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Maize response to elevated plant density combined with lowered N-fertilizer rate is genotype-dependent

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ABSTRACT

Increasing plant density and improving N fertilizer rate along with the use of high density-tolerant genotypes would lead to maximizing maize (*Zea mays* L.) grain productivity per unit land area. The objective of this investigation was to match the functions of optimum plant density and adequate nitrogen fertilizer application to produce the highest possible yields per unit area with the greatest maize genotype efficiency. Six maize inbred lines differing in tolerance to low N and high density (D) [three tolerant (T); L-17, L-18, L-53, and three sensitive (S); L-29, L-54, L-55] were chosen for diallel crosses. Parents and crosses were evaluated in the 2012 and 2013 seasons under three plant densities: low (47,600), medium (71,400), and high (95,200) plants ha^{-1} and three N fertilization rates: low (no N addition), medium (285 kg N ha^{-1}) and high (570 kg N ha^{-1}). The T × T crosses were superior to the S × S and T × S crosses under the low N–high D environment in most studied traits across seasons. The relationships between the nine environments and grain yield per hectare (GYPH) showed near-linear regression functions for inbreds L54, L29, and L55 and hybrids L18 × L53 and L18 × L55 with the highest GYPH at a density of 47,600 plants ha^{-1} and N rate of 570 kg N ha^{-1} and a curvilinear relationship for inbreds L17, L18, and L53 and the rest of the hybrids with the highest GYPH at a density of 95,200 plants ha^{-1} combined with an N rate of 570 kg N ha^{-1}. Cross L17 × L54 gave the highest grain yield in this study under both high N–high-D (19.9 t ha^{-1}) and medium N–high-D environments (17.6 t ha^{-1}).

1. Introduction

Hybrid varieties currently released in Egypt by the National Maize Breeding Program (NMBP) are bred and grown at low plant density (57,000 plants ha^{-1}) around half the density used in developed countries [1]. This lower plant density may be one of the main reasons for lower grain yield per unit land area planted in maize than that in the developed countries. A potential method for maximizing total maize production in Egypt is raising productivity per unit of land area and thus upgrading our global rank in average productivity, especially with the irrigation system used in Egypt and weather and soil conditions better suited to maize cultivation than those of other regions in the world. Grain yield per unit land area is the product of grain yield plant^{-1} and number of plants per unit area [2]. Maximum yield per unit area may be obtained by

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growing maize hybrids that can withstand high plant density, up to 100,000 plants ha^{-1} [3]. Average maize grain yield per unit area in the USA increased dramatically during the second half of the 20th century, owing to improvements in crop management practices and greater tolerance by modern hybrids of high plant densities [4,5].

Growing hybrid varieties released by the NMBP at high plant densities causes a drastic reduction in grain yield per unit area. The reason is probably that these varieties are not tolerant of high plant densities, because of their height, one-eared bearing habit, decumbent leaf, and large-type plants. In contrast, modern maize hybrids in developed countries are characterized by high yielding ability per unit area under high plant densities, owing to morphological and phenological adaptations such as early silking, short anthesis-to-silking interval (ASI), few barren stalks, and prolificacy [6]. Radenovic et al. [7] pointed out that maize genotypes with erect leaves are very desirable for increased population densities, owing to their better light interception.

Maize grain yield per plant decreases as the density per unit area increases [2]. The yield decreases as a response to decreasing light and other environmental resources available to each plant [8]. Reduction in yield is due mainly to fewer cobs (barrenness) [9], fewer grains per cob [10], lower grain weight [11], or a combination of these components [12]. At high densities, 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 [13] and/or owing to a limitation in assimilate supply that causes grain and cob abortion [14]. However, under optimum water and nutrient supply, high plant density can result in an increased number of cobs per unit area, with an eventual increase in grain yield [15]. Liu et al. [16] reported that maize yield differed significantly at varying plant density levels, owing to differences in genetic potential.

Nitrogen is an essential nutrient for maize crop growth [17]. It is the principal raw material required for the growth of plants and is an essential constituent of metabolically active compounds such as amino acids, proteins, enzymes, coenzymes, and some non-proteinaceous compounds [17,18]. Low N stress is one of the factors most frequently occurring under high plant density and limits maize production. Low N availability in soils is an important yield-limiting factor frequently found in farmers' fields where fertilization is not commonly used and organic matter is rapidly mineralized [19]. Ears $plant^{-1}$ and anthesis-to-silking interval are considered the most important low-N adaptive traits [20]. Under these circumstances, given that smallholder farmers cannot afford additional inputs, it is desirable to increase the tolerance of the crop to stresses that occur in their fields [21].

Matching the functions of optimum plant density and adequate nitrogen fertilizer application to produce the highest possible yields with the greatest maize hybrid efficiency has been the aim of many researchers [22–24]. Modern hybrids have shown tendencies to withstand higher levels of stresses (such as low N and high plant densities), allowing them to better sustain suitable photosynthetic rates and sufficient assimilate supplies and to maintain plant growth rates attributable to enhanced nitrogen use efficiency [25]. Along with the prevailing belief that high yields require more plants and that more plants require more N, the idea that different hybrids respond differently to both N and plant density should be considered [26]. Moreover, different hybrids may behave differently in their tolerance to both low N and high-density stresses [26]. The objectives of the present investigation were (i) to evaluate the effects of stresses resulting from elevating plant density combined with lowering N application rate on traits of six inbreds and their diallel F_1 crosses, and (ii) to match the functions of appropriate plant density and adequate nitrogen fertilizer application with greatest maize inbred or hybrid efficiency to produce the highest possible yields per unit area.

2. Materials and methods

This study was performed in the 2011, 2012, and 2013 seasons at the Agricultural Experiment and Research Station of the Faculty of Agriculture, Cairo University, Giza, Egypt. Six maize inbred lines (Table 1) in the sixth selfing generation (S_6), showing clear differences in performance and general combining ability for grain yield per hectare under high plant density were chosen as parents of diallel crosses. In the 2011 season, all possible diallel crosses (except reciprocals) were made among the six parents, so that seeds of 15 direct F_1 progenies were obtained. Two field evaluation experiments were performed in the 2012 and 2013 seasons. The climatic differences over experimental years are shown in Table 2. Each experiment included the 15 F_1 crosses, their six parents, and two check cultivars: SC 10 (with white grains) obtained from the Agricultural Research Center (ARC) and SC 2066 (with yellow grains) obtained from Hi-Tech Company-Egypt.

Evaluation in each season was performed under nine environments (from E1 to E9): three nitrogen levels: high (HN), medium (MN), and low N (LN) by addition of 570, 285, and 0 kg N ha^{-1}, respectively, in two equal doses of urea

Table 1 – Designation, origin, and most important traits of six inbred lines (L) used for making diallel crosses in this study.

Entry designation	Origin	Institution (country)	Prolificacy	Productivity under high density and/or low-N
L17-Y	SC 30 N11	Pioneer	Prolific	High
L18-Y	SC 30 N11	Pioneer	Prolific	High
L53-W	SC 30 K8	Pioneer	Prolific	High
L29-Y	Pop 59	ARC-Thailand	One-eared	Low
L54-W	SC 30 K8	Pioneer	One-eared	Low
L55-W	SC 30 K8	Pioneer	One-eared	Low

ARC: Agricultural Research Center; Pioneer: Pioneer International Company in Egypt; SC: Single cross; W: White grains; Y: Yellow grains.

Table 2 – Some meteorological variables recorded at Giza Agrometeorological Station during two maize growing seasons.

Month	2012 season					2013 season				
	Temperature (°C)			Relative humidity	Precipitation	Temperature (°C)			Relative humidity	Precipitation
	Max.	Min.	Mean	Mean (%)	mm	Max.	Min.	Mean	Mean (%)	mm
April	31.46	19.01	24.99	43.00	0	29.33	14.77	22.50	51.33	0
May	34.37	21.99	27.89	28.33	0	35.70	19.67	27.87	47.00	0
June	35.37	24.30	29.60	56.11	0	35.97	22.40	29.47	53.00	0
July	35.44	25.04	30.09	55.22	0	34.93	22.50	28.87	60.33	0
August	33.64	23.33	28.13	52.44	0	37.07	23.67	30.33	60.67	0

Sowing dates were April 5 and May 2 in the 2012 and 2013 seasons, respectively.

before the first and second irrigations, and three plant densities: high (HD), medium (MD), and low (LD) (95,200, 71,400, and 47,600 plants ha^{-1}, respectively) as follows: E1, HN–LD; E2, HN–MD; E3, HN–HD; E4, MN–LD; E5, MN–MD; E6, MN–HD; E7, LN–LD; E8, LN–MD; and E9, LN–HD. Available soil nitrogen in 30 cm depth was analyzed immediately prior to sowing. Available nitrogen (including soil N and added N) was calculated for each environment and found to be 747, 462, and 177 kg N ha^{-1} in the 2012 season and 732, 447, and 162 kg N ha^{-1} in the 2013 season, with an average across the two seasons of 740, 456, and 170 kg N ha^{-1}, respectively. A split–split plot design in a randomized complete block (RCB) arrangement with three replications was used. Main plots represented nitrogen levels (HN, MN, and LN). Subplots were assigned to plant density (HD, MD, and LD). Sub-subplots were assigned to the 23 maize genotypes (six parents, 15 F_1s, and two checks). Each sub-subplot consisted of one ridge 4 m long and 0.7 m wide. Seeds were sown in hills at 15, 20 and 30 cm apart, and thereafter (before the first irrigation) were thinned to one plant per hill to achieve the three plant densities of 95,200, 71,400, and 47,600 plants ha^{-1}, respectively. The sowing dates in all environments were April 5 and May 2 in the 2012 and 2013 seasons, respectively. The soil analysis of the experimental site is presented in Table 3. All other agricultural practices were followed according to the recommendations of ARC, Egypt. Fertilization with calcium superphosphate was performed with soil preparation and before sowing. Weed control was performed chemically with Stomp herbicide before the first irrigation and just after sowing and manually by hoeing twice, the first before the second irrigation and the second before the third irrigation. Irrigation was applied by flooding after three weeks for the second irrigation and every 12 days for subsequent irrigations. Pest control was performed when required by spraying plants with Lannate (Methomyl) 90% (manufactured by DuPont, USA) against corn borers.

Data were collected for 14 traits: anthesis-to-silking interval (ASI), plant height (PH), barren stalks (BS) percentage, leaf angle (LANG) measured as the angle between stem and blade of the leaf just above ear leaf, and chlorophyll concentration index (CCI) measured with a chlorophyll concentration meter, model CCM 200 (http://www.apogeeinstruments.co.uk/apogee-instruments-chlorophyll-content-meter-technical-information/) as the ratio of transmission between 931 nm and 653 nm through the leaf of the topmost ear. At 80 days from the sowing date, light intensity was measured and the light penetrating the canopy was calculated for each genotype using a Lux-meter equipment Model ACM-DLM-2365, manufactured by ACMAS Technocracy PVT, LTD, India. The light intensity in lux was measured at 12 AM (noon) at the top of the plant and at the base of the topmost ear. Light penetrating the canopy 80 days from sowing (PL-M80) was measured as a percentage of light penetrating from the top of the plant to the base of the topmost ear, as follows: 100 (light intensity at the base of the topmost ear / light intensity at the top of the plant). At harvest, number of ears per plant (EPP), number of kernels per plant (KPP), 100-kernel weight (100-KW), grain yield per plant (GYPP), grain yield per hectare (GYPH), total above-ground dry matter per plant (TDM), harvest index (HI), and

Table 3 – Soil analysis at 0–30 cm depth in the experimental field at Giza during two maize growing seasons.

Soil characteristics	2012 season	2013 season	Soil characteristics	2012 season	2013 season
Physical analysis			Available nutrients (mg kg^{-1})		
Coarse sand %	2.20	4.00	Nitrogen	37.20	34.20
Fine sand %	35.70	30.90	Phosphorus	9.23	8.86
Silt %	29.60	31.20	Potassium	223	242
Clay %	32.50	33.90	Hot water extractable B	0.53	0.49
Texture [a]	C. L.	C. L.	DTPA-extractable Zn	0.44	0.52
Soil bulk density (g cm^{-3})	1.11	1.20	DTPA-extractable Mn	0.89	0.75
Chemical analysis			DTPA-extractable Fe	3.05	3.17
pH (paste extract)	7.61	7.73			
EC (dS m^{-1})	1.87	1.91			
Calcium carbonate (%)	3.67	3.47			
Organic matter (%)	2.25	2.09			

[a] C.L. = clay loam.

economic nitrogen use efficiency (NUE_e), calculated as follows: $NUE_e = GDM / N_s$, where GDM = grain dry matter and N_s = available soil-N according to Moll et al. [27].

A combined analysis of variance of split–split plot across the two seasons was performed if the homogeneity test was nonsignificant, and LSD values were calculated to test the significance of differences between means according to Snedecor and Cochran [28] using SAS (http://www.sas.com/en_us/software/university-edition.html). Rank correlation coefficients were calculated between pairs of the nine studied environments for grain yield per hectare (GYPH). Computation was performed with SPSS 17 (http://www.ibm.com/software/analytics/spss.html) and the significance of the rank correlation coefficient was tested according to Steel et al. [29].

3. Results

3.1. Analysis of variance

The combined analysis of variance across years (Y) of the split–split plot design for the studied 23 genotypes (G) of maize (six inbreds +15 F_1s + two check commercial single-cross hybrids) under three plant densities (D) and three nitrogen (N) levels is presented in Table 4. Mean squares due to years were significant ($P \leq 0.01$) for all studied traits, except for ASI, PH, EPP, and 100-KW, indicating significant effect of climatic conditions on most studied traits (Table 2). Mean squares due to plant densities, N levels and genotypes were significant ($P \leq 0.01$) for all studied characters. Mean

Table 4 – Analysis of variance of split–split plot design for 23 maize genotypes under three levels of nitrogen (N) and three plant densities (D) combined across two years.

SOV	*df*	Mean square						
		ASI	PH	BS	LANG	CCI	PL-M80	EPP
Year (Y)	1	ns	ns	**	**	**	**	ns
Nitrogen level (N)	2	**	**	**	**	**	**	**
N × Y	2	**	**	**	ns	ns	**	ns
Error	8	0.01	308.6	0.04	6.1	53.1	27.0	0.02
Density (D)	2	**	**	**	**	**	**	**
D × Y	2	ns	**	ns	**	**	**	**
D × N	4	**	**	**	**	**	**	**
D × N × Y	4	ns	**	ns	ns	ns	**	**
Error	24	0.005	64.1	0.01	1.6	11.4	4.6	0.01
Genotype (G)	22	**	**	**	**	**	**	**
G × Y	22	ns	**	ns	**	**	**	ns
G × N	44	**	**	**	ns	**	**	**
G × N × Y	44	ns	*	**	ns	ns	**	ns
G × D	44	**	**	**	**	**	**	**
G × D × Y	44	ns	**	ns	**	ns	**	ns
G × D × N	88	**	**	**	ns	**	**	**
G × N × D × Y	88	ns	**	ns	ns	ns	**	ns
Error	792	0.0006	54.1	0.002	0.6	6.5	2.2	0.007

SOV	*df*	KPP	100-KW	GYPP	GYPH	TDM	HI	NUE_e
Year (Y)	1	**	ns	**	**	**	**	**
Nitrogen level (N)	2	**	**	**	**	**	**	**
N × Y	2	**	*	**	*	**	**	**
Error	8	31,899.8	10.6	873.4	26.8	725.4	24.1	14.9
Density (D)	2	**	**	**	**	**	**	**
D × Y	2	**	**	**	**	**	**	**
D × N	4	**	**	**	**	**	**	**
D × N × Y	4	**	ns	**	*	**	**	**
Error	24	28,090.1	2.8	252.4	6.9	175.5	7.5	8.0
Genotype (G)	22	**	**	**	**	**	**	**
G × Y	22	ns	**	**	**	**	ns	ns
G × N	44	**	**	**	**	**	**	**
G × N × Y	44	ns	ns	*	ns	ns	ns	ns
G × D	44	**	**	**	**	**	**	**
G × D × Y	44	*	ns	**	ns	ns	ns	ns
G × D × N	88	**	**	**	**	**	**	**
G × N × D × Y	88	ns	ns	**	ns	**	**	*
Error	792	3228.5	1.5	27.1	0.8	23.3	1.4	1.3

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ns, not significant.

squares due to the first-order interaction, i.e., N × Y, D × Y, G × Y, D × N, G × N, and G × D were significant ($P \leq 0.01$) for all studied traits, except for chlorophyll concentration index (CCI) for N × Y, ASI, and BS for D × Y, DTS, ASI, BS, EPP, KPP, HI, and NUE_e for G × Y and LANG for G × N. Mean squares due to the second-order interactions D × N × Y and G × D × N were significant or highly significant for all studied traits, except ASI, BS, LANG, CCI; and 100-KW for D × N × Y and LANG for G × D × N.

In contrast, mean squares due to G × N × Y and G × D × Y were not significant for all studied traits, except for PH, BS, and light penetration at the topmost ear 80 days from sowing and grain yield per plant for G × N × Y and PH, LANG, PL-M80, KPP, and GYPP for G × D × Y interaction, which were significant. Mean squares due to the third-order interaction G × N × D × Y were significant ($P \leq 0.01$) for PH, PL-M80, GYPP, TDM, HI, and NUE_e.

Combined analysis of variance of a randomized complete block design was performed for 14 traits in one set of diallel crosses among contrasting maize inbreds under each of the nine environments (from E1 to E9), representing combinations of three plant densities × three N levels: E1, high nitrogen and low plant density (HN–LD); E2, high nitrogen and medium plant density (HN–MD); E3, high nitrogen and high plant density (HN–HD); E4, medium nitrogen and low plant density (MN–LD); E5, medium nitrogen and medium plant density (MN–MD); E6, medium nitrogen and high plant density (MN–HD); E7, low nitrogen and low plant density (LN–LD); E8, low nitrogen and medium plant density (LN–MD); and E9, low nitrogen and high plant density (LN–HD) across two seasons (data not presented). Mean squares due to genotypes, parents and crosses under all environments were highly significant for all studied traits, except ASI under E3, E5, E6, and E7; EPP under E8; HI under E7; and E9 and NUE_e under E9 for the inbred parents and BS under E1 through E6 for the F_1 progeny, indicating the significance of differences among the studied parents and among the F_1 diallel crosses in the majority of cases. Mean squares due to parents *vs.* F_1 progenies were highly significant for all studied traits under all nine environments, except for CCI under E6 and 100-KW under E2, E3, E4, and E6. Mean squares due to the interaction parent × year (P × Y) and cross × year (C × Y) were significant and highly significant for all studied traits under all environments, except for ASI under E3, E5, E6, E7, E8, and E9 for parents × years and E1 and E6 for cross × year, PH under E1 for cross × year, BS under E6 and E8 for P × Y, and under E1, E3, E6, and E8 for C × Y, LANG under E7 for P × Y, CCI under all environments for P × Y and E1 through E5 and E7 through E9 for C × Y, EPP under E1, E2, E4 through E7 for P × Y and E2 and E4 for C × Y, KPP under E1, E2, and E4 through E6 for P × Y, GYPP under E5 for P × Y, GYPH under E3, E5 through E8 for P × Y, TDM under E8 for P × Y and HI under E6 for P × Y and under E6 and E8 for C × Y. Mean squares due to parents *vs.* cross × year were significant and highly significant in 101 of 162 cases.

3.2. Effects of combinations of plant density and nitrogen level

The effects of nine combinations of three levels of nitrogen and three plant densities on the studied traits are presented in Table 5. The highest GYPP was obtained from E1 (a combination of highest N level and lowest plant density), which is reasonable, given that available nitrogen was at a maximum across seasons, and accordingly we inferred that this environment was the best one for GYPP. The percent change, either increasing or decreasing, in traits was then calculated relative to this environment. Both stresses (nitrogen and plant density) were exhibited by E9, E8, E6, and E5 environments, in descending order of severity, with minimum severity in E5, whereas the other environments exhibited only one stress (E2, E3, and E7) or no stress (E1 and E4). It can be observed that the severity of the low nitrogen and high density on GYPP was at a maximum (70.9% and 67.6% reduction for inbreds and hybrids, respectively) under environment E9 (LN–HD), where both severe stresses (highest plant density and lowest available nitrogen) were present. The reduction in GYPP due to the effect of both stresses in different combinations showed the descending order E9, E8, E6, and E5 (70.9%, 61.0%, 41.6%, and 32.2%, respectively, for parents and 67.6%, 59.5%, 39.6%, and 29.6%, respectively, for crosses). Significant reductions in GYPH of maize crosses observed in environments E8 and E9 relative to E1 (37.7% and 49.6%, respectively) were due to both N and density stresses. It was observed that reduction in GYPH of both inbreds and crosses was at a maximum under environment E9 (55.5% and 49.6%, respectively) owing to both stresses (highest plant density and lowest available nitrogen).

In contrast, GYPH of both inbreds and hybrids under environments E3 and E2 showed a tendency of increase over that under E1. The highest GYPH was obtained from E3 (the combination of highest density and highest N level) for inbreds and hybrids. The maximum increase (41.1% and 18.1%) in GYPH was shown by F_1 progenies under E3 (HN–HD) and E6 (MN–HD), respectively, owing to high plant density. Reductions in grain yield resulting from both stresses (elevated plant density and reduced N level) in both inbreds and hybrids were associated with reductions in all yield components (EPP, KPP, 100-KW), HI, TDM, CCI, LANG, PL-M50, and DTS. Such reductions were more pronounced in the E9 environment (maximum stresses) followed by E8, E6 and E5, in descending order. Maximum reductions were observed for kernels per plant (81.9% and 82.0%) and CCI (76.5% and 76.8%) for inbreds and hybrids, respectively, under E9, owing to severe stresses of nitrogen and plant density. In contrast, the two stresses together (shown by the four environments E9, E8, E6, and E5) caused increases in BS, ASI, and NUE_e.

Rank correlation coefficients for GYPH estimated for pairs of the nine environments are presented in Table 6. In general, the magnitude and number of significant correlation coefficients for GYPH were much higher in inbreds than in hybrids. In both inbreds and hybrids, environment E7 (low nitrogen and low plant density) and environment E9 (low nitrogen and high plant density) showed no correlation with any other environment for GYPH. The environment E8 was correlated (0.94^{**}) with E9 for GYPH; these two environments were the most stressful. The maximum number of significant correlations (4) in F_1 progenies was found between E4 and each of E1, E2, E5, and E6 (Table 6).

3.3. Genotype × nitrogen × plant density interaction

Mean grain yields per hectare across years under nine combinations of N and D levels for each inbred, hybrid, and

Table 5 – Means of studied traits for nitrogen level × plant density interaction across nine environmental conditions combined across two seasons.

Parameter	E1	E2	E3	E4	E5	E6	E7	E8	E9
	HN–LD	HN–MD	HN–HD	MN–LD	MN–MD	MN–HD	LN–LD	LN–MD	LN–HD
	Anthesis-to-silking interval (ASI) (day)								
Parents	2.3	2.3	2.7	2.2	2.6	2.8	5.1	6.6	8.8
Crosses	1.6	1.4	1.5	1.5	1.7	1.4	4.1	5.0	6.6
$LSD_{0.05}$	N = 0.01, D = 0.01, G = 0.01, N × D = 0.01								
	Plant height (PH) (cm)								
Parents	195.4	200.8	212.4	196.9	204.0	204.7	177.6	178.8	189.9
Crosses	219.9	226.6	242.3	228.9	233.8	255.7	200.9	206.2	218.3
$LSD_{0.05}$	N = 2.65, D = 1.08, G = 2.77, N × D = 1.87								
	Barren stalks (BS) (%)								
Parents	4.3	5.8	9.6	9.3	13.4	13.0	30.4	40.5	43.3
Crosses	0.1	0.0	0.1	0.3	0.4	0.6	16.5	18.9	22.1
$LSD_{0.05}$	N = 0.03, D = 0.02, G = 0.02, N × D = 0.03								
	Leaf angle (LANG) (°)								
Parents	31.3	30.0	28.8	31.1	28.9	27.7	28.8	27.3	26.9
Crosses	35.6	31.2	30.6	34.8	31.0	29.9	32.3	29.1	28.4
$LSD_{0.05}$	N = 0.12, D = 0.33, G = 0.48, N × D = 0.57								
	Chlorophyll concentration index (CCI) (%)								
Parents	56.4	52.0	58.0	57.4	45.0	48.4	28.9	19.5	13.3
Crosses	64.6	62.9	60.5	61.9	57.9	47.8	33.7	21.8	15.0
$LSD_{0.05}$	N = 0.39, D = 0.98, G = 1.28, N × D = 1.70								
	Penetrated light at the base of the topmost ear at 80 day (PL-M80) (%)								
Parents	11.2	10.3	8.7	12.5	11.5	9.9	14.5	12.4	11.2
Crosses	9.5	8.1	6.8	10.9	9.4	7.8	13.7	11.0	9.0
$LSD_{0.05}$	N = 0.32, D = 0.70, G = 0.56, N × D = 1.21								
	Number of ears per plant (EPP)								
Parents	1.2	1.2	1.0	1.1	0.9	0.9	0.9	0.8	0.6
Crosses	1.4	1.3	1.1	1.3	1.1	1.0	1.0	0.9	0.7
$LSD_{0.05}$	N = 0.02, D = 0.02, G = 0.03, N × D = 0.03								
	Number of kernels per plant (KPP)								
Parents	924.1	859.9	680.2	679.5	505.0	479.0	326.3	246.8	167.5
Crosses	1103.3	908.2	742.5	787.2	620.8	518.6	370.7	299.5	198.3
$LSD_{0.05}$	N = 26.97, D = 22.57, G = 21.43, N × D = 39.09								
	100-kernel weight (100-KW) (g)								
Parents	40.2	36.1	33.7	35.8	32.0	29.7	27.1	25.5	21.6
Crosses	39.4	36.9	33.8	35.5	32.9	29.8	27.8	28.7	25.3
$LSD_{0.05}$	N = 0.49, D = 0.23, G = 0.46, N × D = 0.39								
	Grain yield (GYPP) (g $plant^{-1}$)								
Parents	163.8	124.3	110.2	144.6	111.0	95.6	87.8	63.9	47.7
Crosses	224.5	175.4	158.1	199.4	150.1	135.6	119.5	90.0	72.8
$LSD_{0.05}$	N = 4.46, D = 2.14, G = 1.96, N × D = 3.71								
	Grain yield (GYPH) (t ha^{-1})								
Parents	7.2	7.5	8.2	6.3	6.8	7.2	4.0	4.2	3.2
Crosses	10.0	11.5	14.1	8.9	9.9	11.8	5.4	6.2	5.0
$LSD_{0.05}$	N = 0.78, D = 0.35, G = 0.34, N × D = 0.61								
	Total above ground dry matter (TDM) (g $plant^{-1}$)								
Parents	322.3	277.5	256.7	292.5	255.9	236.1	211.3	179.5	151.4
Crosses	391.9	338.3	312.7	360.9	307.7	283.9	256.6	217.7	189.5
$LSD_{0.05}$	N = 4.07, D = 1.78, G = 1.82, N × D = 3.09								
	Harvest index (HI) (%)								
Parents	42.5	37.4	35.9	41.3	36.1	33.7	35.0	29.9	26.5
Crosses	48.0	43.4	42.2	46.3	40.9	39.8	39.0	34.7	32.2
$LSD_{0.05}$	N = 0.74, D = 0.37, G = 0.44, N × D = 0.64								

(continued on next page)

Table 5 (*continued*)

Parameter	E1	E2	E3	E4	E5	E6	E7	E8	E9
	HN–LD	HN–MD	HN–HD	MN–LD	MN–MD	MN–HD	LN–LD	LN–MD	LN–HD
	Economic nitrogen use efficiency (NUE_e) (g g^{-1})								
Parents	8.9	10.1	12.0	12.8	14.7	16.8	20.8	22.6	22.5
Crosses	12.2	14.3	17.2	17.6	19.9	23.9	28.2	31.9	34.4
$LSD_{0.05}$	N = 0.58, D = 0.38, G = 0.44, N × D = 0.66								

H: high; M: medium; L: low; N: nitrogen; D: density.

check are presented in Table 5. The rank of the inbred parents for GYPH was approximately similar in all nine environments, indicating little effect of interaction between inbred, nitrogen level, and plant density on GYPH. The highest GYPH was obtained from E3 (HN–HD) for the first group of inbreds (L17, L18, and L53) and E1 (HN–LD) followed by E2 (HN–MD) for the second group (L29, L54, and L55). With respect to GYPH of the F_1 crosses, the rank varied from one environment (combination of N level with plant density) to another, especially between environments that combined two stresses with those with only one or no stress, indicating the presence of cross × nitrogen × density interaction and indicating that the GYPH of a cross differed from one combination (of N level with plant density) to another. The highest GYPH in this experiment was obtained under E3 (high N, high D) and the highest yielding crosses in this environment were L17 × L54 (19.90 t ha^{-1}), L17 × L18 (19.56 t ha^{-1}), L53 × L54 (18.36 t ha^{-1}), L53 × L55 (17.89 t ha^{-1}), and L29 × L55 (17.33 t ha^{-1}), with significant superiority over SC 10 (the best check under this environment) by 26.9%, 23.3%, 15.8%, 12.8%, and 9.2%, respectively.

The optimum combination of plant density and N level (giving the highest GYPH) in this study was identified for each genotype (Table 7). It differed between inbreds, hybrids, and checks. The optimum environment in this study was E3 (HN–HD) followed by E2 (HN–MD) for the three inbreds L17, L18, and L53, the crosses L18 × L53, L18 × L29, L18 × L55, L29 × L55, and L54 × L55, and the check cultivar SC 10. For the remaining inbreds (L29, L54, and L55), the optimum combination of density and N level was E1 (HN–LD) followed by E2. For crosses L17 × L18, L17 × L53, L17 × L29, L17 × L54, L17 × L55, L53 × L29, L53 × L54, L53 × L55, and L29 × L54 and the check cultivar SC 2066, the optimum combination was E3 (HN–HD) followed by E6 (MN–HD) and for cross L18 × L54 the optimum density was E6 (MN–HD) followed by E3 (HN–HD).

3.4. *Superiority of tolerant (T) to sensitive (S) genotypes*

The higher absolute GYPH and lower proportion of reduction in GYPH under high D combined with low N to yield under low-D combined with high-N were considered as an index of tolerance of the combined stresses. Based on this index, the tolerant inbreds were L17, L18, and L53, while the sensitive inbreds were L29, L54, and L55. The F_1 progenies L18 × L53, L18 × L55, and L18 × L29 were thus considered tolerant and L53 × L54, L17 × L29, and L17 × L18 were considered sensitive crosses. Data averaged for each of the two groups (T and S) for inbreds and hybrids differing in tolerance to both stresses together indicate that GYPH of tolerant (T) was greater than that of the sensitive (S) inbreds and crosses by 17.1% and 36.3%, respectively, under high D combined with low N (no N addition) conditions (Table 8).

Superiority of high-D–low-N tolerant (T) to sensitive (S) inbreds in GYPH under high-D–low-N was associated with superiority in most studied traits, namely GYPP (10.5%), EPP (14.3%), KPP (39.9%), 100-KW (9.0%), HI (2.7%), NUE_e (10.0%), BS (−11.2%), PH (−9.3%), and ASI (−5.8%). Superiority of T to S crosses in GYPH under low-N was due to their superiority in GYPP (28.3%), EPP (23.4%), KPP (8.0%), 100-KW (14.1%), HI (11.6%), NUE_e (32.1%), BS (−62.8%), and ASI (−21.7%). The superiority of T to S under low-N for crosses was greater than that for inbreds.

Table 6 – Rank pairwise correlation coefficients among nine environments for GYPH of parental inbreds (above diagonal) and F_1 progenies (below diagonal) across two seasons.

Environment	E1	E2	E3	E4	E5	E6	E7	E8	E9
	HN–LD	HN–MD	HN–HD	MN–LD	MN–MD	MN–HD	LN–LD	LN–MD	LN–HD
E1		0.94**	0.83*	0.77*	0.77*	0.71*	−0.14	0.94**	1.00**
E2	0.39		0.94**	0.83*	0.71*	0.77*	0.03	1.00**	0.94**
E3	−0.11	0.44*		0.94**	0.60*	0.71*	0.14	0.94**	0.83*
E4	0.56*	0.55*	−0.08		0.54*	0.60*	0.09	0.83*	0.77*
E5	0.27	0.36	−0.14	0.55*		0.94**	0.09	0.71*	0.77*
E6	−0.06	0.06	0.45*	0.39*	0.23		0.26	0.77*	0.71*
E7	−0.01	−0.28	−0.10	0.10	−0.26	0.23		0.03	−0.14
E8	0.22	0.19	−0.47*	0.38	0.49*	−0.20	−0.09		0.94**
E9	−0.17	0.01	−0.01	0.33	−0.09	0.16	−0.25	0.04	

H: high; M: medium; L: low; N: nitrogen; D: density.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

Table 7 – Mean grain yield per hectare (t) under nine environmental conditions.

Genotype	E1	E2	E3	E4	E5	E6	E7	E8	E9
	HN–LD	HN–MD	HN–HD	MN–LD	MN–MD	MN–HD	LN–LD	LN–MD	LN–HD
Parents									
L17	8.96	9.23	10.93	8.13	9.13	9.86	3.87	4.03	3.47
L18	8.50	9.83	11.56	7.53	8.30	10.16	4.17	4.50	3.40
L53	9.00	10.66	12.06	7.93	9.80	10.63	4.30	5.70	3.53
L29	5.43	5.00	4.60	4.63	4.36	3.83	3.60	3.57	2.93
L54	6.23	5.76	5.33	4.80	4.70	4.33	4.23	3.93	3.17
L55	5.23	4.80	4.43	4.53	4.43	4.26	3.87	3.50	2.80
Crosses									
L17 × L18	11.56	13.49	19.56	10.23	11.76	15.23	5.56	6.93	3.93
L17 × L53	8.86	10.10	11.83	7.93	8.70	10.33	5.93	6.93	5.60
L17 × L29	8.00	9.33	10.66	7.50	8.43	9.80	5.23	7.00	3.63
L17 × L54	13.73	17.60	19.90	12.59	13.33	17.63	5.36	5.83	4.86
L17 × L55	8.23	9.16	10.30	7.96	8.40	9.46	4.66	5.80	4.73
L18 × L53	10.76	12.16	14.06	8.20	8.43	9.70	6.43	7.66	7.10
L18 × L29	8.16	10.50	11.96	7.73	8.70	9.86	5.46	6.66	6.10
L18 × L54	9.73	8.83	10.40	8.30	7.76	10.66	6.30	6.23	5.06
L18 × L55	10.86	12.46	15.29	8.13	9.53	11.50	5.36	6.30	6.50
L53 × L29	8.86	10.96	12.56	8.70	10.06	11.63	6.56	6.36	5.86
L53 × L54	12.03	13.96	18.36	10.60	12.66	14.76	3.83	3.30	3.20
L53 × L55	10.56	12.83	17.89	9.53	11.43	14.43	3.30	5.50	3.63
L29 × L54	8.00	8.70	10.26	7.40	8.13	8.70	6.23	6.33	5.83
L29 × L55	12.36	14.99	17.33	11.60	12.96	14.86	5.63	5.50	4.76
L54 × L55	8.20	9.33	10.96	6.73	8.20	8.43	5.13	7.10	4.80
Checks									
SC 10	11.83	13.59	15.86	9.16	10.96	12.89	7.03	7.73	3.83
SC 2066	11.80	12.43	13.43	12.00	12.40	12.93	5.83	7.16	6.90
$LSD_{0.05}$	G = 0.11, G × D × N = 0.34								

3.5. *Differential response of T × T, T × S, and S × S crosses*

Mean performance of traits were averaged across three groups of F_1 crosses, i.e., T × T, T × S, and S × S groups based on grain yield per hectare of their parental lines under stress and non-stress conditions—that is, both high-D and low-N stresses together—and are presented in Table 9. The numbers of crosses were three, nine and three for the T × T, T × S, and S × S groups, respectively. In general, T × T crosses had more favorable (higher) values for grain yield and its attributes and lower (more favorable) values for DTS, ASI, BS and LANG than S × S and T × S crosses under each stress and both stresses. In general, low-N and high density T × T crosses were the most superior for all studied traits (Table 9), under the most severe environment (E9) where both severe stresses (low-N and density of 40,000 plants ha^{-1}) were present. The T × S crosses for both stresses ranked second for superiority in ASI, PH, PL-M80, EPP, KPP, and 100-KW and the S × S crosses for both stresses ranked second for superiority in the remaining traits (BS, LANG, 100-KW, GYPP, GYPH, TDM, HI, and NUE_e).

Under low-N and high-D stresses together (E9), grain yield per hectare of low-N and high-D T × T crosses (5.53 t) was

Table 8 – Superiority (%) in selected traits of the most three tolerant (T) to the most three sensitive (S) inbreds and crosses to high-D–low-N under high-D–low-N combined across two seasons.

Trait	Inbreds			Crosses		
	T	S	% Superiority	T	S	% Superiority
GYPH (t ha^{-1})	4.1	3.5	17.1	6.5	4.7	36.3
GYPP (g)	69.8	63.2	10.5	107.4	83.7	28.3
EPP	0.80	0.70	14.3	0.95	0.77	23.4
KPP	287.9	205.8	39.9	299.8	277.7	8.0
100-KW (g)	25.8	23.7	9.0	29.7	26.0	14.1
TDM (g)	187.2	174.3	7.4	242.5	207.0	17.1
HI (%)	30.9	30.1	2.7	37.1	33.2	11.6
NUE_e (g g^{-1})	23.0	20.9	10.0	36.4	27.6	32.1
BS (%)	35.8	40.3	−11.2	10.8	28.9	−62.8
PH (cm)	173.2	190.9	−9.3	206.9	202.7	2.1
ASI (day)	6.6	7.1	−5.8	4.4	5.6	−21.7

% Superiority = 100 × [(T − S) / S].

Table 9 – Trait differences averaged across the T × T, T × S, and S × S groups of F_1 progenies for both stresses under the low nitrogen–high plant density environment (E9) across two seasons.

Trait	T × T	T × S	S × S	Trait	T × T	T × S	S × S
ASI (day)	5.2	6.7	7.6	KPP	245.6	190.2	75.5
PH (cm)	211.1	219.4	21.9	100-KW (g)	26.6	25.0	25.0
BS (%)	13.1	25.8	19.8	GYPP (g)	81.8	70.1	72.2
LANG (°)	27.6	28.9	27.7	GYPH (t ha^{-1})	5.5	4.8	5.1
CCI (%)	11.2	16.6	13.8	TDM (g)	203.3	184.8	190.0
PL-M80 (%)	9.8	8.9	8.6	HI (%)	33.7	31.7	32.0
EPP	0.8	0.7	0.7	NUE_e (g g^{-1})	38.7	33.1	34.1

T: tolerant; S: sensitive.

greater than that of S × S (5.13 t) and T × S (4.8 t) by 7.79% and 14.48%, respectively. This finding indicates that to obtain a cross tolerant of both stresses at the same time, the two parental inbred lines should be tolerant of the same stresses. The superiority of low-N and high-D T × T to S × S and T × S crosses in GYPH under low-N and high-D stresses was due to their superiority in GYPP by 9.5 and 11.7 g, KPP by 70.4 and 55.4, 100-KW by 1.6 and 1.6 g, EPP by 0.1 and 0.1, TDM by 13.3 and 18.5 g $plant^{-1}$, HI by 1.7 and 2.0%, NUE_e by 4.6 and 5.6 g g^{-1}, and PL-M80 by 1.2% and 0.9%, respectively. Moreover, low-N and high-D T × T crosses were earlier in DTS by 4.7 and 1.9 days, had ASI shorter by 2.1 and 1.5 days, PH shorter by 10.8 and 8.3 cm, BS lower by 6.7% and 12.7%, and LANG narrower by 0.1° and 1.3° than S × S and T × S crosses, respectively, under the most severe stresses in this experiment, which were present in the E9 environment.

3.6. *Grouping genotypes based on tolerance and responsiveness*

The mean grain yield per plant or per hectare across years of the studied crosses under low-N–high-D together was plotted against those of the same trait of the same genotypes under high-N and low-D together (Figs. 1 and 2) where numbers from 1 to 15 refer to F_1 hybrid names: 1, L17 × L18; 2, L17 × L53; 3, L17 × L29; 4, L17 × L54; 5, L17 × L55; 6, L18 × L53; 7, L18 × L29; 8, L18 × L54; 9, L18 × L55; 10, L53 × L29; 11, L53 × L54; 12, L53 × L55; 13, L29 × L54; 14, L29 × L55; and 15, L54 × L55; making it possible to distinguish between efficient and inefficient genotypes on the basis of above-average and below-average grain yield under low-N and high-D together and responsive and non-responsive genotypes on the basis of above-average and below-average grain yield under high-N and low-D together. According to tolerance to both stresses (high density and low nitrogen together and responsiveness to high nitrogen and low-density conditions), the 15 studied crosses were classified into four groups: efficient (tolerant) and responsive, efficient (tolerant) and nonresponsive, inefficient (sensitive) and responsive, and inefficient (sensitive) and nonresponsive. Based on grain yield per plant (Fig. 1) or per hectare (Fig. 2), the two cross numbers 6 (L18 × L53) and 9 (L18 × L55) had the highest GYPP or GYPH under high-N low-D (E1) and low-N high-D (E9), and could thus be considered tolerant (efficient) to both stresses and responsive to the non-stressed environment.

The five cross numbers 4 (L17 × L54), 14 (L29 × L55), 1 (L17 × L18), 12 (L53 × L55), and 11 (L53 × L54) were assigned as inefficient (sensitive) but responsive based on GYPP and GYPH. The group of efficient (tolerant to both stresses but not responsive) crosses included crosses 7 (L18 × L29), 2 (L17 × L53), 10 (L53 × L29), and 13 (L29 × L54) based on both GYPP and GYPH but included one more cross, 8 (L18 × L54) based on GYPH alone.

In contrast, the group of inefficient (sensitive to both stresses) and nonresponsive to high-N and low-D included

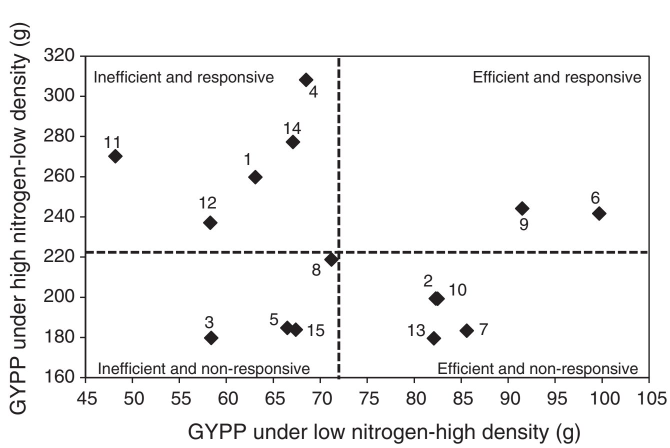

Fig. 1 – Relationships among grain yields per plant (GYPP) of 15 F_1 maize hybrids under high nitrogen–low density and low nitrogen–high density combined across two seasons. Broken lines represent means of GYPP (numbers from 1 to 15 refer to F_1 hybrids in Table 7).

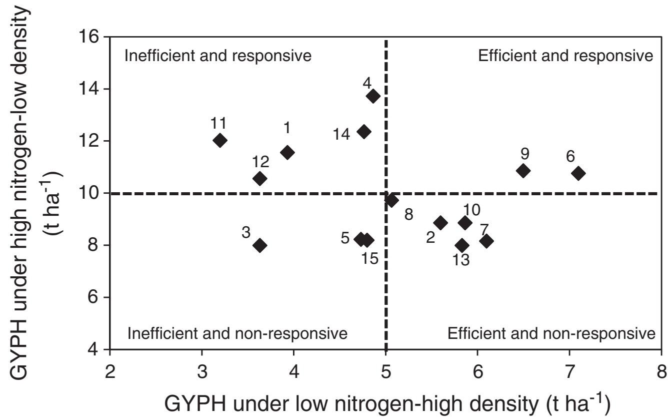

Fig. 2 – Relationships between grain yield per hectare (GYPH) of 15 F_1 maize hybrids under high nitrogen–low density and low nitrogen–high density combined across two seasons. Broken lines represent mean of GYPH (numbers from 1 to 15 refer to F_1 hybrids in Table 7).

crosses 3 (L17 × L29), 5 (L17 × L55), and 15 (L54 × L55) based on both GYPP (Fig. 1) and GYPH, but included one more cross, 8 (L18 × L54) based on GYPH alone (Fig. 2).

3.7. *Identifying appropriate density and/or adequate N application*

Data were reanalyzed to evaluate GYPH responses of inbreds and hybrids across varying levels of stress. For each genotype or group of genotypes, a quadratic regression was fitted for N rate × plant density interaction. The regression functions were used to identify the treatments showing optimum value for each genotype (or group of genotypes). The relationships between the nine environments (combinations of three N levels and three plant densities) and grain yield per hectare ha across seasons are illustrated in Fig. 3 for inbreds and Fig. 4 for F_1 crosses. The nine environments are arranged in Figs. 3 and 4 based on the severity of both N and plant density stresses together, where the poorest environment (E9) represents maximum stress (lowest N and highest plant density), while the best environment (E1) represents the nonstress one (highest N and lowest plant density). The three inbred parents (L17, L18, and L53) showed a quadratic relationship, with the highest GYPH at a density of 95,200 plants ha^{-1} plant density combined with N rate of 570 kg N ha^{-1}. In contrast, the inbreds L54, L29, and L55 showed a weak quadratic relationship, very close to a linear response (Fig. 3), with the highest GYPH at a density of 47,600 plants ha^{-1} and N rate of 570 kg N ha^{-1}. The grain yield ha^{-1} across years of all groups of F_1 progenies showed a quadratic relationship under the nine combinations of plant densities and N levels (Fig. 4), except crosses of E–R group, which showed a near-linear relationship. The highest GYPH was achieved under a combination of 95,200 plants ha^{-1} and a fertilization rate of 570 kg N ha^{-1} across the four groups of F_1 crosses. The group of hybrids most responsive to the improvement of environmental conditions was the E–R group and the least responsive group was I–NR.

4. Discussion

To increase maize grain yield per unit area in Egypt, breeding programs should be directed towards the development of inbreds and hybrids with traits adapted to high plant density

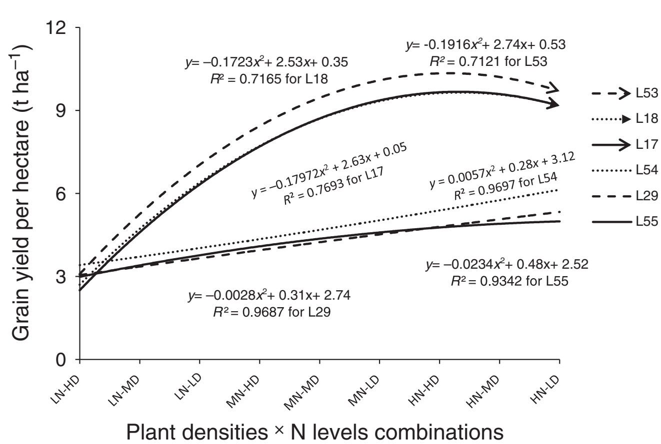

Fig. 3 – Relationship between GYPH of inbreds and nine environment combinations between three plant densities and three N levels across two seasons.

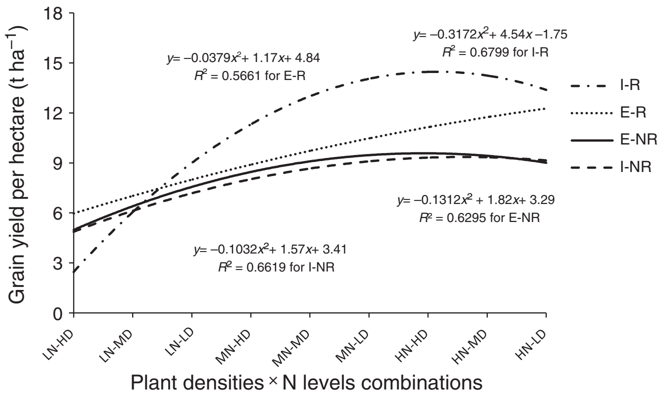

Fig. 4 – Relationship between GYPH of four groups of F_1 crosses: five inefficient and responsive (I–R), two efficient and responsive (E–R), four efficient and non-responsive (E–NR) and four inefficient and non-responsive (I–NR) crosses and nine combinations between three plant densities and three N levels.

tolerance. Although high plant density results in interplant competition (especially for light, water, and nutrients), which affects vegetative and reproductive growth of maize [5,10], the use of hybrids tolerant of high density and improvement of fertilization management practices would overcome the negative impacts of such competition and lead to maximizing maize productivity per unit area [23]. As an alternative breeding strategy, tolerance to high plant population density has been suggested to improve performance under diverse abiotic stresses including drought and low N [30].

In the present study, analysis of variance indicated that each of the three main factors, plant density, N level, and genotype, has a marked effect on all studied traits. In that context, the ranks of maize genotype differ from one nitrogen level to another, from one density to another, and from one year to another. Selection for improved performance under a specific combination of soil nitrogen and plant density is possible, as proposed by several investigators [31–33]. Significance of mean squares due to parents *vs.* F_1 progenies and those due to parents *vs.* cross × year indicated the presence of heterosis and indicated that heterosis differed from season to season for most studied characters.

The highest GYPP for all genotypes was achieved in environment E1 (a combination of highest N level and lowest plant density). This result may be attributed to the high CCI, which promotes photosynthesis and high TDM, an indicator of high N absorption by plants (Table 5). However, the highest GYPH was obtained from E3 (a combination of highest density and highest N level) for the inbreds and hybrids. This result could be attributed to the highest values of CCI and TDM and the lowest values of ASI and BS (Table 5). However the economic application of fertilizer should be taken into consideration and awaits a separate study.

Reductions in grain yield resulting from both stresses (elevated plant density and reduced N level) incurred by the four environments E9, E8, E6, and E5 in both inbreds and hybrids were associated with reductions in all yield components (EPP, KPP, 100-KW), harvest index, TDM, CCI, LANG, PL-M50, and DTS. However, the two stresses together caused increases in BS, ASI (unfavorable), and NUE_e (favorable). Maximum increases appeared under E9 followed by E8 environment and by BS trait (Table 5). It is noteworthy that plant height of both parents and crosses showed a tendency to increase under the E5 and E6 environments, but to decrease under E8 and E9. The PH increase under E5 and E6 may be attributed to elevated levels of plant density, whereas the reduction under E8 and E9 may be due to the severe N stress.

Correlation analysis among environments indicated that the interaction of inbreds with different environments (combinations of three plant densities × three N-levels) was much lower than that of F_1 crosses. The crosses thus have higher sensitivity to differences between environments than the inbreds, because heterozygotes are more responsive to improved environments than homozygotes, expressed in grain yield per hectare. This conclusion agrees with those of Rodrigues et al. [34] and Monneveux et al. [35].

The percent reduction in GYPH due to both stresses, relative to E3 (HN–HD), which gave the highest GYPH, was smaller in the low-performing lines (L29, L54, and L55) than in the high-performing ones (L17, L18, and L53), a finding that could be attributed to the lower yield potential of the first than the second group of lines, under favorable environmental conditions. The first group of lines was accordingly considered tolerant (T) to both stresses expressed in GYPH, while the second one was considered sensitive (S).

Some hybrids in this experiment showed significant superiority to the best check in the respective environment (one cross under E9, five crosses under E6, and two crosses under E5). These superiorities reached 36.65% over SC 2066 under E6 for the cross L17 × L54 (the best cross in this experiment). It is noteworthy that the five crosses (L17 × L54, L17 × L18, L53 × L54, L53 × L 55, and L29 × L55) were considered the most responsive, while other crosses (L18 × L53, L18 × L55, L18 × L29, L53 × L29, and L29 × L54) were considered the most tolerant to both stresses (low N combined with high density).

Superiority of T to S inbreds and crosses may be attributed to the high nitrogen use efficiency traits of the hybrids, due to heterosis, relative to their inbred parents. These results are in agreement with those reported by several investigators [36–39]. The superiority of modern maize hybrids tolerant of high plant density has also been attributed to decreased barrenness [40], more leaf erectness [7], synchronization of 50% anthesis with 50% silking [41] and increased prolificacy (more ears per plant) [42]. A shortened ASI is considered an indication of higher flow of assimilates to the developing ears during the early reproductive stage under conditions of high density stress [43,44]. High plant density-tolerant genotypes display shorter ASI than intolerant ones [45–47]. Al-Naggar et al. [33] also reported that under high plant density, tolerant testcrosses showed 314.4% more GYPP, 115.0% more KPP, 48.4% heavier 100-KW, 42.9% more EPP, 98.2% less BS and 63.3% shorter ASI than sensitive testcrosses. Mansfield and Mumm [48] reported that in U.S. maize germplasm evaluated for plant density tolerance, a subset of traits including leaf angle, upper stem diameter, leaf area required to produce a gram of grain, kernel rows per ear, days to canopy closure, barrenness, kernels plant^{-1}, kernel length, leaf number, upper leaf area, staygreen, zipper effect, kernels per row, and anthesis-to-silking interval were associated with grain yield across plant densities ranging from 47,000 to 133,000 plants ha^{-1}.

CIMMYT breeders found that maize grain yield under low N was closely related to some secondary traits such as improved N-uptake, high plant nitrate content, large leaf area, high specific leaf-N content, ears plant^{-1}, ASI and leaf senescence [19,44,49]. These results are consistent with those reported by Al-Naggar et al. [32]. Reduction in barren stalks and shortening in ASI of tolerant as compared to sensitive inbreds and hybrids in the present study are desirable and may be considered as important contributors to low-N as well as to high-density tolerance. Similar conclusions have been reported by several investigators [33,43–48,50].

In general, low-N and high density T × T crosses were the most superior for all studied traits (Table 9), under the most severe environment (E9) where both severe stresses (low N and density of 40,000 plants ha^{-1}) were present. The T × S crosses for both stresses ranked second for superiority in ASI, PH, PL-M80, EPP, KPP, and 100-KW and the S × S crosses for both stresses ranked second for superiority in the remaining traits (BS, LANG, 100-KW, GYPP, GYPH, TDM, HI, and NUE_e). In general, crosses classified as low-N and high-density tolerant × low-N and high-density tolerant crosses in terms of grain yield under low-N and high-D stresses showed better nitrogen use efficiency traits and high density-adaptation traits such as lower values of DTS, ASI, PH, BS, and LANG than low N- and high density-sensitive × low N- and high density-sensitive crosses.

Based on the grouping of genotypes proposed by Sattelmacher et al. [51], the 15 crosses in this study could be classified into four groups: efficient (tolerant) and responsive, efficient (tolerant) and nonresponsive, inefficient (sensitive), and responsive and inefficient (sensitive) and nonresponsive. Based on grain yield per plant (Fig. 1) or per hectare (Fig. 2), the two crosses L18 × L53 and L18 × L55 had the highest GYPP or GYPH under high-N low-D (E1) and low-N high-D (E9), and could thus be considered tolerant (efficient) to both stresses and responsive to the nonstress environment.

The relationships between the nine environments and grain yield per hectare (GYPH) showed near linearity for inbreds L54, L29, and L55 and hybrids L18 × L53 and L18 × L55, with the highest GYPH at a density of 47,600 plants ha^{-1} and N rate of 570 kg N ha^{-1} and curvilinearity for inbreds L17, L18, and L53 and the rest of the hybrids with the highest GYPH at a density of 95,200 plants ha^{-1} combined with an N rate of 570 kg N ha^{-1}. The cross L17 × L54 showed the highest grain yield in this study under both high N- high-D (19.9 t ha^{-1}) and medium N–high-D environments (17.6 t ha^{-1}).

In this context, Shapiro and Wortmann [52] reported that corn grain yield typically exhibits a quadratic response to plant density with a near-linear increase across a range of low densities, a gradually decreasing rate of yield increase relative to density increase, and finally a yield plateau at relatively high plant density. Clark [23] reported little yield response to N rates above 90 kg N ha^{-1} at low and high densities, as there was a curvilinear increase until a yield plateau at low density (8.1 Mg ha^{-1} at 133.0 kg N ha^{-1}) and high density (5.9 Mg ha^{-1} at 102.0 kg N ha^{-1}). He added that response to N was greatest at the middle density (83,980 plants ha^{-1}), as there was a quadratic response with maximum yield at 188.0 kg N ha^{-1} (8.7 Mg ha^{-1}). He found that across the low-stress environments, the lowest density (44,460 plants ha^{-1}) responded little to N rates above 90 kg N ha^{-1}, whereas there was greater response to N rates at the middle density (13.5 Mg ha^{-1} at 162.0 kg N ha^{-1}) and the high density (13.4 Mg ha^{-1} at 174.0 kg N ha^{-1}). He found no support for the idea that increasing corn yield requires increases in both plant density and N rate above rates typically used. In a recent Indiana study, Boomsma et al. [26] showed that under large ranges of plant density (54,000–104,000 plants ha^{-1}) and N rate (0–330 kg N ha^{-1}), higher densities required more N. This finding seems reasonable, given the prevailing belief that high yields require more plants and that more plants require more N. These and our results advance our understanding of N rate–plant density interaction under contrasting environmental conditions, but understanding the complexities of hybrid interactions with N rate and plant density will require additional work.

5. Conclusion

Some newly developed maize genotypes could double maize productivity, reaching 19.9 t ha^{-1} in the cross L17 × L54 on the same land unit area, if they are grown at twice the plant population density of 95,200 plants ha^{-1} used in Egypt, but provided they are given the highest N fertilization tested in this experiment (570 kg N ha^{-1}). Fortunately, the same cross also gave the highest grain yield (17.6 t ha^{-1}) under medium N (285 kg N ha^{-1}) and high plant density (95,200 plants ha^{-1}). A cost-return analysis for extra yield and extra N for HN–HD *vs.* MN–HD of this cross, based on Egyptian market prices, revealed that the additional cost of extra unsubsidized N (284 kg N ha^{-1}) was 258.8 U.S. dollars and the return due to grain yield increase (2.3 t ha^{-1}) was 638.9 U.S. dollars, with a profit of ca. 380 U.S. dollars ha^{-1} that deserves to be considered. In this study, the best combination of plant population density and N level for giving the highest grain yield per unit land area was identified for the studied maize genotypes. The best combination in the present study was high N (570 kg N ha^{-1}) × high density (95,200 plants ha^{-1}) for three of six inbreds and 14 of 15 F_1 crosses, whereas it was high N (570 kg N ha^{-1}) × low density (47,600 plants ha^{-1}) for the remaining three inbreds and medium

N (285 kg N ha^{-1}) × high density (95,200 plants ha^{-1}) for the remaining cross (L18 × L54).

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