

In Season 2, hot and dry growth conditions prevailed for the majority of the latter vegetative development stages and early reproductive stages (**Figure 1**) which lowered yield potential by inhibiting photosynthesis, pollination, and carbohydrate translocation to kernels (Boyer, 1982; Schussler and Westgate, 1991). Soil water availability per plant was very low during the linear phase of kernel filling and ceased kernel filling. This slowdown in the crop's life cycle was exacerbated with higher interplant competition exerted by the higher plant populations and narrower row spacing, resulting in low kernel weight and consequently poor grain yields despite increasing biomass production (Setter et al., 2001; **Table 5**). This explains the negative response of grain yield to crop ET in Season 2 at 0.52 m row spacing.

Cautious consideration must be given not only to plant population, but also the combination of plant population and row spacing in semi-arid environments. A maize grain yield of between 6,000 and 7,000 kg ha^{-1} is possible with plant populations of between 20,000 and 40,000 plants ha^{-1} irrespective of the row spacing. To achieve maize grain yields greater than 7,000 kg ha^{-1}, it appears that a plant population in excess of 40,000 plants ha^{-1} is required at a row spacing of 0.76 m. The evidence of improved sunlight interception and ultimately higher biomass and maize grain yields at high plant populations and 0.76 m row spacing in seasons with more timely rainfall is clear. However, in semi-arid environments, deciding on a more optimal plant population and row spacing will ultimately be a compromise between obtaining high maize grain yield and minimizing the potential for stress-induced yield losses. In seasons with low rainfall, lower plant populations (<40,000 plants ha^{-1}) will be associated with lower risk, but in seasons with adequate or plentiful rainfall a maize grain yield penalty could be expected (Birch et al., 2008). Although producers can use seasonal forecasts to adjust plant population at a given row spacing before planting, rainfall amount, and distribution throughout the particular season will ultimately determine if the approach is successful or not (Adisa et al., 2018, 2019). The higher seed costs associated with increased plant populations have a further impact on the decision-making process of producers (Lenssen et al., 2018), as economic losses increase when higher plant populations are established in dry seasons. Combining the economic (variable costs such as seed, labor, and fertilizer) and weather factors into a predictive model could produce a probability distribution of profit margin for each plant population management option.

CONCLUSION

Vegetative growth, biomass production, and grain yield responded inconsistently to plant population and row spacing between seasons due to timing of rainfall in relation to growth stage. In seasons with low and poorly distributed rainfall, there was no clear indication of benefits in terms of biomass production, grain yield, or water productivity with increased plant population at both 0.52 and 0.76 m row spacings, although plant population treatments at 0.76 m row spacing outperformed plant population treatments at 0.52 m row spacing. This was mainly attributed to poorer growth during the vegetative development stages, enabling plants to utilize available soil resources more effectively later in the season. In low-rainfall seasons lower plant populations (<40,000 plants ha^{-1}) will be associated with lower risk for crop failure, however, in seasons with plentiful rainfall a yield penalty could be expected. Although producers can use seasonal forecasts to adjust plant population at a given row spacing before planting, rainfall amount and distribution throughout the particular season will ultimately determine if the approach is successful or not. Developing prediction models by incorporating economic factors with weather-related factors such as rainfall amount and timing, and daily temperatures using long-term weather data (or generated weather for future scenarios) will serve as useful support tools. Producers and agronomists will be able to make better informed decisions when deciding on the optimal plant populations for a specific region and season.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

SH and PS: conceptualization, methodology, data curation, and investigation. SH: draft preparation. PS: supervision, project administration, funding acquisition, and review and editing. All authors contributed to the article and approved the submitted version.

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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.2022.761121/full#supplementary-material

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Nitrogen and Chemical Control Management Improve Yield and Quality in High-Density Planting of Maize by Promoting Root-Bleeding Sap and Nutrient Absorption

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High-density planting aggravates competition among plants and has a negative impact on plant growth and productivity. Nitrogen application and chemical control can improve plant growth and increase grain yield in high-density planting. Our experiment explored the effects of nitrogen fertilizer and plant growth regulators on maize root-bleeding sap, phosphorus (P) and potassium (K) accumulation and translocation, and grain yield and quality in high-density planting. We established a field study during the 2017 and 2018 growing seasons, with three nitrogen levels of N100 (100 kg ha^{-1}), N200 (200 kg ha^{-1}), and N300 (300 kg ha^{-1}) at high-density planting (90,000 plants ha^{-1}), and applied Yuhuangjin (a plant growth regulator mixture of 3% DTA-6 and 27% ethephon) at the 7th leaf. Our results showed that N200 application combined with chemical control could regulate amino acid and mineral nutrient concentration delivery rates in root-bleeding sap and improve its sap rate. Also, the treated plant exhibited higher P and K uptake and translocation ability. Furthermore, chemical control and N200 treatment maintained a high level of ribulose-1,5-bisphosphate carboxylase (RuBPCase), phosphoenolpyruvate carboxylase (PEPCase), nitrate reductase (NR), and glutamine synthetase (GS) enzymatic activities in leaves. In addition, plant growth regulator and nitrogen application improved the enzymatic activities of GS, glutamate dehydrogenase (GDH), and glutamic pyruvic transaminase (GPT) and the contents of crude protein, lysine, sucrose, and soluble sugar in grain and ultimately increased maize yield. This study suggests that N200 application in combination with chemical control promotes root vitality and nutrient accumulation and could improve grain yield and quality in high-density planting.

Keywords: nitrogen fertilizer, chemical control, root bleeding sap, nutrient absorption, maize

INTRODUCTION

The root is an essential absorption system, and its function is to maintain the supply of nutrients and soil moisture for crop growth and development (Xu et al., 2009; Fan et al., 2021). The root system of crops greatly influences the above-ground growth and biomass yield, which play an important role in yield formation (Yang et al., 2004; Chen et al., 2022). The capacity for nutrient and soil moisture uptake by crops is directly influenced by root development and root activity strength (Li et al., 2019). Well-developed root systems are always accompanied by vigorous above-ground growth and high yields. Root-bleeding sap is a sign of root pressure, and its change is consistent with root activity (Xu et al., 2016). The root-bleeding sap is directly correlated to the uptake of nutrients and water and reflects the root system's potential for plant growth and root activity (Ansari et al., 2004; Noguchi et al., 2005). The concentration of nutrients in root-bleeding sap represents the nutritional status and reflects root absorption and translocation rates in crops (Noguchi et al., 2005; Nishanth and Biswas, 2008). Hence, an appropriate rate of root-bleeding sap is vital to optimizing maize yield and directly influencing maize growth and development.

Nutrient absorption and translocation in crops are the physiological basis for dry matter accumulation and yield formation, influencing crop growth and development (Wu et al., 2018; Li et al., 2021). The difference in biomass yield is closely correlated to the plant's nutrient uptake and utilization characteristics. It is generally believed that obtaining a higher yield requires crops to absorb a large amount of nutrients from the soil (Wu et al., 2015; Zhan et al., 2016). Phosphorus promotes carbohydrate and starch synthesis in stems and leaves and increases the nutrient transport to the grains, thereby improving grain weight and quality (Wang and Ning, 2019). Potassium can stimulate the synthesis and transport of carbohydrates and promote the growth of maize ear (Shahzad et al., 2017). Phosphorus and potassium are nutrient elements in great demand for maize. Adequate P and K supply promotes root development and dry matter accumulation and enhances maize's resistance to stress (Xie et al., 2011; Iqbal et al., 2020). Furthermore, maize's adequate P and K contents promote the grain development process and help in obtaining a relatively high grain number per ear and weight (Liu et al., 2011). Therefore, the absorption and translocation of P and K play an important role in maize growth and yield potential in the process of yield formation.

Maize (*Zea mays* L.) is one of the most essential cereal feeds worldwide and occupies a prominent place in global food security and sustainable development (Palacios-Rojas et al., 2020). Since the mid-1990s, with the improvement of the economy and dietary structure in China, the consumption of animal-derived foods, such as meat, milk, and eggs, has increased, which rapidly increased the demand for maize. Maize is the most widely cultivated crop in China, and its production reflects people's need (Liu S. Q. et al., 2021). Northeast China is a major maize producing region, and its planting area and yield account for 31 and 34%, respectively, of the total maize production in China (Liu and Ye, 2020). The current maize planting density in Northeast China is relatively low, resulting in fewer grain yields (Luo et al., 2020). Maize yield in this region has only reached 50% of its yield potential, which offers an excellent opportunity for increasing yield. It is generally accepted that relying on high-density planting to enhance population productivity is one of the most important measures to increase yield potential (Tang et al., 2018). However, high-density planting increases resource competition among maize plants, leading to a decline in individual plant productivity and negatively affecting yield potential (Rossini et al., 2011). This inevitably intensifies the competition betwen the root systems as it is an important organ for maize to obtain environmental resources. Increased planting density leads to decreased row spacing, resulting in increased nutrients, water, and space competition between maize plants. It also severely limits the spatial distribution of the root system and restricts the capacity of nutrient absorption and utilization, ultimately leading to a decline in root quality and grain yield (Gao et al., 2021). According to Shao et al. (2018), root length and root number per plant decrease significantly as planting density increases. The increase in planting density not only inhibits the growth, quantity, and quality of maize roots but also reduces nutrient absorption and translocation in maize (Li et al., 2020; Gao et al., 2021). Therefore, enhancing root physiological characteristics and nutrient absorption capacity in high-density planting for optimal maize growth and high yield has become a significant problem in maize production.

A sufficient supply of nutrients has become essential to achieving high crop yield under high-density planting. Nitrogen, one of the most critical nutrient elements during the maize growing period, greatly affects the root morphological characteristics and physiological activities (Li et al., 2019). It is reported that nitrogen application could significantly increase the total length, volume, and effective absorption area of roots, thereby improving root nutrient absorption capacity (Liu et al., 2017). Furthermore, nitrogen fertilizer plays an important role in the crop's nutrient accumulation and transport activity. Appropriate nitrogen application can increase the grain yield by increasing nutrient accumulation post-anthesis and nutrient translocation to grains (Zhang et al., 2021). Chemical control is one of the efficient cultivation measures, which regulates plant growth and development process, enhances nutrient utilization capacity and environment adaptability, and improves grain yield and quality (Hutsch and Schubert, 2017; Stutts et al., 2018). The application of plant growth regulators can enhance the capacity of crops to absorb nutrients and soil moisture by improving their root growth characteristics (Lin et al., 2019; Nawaz et al., 2020). Yuhuangjin is a type of plant growth regulator that is widely used in maize production in China. The main component is ethephon and diethyl aminoethyl hexanoate DTA-6, which improves plant growth, enhances lodging resistance, optimizes yield component, and increases yield (Zhang et al., 2014). Therefore, we hypothesized that chemical control and nitrogen fertilizer could improve root growth, increase nutrient absorption, and promote yield formation in maize. To prove this hypothesis, this study investigated the effects of chemical control and nitrogen fertilizers on root-bleeding sap characteristics, P and K accumulation and translocation, and grain yield and

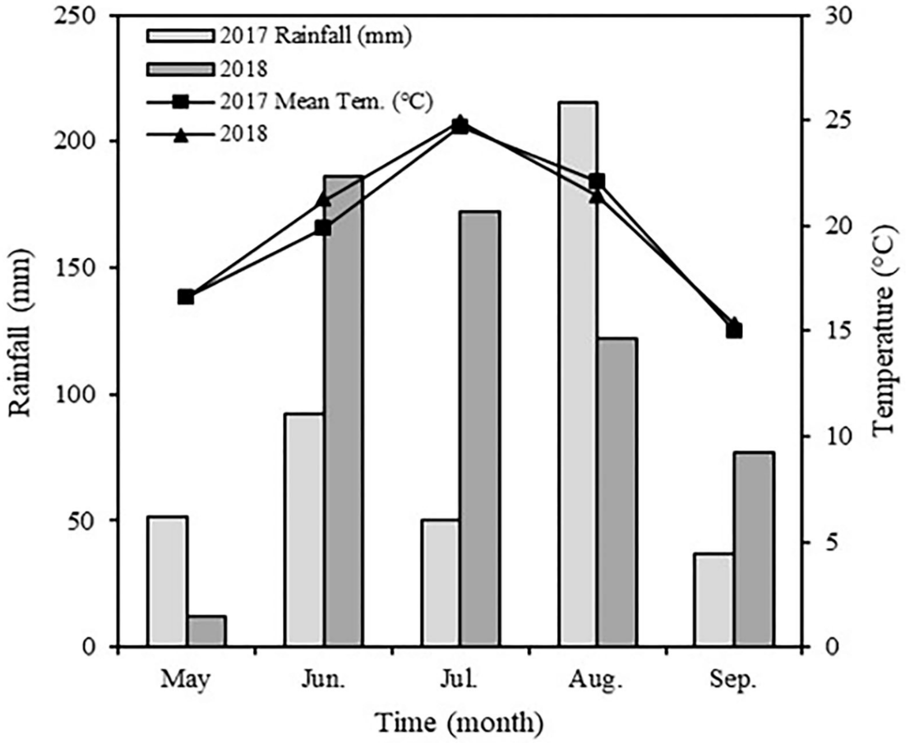

FIGURE 1 | Monthly rainfall distribution and mean temperature during spring maize growing stage in 2017 and 2018.

quality in high plant density. This study aimed to provide a theoretical basis for increasing maize yield and quality in future high-density planting management practices.

MATERIALS AND METHODS

Site Description

The experiment was conducted from April to September in 2017 and 2018 at the experimental station of Northeast Agricultural University, Harbin, Heilongjiang Province, China (126°54′E, 45°46′N). The region has a typical warm temperate monsoon climate with an annual mean temperature of 4.5°C and annual mean precipitation of 569 mm. The crop rotation system is continuous maize cropping, and the soil type at the experimental site is chernozem. The physical and chemical characteristics of tillage layer soil were pH 6.85; organic matter 25.25 g kg^{-1}; total nitrogen 1.70 g kg^{-1}; available phosphorus 65.34 mg kg^{-1}; and available potassium 179.35 mg kg^{-1}. Temperature and rainfall during the growth stage of spring maize in 2017 and 2018 are shown in **Figure 1**.

Experimental Design and Field Management

The experiment was laid out as a split-plot design with three replicates. Two chemical treatments (Y, Yuhuangjin; Control (CK), water) were used as the main plots, and three nitrogen fertilizer levels were used as the subplots: 100 kg ha^{-1} (N100), 200 kg ha^{-1} (N200), and 300 kg ha^{-1} (N300). The plant growth regulator Yuhuangjin (the mixture of 3% DTA-6 and 27% ethephon) was provided by Haolun Co., Ltd., Fujian, China. About 0.83 mL L^{-1} of Yuhuangjin solution was sprayed on the foliar surface at the seven-leaf stage in the afternoons between 16:00 and 18:00 h. Yuhuangjin was applied at 450 L ha^{-1}, and the same volume of water was applied to the control plants. Spring maize Longyu 365, a high-yielding variety in Heilongjiang province, was sown manually at 90,000 plants ha^{-1} on 30 April and harvested on 25 September in 2017 and 2018. The size of each plot was 5.2 × 8 m with 0.65 m row spacing. All plots were supplied with 100 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O. The total phosphorus and potassium and half of the nitrogen (urea, 46% N) were applied at the sowing. The balance half of the nitrogen was applied at the jointing stage. No irrigation was applied during the maize growing season. Pests, weeds, and diseases were controlled in a timely manner, and tillage management was conducted according to local farmer management.

Collection of Root-Bleeding Sap

Three representative plants were sampled from each plot at jointing, tasseling, early grain filling, and milking stages. The plants were cut at the third basal internode using lopping shears at 19:00 h. The incision was washed with distilled water, covered with a centrifuge tube containing degreasing cotton (≈2/3 of the centrifugal tube volume), and secured with plastic wrap to collect the root-bleeding sap. The centrifuge tubes were collected at 6:00 h the next day, and the weight was measured (Wang H. et al., 2019). The bleeding sap rate was calculated as the weight increase of the centrifuge tube per hour per plant (g h^{-1} $plant^{-1}$).

Analysis of Root-Bleeding Sap Components

Concentrations of serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), valine (Val), lysine (Lys), methionine (Met), arginine (Arg), and leucine (Leu) in the root-bleeding sap were measured using high-performance liquid chromatography with pre-column derivatization (Li H. W. et al., 2012). Concentrations of P, K, Ca, Mg, Fe and Zn were measured using inductively coupled plasma optical emission spectroscopy (ICP-AES, OPTIMA 3300 DV, Perkin-Elmer, USA).

Determination of Photosynthesis and N Metabolism Enzyme Activities in Ear Leaf

Approximately 0.5 g of fresh ear leaf was homogenized with an extraction medium (pH 8.4, 0.1 mmol L^{-1} Tricine-HCl, 10 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA, 7 mmol L^{-1} β-mercaptoethanol, 5% glycerol (v/v) and 1% PVP) in an ice-cold mortar with a pestle. The homogenate was centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was used for the RuBPCase and PEPCase assays following the methods of Lilley and Walker (1974) and Arnozis et al. (1988), respectively.

Approximately 1 g of fresh ear leaf was homogenized with the extraction medium (pH 7.5, 0.1 mol L^{-1} Tris-NaOH, 5 mmol L^{-1} $MgCl_2$ and 1 mmol L^{-1} DTT) precooled in ice, followed by centrifugation at 20,000 × g for 15 min at 4°C. The supernatant was used for enzyme assays. Nitrate reductase (NR) activity was determined by the method of Lewis et al. (1982), and glutamine synthetase (GS) activity was determined by the method of Canovas et al. (1991).

Determination of N Metabolism Enzyme Activity in Grain

Three ears per plot were randomly sampled at 10, 15, 20, 25, and 30 days after silking. Approximately 100 grains in the middle of the ear were collected and frozen in liquid N_2 and stored at −80°C for enzyme assays. About 0.5 g of frozen grain was homogenized with phosphate buffer (pH 7.2), followed by centrifugation at 10,000 × g for 20 min. The supernatant was used for enzyme assays of GS and glutamate dehydrogenase (NADH-GDH and NAD-GDH) activities following the method of Wang et al. (2016).

About 0.2 g of frozen grain was homogenized with Tris-HCl extraction buffer (pH 7.2, 50 mmol L^{-1} trihydroxymethyl aminomethane) precooled in ice, followed by centrifugation at 20,000 × g for 20 min at 4°C. The supernatant was used for the glutamic-pyruvic transaminase (GPT) assay following the method of Wang et al. (2016).

Analysis of Nutrients Concentration in Grain

The grains were sampled and oven-dried at 40°C for 24 h and ground to powder at harvest. The resulting grain powder was passed through a 0.25 mm mesh and stored at 4°C for analysis. Crude protein in grain was assayed by the micro-Kjeldahl method described by the Association of Official Agricultural Chemists AOAC (1975). Crude fat was assayed following the method of AOAC (1984). Starch was assayed by the colorimetric method described by Boros et al. (2004). Lysine was assayed using the colorimetric method described by Reddy et al. (2013).

Approximately 1 g of fresh grain was ground in a mortar with liquid nitrogen, and 10 ml of distilled water was added to the sample and incubated in boiling water for 60 min. The mixture was centrifuged at 12,000 × g for 20 min at 4°C. The supernatant was used for soluble sugar and sucrose measures. Soluble sugar was measured by the anthrone colorimetric method described by Liu et al. (2007). Sucrose was measured by the anthrone method described by Van (1968).

Determination of P and K Accumulation and Translocation

Three plants were sampled from each plot and separated into stems, leaves, and grains during harvest. The samples were dried in an oven at 105°C for 30 min and afterward at 80°C to a constant weight. Dried samples were weighed and ground to pass through a 1-mm sieve and digested by an H_2SO_4-H_2O_2 mixture (Wolf, 1982). The P concentration was determined by the ammonium molybdate ascorbic acid reduction method (Murphy and Riley, 1962). The K concentration was determined by the flame photometer method. Nutrient (P or K) accumulation was calculated based on the sum of the dry matter and P or K concentration in plant parts.

Nutrient (P or K) translocation amount of pre-silking (TAE, kg ha^{-1}) = vegetative organ nutrient (P or K) content at silking—vegetative organ nutrient (P or K) content at maturity.

Nutrient (P or K) translocation rate of pre-silking (TRE, %) = TAE/vegetative organ nutrient (P or K) content at silking × 100.

Contribution rate of nutrient (P or K) translocation amount of pre-silking (CTAE, %) = TAE/grain nutrient (P or K) content at maturity × 100.

Nutrient (P or K) accumulation amount of post-silking (AAT, kg ha^{-1}) = plant nutrient (P or K) content at maturity – plant nutrient (P or K) content at silking.

Contribution rate of nutrient (P or K) accumulation amount of post-silking (CAAT, %) = AAT/grain nutrient (P or K) content at maturity × 100.

Statistical Analysis

The data were summarized to calculate the mean value and standard error (SE). The mean value was compared by the analysis of variance (ANOVA) to analyze the significant differences between samples with different treatments ($P < 0.05$). All statistical analyses were performed by SPSS 19.0 procedures (SPSS Inc., Chicago, IL, USA). Microsoft Excel 2010 was used to draw tables.

TABLE 1 | Effects of chemical control and nitrogen fertilizers on root-bleeding sap rate (μg h^{-1} $plant^{-1}$) during the maize growing period in 2017 and 2018.

Year	Treatment	Jointing stage	Tasseling stage	Early filling stage	Milk stage	Maturing stage
2017	N100+CK	1.42d	1.75d	1.99d	2.75cd	0.76d
	N200+CK	1.56c	1.96c	2.17c	2.79c	0.86c
	N300+CK	1.47d	1.85cd	2.08cd	2.71d	0.82c
	N100+Y	1.58c	2.07b	2.31b	2.99b	0.92b
	N200+Y	1.77a	2.26a	2.49a	3.10a	1.03a
	N300+Y	1.67b	2.20a	2.38b	2.98b	0.98a
2018	N100+CK	1.36c	1.65c	2.03d	2.50c	0.70c
	N200+CK	1.49b	1.87b	2.24bc	2.64b	0.80b
	N300+CK	1.47b	1.75c	2.15c	2.56c	0.77b
	N100+Y	1.53b	1.95b	2.32b	2.70b	0.82b
	N200+Y	1.67a	2.10a	2.50a	2.85a	0.91a
	N300+Y	1.61ab	1.98b	2.45a	2.77ab	0.90a

N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same year followed by the different letters indicate a significant difference at $P < 0.05$.

RESULTS

Root-Bleeding Sap and Nutrients Composition Delivery Rate

The chemical control and nitrogen fertilization exhibited a significant influence on the rate of root-bleeding sap during the maize growing period in 2017 and 2018 (**Table 1**). At the same N levels, chemical control increased root-bleeding sap rate with an average augment of 12.26, 15.99, 14.21, 8.97, and 18.46% from the jointing stage to the maturing stage compared with water treatment. Root-bleeding sap rate first increased and then decreased with the increase of nitrogen application under the same chemical treatment, and the highest value was measured under N200 treatment. The results show that a high N level inhibited the increase of root-bleeding. An analysis of the synthetic effect revealed that the highest root-bleeding sap rate was obtained from N200 application under chemical control.

The delivery rate of free amino acids in root-bleeding sap was influenced by chemical control and nitrogen fertilizer, which decreased after the jointing stage in maize (**Table 2**). At the same N levels, chemical control increased the delivery rate of Ser, Glu, Gly, Ala, Val, Lys, Met, Arg, and Leu with an average augment of ≈11.45–19.04% than water treatment at the tasseling stage in both years, which was consistent at different growth stages. Under the same chemical treatment, the free amino acid delivery rate obtained the highest value under N200 treatment, which showed an average augment of 6.54–15.04% and of 4.15–6.97% compared with N100 and N300 nitrogen rates in both years. From the analysis of synthetic effect, the delivery rate of free amino acids in root-bleeding sap was optimal in N200 application under chemical control.

A similar change trend was observed in the mineral nutrient concentrations in bleeding sap during the maize growing period in 2017 and 2018 (**Table 3**). The mineral nutrient concentrations were significantly affected by chemical control and nitrogen fertilizer. The delivery rate of mineral nutrients first increased and then decreased with the increase of nitrogen application under the same chemical treatment. At the same N levels, chemical control obviously increased the delivery rate of mineral nutrients at different growth stages. From the analysis of synthetic effect, the delivery rate of mineral nutrients in root-bleeding sap was optimal in N200 application under chemical control.

P and K Accumulation and Translocation

Changes between the P and K accumulation in maize plants followed similar trends; both P and K increased gradually from the jointing stage to thewe maturing stage (**Table 4**). Chemical control and N fertilization level exhibited a marked influence on P and K accumulation amount during the maize growing period in both years. At the same N levels, chemical control increased P accumulation amount with an average augment of 4.48, 15.34, 22.07, 23.52, and 24.32% and K accumulation amount with an average augment of 6.30, 14.43, 17.60, 18.94, and 19.55% from the jointing stage to the maturing stage in 2017 and 2018. Under both water and chemical control conditions, P and K accumulation amount increased by increasing the N level from N100 to N300, but there was no significant difference between N200 and N300 treatments in both years. Compared with N100, N200 and N300 treatments increased P and K accumulation amount with an average augment of 22.41 and 24.26%, respectively.

Changes in the proportion of P and K accumulation in maize plants during various growth stages seemed to follow similar trends (**Table 5**). Proportions of P and K accumulation had a higher value at emerging (VE) —jointing (JT) and JT—tasseling (TS) stages and decreased gradually from TS—early-filling (EF) to milk (MK)—maturing (MT) stage. The proportions of P and K accumulation were significantly affected by chemical control and N fertilization level. At the same N levels, chemical control increased the proportions of P and K accumulation at TS-EF, EF-MK, and MK-MT stages, while the proportions decreased at the VE-JT stage and remained relatively constant at the JT-TS

TABLE 2 | Effects of chemical control and nitrogen fertilizers on amino acids concentrations (μg h^{-1} $plant^{-1}$) in root-bleeding sap during the maize growing period in 2017 and 2018.

Growth stage	Treatment	2017									2018								
		Ser	Glu	Gly	Ala	Val	Lys	Met	Arg	Leu	Ser	Glu	Gly	Ala	Val	Lys	Met	Arg	Leu
Jointing stage	N100+CK	479.35d	284.97d	1.34d	13.91d	57.59d	92.92e	5.46c	85.15d	17.88e	468.15d	279.68d	1.27d	13.39d	54.58d	89.06d	4.95d	83.29d	16.63d
	N200+CK	506.23c	310.99c	1.44c	15.38c	62.68bc	101.63cd	5.87b	95.36b	19.47cd	495.77bc	303.61c	1.38c	14.56bc	59.63bc	96.43c	5.26c	90.14c	18.15c
	N300+CK	496.70cd	307.06c	1.40cd	14.26d	59.52cd	96.61de	5.30c	89.93c	18.36de	482.89cd	295.27c	1.31d	14.07c	57.71c	91.45d	5.13cd	87.15cd	17.36cd
	N100+Y	539.22b	334.64b	1.56b	16.01bc	66.32b	105.60bc	6.00b	99.17b	20.86bc	516.74b	327.46b	1.45b	15.22b	62.46b	101.54b	5.59b	94.82b	19.43b
	N200+Y	568.89a	359.67a	1.65a	17.53a	71.86a	116.71a	6.48a	105.17a	22.92a	543.23a	346.84a	1.57a	16.24a	66.87a	109.18a	6.11a	100.48a	21.32a
	N300+Y	544.89ab	339.95b	1.56b	16.51ab	67.04ab	108.43b	6.17ab	99.18b	21.74ab	525.28ab	335.29ab	1.49b	15.48ab	63.52b	104.77ab	5.78b	96.57ab	20.08b
Tasseling stage	N100+CK	377.14d	227.46d	1.13d	11.69d	49.83d	73.17d	4.37d	74.47e	13.91c	365.26d	212.76d	1.04d	11.24d	45.62d	70.33d	4.05d	69.02d	13.34d
	N200+CK	403.18c	242.68c	1.22c	12.64c	53.64cd	82.09c	4.72c	80.27cd	15.97b	386.53c	230.53c	1.12c	12.29c	50.57c	78.05c	4.48bc	75.24bc	15.36b
	N300+CK	383.42d	226.22d	1.13d	11.74d	49.96d	73.17d	4.28d	76.75de	14.39c	370.72cd	218.42d	1.07cd	11.73cd	47.45d	72.48d	4.29c	72.65c	14.21c
	N100+Y	424.42b	255.84b	1.26bc	13.55b	55.78bc	86.38b	5.07b	83.34bc	17.40a	409.58b	245.84b	1.19b	13.26b	52.63c	82.09b	4.66b	78.73b	16.02b
	N200+Y	453.42a	278.94a	1.36a	14.70a	61.24a	94.45a	5.33a	89.53a	18.28a	435.21a	264.39a	1.30a	14.02a	59.29a	88.84a	5.03a	85.82a	17.27a
	N300+Y	438.67ab	265.77b	1.31ab	14.05ab	58.21ab	89.34b	4.97b	85.13b	17.02ab	422.47ab	254.56ab	1.23b	13.68ab	56.34b	84.27b	4.83ab	80.56b	16.29b
Early filling stage	N100+CK	318.24c	173.80c	0.71c	9.88e	44.29c	61.05e	3.99d	66.60c	7.71c	302.85c	169.82d	0.69d	9.43d	40.03d	60.61d	3.68d	62.14d	7.06d
	N200+CK	342.15bc	197.32b	0.77b	11.05cd	48.62b	69.74c	4.31c	72.01b	9.14b	333.52b	185.35c	0.74c	10.24c	44.67c	66.44c	4.04bc	70.47b	8.23c
	N300+CK	329.59bc	184.54c	0.71c	10.36de	44.05c	65.30d	4.01d	66.90c	7.75c	315.62c	177.47cd	0.70d	9.77cd	42.98c	63.25d	3.89c	66.44c	7.32d
	N100+Y	367.79ab	209.01b	0.88a	11.59bc	51.53a	72.88c	4.42bc	74.67b	9.47b	345.07b	198.09b	0.83b	10.88b	47.44b	72.96b	4.21b	73.92ab	8.98b
	N200+Y	383.05a	230.34a	0.90a	12.86a	54.37a	83.57a	4.90a	79.70a	10.80a	367.26a	221.53a	0.88a	12.02a	51.85a	78.37a	4.65a	77.25a	9.75a
	N300+Y	365.08ab	222.42a	0.87a	12.25ab	52.57a	77.77b	4.61b	78.99a	9.84ab	351.63ab	207.04b	0.85ab	11.34b	48.62b	76.72a	4.36b	75.34a	9.52a
Milk stage	N100+CK	147.14d	108.01e	0.50e	6.00d	23.39d	39.69d	2.02cd	29.19c	3.86c	148.53d	110.84d	0.55e	5.89d	22.08e	39.82d	1.78d	27.14e	4.14e
	N200+CK	169.19c	123.96cd	0.69c	7.08c	29.31b	47.88c	2.17c	36.59b	4.99b	163.29c	120.06c	0.64c	6.78c	25.31c	45.47c	1.92c	32.19c	5.05c
	N300+CK	162.04c	119.80de	0.63d	6.68c	26.81c	44.55c	1.95d	31.15c	4.25c	157.24c	114.26d	0.59d	6.14d	23.86d	41.19d	1.83cd	30.18d	4.63d
	N100+Y	183.75b	134.16bc	0.71bc	8.04b	30.24b	52.28b	2.51b	39.02b	5.33b	179.08b	130.32b	0.70b	7.66b	28.75b	50.95b	2.27b	34.63b	5.51b
	N200+Y	209.35a	148.63a	0.82a	8.89a	33.84a	60.34a	2.77a	45.58a	6.46a	192.41a	142.89a	0.76a	8.25a	31.87a	56.21a	2.49a	40.52a	6.11a
	N300+Y	190.15b	144.44ab	0.75b	8.14b	32.59a	55.58b	2.46b	39.37b	6.07a	184.47ab	136.93a	0.72b	7.62b	29.24b	53.02b	2.35b	35.79b	5.78b

N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same growth stage followed by the different letters indicate a significant difference at $P < 0.05$.

TABLE 3 | Effects of chemical control and nitrogen fertilizers on mineral nutrients concentrations (μg h^{-1} $plant^{-1}$) in root-bleeding sap during the maize growing period in 2017 and 2018.

Growth period	Treatment	2017											2018										
		Fe	Mn	Cu	Zn	Ca	Mg	Mo	K	P	B	Si	Fe	Mn	Cu	Zn	Ca	Mg	Mo	K	P	B	Si
Jointing stage	N100+CK	1.62d	4.37d	0.046c	10.93c	318.23c	299.46cd	0.057d	1747.53e	113.76c	1.14cd	50.45c	1.55d	4.15d	0.043d	10.08d	302.98d	259.46d	0.051d	1682.49d	104.07d	1.01d	44.37d
	N200+CK	1.80c	4.75c	0.050bc	11.55bc	347.74b	314.76bc	0.064c	1920.50cd	123.26bc	1.21c	53.66b	1.79c	4.62c	0.049c	11.39bc	332.63bc	290.56c	0.058c	1801.37c	115.48c	1.11c	48.62c
	N300+CK	1.87c	4.80c	0.051bc	11.66b	339.26b	284.37d	0.055d	1833.47de	118.52c	1.07d	53.96b	1.77c	4.51c	0.047c	10.92c	320.06c	278.85c	0.056c	1754.88cd	110.75c	1.04d	45.89d
	N100+Y	2.06b	5.22b	0.056ab	12.43a	350.32b	332.33ab	0.070b	1998.30bc	134.01ab	1.37b	55.22b	1.96b	5.07b	0.053b	11.85b	346.45b	307.82b	0.064b	1895.03b	122.62b	1.26b	51.84b
	N200+Y	2.17a	5.50ab	0.060a	12.82a	383.02a	345.53a	0.075a	2124.70a	142.78a	1.49a	58.39a	2.12a	5.43a	0.058a	12.77a	370.82a	333.13a	0.071a	2093.27a	136.59a	1.39a	56.25a
	N300+Y	2.20a	5.57a	0.062a	13.05a	374.64a	328.45ab	0.068b	2087.37ab	138.60a	1.37b	58.97a	2.08a	5.39a	0.057a	12.64a	362.17ab	316.28b	0.066b	1956.36b	127.94b	1.28b	53.08b
Tasseling stage	N100+CK	0.49d	4.29d	0.063e	9.26d	300.46e	292.80d	0.062d	1538.44d	110.49c	1.09d	44.41d	0.52e	4.05d	0.58d	8.98d	282.94d	267.25d	0.059d	1496.05d	98.72c	1.00d	42.67d
	N200+CK	0.65c	4.73c	0.069d	9.97c	320.38cd	310.57bc	0.075c	1671.27c	118.37bc	1.18c	46.93c	0.62c	4.52bc	0.64c	9.53c	303.39c	285.03bc	0.069c	1602.88c	109.46b	1.10c	45.42c
	N300+CK	0.71c	4.90c	0.071cd	9.92c	307.73de	297.60cd	0.071c	1608.97cd	110.54c	1.09d	45.82cd	0.58d	4.37c	0.62c	9.39cd	287.33d	276.29cd	0.066c	1579.14c	102.78c	1.06c	44.68cd
	N100+Y	0.77b	5.04bc	0.074bc	10.93b	344.16ab	328.07a	0.086b	1760.22b	123.53b	1.28b	49.99b	0.74b	4.68b	0.69b	10.21b	320.17b	297.42b	0.077b	1693.49b	112.53b	1.17b	48.39b
	N200+Y	0.90a	5.40ab	0.078ab	11.48a	359.44a	335.49a	0.094a	1874.43a	135.76a	1.39a	53.39a	0.86a	5.23a	0.76a	11.15a	343.48a	325.28a	0.086a	1819.53a	125.85a	1.31a	52.05a
	N300+Y	0.92a	5.55a	0.079a	11.74a	335.66bc	323.83ab	0.088b	1822.54ab	126.88ab	1.29b	54.78a	0.83a	5.06a	0.74a	10.92a	325.84b	316.17a	0.084a	1786.67a	117.09b	1.22b	50.83a
Early filling stage	N100+CK	1.55c	5.87d	0.039c	4.85d	456.81c	361.88d	0.095e	1036.46c	143.64c	0.96d	25.91d	1.47d	5.79d	0.34d	5.17d	450.24e	333.92d	0.088d	1087.65d	128.95d	0.90d	22.07e
	N200+CK	1.68b	6.65c	0.045bc	5.83c	486.52b	377.28c	0.103cd	1251.03b	155.73bc	1.07c	28.92c	1.63c	6.27c	0.38c	5.59c	477.91cd	354.38c	0.096c	1174.59c	141.63c	0.99c	28.58c
	N300+CK	1.73b	6.75c	0.044bc	5.95c	475.80b	358.97d	0.098de	1167.03b	151.38bc	1.03c	30.43c	1.59c	6.05cd	0.37c	5.42cd	468.17de	340.03cd	0.093c	1106.27d	134.07d	0.93d	24.94d
	N100+Y	1.77b	7.30b	0.044bc	6.73b	524.52a	392.79b	0.109bc	1248.27b	163.15ab	1.16b	34.17b	1.75b	6.91b	0.41b	6.58b	496.87bc	377.49b	0.104b	1256.76b	152.19b	1.06b	32.31b
	N200+Y	1.94a	7.98a	0.050ab	7.12a	537.46a	411.63a	0.122a	1396.47a	173.51a	1.22a	37.13a	1.85a	7.64a	0.46a	6.94a	525.75a	403.67a	0.115a	1362.09a	169.72a	1.14a	35.85a
	N300+Y	1.98a	7.91a	0.051a	7.28a	525.22a	399.12ab	0.113b	1380.29a	165.35ab	1.16b	37.88a	1.81ab	7.38a	0.45a	6.85ab	520.33ab	396.54a	0.108b	1283.15b	157.94b	1.12a	33.67b
Milk stage	N100+CK	0.37d	1.26d	0.021c	2.64d	117.79c	20.18d	0.067d	481.15d	41.52d	0.17d	15.89c	0.35d	1.31d	0.23d	3.42d	113.06d	24.31e	0.065d	493.17d	42.35d	0.20d	13.77d
	N200+CK	0.46c	1.66c	0.026bc	3.26c	130.83b	30.82c	0.076c	560.38c	48.58c	0.25c	16.56c	0.43c	1.62c	0.26c	3.89c	126.74c	31.38d	0.073c	545.39c	46.88c	0.24c	15.85c
	N300+CK	0.48c	1.80bc	0.028bc	3.54c	129.95b	32.04c	0.069d	539.03c	46.93cd	0.25c	17.03c	0.41c	1.57c	0.24d	3.57d	118.38d	25.47e	0.068d	516.28d	44.27d	0.21d	14.42d
	N100+Y	0.59b	1.92b	0.026b	4.31b	155.06a	38.73b	0.085b	622.59b	57.07b	033a	20.97b	0.56b	1.85b	0.30b	4.36b	139.02b	36.79c	0.079b	603.05b	52.53b	0.29b	20.51b
	N200+Y	0.68a	2.33a	0.031ab	4.65ab	167.65a	49.55a	0.091a	712.55a	64.38a	0.35a	24.25a	0.64a	2.26a	0.33a	4.73a	158.85a	45.32a	0.088a	684.91a	57.96a	0.32a	22.69a
	N300+Y	0.69a	2.44a	0.034a	4.97a	162.60a	46.57a	0.089ab	676.37a	58.75ab	0.27b	25.50a	0.62a	2.18a	0.31b	4.48b	152.37a	42.68b	0.085a	627.56b	54.19b	0.30b	21.18b

N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same growth stage followed by the different letters indicate a significant difference at $P < 0.05$.

TABLE 4 | Effects of chemical control and nitrogen fertilizers on P and K accumulation (kg ha^{-1}) during the maize growing period in 2017 and 2018.

Nutrient	Treatment	2017					2018				
		Jointing stage	Tasseling stage	Early filling stage	Milk stage	Maturing stage	Jointing stage	Tasseling stage	Early filling stage	Milk stage	Maturing stage
P	N100+CK	9.88c	22.02c	26.96c	29.08d	30.31d	9.55c	20.78c	25.81c	27.82c	28.95c
	N200+CK	10.26bc	25.55b	32.52b	36.78bc	39.09bc	10.02bc	23.74b	30.64b	34.35b	36.37b
	N300+CK	10.45b	26.32b	33.57b	37.83b	39.85b	10.38b	24.66b	31.89b	35.77b	37.81b
	N100+Y	10.20b	25.48b	32.78b	36.03c	38.01c	10.04b	23.62b	31.16b	34.29b	36.13b
	N200+Y	10.76a	29.85a	40.08a	45.65a	48.64a	10.61a	27.81a	38.27a	43.43a	46.18a
	N300+Y	10.96a	30.10a	40.37a	45.86a	48.65a	10.68a	28.17a	38.75a	43.77a	46.38a
K	N100+CK	37.0.88c	72.56c	93.49c	105.01d	114.71d	37.49c	70.15c	89.64c	100.87c	109.61c
	N200+CK	39.53bc	81.61b	107.64b	123.89bc	137.60bc	39.36bc	77.42b	101.27b	114.92b	125.88b
	N300+CK	41.21b	85.18b	112.92b	129.75c	143.62c	39.91b	78.79b	103.25b	117.38b	128.53b
	N100+Y	39.95b	82.01b	107.51b	122.60b	134.81b	38.67bc	76.04b	99.21b	112.45b	123.04b
	N200+Y	42.89a	96.47a	130.70a	152.04a	169.10a	42.52a	89.88a	120.96a	139.24a	153.72a
	N300+Y	43.05a	97.22a	133.48a	154.80a	172.73a	43.13a	91.37a	123.52a	141.91a	156.29a

N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same nutrient followed by the different letters indicate a significant difference at $P < 0.05$.

stage in 2017 and 2018. Under both water and chemical control conditions, N supply significantly increased the proportions of P and K accumulation. However, there was no significant difference between N200 and N300 treatments, and the highest proportions were obtained under N200 treatment at EF-MK and MK-MT stages in both years.

Chemical control and nitrogen fertilizer significantly influenced the nutrient (P and K) translocation and contribution, including the vegetative organ nutrient content at the silking stage (VCS), the vegetative organ nutrient content at the maturing stage (VCM), and the grain nutrient content at the maturing stage (GCM), the nutrient translocation amount of pre-silking (TAE), the nutrient translocation rate of pre-silking (TRE), the contribution rate of nutrient translocation amount of pre-silking (CTAE), the nutrient accumulation amount of post-silking (AAT), and the contribution rate of nutrient accumulation amount of post-silking (CAAT) (**Table 6**). At the same N levels, VCS, VCM, GCM, TAE, AAT, and CAAT of P and K in maize plants under chemical control were markedly higher than those under water treatment. In contrast, TRE and CTAE of P and K in maize plants under chemical control were markedly lower than those under water treatment. Under both water and chemical control conditions, VCS, VCM, GCM, and TAE of P and K in maize plants were significantly increased by increasing N levels; however, TRE and CTAE were decreased. While N supply in general significantly increased AAT and CAAT of P and K in maize plants, there is no significant difference between N200 and N300 treatments, and the highest values were obtained under N200 treatment in both years.

RuBPCase and PEPCase Activities in Leaf

Chemical control and N fertilization level exhibited a marked influence on RuBPCase activity in leaves during the maize growing period in 2017 and 2018 (**Figure 2**). At the same N levels, chemical control increased RuBPCase activity with an average augment of 12.45, 12.91, 11.03, and 13.02% from the jointing stage to the milk stage in 2017 and 2018, respectively. Under both water and chemical control conditions, RuBPCase activity increased with an average augment of 6.78% by increasing the N supply level from N100 to N200 in both years, but further increasing the N supply level from N200 to N300 decreased RuBPCase activity at different stages. From the analysis of synthetic effect, RuBPCase activity in maize leaf was optimal in N200 application under chemical control.

A similar trend was also observed for PEPCase activity in maize leaf, and the activity was significantly affected by chemical control and N fertilization levels (**Figure 2**). At the same N levels, chemical control increased PEPCase activity with an average augment of 15.46, 11.98, 15.13, and 17.43% from the jointing stage to the milk stage in 2017 and 2018, respectively. Under both water and chemical control conditions, PEPCase activity under N200 treatment was higher than those under N100 and N300 treatments, with an average augment of 7.87 and 4.46% at different stages, respectively. From the analysis of synthetic effect, PEPCase activity in maize leaf was optimal in N200 application under chemical control.

TABLE 5 | Effects of chemical control and nitrogen fertilizers on the proportion of P and K accumulation (%) at different maize growing stages in 2017 and 2018.

Nutrient	Treatment	2017					2018				
		VE-JT	JT-TS	TS-EF	EF-MK	MK-MT	VE-JT	JT-TS	TS-EF	EF-MK	MK-MT
P	N100+CK	32.60a	40.04a	16.31d	6.98d	4.07d	32.99a	38.79a	17.37d	6.94d	3.90d
	N200+CK	26.25b	39.12a	17.82c	10.91b	5.90b	27.55b	37.72a	18.97c	10.20b	5.55b
	N300+CK	26.23b	39.83a	18.19c	10.69b	5.05c	27.45b	37.77a	19.12c	10.26b	5.40b
	N100+Y	26.85b	40.19a	19.21b	8.55c	5.21c	27.79b	37.59a	20.87b	8.66c	5.09c
	N200+Y	22.12c	39.26a	21.02a	11.46a	6.14a	22.98c	37.25a	22.65a	11.17a	5.96a
	N300+Y	22.54c	39.34a	21.11a	11.28a	5.73b	23.03c	37.71a	22.81a	10.82a	5.63b
K	N100+CK	33.02a	30.23a	18.25d	10.04c	8.46d	34.20a	29.80a	17.78c	10.25c	7.97c
	N200+CK	28.73b	30.58a	18.92cd	11.81b	9.96c	31.27b	30.24a	18.95b	10.84b	8.71b
	N300+CK	28.69b	30.62a	19.31bc	11.72b	9.66c	31.05b	30.25a	19.03b	10.99b	8.68b
	N100+Y	29.63b	31.20a	18.92cd	11.19b	9.06b	31.43b	30.37a	18.83b	10.76b	8.61b
	N200+Y	25.36c	31.69a	20.24ab	12.62a	10.09a	27.66c	30.81a	20.22a	11.89a	9.42a
	N300+Y	25.07c	31.54a	21.12a	12.41a	9.86a	27.60c	30.87a	20.57a	11.77a	9.20a

VE, emerging stage; JT, jointing stage; TS, tasseling stage; EF, Early filling stage; MK, milk stage; MT, maturing stage. N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same nutrient followed by the different letters indicate a significant difference at $P < 0.05$.

NR and GS Activities in Leaf

Chemical control and N fertilization level exhibited a marked influence on NR and GS activities in leaves during the maize growing period in 2017 and 2018 (**Figure 3**). At the same N levels, chemical control increased NR activity with an average augment of 18.23, 17.11, 14.32, and 14.71% and increased GS activity with an average augment of 20.28, 24.12, 17.41, and 25.69% from the jointing stage to the milk stage in both years, respectively. Under water and chemical control conditions, NR and GS activities were significantly increased by increasing the N level from N100 to N200, but further increasing the N supply level from N200 to N300 caused a decrease in NR and GS activities at different stages. From the analysis of synthetical effect, NR and GS activities in maize leaf were optimal in N200 application under chemical control.

N Metabolism Enzyme Activity in Grain

Chemical control and N fertilization level exerted a marked effect on grain GS, GDH, and GPT activities from 10 to 30 days after silking in 2017 and 2018 (**Figure 4**). Of these, GS and GDH activities increased between 10 and 20 days after silking and then decreased until 30 days after silking. However, GPT activity fluctuated with grain growth, which was highest and lowest at 25 and 30 days after silking, respectively. At the same N levels, chemical control increased GS, GDH, and GPT activities with an average augment of 15.22, 12.76, and 14.21% from 10 to 30 days after silking in both years, respectively. Under both water and chemical control conditions, GS, GDH, and GPT activities in grain were significantly increased by increasing the N supply level from N100 to N200 in both years, but further increasing the N supply level from N200 to N300 caused a slight decrease in grain N metabolism enzyme activities. From the analysis of synthetic effect, N metabolism enzyme activities in grain were optimal in N200 application under chemical control.

Nutrients Concentrations in Grain

At the same N levels, chemical control significantly increased crude protein, lysine, sucrose, and soluble sugar concentrations of maize compared with water treatment in 2017 and 2018 (**Table 7**). Crude protein and lysine concentrations were significantly increased by increasing the N supply level from N100 to N200, but further increasing the N supply level from N200 to N300 caused a significant decrease in 2017 and a slight decrease in 2018. Similar trends were also observed for sucrose and soluble sugar concentrations of maize grain. Crude fat and starch concentrations were unaffected by chemical control and N fertilization level. The results show that nutrient concentrations in maize grain were optimal in N200 application under chemical control.

Yield and Yield Components

Chemical control and N fertilization level exhibited a marked influence on yield and yield components of maize in 2017 and 2018 (**Table 8**). Chemical control significantly increased the number of grains per ear and 1,000-grain weight compared with maize under water treatment in 2017 and 2018. Grain number per ear and 1,000-grain weight significantly increased by increasing the N supply level from N100 to N200, but further increasing the N supply level from N200 to N300 caused a slight decrease in 2017 and 2018. The highest grain yields were obtained from the N200 application under chemical control in 2017 and 2018.

Correlation Analysis

As shown in **Figure 5**, correlation analysis indicated that grain yield was positively correlated with the rate of root-bleeding sap, the delivery rate of amino acids and mineral nutrients in

TABLE 6 | Effects of chemical control and nitrogen fertilizers on maize nutrient (P and K) translocation and contribution during the maize growing period 2017 and 2018.

Nutrient	Treatment	2017								2018							
		VCS (kg ha^{-1})	VCM (kg ha^{-1})	GCM (kg ha^{-1})	TAE (kg ha^{-1})	TRE (%)	CTAE (%)	AAT (kg ha^{-1})	CAAT (%)	VCS (kg ha^{-1})	VCM (kg ha^{-1})	GCM (kg ha^{-1})	TAE (kg ha^{-1})	TRE (%)	CTAE (%)	AAT (kg ha^{-1})	CAAT (%)
P	N100+CK	21.19d	7.54e	22.27c	13.64d	64.40a	61.27a	8.62c	38.73d	20.37c	7.03d	21.02c	13.35d	65.51a	63.49a	7.68c	36.51c
	N200+CK	26.33c	10.26c	28.63b	16.07c	61.04b	56.13c	12.56b	43.87b	24.60b	9.52bc	25.73b	15.09c	61.32b	58.63b	10.65b	41.37b
	N300+CK	27.82b	11.05b	28.80b	16.77b	60.27b	58.24b	12.03b	41.76c	25.49b	9.97b	26.01b	15.52bc	60.90b	59.69b	10.48b	40.31b
	N100+Y	26.08c	9.17d	29.13b	16.91b	64.84a	58.05b	12.22b	41.95c	25.10b	9.07c	26.83b	16.03b	63.87a	59.77b	10.79b	40.23b
	N200+Y	32.16a	13.50a	34.94a	18.66a	58.01c	53.39d	16.29a	46.61a	31.29a	12.92a	33.24a	18.38a	58.73b	55.29c	14.86a	44.71a
	N300+Y	32.24a	13.72a	34.11a	18.52a	57.43c	54.29d	15.59a	45.71a	31.46a	12.89a	33.35a	18.58a	59.04b	55.69c	14.78a	44.31a
K	N100+CK	82.58e	35.07d	78.64e	47.51e	57.53b	60.41a	31.13d	39.59d	79.59d	30.88d	76.73c	48.70c	61.20a	63.48a	28.02d	36.52d
	N200+CK	96.39c	44.87b	94.73d	51.51d	53.44d	54.38c	43.21c	45.62b	89.70bc	39.76b	88.12b	49.94bc	55.67b	56.68c	38.18b	43.32b
	N300+CK	100.76b	44.26b	103.36b	56.50b	56.08c	54.67bc	46.86b	45.33bc	92.03b	40.56b	89.97b	51.47b	55.93b	57.21c	38.50b	42.79b
	N100+Y	91.77d	37.38c	97.43c	54.39c	59.27a	55.82b	43.04c	44.18c	87.11c	35.31c	86.13b	51.80b	59.46a	60.14b	34.33c	39.86c
	N200+Y	111.62a	52.11a	118.51a	59.51a	53.31d	50.22d	59.00a	49.78a	103.24a	46.12a	107.60a	57.12a	55.33b	53.08d	50.48a	46.92a
	N300+Y	112.83a	51.85a	121.03a	60.98a	54.05c	50.38d	60.05a	49.62a	105.96a	46.89a	109.40a	59.08a	55.75b	54.00d	50.33a	46.00a

VCS, vegetative organ nutrient (P or K) content at silking stage; VCM, vegetative organ nutrient (P or K) content at maturing stage; GCM, grain nutrient (P or K) content at maturing stage; TAE, nutrient (P or K) translocation amount of pre-silking; TRE, nutrient (P or K) translocation rate of pre-silking; CTAE, contribution rate of nutrient (P or K) translocation amount of pre-silking; AAT, nutrient (P or K) accumulation amount of post-silking; and CAAT, contribution rate of nutrient (P or K) accumulation amount of post-silking. N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under Yuhuangjin treatment, respectively. Means within a column for the same nutrient followed by the different letters indicate a significant difference at $P < 0.05$.

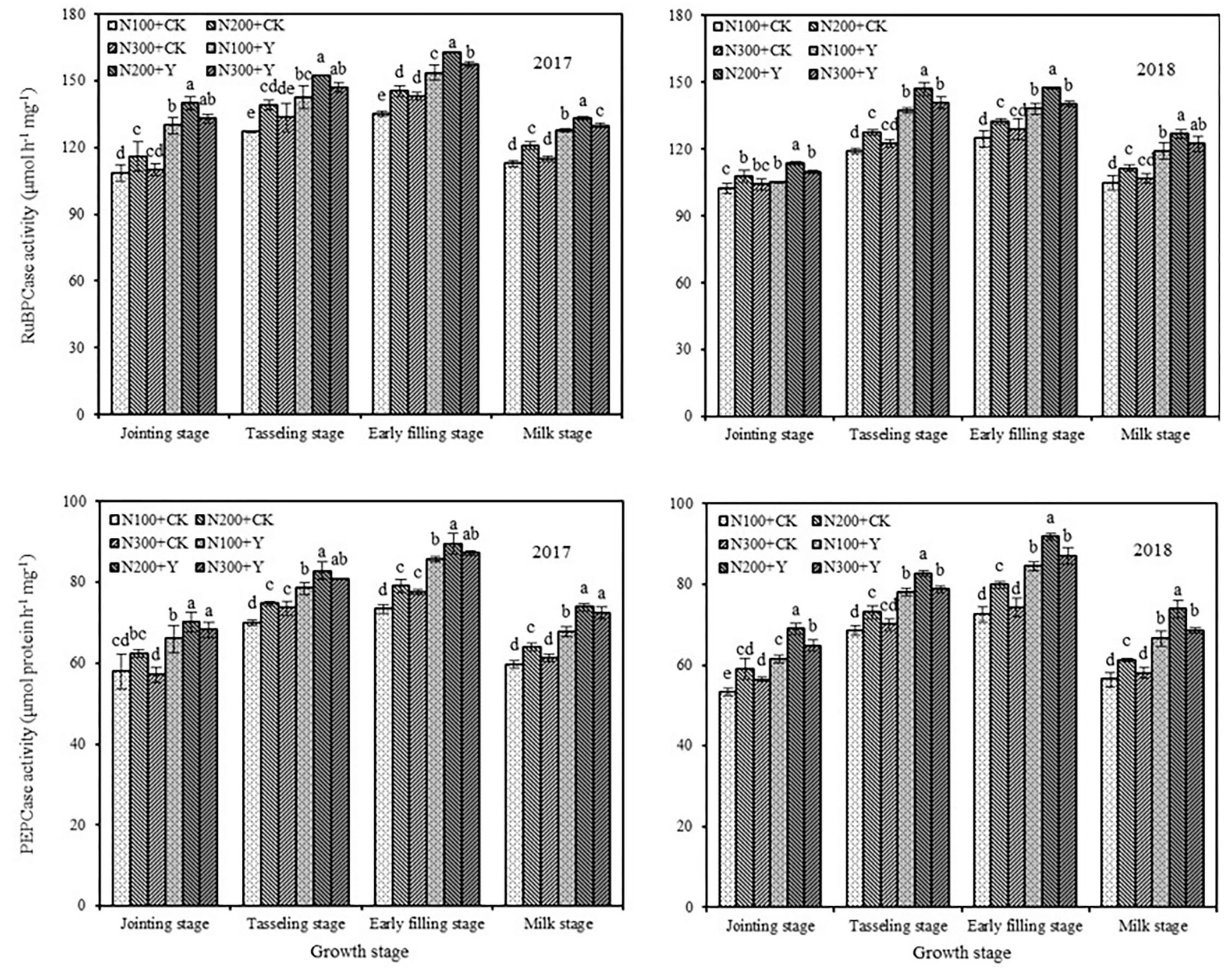

FIGURE 2 | Effects of chemical control and nitrogen fertilizers on RuBPCase and PEPCase activities in ear leaf during the maize growing period in 2017 and 2018. N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Error bars indicate the value of standard error. Different letters within a growth stage indicate a significant difference at $P < 0.05$.

the bleeding sap, and CAAT of P and K. Besides, the CAAT of P and K were positively correlated with the rate of root-bleeding sap.

DISCUSSION

The root system is an essential source for uptake of water and nutrients, and its physiological activity is closely correlated to the development of the plant's parts above ground and the yield formation of crops (Yang et al., 2004; Fan et al., 2021). Root-bleeding sap reflects the capacity of roots to uptake water and nutrients, and it represents the physiological activity of the root system (Ansari et al., 2004; Wang P. et al., 2019). It has been found that root growth is closely associated with root-bleeding sap rate. The reduction of root quality in high-density planting seriously affects yield formation (Yu et al., 2019; Liu Z. et al., 2021). A balanced application of nitrogen can enhance root activity by supplying nutrients to form a robust root system (Wang H. et al., 2019). Equally, chemical control can optimize root morphological construction and improve the absorption ability of the root system (Lin et al., 2019).

In this study, N200 application in combination with chemical control significantly enhanced the rate of root-bleeding sap to enhance the strength of root activity. The nutrient concentrations in root-bleeding sap are closely associated with the absorption and transformation capacity of the root system, and its variation reflects the interaction intensity of nutrients in the aboveground

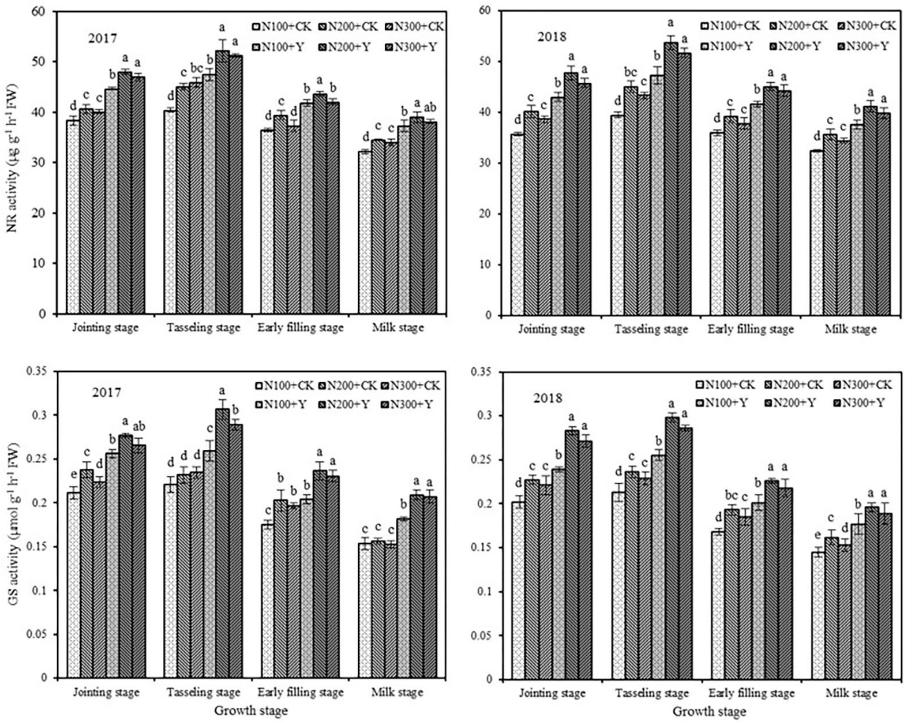

FIGURE 3 | Effects of chemical control and nitrogen fertilizers on NR and GS activities in ear leaf during the maize growing period in 2017 and 2018. N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Error bars indicate the value of standard error. Different letters within a growth stage indicate a significant difference at $P < 0.05$.

and underground plant parts (Nishanth and Biswas, 2008). The xylem sap can transport nutrients upward to the aboveground tissues. The nutrient concentrations in root-bleeding sap are generally recognized as indicators of the plant's nutrient status (Ansari et al., 2004). Amino acids are essential for maintaining plant growth and, when contained in root-bleeding sap, promote root growth (Zheng et al., 2020). Mineral nutrient concentration is considered a primary factor for plant growth and grain yield. The delivery rate of mineral nutrients primarily depends on the root physiological activity and the nutrient concentrations across the root zone (Liang et al., 2020). High-density planting reduces root physiological activity and intensifies the depletion of nutrients in the root zone, resulting in the reduction of free amino acids and mineral nutrient concentrations (Yu et al., 2012; Liang et al., 2020). The content of free amino acids varied significantly with different nitrogen nutrient levels. It is believed that the delivery rate of free amino acids in root-bleeding sap increases with an increasing rate of nitrogen application (Li et al., 2009). In the present study, we found that N200 application combined with chemical control increased the delivery rate of amino acids and mineral nutrients in root-bleeding sap. The proper cultivation measure can improve the capacity of roots to absorb, synthesize, and transport carbohydrates, auxin, and other substances, thereby promoting root activity and root growth (Wang H. et al., 2019). The increase in root activity and its capacity for water and nutrients could lay the foundation for the increase in maize yield under high-density planting.

Nutrient absorption and accumulation are the basis of crop yield formation, and it directly affects the growth process of crops (Wu et al., 2018; Gorlach et al., 2021). Nutrient absorption in maize increases with plant growth. Sufficient nutrient supply during the growth period is the key to obtaining a high maize yield (Ray et al., 2020). Phosphorus and potassium are essential macronutrient elements for maize growth, which play an important role in the yield potential (Wu et al., 2015; Zhan et al., 2016). Nitrogen fertilizer is recognized to be an important factor affecting nutrient accumulation and transportation in addition to chemical control, which also impacts plant nutrient absorption capability (Van Oosten et al., 2019; Ray et al., 2020). In the present study, chemical control increased P and K accumulation amounts at different N levels. P and K accumulation amount increased with increasing level of N application, but the differences between N200 and N300 treatments were not significant. The nutrient accumulation by plants during different growth stages may impact crop yield. It is believed that the high nutrient absorption of N, P, and K in the middle growth stage of crops can promote pre-anthesis non-structural carbohydrate (NSC) reserves in the stem and accordingly enhance grain sink strength during grain filling (Fu et al., 2011; Li W. H. et al., 2012). Liu et al. (2019) considered that the P and K nutrient absorption

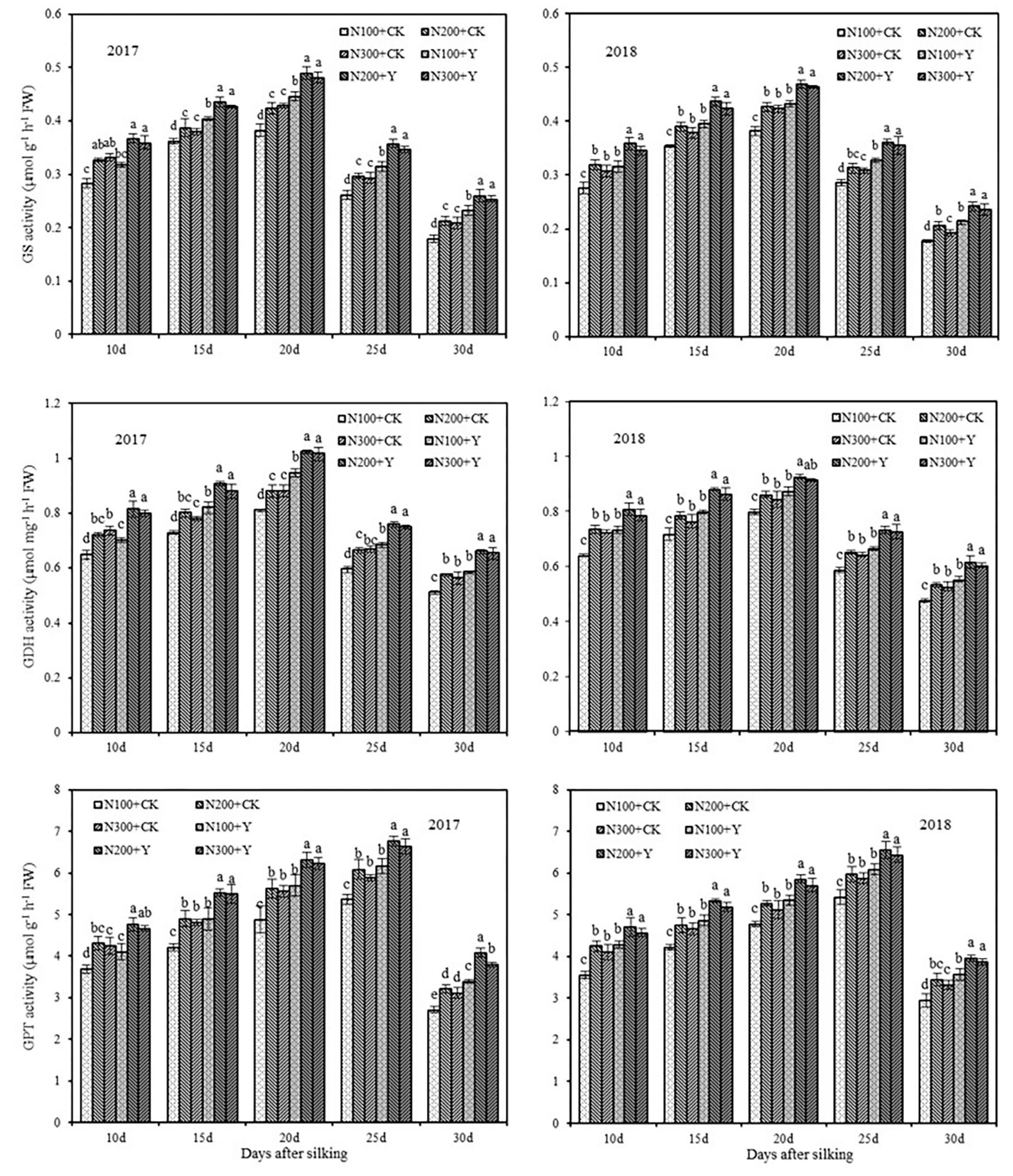

FIGURE 4 | Effects of chemical control and nitrogen fertilizers on GS, GDH, and GPT activities in grain from 10 to 30 days after silking in 2017 and 2018. N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Error bars indicate the value of standard error. Different letters within a growth stage indicate a significant difference at $P < 0.05$.

TABLE 7 | Effects of chemical control and nitrogen fertilizers on grain nutrients concentrations (%) of maize during maize growing period 2017 and 2018.

Year	Treatment	Crude protein	Crude fat	Starch	Lysine	Sucrose	Soluble sugar
2017	N100+CK	9.53e	5.16a	71.81a	0.43d	1.02e	1.68d
	N200+CK	10.67c	5.20a	73.14a	0.47b	1.11b	1.78b
	N300+CK	10.06d	5.16a	72.56a	0.45c	1.07d	1.72cd
	N100+Y	10.78c	5.14a	71.69a	0.45c	1.09c	1.74bc
	N200+Y	11.78a	5.26a	73.18a	0.49a	1.15a	1.85a
	N300+Y	11.33b	5.22a	72.97a	0.47b	1.12b	1.82a
2018	N100+CK	9.05e	5.21a	71.63a	0.42c	1.02c	1.67c
	N200+CK	10.12cd	5.28a	73.57a	0.45b	1.14b	1.80b
	N300+CK	9.67d	5.23a	72.35a	0.45b	1.09b	1.75bc
	N100+Y	10.29bc	5.24a	72.06a	0.45b	1.10b	1.79b
	N200+Y	11.18a	5.34a	73.94a	0.49a	1.17a	1.93a
	N300+Y	10.74ab	5.29a	72.68a	0.48a	1.11ab	1.88ab

N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same year followed by the different letters indicate a significant difference at $P < 0.05$.

TABLE 8 | Effects of chemical control and nitrogen fertilizers on yield and yield components of maize during the maize growing period 2017 and 2018.

Year	Treatment	Ears number per ha	Grains number per ear	1,000-grain weight (g)	Yield (kg ha^{-1})
2017	N100+CK	81,078a	541c	332b	10511c
	N200+CK	81,654a	568b	327b	11548b
	N300+CK	81,782a	560b	316c	11053bc
	N100+Y	81,657a	571b	340ab	11427b
	N200+Y	81,683a	591a	351a	12646a
	N300+Y	82,150a	570b	339ab	11921b
2018	N100+CK	80,325a	531c	294c	9840bc
	N200+CK	80,793a	550bc	298bc	10430b
	N300+CK	78,685b	533c	298bc	9204c
	N100+Y	81,052a	556abc	306bc	9990bc
	N200+Y	81,184a	581a	327a	11704a
	N300+Y	81,167a	566ab	314ab	10732ab

N100+CK, N200+CK, and N300+CK indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under water treatment, respectively; N100+Y, N200+Y, and N300+Y indicate nitrogen applied levels at 100, 200, and 300 kg ha^{-1} under chemical control, respectively. Means within a column for the same year followed by the different letters indicate a significant difference at $P < 0.05$.

in the late growth stage played an important role in improving maize production. In the present study, chemical control and nitrogen fertilizer greatly influenced the proportion of P and K accumulation during different growth stages in maize plants. Similarly, chemical control significantly increased the proportion of P and K accumulation during different growth stages except for the VE-JT and JT-TS stages. The proportion of P and K accumulation after the tasseling stage was obviously increased with increasing levels of N application. Chemical control and nitrogen fertilizer application substantially improved the CAAT of P and K in maize plants, and the highest CAAT of P and K were recorded under N200 application in combination with chemical control. The above results indicate that chemical control and nitrogen fertilizers can improve nutrient accumulation in maize after tasseling and increase the transfer of nutrients from vegetative organs to grains, consequently providing a material basis for yield formation. This result is similar to the study by Ray et al. (2020), which found that appropriate nutrient accumulation and translocation after silking created good conditions for maintaining the supply of nutrients to the grains, resulting in increased yields.

Carbon and nitrogen metabolism determines the level of crop production and function to provide the main energy and basic nutrients for plants (Cui et al., 2019). RuBPCase, PEPCase, NR, and GS are key enzymes involved in carbon and nitrogen metabolism in plants. In the present study, chemical control combined with N200 treatment increased RuBPCase, PEPCase, NR, and GS activities, leading to more assimilate accumulation and higher grain yield (Cheng et al., 2019; Yang et al., 2020). The plants maintained a high carbon and nitrogen metabolism and nutrient accumulation, which was the basis for assimilate accumulation in the grains. Main enzymes such as GS, GPT, and GDH are involved in the nitrogen metabolism in grains, and their activities directly affect the synthesis of amino acids

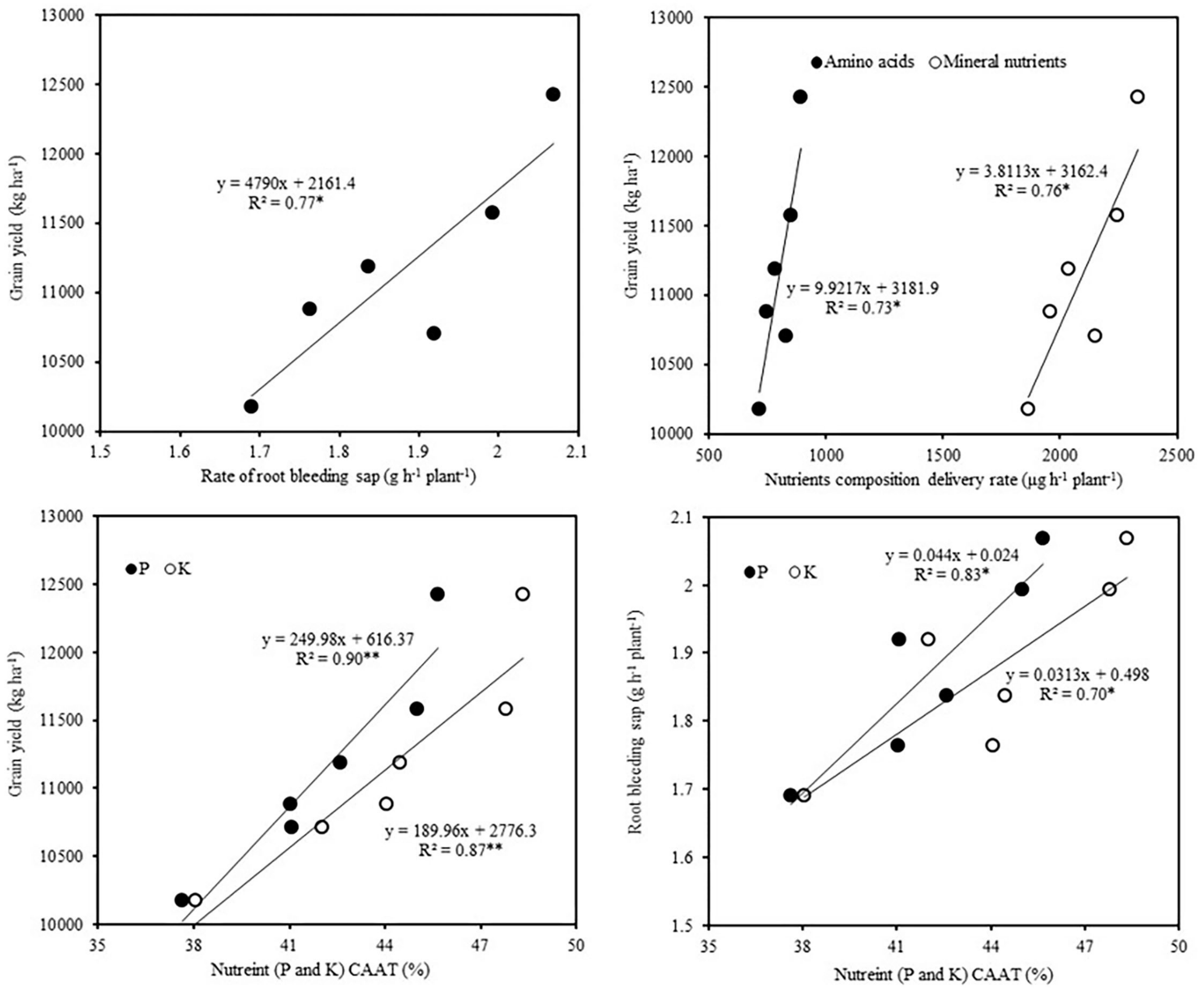

FIGURE 5 | Correlation analysis of root-bleeding sap, nutrient contribution, and grain yield (values are the average in both years). CAAT, contribution rate of nutrient (P or K) accumulation amount post-silking.

and protein in grains (Wang et al., 2016). The N200 application, in combination with chemical control, significantly increased amino acid and protein content in grains, which in turn increased GS, GPT, and GDH activities. Chemical control and N200 treatments also increased the sucrose and soluble sugar contents of grains. This may be due to its association with higher sucrose metabolism and key enzyme activities (Kaur et al., 2018).

Increasing planting density is one of the important practices to increase maize yield per unit area in agricultural production (Tang et al., 2018). However, high-density planting intensifies the competition for light, nutrients, moisture, and space between maize plants, which restricts the growth of shoot and root systems, resulting in reduced crop yield (Rossini et al., 2011). The root system is the crop organ responsible for the uptake of nutrients, and a higher root activity enhances the nutrient absorption capacity in the root system (Yang et al., 2004). In the present study, the rate of root-bleeding sap was positively correlated with the contribution rate of nutrient (P or K) accumulation amount post-silking. It showed that the enhancement of root activity might be an effective method to develop the absorption and utilization capacity of P and K. Maintaining a relatively high level of root activity is an important approach to improving maize production. Niu et al. (2020) showed that increased root activity ensured the availability of soil nutrients and boosted photosynthetic capacity and biomass production, which are critical for grain filling and yield formation. In the present study, the grain yield was positively correlated with the rate of root-bleeding sap, the delivery rate of amino acids and mineral nutrients in bleeding sap, and the CAAT of P and K. It further confirmed that maintaining higher root activity and absorption and utilization capacity of P and K are the important approaches to obtaining high yields. Establishing

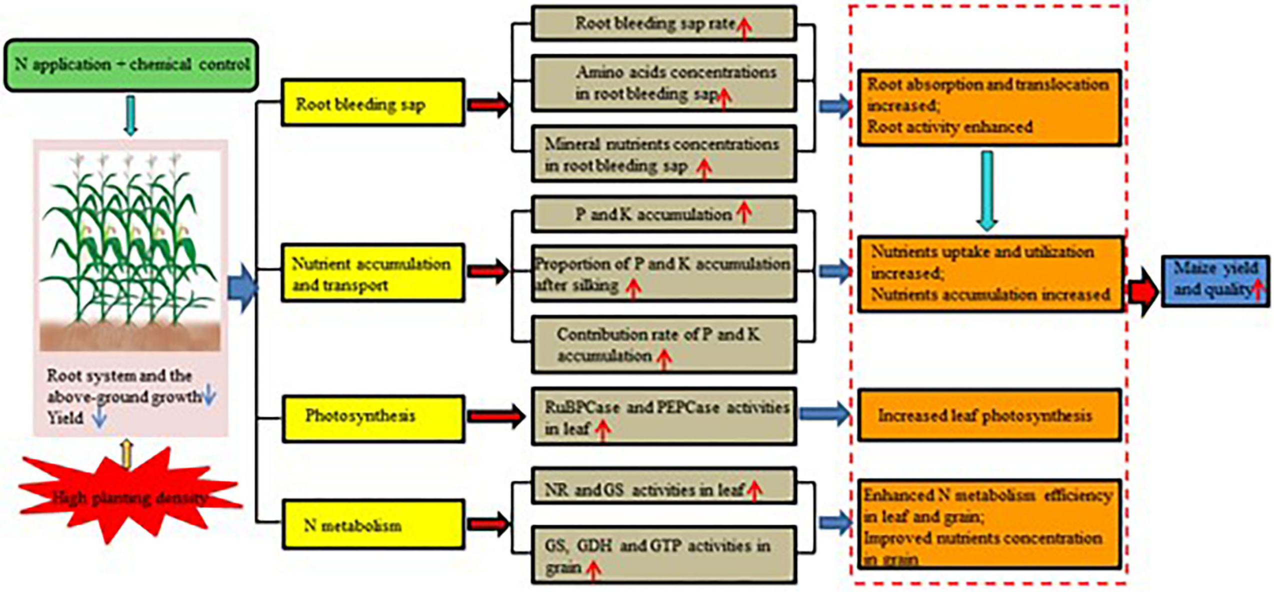

FIGURE 6 | The schematic representation of nitrogen fertilizers and chemical control regulated maize yield. The red arrows (↑) and the blue arrows (↓) represent the positive and passive roles of treatment, respectively.

a well-developed root system and efficient plant population can promote photosynthate production and nutrient accumulation and improve phosphorus and potassium distribution ratios after silking. Excessive nutrient transfer after silking usually affects the photosynthesis in leaves at a later growth stage, resulting in acceleration of leaf and root senescence and limiting yield improvement. However, deficient nutrient transfer after silking is harmful to grain filling, making it difficult to achieve a high yield. Therefore, appropriate cultivation methods can coordinate nutrient transfer and nutrient accumulation after silking and optimize the source-sink relationship, which plays an important role in improving yield. Our study on maize cultivation in Northeast China indicated that N200 combined with chemical control could optimize P and K absorption and translocation in the later growth stage by increasing root activity, thereby improving grain yield and quality.

CONCLUSION

N200 application in combination with chemical control significantly increased the root-bleeding sap rate, amino acid delivery rate, and mineral nutrient delivery rate. It promoted the accumulation and translocation of P and K nutrients after the tasseling stage, and as a result, it provided a material basis for yield formation. Moreover, N200 combined with chemical control obviously enhanced enzyme activities of carbon and nitrogen metabolism in leaves, increased nitrogen metabolism enzyme activities in grains during the early and middle grain filling stage, and improved amino acid and protein content in grains, thereby increasing the grain yield and quality of maize in high-density planting. The schematic representation indicates that nitrogen fertilizers and chemical control increased the grain yield and quality by optimizing root-bleeding sap, nutrient accumulation and transport, photosynthesis, and N metabolism in maize under high-density planting (**Figure 6**). Therefore, attention should be paid to promoting nitrogen fertilizer and chemical control management in high-density planting of maize in future agricultural production in Northeast China as it plays a crucial role in improving maize yield and quality.

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 author/s.

AUTHOR CONTRIBUTIONS

XL and LZ collected and analyzed the samples and wrote the manuscript. YY, CQ, and CoL contributed to the writing and editing of the manuscript. SW, CaL, and WG contributed to the design of the work and analysis and revised the manuscript. All authors read and approved the article.

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