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# EFFECTS OF ELEVATED PLANT DENSITY AND REDUCED NITROGEN ON AGRONOMIC AND YIELD ATTRIBUTES OF MAIZE INBRED LINES AND THEIR DIALLEL CROSSES

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## SUMMARY

Elevating plant density and improving N fertilizer rate for high density-tolerant genotype can maximize maize (*Zea mays* L.) grain productivity per unit land area. This investigation's objective sought to evaluate the effects of stresses resulting from increasing plant density combined with reducing N application rate on traits of eight inbred lines and their diallel $F_1$ crosses. Choosing eight maize inbred lines differing in tolerance to low N and high density (D) were samples for diallel crosses. Parents and crosses' evaluation ensued in the 2020 and 2021 seasons under three plant densities: low (47,600), medium (71,400), and high (95,200) plants/ha, and three N fertilization rates: low (95 kg N/ha), medium (285 kg N/ha), and high (476 kg N/ha). Elevating plant density from 47,600 to 71,400 and 95,200 plants/ha caused a significant decrease in grain yield/plant by 25.43% and 30.15% for inbred parents and 17.92% and 25.65% for $F_1$ crosses, respectively. This reduction correlated with significant decreases in all yield components but caused a notable increase in grain yield/ha by 13.69% and 27.33% for inbreds and 20.99% and 44.69% for $F_1$ crosses, respectively. The best combination of plant population density and N level for giving the highest grain yield/ha was high N (476 kg N/ha) × high density (95,200 plants/ha) for all inbreds and all $F_1$ crosses.

**Key words**: Maize (Zea mays L.), high-density, low-N, unit area productivity

**Key findings**: The results will help maize (*Zea mays* L.) breeders match the functions of optimum plant density with adequate nitrogen fertilizer application to produce the highest possible yields per land unit area with the supreme maize genotype efficiency.

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## INTRODUCTION

Cultivation of maize (*Zea mays* L.) commercial hybrid varieties currently released in Egypt has low plant density (ca. 50,000 plants $ha^{-1}$). One of the main causes of a lower grain production per unit of land area in Egypt planted with maize compared with developed countries may be this lower plant density. Raising productivity per land area unit to improve Egypt's standing in terms of average productivity could help increase the country's overall maize production. The product of the number of plants per unit area and the grain yield per plant is called the grain yield per unit land area (Hashemi *et al*., 2005). Growing maize hybrids that can tolerate high plant densities, up to 100,000 plants per ha, may produce the maximum yield per unit area (Huseyin *et al*., 2003; Younis *et al*., 2021).

In the USA, average maize grain production per unit area has improved significantly in the second half of the 20th century as a result of advancements in crop management techniques and new hybrids' increased tolerance for dense plant growth (Duvick and Cassman, 1999; Tollenaar and Wu, 1999). Grain yield per unit area drastically declined when growing the Egyptian hybrids at high plant densities. Given their height, one-ear bearing habit, decumbent leaf, and large-type plants, these cultivars are probably intolerant of high plant densities (Mushtaq *et al*., 2016; Chozin *et al*., 2017; Al-Naggar *et al*., 2022). However, due to morphological and phenological adaptations like early silking, a short anthesis-to-silking interval (ASI), a few barren stalks, and prolificacy, current maize hybrids in developed countries are distinct by their high producing capacity per unit area under high plant densities (Duvick, 1999). With their superior light absorption, Radenovic *et al*. (2007) noted that maize genotypes with erect leaves are highly desirable for higher population densities. As density per unit area rises, the yield of maize grains per plant declines (Hashemi *et al*., 2005).

The yield declines from each plant's need for less light and other environmental resources (Widdicombe and Thelen, 2002). Fewer cobs (barrenness) are the chief cause of a decreased yield, fewer grains per cob (Tetio-Kagho and Gardner, 1988), lower grain weight (Poneleit and Egli, 1979), or a combination of these (Betran *et al*., 2003). High densities can lead to many undeveloped kernels, which happens in some hybrids after poor pollination due to a delayed silking period relative to tassel emergence (Otegui, 1997), or a shortage of assimilates, which results in grain and cob abortion (Karlen and Camp, 1985). High plant density can, however, lead to more cobs per unit area and a corresponding increase in grain output when supplied with water and nutrients optimally (Bavec and Bavec, 2002).

For the growth of maize crops, nitrogen is a crucial nutrient (Biswas and Mukherjee, 1993). In addition to being a vital component of metabolically active substances, such as, amino acids, proteins, enzymes, coenzymes, and some non-proteinaceous chemicals, it is the primary raw material needed for plant growth (Brady and Weil, 2002). Low N stress, one of the conditions most usually present during dense plant growth, reduces the possible maize yields to produce. Low N availability is a significant yield-limiting factor typically visible in agricultural settings where organic matter mineralization is fast, and fertilization is uncommon (Banziger and Lafitte, 1997). The two most notable low-N adaptation features are ears per plant and anthesis-to-silking interval (Banziger *et al*., 2000).

From the above, raising the crop's tolerance to stresses in their fields is most appropriate because smallholder farmers cannot afford extra inputs (Bhatt, 2012). Many researchers have sought to match the functions of optimal plant density and enough nitrogen fertilizer application to achieve the best yields with the utmost maize hybrid efficiency (Bhatt, 2012; Clark, 2013; Tajul *et al*., 2013). In maintaining adequate photosynthetic rates, sufficient assimilation supplies, and plant growth rates attributable to improved nitrogen use efficiency, modern hybrids tend to withstand higher levels of stresses, such as, low N and high plant densities (O'Neill *et al*., 2004). This study's prime goal was to evaluate the effects of pressures from elevating plant density and lowering N application rate on traits of eight inbreds and their diallel $F_1$ crosses.

## MATERIALS AND METHODS

This study proceeded at the Agricultural Experiment and Research Station of the Faculty of Agriculture, Cairo University, Giza, Egypt (30° 02′ N latitude and 31° 13′ E longitude with an altitude of 22.50 masl). Based on the results of a previous study (Al-Naggar *et al*., 2017), eight maize (*Z. mays* L.) inbred lines differing in tolerance to low N and high density (D) (four tolerant: L-14, L-17, L-21, and L-28, and four sensitive: IL51, IL53, IL-80, and IL84) became samples in this study as parents of diallel crosses. In the 2019 season, producing all possible diallel crosses (except reciprocals) among the eight parents ensued, hence obtaining seeds of 28 direct $F_1$ crosses. Two field evaluation experiments ran in the 2020 and 2021 seasons. Each experiment included 28 $F_1$ crosses, their eight parents, and two check cultivars, namely, SC-132 (white) and SC-168 (yellow), obtained from the Agricultural Research Center (ARC).

Evaluation in each season was under nine environments (E1 to E9), i.e., three nitrogen levels, namely, high-, medium-, and low-N by adding 476, 285, and 95 kg N/ha, respectively, in two equal doses in urea form before first and second irrigations, and three plant densities, namely, high-, medium-, and low-plant density (95,200, 71,400 and 47,600 plants/ha) as follows: E1 - High nitrogen-low plant density (HN-LD), E2 - High nitrogen-medium plant density (HN-MD), E3 - High nitrogen-high plant density (HN-HD), E4 - Medium nitrogen-low plant density (MN-LD), E5 - Medium nitrogen-medium plant density (MN-MD), E6 - Medium nitrogen-high plant density (MN-HD), E7 - Low nitrogen-low plant density (LN-LD), E8 - Low nitrogen-medium plant density (LN-MD), and E9 - Low nitrogen-high plant density (LN-HD).

Using a split-split plot design in randomized complete blocks (RCB) arrangement with three replications had the main plots devoted to nitrogen levels (high-N, medium-N, and low-N). Sub-plot assignments were for plant density (high-D, medium-D, and low-D) and sub-sub-plots for 38 maize genotypes (eight parents, 28 $F_1$s, and two checks). Each sub-sub-plot consisted of one ridge, 4 m long and 0.7 m wide, i.e., the experimental plot area was 2.8 $m^2$. Seeds sowing in hills had a spacing of 15, 20, and 30 cm, followed (before the first irrigation) with thinning to one plant/hill to achieve the three-plant densities, i.e., 95,200, 71,400, and 47,600 plants/ha, respectively. Each main plot comprised a wide alley surrounding (1.5 m width) to avoid interference of the three N treatments with irrigation water. All environments' sowing dates per season occurred on April 29 and May 13 in the 2019 and 2020 seasons, respectively. All other agricultural practices continued according to the recommendations of ARC, Egypt.

Data collection for 13 traits included (1) days to 50% silking (DTS), (2) anthesis-silking interval (ASI) as the number of days between 50% silking and 50% anthesis, (3) plant height (PH), (4) leaf angle (LANG) measured as leaf angle between blade and stem for the leaf just above the ear (using a protractor on 10 guarded plants/plot according to Zadoks *et al*. [1974]), (5) leaf area to produce 1 g of grain (LA/1gG) measured as leaf area per plot/grams of grains per plot, (6) penetrated light at the base of the top-most ear (PLE) as follows: PLE = 100 (light intensity at the base of the top-most ear/light intensity at the top of the plant), (7) chlorophyll concentration index (CCI) measured by a chlorophyll concentration meter, as the ratio of transmission at 931 nm to 653 nm through the leaf of the top-most ear, (8) the number of ears/plant (EPP), (9) the number of rows/ear

(RPE), (10) the number of kernels/plant (KPP), (11) 100-kernel weight (100KW), (12) grain yield/plant (GYPP) adjusted to 15.5% grain moisture at harvest, and (13) grain yield/ha (GYPH) estimated by adjusting grain yield/plot at 15.5% grain moisture to grain yield/ha in tons.

### Statistical analysis

Performing a combined analysis of the variance of a split–split plot across the two seasons continued using Bartlet's equation if the homogeneity test was nonsignificant, and calculating LSD values tested the significance of differences between means according to Steel *et al*. (1997) using SAS (http://www.sas.com/en_us/software/university-edition.html).

## RESULTS AND DISCUSSION

### Effect of elevated plant density

The effects of elevating plant density on the means of studied traits across all the maize (*Z. mays* L.) inbreds and $F_1$ crosses are available in Table 1. Environment D1 (47,600 plants/ha) represents the non-stressed one, while D2 and D3 represent elevated plant density (stressed) environments (71,400 and 95,200 plants/ha, respectively). Reduction in mean grain yield/plant was significant ($P \leq 0.01$) due to elevating plant density from 47,600 plants/ha (D1) to 71,400 plants/ha (D2) and 95,200, plants/ha (D3) by 25.43% and 30.15% for inbred parents and 17.92% and 25.65% for $F_1$ crosses, respectively. This reduction correlated with significant decreases in all yield components, namely, ears/plant (12.92% and 12.92% for parents and 14.93% and 16.15% for crosses), kernels/plant (41.54% and 57.72% for parents and 31.44% and 40.93% for $F_1$s), and kernels/plant (14.61% and 22.33% for parents and 10.51% and 14.14% for $F_1$ crosses) at plant density of 71,400 and 95,200 plants/ha, respectively, compared with 47,600 plants/ha, indicating the importance of the number of kernels, followed by kernel weight and the number of ears/plant as measures of tolerance to high-density. This conclusion has a previous report from Vega *et al*. (2001), Sangoi *et al*. (2002), and Al-Naggar *et al.* (2011, 2015, 2017, and 2021).

**Table 1.** Effect of elevated plant density on maize traits across 2020 and 2021 seasons.

| Traits | Inbreds | | | | | Crosses | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | D1 (47,600 plants/ha) | D2 (71,400 plants/ha) | Ch % | D3 (95,200 plants/ha) | Ch % | D1 (47,600 plants/ha) | D2 (71,400 plants/ha) | Ch % | D3 (95,200 plants/ha) | Ch % |
| DTS (day) | 65.4 | 67.3 | -2.83** | 68.2 | -4.25** | 63.3 | 64.6 | -2.21** | 66.6 | -5.27** |
| ASI (day) | 2.1 | 2.5 | -16.91** | 2.91 | -36.42** | 1.9 | 2.7 | -44.90** | 2.93 | -54.79** |
| PH (cm) | 174.3 | 178.73 | -2.54** | 189.44 | -8.68** | 231.19 | 241.6 | -4.50** | 259.03 | -12.04** |
| LANG (°) | 30.28 | 23.81 | 21.36** | 21.76 | 28.14** | 31.41 | 23.73 | 24.44** | 21.32 | 32.13** |
| CCI% | 37.3 | 42.83 | -14.84** | 52.09 | -39.66** | 42.98 | 47.63 | -10.83** | 55.56 | -29.27** |
| PLE% | 25.42 | 17.69 | 30.40** | 16.26 | 36.02** | 31.33 | 21.48 | 31.44** | 18.95 | 39.51** |
| LA/1g ($cm^2$) | 49.66 | 62.75 | -26.37** | 93.35 | -87.98** | 40.16 | 48.34 | -20.37** | 69.68 | -73.50** |
| RPE | 14.36 | 13.12 | 8.65** | 12.1 | 15.79** | 16.3 | 15.31 | 6.08** | 14.49 | 11.13** |
| EPP | 1.15 | 1.0 | 12.92** | 1.0 | 12.92** | 1.2 | 1.02 | 14.93** | 1.01 | 16.15** |
| KPP | 626.62 | 366.32 | 41.54** | 264.94 | 57.72** | 899.32 | 616.59 | 31.44** | 531.24 | 40.93** |
| 100KW(g) | 33.56 | 28.65 | 14.61** | 25.73 | 23.33** | 33.45 | 29.94 | 10.51** | 28.05 | 16.14** |
| GYPP(g) | 122.43 | 91.3 | 25.43** | 85.51 | 30.15** | 187.72 | 154.09 | 17.92** | 139.57 | 25.65** |
| GYPH (t) | 6.11 | 6.95 | -13.69** | 7.79 | -27.33** | 9.1 | 10.97 | -20.99** | 13.11 | -44.69** |

Change % = 100 × (D1-D2 or D3)/D1, Negative Ch (-) refers to increase, DTS = days to 50% silking, ASI = anthesis-silking interval, PH = plant height, LANG = leaf angle, CCI = chlorophyll concentration index, PLE = penetrated light at the base of top-most ear, LA/1Gg = leaf area producing 1 gram of grain, RPE = rows/ear, EPP = number of ears per plant, KPP = number of kernels per plant, 100KW = 100-kernel weight, GYPP = grain yield per plant, GYPH = grain yield per ha, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively.

An observation noted the reduction in number of kernels/plants was 2.58 and 2.89-fold higher than the reduction in 100-kernel weight under high density (95,200, plants/ha) for inbreds and hybrids, respectively, which is consistent with previous investigators (Sarlangue *et al.,* 2007; Sharifi *et al.,* 2009; Al-Naggar *et al.,* 2011 and 2021).

Several studies have investigated the impact of increased plant density on yield and yield components. Lashkari *et al*. (2011) reported that kernels per plant and ear diameter decreased as plant density increased. Sangoi *et al*. (2002) reported reduced kernels per plant and a 16% decrease in kernel weight as plant density increased from 25,000 to 100,000 plants ha$^{-1}$. Likewise, Hashemi *et al*. (2005) reported that kernels per row and weight decreased as plant density increased, suggesting a complex interaction between the sink and assimilate supply. The reductions in yield components are logical and could refer to the increase in competition between plants at higher densities for sunlight, nutrients, and water. This conclusion has earlier accounts from several investigators (Tokatlis *et al*., 2005; Has *et al*., 2008; Mashiqa *et al*., 2013; and Al-Naggar *et al*., 2017, 2021).

Increasing plant density from 47,600 plants/ha to 71,400 and 95,200 plants/ha also resulted in significant reductions in leaf angle (21.36% and 28.14% for parents and 24.44% and 32.13% for crosses, respectively) and the penetrated light at the base of the top-most ear (30.40% and 36.02% for parents and 31.44% and 39.51% for crosses, accordingly). A substantial decline in leaf angle (erectness) is the result of rising plant density in this study, which is consistent with Edmeades *et al.* (2000) and Al-Naggar *et al.* (2012).

In contrast, elevating plant density to 71,400 and 95,200 plants/ha caused a significant increase in grain yield/ha compared with the low-density (47,600 plants/ha) by 13.69% and 27.33% for inbreds and 20.99% and 44.69% for $F_1$ crosses, respectively (Table 1). The increase in grain yield/ha due to increasing plant density for $F_1$s was 1.51 and 1.64-fold higher than the increase for inbred parents under 71,400 and 95,200 plants/ha, respectively, indicating that heterozygotes are more adapted to high plant density than homozygotes in maize. This conclusion was analogous to previous outcomes from several researchers (Widdicombe and Thelen, 2002; Ipsilandis and Vafias, 2005; Has *et al.,* 2008; and Al-Naggar *et al.,* 2012, 2021). On the contrary, Monneveux *et al.* (2005) reported that lines yielded more than open-pollinated varieties and hybrids under high plant population density, probably because of lower vigor and lower competition between plants.

Moreover, high density caused a significant increase in the anthesis-silking interval (ASI) (elongation) by 0.4 and 0.8 days (16.91% and 36.42%) in parents and 0.8 and 1.0 days (44.90% and 54.79%) in crosses, plant height by 4.4 and 15.1 cm (2.54% and 6.68%) for parents and 10.4 and 27.4 cm (4.50% and 12.04%) for crosses, chlorophyll concentration index by 14.84% and 39.66% for parents and 10.83% and 29.27% for crosses, and the leaf area to produce 1 gram of grain by 26.37% and 87.98% for parents and 20.37% and 73.50% for crosses. A small but significant increase in the trait days-to-silking (DTS) was evident due to an increase in plant density from low density (47,600 plants/ha) to 71,400 and 95,200 plants/ha.

In general, the elongation of ASI due to high plant density in this study was less than the results reported by other investigators. Such ASI elongation ranged from -4 to 10 days (Bolanos and Edmeades, 1996). Tokatlis *et al*. (2005) reported that the time gap between pollen shedding and silking increased from 0 to 9 days by increasing plant density from 5 plants m$^{-2}$ to 20 plants m$^{-2}$. Increased days to silking and ASI as symptoms of interplant competition came from several investigators (Helland, 2012; Al-Naggar *et al.,* 2012 and 2021). These traits (DTS and long ASI) also indicate barrenness or high-density intolerance (Gebre, 2006; Al-Naggar *et al.,* 2012 and 2021). Several authors implied that the separation of reproductive organs in maize may also account for this susceptibility to stress at flowering (Haegele *et al.,* 2013). Delayed silking under drought or high-density conditions is relative to less assimilates being partitioned to growing ears around anthesis, which results in lower ear growth rates, increased ear abortion, and more barren plants (Edmeades and Lafitte, 1993). When assimilate supply is inadequate under stress, its preferential distribution is usually to the stem and tassel at the expense of ear nutrition, leading to poor pollination and partial or complete failure of the seed set. It often occurs with all kinds of stress, including drought, low soil N and P, excess moisture, low soil pH, iron deficiency, and high population density (Monneveux *et al.,* 2005). Sufficient evidence indicates that maize plants exposed to these stresses have lesser ears/plant and kernels/plant (Has *et al.,* 2008).

Elongation of plant stalks and ear position increase exhibited in this study due to elevating the plant densities could be attributable to lower light levels and greater competition between plants for light. This conclusion has previous reports from other investigators (Carena and Cross, 2003; Monneveux *et al.,* 2005; and Al-Naggar *et al*., 2012, 2021).

## Effect of reduced N level

A comparative summary of means of all studied traits across all inbreds and crosses subjected to low-, medium-, and high-N conditions and across two years appears in Table 2. Mean grain yield/plant (GYPP)'s reduction was significant ($P \le 0.01$) due to decreasing N level from 476 kg N/ha (HN) to 285 kg N/ha (MN) and 95 kg/ha (LN) by 14.0% and 22.9% for parental inbreds and 7.7% and 21.9% for $F_1$ crosses. Moreover, mean grain yield/ha was significantly ($P \le 0.01$) lower due to reducing N level by 14.4% and 45.9% for inbreds and 9.7% and 43.8% for crosses. Consistent with these results are reports of a reduction in grain yield due to N stress by several investigators (Presterl *et al.,* 2003; Al-Naggar *et al.,* 2010, 2020).

Reduction in grain yield due to medium-N and low-N levels has linkage with substantial decreases in kernels/plant by 23.2% and 48.9% for inbreds and 15.0% and 32.0% for $F_1$ crosses, 100-KW by 6.6% and 17.7% for inbreds and 3.9% and 11.9% for crosses, rows/ear by 7.9% and 20.8% for inbreds and 4.1% and 11.2% for crosses, and ears/plant by 6.5% and 9.1% for inbreds and 4.8% and 8.5% for crosses, respectively. Declining yield components caused by low-N were maximum for the number of kernels/plant and minimum for the other two yield components, i.e., ears/plant and 100-KW. It indicates that number of kernels per plant is

**Table 2.** Effect of reduced N level on maize traits across 2020 and 2021 seasons.

| | Inbreds | | | | | Crosses | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Traits | HN (476 kg/ha) | MN (285 kg/ha) | Ch % | LN (95 kg/ha) | Ch % | HN (476 kg/ha) | MN (285 kg/ha) | Ch % | LN (95 kg/ha) | Ch % |
| DTS (day) | 67.8 | 67.1 | 1.0* | 65.9 | 2.8** | 65.7 | 65.1 | 1.0* | 63.7 | 3.0** |
| ASI (day) | 2.3 | 2.8 | -24.0** | 2.4 | -4.5 | 2.4 | 2.6 | -6.1* | 2.5 | -3.4 |
| PH (cm) | 183.75 | 183 | 0.4 | 175.71 | 4.4** | 250.79 | 245.63 | 2.1** | 235.41 | 6.1** |
| LANG (°) | 22.12 | 25.82 | -16.7** | 27.92 | -26.2** | 22.02 | 25.62 | -16.3** | 28.81 | -30.8** |
| CCI% | 48.59 | 43.85 | 9.8** | 39.77 | 18.2** | 52.53 | 48.53 | 7.6** | 45.12 | 14.1** |
| PLE% | 16.68 | 19.19 | -15.0** | 23.5 | -40.9** | 20.13 | 23.79 | -18.2* | 27.83 | -38.2** |
| LA/1gG (cm$^2$) | 82.22 | 67.59 | 17.8** | 55.95 | 31.9** | 61.68 | 52.45 | 15.0** | 44.06 | 28.6** |
| RPE | 14.59 | 13.44 | 7.9** | 11.55 | 20.8** | 16.19 | 15.53 | 4.1** | 14.38 | 11.2** |
| EPP | 1.11 | 1.03 | 6.5** | 1.01 | 9.1** | 1.13 | 1.08 | 4.8** | 1.03 | 8.5** |
| KPP | 551.82 | 423.97 | 23.2** | 282.09 | 48.9** | 809.17 | 687.79 | 15.0** | 550.18 | 32.0** |
| 100KW (g) | 31.9 | 29.79 | 6.6** | 26.25 | 17.7** | 32.17 | 30.91 | 3.9** | 28.36 | 11.9** |
| GYPP (g) | 113.72 | 97.84 | 14.0** | 87.69 | 22.9** | 177.98 | 164.36 | 7.7** | 139.03 | 21.9** |
| GYPH (t) | 8.7 | 7.45 | 14.4** | 4.7 | 45.9** | 13.44 | 12.14 | 9.7** | 7.6 | 43.8** |

Change % = 100 × (HN-MN or LN)/HN, Negative Ch (-) refers to increase, DTS = days to 50% silking, ASI = anthesis-silking interval, PH = plant height, LANG = leaf angle, CCI = chlorophyll concentration index, PLE = penetrated light at the base of top-most ear, LA/1Gg = leaf area producing 1 gram of grain, RPE = rows/ear, EPP = number of ears per plant, KPP = number of kernels per plant, 100KW = 100-kernel weight, GYPP = grain yield per plant, GYPH = grain yield per ha, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively.

the most determining component of grain yield/plant under low- and medium-N levels, similar to that observed under high-plant density stress. These results fully agree with those reported by Al-Naggar *et al*. (2010 and 2021).

Moreover, lower levels of nitrogen, i.e., medium- and low-N, caused a significant decrease in the leaf area, producing 1 gram of grain by 17.8% and 31.9% for inbreds and 15.0% and 28.6% for crosses and chlorophyll concentration index (CCI) by 9.8% and 18.2% (inbreds) and 7.6% and 14.1% (crosses) compared with high N, respectively. The reduction observed in CCI was comparable to that observed in kernels/plants; they were more pronounced under a low-N environment. Thus, the physiological (CCI) and kernels/plant traits could be the most determining indicator for grain yield/plant under N stress conditions. Previous reports from Vanyine *et al.* (2012) and Al-Naggar *et al.* (2010) declared the importance of chlorophyll concentration in maize tolerance to low-N. Moreover, an earlier study reported the number of kernels per plant is vital for maize tolerance to low and moderate N stress (Al-Naggar *et al.*, 2010). Generally, low-N caused a significant but small magnitude reduction in days-to-silking, plant height, and ears/plant. The observed decline in the leaf area producing 1 gram of grain (LA/1gG) was more pronounced under a low-N environment and reached 31.9% (inbreds) and 28.6% (crosses), which was very close to the reduction in GYPP and kernels/plant under low-N. This trait (LA/1gG) has the potential as a determining physiological trait for grain yield/plant under low-N stress.

Inversely, medium-N and low-N caused significant ($P \leq 0.01$) increases in the means of leaf angle by 16.7% and 26.2% (inbreds) and 16.3% and 30.8% (crosses), the penetrated light at the base of the top-most ear by 15.0% and 40.9% (inbreds) and 18.2% and 38.2% (crosses) (Table 2). Furthermore, low-N and medium-N stresses caused the elongation of the anthesis-silking interval (ASI). The lengthening of ASI in this study due to N-stress fully aligns with Monneveux *et al.* (2005) and Al-Naggar *et al.* (2011). Monneveux *et al*. (2005) reported that ASI was < 1 day under optimal conditions but increased to > 3 days under low-N conditions.

## Effect of different levels of N combined with different levels of plant density

The effect of nine combinations between three nitrogen levels and three plant densities on the studied traits occurs in Table 3. The highest grain yield/plant came from E1 (a combination of the highest N level and lowest plant density), which is logical since available nitrogen for each plant was at maximum across seasons; therefore, this environment is the best for grain yield/plant, and the percent change, in different studied traits showed calculations relevant to this environment, either in case of increase (-) or decrease (+). Both stresses (nitrogen and plant density) occurred in E9, E8, E6, and E5 environments, in descending order of severity, with a minimum severity in E5. Other environments exhibited only one (E2, E3, and E7) or no stress (E1). It was evident that the rigidity of the stress combinations on grain yield/plant was at maximum (45.41% and 41.94% reduction for inbreds and hybrids, respectively) under environment E9 (LN-HD), where both severe stresses (highest plant density and lowest available nitrogen) existed. The reduction in grain yield/plant due to the effect of both stresses in different combinations showed a descending order, from E9 to E8, E6, and E5 (45.41%, 43.68%, 39.81%, and 36.36%, respectively) for parents and (41.94%, 37.30%, 30.33%, and 20.50%, respectively) in crosses.

Significant reductions in grain yield/ha of maize crosses observed in environments E8 and E9 relative to E1 (45.40% and 53.18%) were due to N and density stresses. Notably, the decline in grain yield/ha of inbreds and crosses were at maximum under environment E9 (53.18% and 43.93%, respectively) due to both stresses (the highest plant density and the lowest available nitrogen for each plant). Contrastingly, grain yield/ha of both inbreds and hybrids under the environments E3 and E2 tended to increase over that under E1. Maximum increase (41.46% and 27.19%) in grain yield/ha resulted in $F_1$ crosses under E3 and E6, respectively, due to high plant density. The best combination of plant population density and N level for giving the highest grain yield/ha was high N (476 kg N ha$^{-1}$) × high density (95,200 plants ha$^{-1}$) for all inbreds and all F$_1$ crosses.

Decreases in grain yield resulting from both stresses (elevated plant densities and reduced N levels) in both inbreds and hybrids were parallel with reductions in all yield components (ears/plant, rows/ear, kernels/plant, 100-kernel weight), the leaf angle, and the penetrated light at the base of the top-most ear. Such reductions were more evident in the E9 environment (maximum stresses), followed by E8, E6, and E5, in descending order. The highest reductions appeared from kernels/plant (80.6% and 62.2%) and chlorophyll concentration index (76.5% and 76.8%) for inbreds and hybrids, respectively, under E9, due to severe stresses of low nitrogen and high plant density.

On the other hand, the two stresses together (shown by the four environments E9, E8, E6, and E5) caused increases in the anthesis-silking interval (ASI). Maximum surges appeared under E9, followed by the E8 environment (Table 3).

Remarkably, the plant height (PH) of both parents and crosses tended to increase under E2, E5, and E6 environments but showed a reducing tendency under E7. The reason for the PH increase under E2, E5, and E6 may refer to elevated plant density levels, whereas the reduction under E7 may be due to the severe stress of nitrogen. In general, the reduction in magnitude in grain yields due to either one stress or both stresses combined is more pronounced in the parental inbreds than in their F$_1$ crosses. Therefore, the crosses have a higher ability to tolerate stresses than the inbreds since heterozygotes are more adaptive to different environments than homozygotes, expressed in grain yield. Rodrigues *et al.* (2003) and Monneveux *et al.* (2005) previously reported this conclusion.

**Table 3.** Means of studied traits for nitrogen levels × plant densities interaction across nine environmental conditions (Data are combined across 2020 and 2021 seasons).

| Genotype | E1 HN-LD | E2 HN-MD | E3 HN-HD | E4 MN-LD | E5 MN-MD | E6 MN-HD | E7 LN-LD | E8 LN-MD | E9 LN-HD |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Days to silking | | | | |
| Parents | 66.45 | 68.59 | 68.43 | 65.71 | 67.46 | 68.22 | 64.06 | 65.73 | 67.92 |
| Change % | | -3.22** | -2.98** | 1.11** | -1.53** | -2.66** | 3.59** | 1.08** | -2.21** |
| Crosses | 64.42 | 65.62 | 67.1 | 63.29 | 64.81 | 67.07 | 62.05 | 63.51 | 65.59 |
| Change % | | -1.86** | -4.15** | 1.76** | -0.61 | -4.10** | 3.68** | 1.41** | -1.82** |
| | | | | | Anthesis-silking interval | | | | |
| Parents | 2.08 | 1.73 | 3.07 | 2.27 | 2.97 | 3.3 | 2.05 | 2.78 | 2.36 |
| Change % | | 16.75 | -47.50** | -9 | -42.75** | -58.50** | 1.5 | -33.50** | -13.5 |
| Crosses | 1.92 | 2.13 | 3.29 | 1.75 | 3.08 | 2.96 | 2.01 | 3.03 | 2.55 |
| Change % | | -10.99 | -71.05** | 8.98 | -60.22** | -54.18** | -4.8 | -57.43** | -32.66** |
| | | | | | Plant height (cm) | | | | |
| Parents | 178.98 | 181.71 | 190.56 | 175.76 | 182.04 | 191.2 | 168.16 | 172.43 | 186.54 |
| Change % | | -1.52** | -6.47** | 1.80** | -1.71** | -6.83** | 6.05** | 3.66** | -4.22** |
| Crosses | 237.78 | 248.87 | 265.7 | 233.02 | 245.62 | 258.25 | 222.77 | 230.31 | 253.14 |
| Change % | | -4.66** | -11.74** | 2.00** | -3.29** | -8.61** | 6.31** | 3.14** | -6.46** |
| | | | | | Leaf angle (°) | | | | |
| Parents | 26.94 | 19.95 | 19.48 | 30.68 | 25.15 | 21.63 | 33.23 | 26.34 | 24.18 |
| Change % | | 25.95** | 27.69** | -13.88** | 6.65** | 19.72** | -23.36** | 2.2 | 10.25** |
| Crosses | 28.57 | 18.35 | 19.15 | 30.64 | 25.18 | 21.04 | 35.02 | 27.66 | 23.76 |
| Change % | | 35.76** | 32.95** | -7.26** | 11.84** | 26.33** | -22.60** | 3.17 | 16.84** |
| | | | | | Chlorophyll concentration index (%) | | | | |
| Parents | 41.72 | 46.9 | 57.17 | 37.32 | 43.44 | 50.78 | 32.84 | 38.15 | 48.31 |
| Change % | | -12.41** | -37.03** | 10.54** | -4.12** | -21.72** | 21.27** | 8.55** | -15.79** |
| Crosses | 46.98 | 51.29 | 59.33 | 43.13 | 47.6 | 54.85 | 38.83 | 44.01 | 52.51 |
| Change % | | -9.17** | -26.27** | 8.20** | -1.32 | -16.73** | 17.36** | 6.33** | -11.76** |

**Table 3** (cont'd).

| Genotype | E1<br>HN-LD | E2<br>HN-MD | E3<br>HN-HD | E4<br>MN-LD | E5<br>MN-MD | E6<br>MN-HD | E7<br>LN-LD | E8<br>LN-MD | E9<br>LN-HD |
|---|---|---|---|---|---|---|---|---|---|
| Penetrated light at the base of top-most ear (%) | | | | | | | | | |
| Parents | 22.58 | 14.79 | 12.68 | 22.52 | 18.5 | 16.54 | 31.16 | 19.78 | 19.57 |
| Change % | | 34.50** | 43.85** | 0.28 | 18.05** | 26.74** | -38.03** | 12.39* | 13.33* |
| Crosses | 27.22 | 17.83 | 15.35 | 28.91 | 22.38 | 20.08 | 37.86 | 24.21 | 21.42 |
| Change % | | 34.48** | 43.61** | -6.21 | 17.75** | 26.21** | -39.10** | 11.04* | 21.31** |
| Change % | | 25.69** | 33.68** | -5.80** | 10.55** | 20.23** | -16.21** | 4.89** | 15.17** |
| Leaf area producing 1 gram of grain (cm$^2$) | | | | | | | | | |
| Parents | 66.73 | 78.47 | 101.46 | 48.08 | 63.02 | 91.67 | 34.15 | 46.77 | 86.92 |
| Change % | | -17.58** | -52.03** | 27.95** | 5.56* | -37.36** | 48.82** | 29.92** | -30.25** |
| Crosses | 48.54 | 55.87 | 80.63 | 40.53 | 48.85 | 67.96 | 31.41 | 40.31 | 60.45 |
| Change % | | -15.10** | -66.12** | 16.49** | -0.63** | -40.02** | 35.29** | 16.95** | -24.54** |
| Rows/ear | | | | | | | | | |
| Parents | 15.64 | 14.55 | 13.58 | 14.63 | 13.43 | 12.26 | 12.82 | 11.39 | 10.45 |
| Change % | | 6.97** | 13.22** | 6.50** | 14.17** | 21.61** | 18.03** | 27.22** | 33.21** |
| Crosses | 17.37 | 16 | 15.21 | 16.39 | 15.55 | 14.66 | 15.14 | 14.39 | 13.6 |
| Change % | | 7.91** | 12.46** | 5.65** | 10.49** | 15.60** | 12.82** | 17.18** | 21.73** |
| Ears/plant | | | | | | | | | |
| Parents | 1.32 | 1.0 | 1.0 | 1.1 | 1.0 | 1.0 | 1.02 | 1.0 | 1.0 |
| Change % | | 24.29** | 24.29** | 16.38** | 24.29** | 24.29** | 22.80** | 24.29** | 24.29** |
| Crosses | 1.33 | 1.04 | 1.01 | 1.19 | 1.03 | 1.01 | 1.09 | 1.0 | 1.0 |
| Change % | | 21.47** | 23.68** | 10.55** | 22.89** | 23.85** | 17.73** | 24.50** | 24.65** |
| Kernels/plant | | | | | | | | | |
| Parents | 824.1 | 472.9 | 358.4 | 592.1 | 403.3 | 276.5 | 463.7 | 222.7 | 159.8 |
| Change % | | 42.6** | 56.5** | 28.2** | 51.1** | 66.4** | 43.7** | 72.9** | 80.6** |
| Crosses | 1102.3 | 707.2 | 617.95 | 867.2 | 637.3 | 558.7 | 728.3 | 505.17 | 417 |
| Change % | | 35.8** | 43.9** | 21.3** | 42.2** | 49.3** | 33.9** | 54.2** | 62.2** |
| 100-kernel weight (g) | | | | | | | | | |
| Parents | 36.04 | 31.41 | 28.26 | 33.25 | 29.62 | 26.5 | 31.39 | 24.93 | 22.42 |
| Change % | | 12.83** | 21.59** | 7.73** | 17.80** | 26.45** | 12.90** | 30.83** | 37.78** |
| Crosses | 35.34 | 31.82 | 29.37 | 33.23 | 30.46 | 29.03 | 31.79 | 27.53 | 25.76 |
| Change % | | 9.96** | 16.90** | 5.98** | 13.80** | 17.84** | 10.03** | 22.11** | 27.11** |
| Grain yield/plant (g) | | | | | | | | | |
| Parents | 140.48 | 105.38 | 95.31 | 119.56 | 89.4 | 84.55 | 107.25 | 79.12 | 76.69 |
| Change % | | 24.98** | 32.16** | 14.89** | 36.36** | 39.81** | 23.65** | 43.68** | 45.41** |
| Crosses | 204.21 | 171.86 | 157.86 | 188.47 | 162.35 | 142.27 | 170.49 | 128.05 | 118.57 |
| Change % | | 15.84** | 22.70** | 7.71** | 20.50** | 30.33** | 16.51** | 37.30** | 41.94** |
| Grain yield/ha (t) | | | | | | | | | |
| Parents | 8.06 | 8.81 | 9.23 | 6.51 | 7.64 | 8.19 | 5.93 | 4.4 | 3.77 |
| Change % | | -9.33** | -14.51** | 19.19** | 5.14** | -1.67 | 26.35** | 45.40** | 53.18** |
| Crosses | 11.28 | 13.09 | 15.96 | 9.59 | 12.5 | 14.35 | 9.04 | 7.31 | 6.33 |
| Change % | | -16.00** | -41.46** | 15.02** | -10.82** | -27.19** | 19.90** | 35.17** | 43.93** |

* and ** indicate significance at 0.05 and 0.01 probability levels, respectively. Change % = 100 × (E1-E2, E3, or E9) / E1, Negative Ch (-) refers to increase. E1: High nitrogen-low plant density (HN-LD), E2: High nitrogen-medium plant density (HN-MD), E3: High nitrogen-high plant density (HN-HD), E4: Medium nitrogen-low plant density (MN-LD), E5: Medium nitrogen-medium plant density (MN-MD), E6: Medium nitrogen-high plant density (MN-HD), E7: Low nitrogen-low plant density (LN-LD), E8: Low nitrogen-medium plant density (LN-MD), and E9: Low nitrogen-high plant density (LN-HD).

## CONCLUSIONS

Elevating maize (*Z. mays* L.) plant density from 47,600 to 71,400 and 95,200 plants/ha caused a significant decrease in grain yield/plant but caused a significant increase in grain yield/ha by 13.69% and 27.33% for inbreds and 20.99% and 44.69% for $F_1$ crosses, respectively. In this study, the best combination of plant population density and N level for giving the highest grain yield/ha was high N (476 kg N ha$^{-1}$) × high density (95,200 plants ha$^{-1}$) for all inbreds and all $F_1$ crosses.

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