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Plant Density Tolerance of 23 Inbred Lines of Maize (*Zea mays* L.) and Their 69 Testcrosses

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMMAN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AMMAN, RS, MSH and TAE supervised the study and managed the literature searches. Authors AMAM and ASMY managed the experimental process and performed data analyses. All authors read and approved the final manuscript.

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ABSTRACT

Maximum yield per unit area may be obtained by growing maize hybrids that can withstand high plant density. Maize genotypes differ in plant density tolerance (PDT). The objectives of the present investigation were to identify the density tolerant genotypes, to estimate the superiority of tolerant (T) over sensitive (S) inbreds and testcrosses and to identify the trait(s) of strongest association with PDT. Ninety-six testcrosses were produced between 23 inbreds and three testers. All genotypes were evaluated under low (LD), medium (MD) and high (HD) density (47,600, 71,400 and 95,200 plants/ha, respectively). The highest stress tolerance index (STI) under HD and MD was exhibited by the inbred lines L21, IL15, IL53, Inb176, IL80, L28, IL151 and L14 and the testcrosses IL51 × Giza2 , IL51 × SC10, L14 × SC10, L28 × Sd7, IL53 × SC10 and L28 × SC10, in descending order. Grain yield/ha (GYPH) of density tolerant (T) was greater than the sensitive (S) inbreds and

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testcrosses by 100.6 and 89.3%, respectively under HD. Superiority in GYPH was associated with superiority in all yield components, earliness in anthesis, shortening of anthesis-silking interval and plant height, thickness of lower and upper stem diameter, decrease in leaf angle and leaf area to produce 1g grain, increase in penetrated light to ear and in chlorophyll concentration index than the sensitive ones. The study concluded that to have a density tolerant cross, both parents should be tolerant.

Keywords: Density tolerance index; penetrated light; chlorophyll concentration.

1. INTRODUCTION

One of the potential methods to maximize total production of maize (*Zea mays* L.) in Egypt is through raising productivity per land unit area [1]. Grain yield per land unit area is the product of grain yield per plant and number of plants per unit area [2,3]. Maximum yield per unit area may be obtained by growing maize hybrids that can withstand high plant density up to 100,000 plants ha^{-1} [4]. There is a lack of information in Egypt on utilization of high density tolerant maize hybrids to increase crop yield from land unit area. Modern maize hybrids in North America and Europe are tolerant to high density stress because of decreased lodging and decreased barrenness [5]. Radenovic et al. [6] found that erect leaves of maize genotypes are very desirable when increasing the plant density due to better light interception. On the other hand, a negative association was reported between anthesis-silking interval (ASI) and yield under high plant population density [7]. Moreover, prolific genotypes tended to produce fewer barren plants at higher plant densities than non-prolific ones [8].

Maize genotypes differ in tolerance to high plant density [9-11]. Maize grain yield is more affected by variations in plant density than other members of the grass family due to its monoecious floral organization, its low tillering ability, and its short flowering period [12]. At lower plant densities, the differences between older and modern hybrids were smaller, becoming greater as plant density increased [13]. Mansfield and Mumm [14] reported that in US maize germplasm evaluated for plant density tolerance, a subset of traits including leaf angle, upper stem diameter, leaf area required to produce one gram of grain, kernel rows per ear, days to canopy closure, barrenness, kernels plant^{-1}, kernel length, leaf number, upper leaf area, stay green, zipper effect, kernels per row, and anthesis-silking interval were associated with grain yield across plant densities ranging from 47,000 to 133,000 plants ha^{-1}. Al-Naggar et al. [15,16] reported strong favorable and significant genetic correlations between density tolerance index and each of yield components for inbreds and hybrids and days to anthesis, anthesis silking interval, plant height, ear height, and leaf angle for hybrids; they considered these traits as secondary traits to plant density tolerance. The objectives of the present investigation were: (*i*) to assess plant density tolerance of the studied inbreds and testcrosses in order to identify the best ones for future use, (*ii*) to estimate the superiority of tolerant (T) over sensitive (S) inbreds and T×T and T×S over S×S testcrosses and (*iii*) to identify secondary trait(s) for high plant density tolerance in maize inbreds and testcrosses.

2. MATERIALS AND METHODS

This study was carried out 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 meters above sea level) in 2015 and 2016 seasons [17].

2.1 Genetic Materials

Twenty three maize inbred lines, of different origins were chosen on the basis of their adaptive traits to high plant density and/or drought, to be used as females in this study. Seven of them (L14, L17, L18, L20, L21, L28 and L53) were obtained from Agronomy Department, Faculty of Agriculture, Cairo University and 16 inbreds (IL115, IL17, IL24, IL51, IL53, IL80, IL84, IL151, IL171, Sk9, CML67, CML104, Inb174, Inb176, Inb208 and Inb213) were obtained from Agricultural Research Center, Egypt. Three testers of different genetic base were used as males to make all possible testcrosses with the 23 inbred females, namely the commercial inbred line Sd7, the commercial single cross hybrid SC 10 and the commercial synthetic Giza 2 (open-pollinated variety).

2.2 Making the Testcrosses

In 2015 summer season, the 23 inbred lines (females) and the three testers (males) were planted at the Agricultural Experiment and Research Station of Faculty of Agriculture, Cairo University, Giza, Egypt at three sowing dates (May 4^{th} , May 11^{th} and May 18^{th}) in order to grant flower matching among males and females. For each sowing date, each tester was sown in 25 rows and each inbred line was sown in 4 rows (one row for making testcross seed with each of the three testers and the fourth row for making selfing). For both testers and inbred lines, rows were 5 m long and 0.70 cm wide. Two seeds hill^{-1} were sown in hills spaced 25 cm apart along the row. Hills were thinned to one plant hill^{-1} before the first irrigation. In the day before pollination, tassels of tester plants and lines were bagged in the afternoon. Pollen grains of the tester plants were collected the next morning between 10 and 12 am from each tester (as male) and used to hand pollinate silks of all tested inbred lines (as females). Pollen from at least 50 tassels tester^{-1}were sampled for hand pollination of the female inbred lines. Consequently, seeds of 69 F_1 testcrosses were obtained. Parental inbred lines and the inbred tester Sd 7 were also self-pollinated at the same season to obtain enough quantities of seeds for the evaluation experiment in the next season.

2.3 Experimental Design and Treatments

In 2016 season, one field experiment was carried out during the early summer. The experiment was conducted to evaluate 100 genotypes, namely 23 inbred lines, three testers, 69 testcrosses and five high-yielding commercial hybrids as checks (the single crosses SC 168, SC 2031, SC 30K9, SC30N11and the three-way cross TWC 1100). A split-plot design in RCB arrangement with three replications was used. The main plots were allotted to three plant densities (low, medium and high) and the sub-plots were devoted to genotypes (100 genotypes). The inbred lines were separated from other studied material in each block, because of their differences in plant height and vigor. The date of planting was the 20^{th} of May. Sub-plots were single rows 4.0 m long and 0.70 m wide, with hills spaced at a distance of 15 cm for the high density (HD), 20 cm for the medium density (MD) and 25 cm for the low plant density (LD) with two plants hill^{-1} and plants were thinned to one plant hill^{-1} before the first irrigation to achieve the plant densities 95,200, 71,400 and 47,600 plants/ha, respectively. All other agricultural practices were followed according to the recommendations of ARC, Egypt. Nitrogen fertilization at the rate of 285.6 kg N/ha was added in two equal doses of Urea before the first and second irrigation. 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.

2.4 Soil Analysis and Meteorological Data

The analysis of the experimental soil, indicated that the soil is clay loam (5.50% coarse sand, 22.80% fine sand, 36.40% silt, and 35.30% clay), the pH (paste extract) is 7.92, the EC is 1.66 dSm^{-1}, soil bulk density is 1.2 g cm^{-3}, calcium carbonate is 7.7%, the available nutrients in mg kg^{-1}were Nitrogen (371.0), Phosphorous (0.4), Potassium (398), DTPA-extractable Zn (4.34), DTPA-extractable Mn (9.08) and DTPA-extractable Fe (10.14). Meteorological variables in the 2016 growing season of maize were obtained from Agro-meteorological Station at Giza, Egypt. For May, June, July and August, mean temperature was 27.87, 29.49, 28.47 and 30.33°C, maximum temperature was 35.7, 35.97, 34.93 and 37.07°C and relative humidity was 47.0, 53.0, 60.33 and 60.67%, respectively.

2.5 Parameters Recorded

1. **Days to 50% anthesis (DTA):** (Number of days from planting to anthesis of 50% of plants), it was measured on all plants plot^{-1}.
2. **Anthesis-silking interval (ASI) (day):** (Number of days between 50% silking and 50% anthesis), it was measured on all plants plot^{-1}.
3. **Plant height (PH) (cm):** It was measured on 10 guarded plants plot^{-1} from ground to the point of flag leaf insertion.
4. **Leaf angle (LANG) (^{o}):** It was measured as leaf angle between blade and stem for the leaf just above ear using a protractor on 10 guarded plants plot^{-1}according to Zadoks et al. [18].

5. **Lower stem diameter (SDL) (mm):** It was measured with caliper from 10 guarded plants/plot as the stem diameter above second node; two measurements were taken. The first measurement was used as a base line with the second measurement recorded after a 90 degree turn of the caliper.
6. **Upper stem diameter (SDU) (mm):** It was measured with caliper from 10 guarded plants/plot as the stem diameter on third internode below flag leaf.
7. **Leaf area to produce 1 g of grain (LA/1gG) (cm^2):** It was measured as leaf area per plot /grams of grains per plot.
8. **Penetrated light at the base of top-most ear (PLE) (%):** At 70 days from sowing date light intensity was measured and then penetrated light inside the canopy was calculated for each genotype. The Lux-meter apparatus was used. The light intensity in (lux) was measured at 12 am (noon time) at the top of the plant, and at the base of top-most ear. Penetrated light inside the canopy was measured as a percentage of light penetrated from the top of the plant to the base of top-most ear as follows: PLE =100 (light intensity at the base of top-most ear/light intensity at the top of the plant).
9. **Chlorophyll concentration index (CCI) (%):** It was measured by Chlorophyll Concentration Meter, Model CCM200 as the ratio of transmission at 931 nm to 653 nm through the leaf of top-most ear. It was measured on 5 guarded plants/plot.
10. **Number of ears plant^{-1} (EPP):** It was estimated by dividing number of ears plot^{-1} on number of plants plot^{-1}.
11. **Number of rows ear^{-1} (RPE):** Using 10 random ears plot^{-1} at harvest.
12. **Number of kernels row^{-1} (KPR):** Using the same 10 random ear plot^{-1}.
13. **Number of kernels plant^{-1} (KPP):** Calculated by multiplying number of ears plant^{-1} by number of rows ear^{-1} by number of kernels row^{-1}.
14. **100-kernel weight (100KW) (g):** Adjusted at 155g water kg^{-1} grain.
15. **Grain yield plant^{-1} (GYPP) (g):** It was estimated by dividing the grain yield plot^{-1} (adjusted at 15.5% grain moisture) on number of plants plot^{-1} at harvest.
16. **Grain yield ha^{-1} (GYPH) (ton):** It was estimated by adjusting grain yield plot^{-1} at 15.5% grain moisture to grain yield ha^{-1}.

2.6 Biometrical Analyses

Analysis of variance of the split-plot design in RCB arrangement was performed on the basis of individual plot observation using the MIXED procedure of SAS ® [19]. The data collected from the experiment was subjected to the standard analysis of variance of split-plot design. Least significant difference (LSD) was calculated to test significance of differences between means according to Steel *et al.* [20]. Stress tolerance index (STI) modified from equation suggested by Fageria [21] was used to classify genotypes for tolerance to density stress. The formula used is as follows: STI= (Y_1/AY_1) X (Y_2/AY_2), Where, Y_1 = grain yield mean of a genotype at non-stress. AY_1 = average yield of all genotypes at non-stress. Y_2 = grain yield mean of a genotype at stress. AY_2 = average yield of all genotypes at stress. Rank correlation coefficients were calculated between each STI and each of studied traits under each stress environment (medium and high density) for inbreds and testcrosses according to Steel et al. [20].

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance of split plot design (Table 1) showed that mean squares due to plant density (D) for all studied traits were significant ($P\leq 0.01$) for all studied traits, indicating that the plant density stress has an obvious effect on most studied traits of all studied genotypes in the present experiment. Mean squares due to genotypes (G) were significant ($P\leq 0.01$) for all studied traits, indicating genetic-background differences among genotypes for all studied traits across the three plant densities (high, medium and low).

Mean squares due to genotype × plant density interaction were significant ($P \leq 0.01$) for all studied traits, except lower and stem diameter, indicating the possibility of selecting genotypes for improved performance under a specific plant density as proposed by previous investigators [22-25]. Mean squares due to genotypes (data not presented) under all environments were significant ($P\leq 0.01$ or $P\leq 0.05$)) for all studied traits, indicating the significance of differences among studied genotypes under each of the three plant densities.

3.2 Stress Tolerance Index

The highest stress tolerance index (STI) under both stressed environments (MD and HD) was exhibited by the inbred line L21 followed by inbreds IL15, IL53, Inb176, IL80, L28, IL151 and then L14 in descending order (Table 2). These inbreds had STI value greater than unity under both studied stresses and therefore could be considered tolerant to medium (71,400 plants/ha) and high (95,200 plants/ha) plant density stress. On the contrary, the nine inbred lines Inb208, CML104, Inb213, Inb174, CML67, L18, L53, L17 and IL84 exhibited STI values ranging from close to zero to less than unity under both stressed environments and therefore could be considered sensitive to medium and high plant density stress; with the most sensitive ones were the inbreds Inb208, CML104 and Inb213 under both environments. For the testers, SC10 and Giza 2 were tolerant (STI >1), but Sd7 was sensitive (STI<1) to both MD and HD.

For testcrosses, the highest STI value was recorded by the cross IL51 × Giza2 (T×T) under medium stress and (S×T) under high density stressed environment followed by the cross IL51 × SC10 (T×T), L14 × SC10 (T×T), L28 × Sd7 (T×T), IL53 × SC10 (T×T) and L28 × SC10 (T×T) under both medium and high density stresses. On the other hand, the most sensitive crosses under both stressed environments (MD and HD) were between the sensitive inbreds Inb213, Inb208, Inb174, CML104, CML67 and L53 and the testers Sd7 (S), SC10 (T) and Giza2 (T) under both medium and high density stress. For the checks, the single cross SC 168 was tolerant under both medium and high density, the single

Table 1. Analysis of variance of split plot design for 16 traits of 100 maize genotypes (G) under three plant densities (D) in 2016 season

SOV	df	Mean squares			
		DTA	**ASI**	**PH**	**LANG**
Density (D)	2	1838**	8.70**	117953**	8536**
Error a	4	1.71	0.18	1789	58.57
Genotype (G)	99	23.4**	1.00**	13136**	106.2**
G × D	198	4.85**	0.63**	284.7**	13.05**
Error b	594	0.53	0.33	127.4	3.11
CV%		1.17	19.58	4.71	7.86
		SDL	**SDU**	**LA/1gG**	**PL-E**
Density (D)	2	2852**	1641**	29199**	7805**
Error a	4	24.63	17.8	80.16	410.4
Genotype (G)	99	51.59**	36.85**	646.3**	103.3**
G × D	198	3.51**	1.89**	68.96**	51.69**
Error b	594	1.06	0.55	10.8	10.89
CV%		4.81	5.98	7.33	13.87
		CCI	**EPP**	**RPE**	**KPR**
Density (D)	2	7814**	0.22**	258.9**	2401**
Error a	4	38.55	0.004	0.42	7.15
Genotype (G)	99	205.1**	0.01**	8.76**	425.1**
G × D	198	11.61**	0.01**	0.85**	4.86**
Error b	594	2.54	8.43	0.14	1.32
CV%		3.61	2.87	2.81	3.33
		KPP	**100-KW**	**GYPP**	**GYPH**
Density (D)	2	1973910**	1279**	317383**	2234**
Error a	4	9559	1.04	1541	6.41
Genotype (G)	99	112556**	103.3**	15593**	665.3**
G × D	198	2751**	5.71**	481.0**	20.1**
Error b	594	617.9	0.64	78.23	2.76
CV%		5.31	2.93	6.71	6.2

*DTA = Days to 50% anthesis, ASI = Anthesis-silking interval, PH = Plant height, LANG = Leaf angle, SDL= Lower stem diameter, SDU = Upper stem diameter, LA/1gG = Leaf area to produce 1 g of grain, PL-E = Penetrated light at top-most ear, CCI = Chlorophyll concentration, EPP = ears/ plant, RPE = rows/ ear, KPR = kernel/ row, KPP = kernels/plant, 100-KW = 100-kernel weight, GYPP = grain yield/ plant, GYPH = grain yield/ ha, and * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

Table 2. Stress tolerance index (STI) of maize inbred lines, testers, testcrosses and checks under medium (MD) and high (HD) plant density in 2016 season

	Inbreds		Testcrosses					
			MD			HD		
	MD	HD	Sd7	SC10	Giza2	Sd7	SC10	Giza2
L 14	1.00	1.16	1.10	1.63	1.31	1.22	1.51	1.26
L 17	0.92	0.98	1.04	1.18	1.54	1.08	1.18	1.77
L 18	0.79	0.85	1.08	1.16	0.95	1.12	1.08	0.95
L 20	1.25	0.98	1.04	0.68	0.84	1.02	0.66	0.84
L 21	1.97	2.11	1.11	1.08	0.91	1.48	1.04	1.04
L28	1.17	1.24	1.54	1.25	1.33	1.71	1.35	1.47
L 53	0.83	0.94	0.73	0.74	0.82	0.78	0.72	0.77
IL 15	1.86	1.94	1.19	1.12	0.87	1.28	1.16	0.83
IL 17	1.11	0.97	0.70	0.79	0.75	0.68	0.74	0.76
IL 24	1.13	0.98	0.90	1.25	0.85	0.81	1.13	0.79
IL 51	1.09	0.85	1.03	1.82	2.03	1.02	1.61	2.06
IL 53	1.66	1.71	1.17	1.38	1.45	1.12	1.45	1.47
IL 80	1.24	1.28	1.12	1.04	1.18	1.10	1.01	1.04
IL 84	0.94	0.99	0.83	1.58	0.89	0.81	1.75	0.90
IL 151	1.19	1.27	0.65	1.01	1.46	0.62	0.97	1.46
IL 171	1.01	0.91	0.79	0.81	0.95	0.83	0.82	0.93
Sk 9	1.01	0.88	0.85	0.77	0.97	0.82	0.70	0.89
CML 67	0.70	0.67	0.92	0.69	0.87	0.85	0.72	0.81
CML 104	0.39	0.43	0.74	0.65	0.96	0.75	0.68	0.89
Inb 174	0.51	0.53	0.89	0.84	0.74	0.85	0.83	0.72
Inb 176	1.29	1.39	0.95	0.88	1.06	0.90	0.84	1.09
Inb 208	0.38	0.39	0.51	0.81	0.94	0.48	0.80	0.96
Inb 213	0.47	0.51	0.74	0.86	0.78	0.78	0.89	0.78
Testers								
			0.58	1.38	1.12	0.51	1.44	1.17
Checks								
SC 2031				0.92			0.96	
TWC 1100				1.13			0.95	
SC 30K9				0.89			1.04	
SC 30N11				0.71			0.76	
SC 168				1.40			1.30	

cross SC30K9 was tolerant under HD and the three-way cross TWC 1100 was tolerant under MD only. The other two checks SC 2031 and SC 30N11 were sensitive under both MD and HD environments.

3.3 Superiority of Tolerant (T) Over Sensitive (S) Inbreds and Testcrosses

To describe the differences between tolerant (T) and sensitive (S) inbreds and testcrosses, data of the selected characters were averaged for the two groups of inbreds and testcrosses differing in their density tolerance (both high and medium), as well as in grain yield/plant under high and medium density stress (Table 3). Based on STI, the three high- and medium-density tolerant (T) inbred lines were L21, IL15 and IL53 and the three high- and medium-density sensitive (S) inbred lines were CML104, Inb208 and Inb213. Moreover, the five F_1 testcrosses L28 × Sd7, L21 × Sd7, IL51 × Giza2, IL84 ×SC10 and L28 × SC10 were considered the most tolerant to high density, while the testcrosses Inb208 × Sd7, IL151 × Sd7, IL17 × Sd7, L20 × SC10 and L53 × Giza2 were considered as the most high-density sensitive crosses.

Results averaged for each of the two groups (T and S) of inbreds and testcrosses differing in tolerance to high/medium density indicated that grain yield/ha of high density tolerant (T) was greater than that of the sensitive (S) inbreds and testcrosses by 100.6 and 89.3%, respectively under high density (95,200 plants/ha) conditions. Superiority of high-density tolerant (T) over sensitive (S) inbreds in GYPF under high density was due to their superiority in GYPP (101.9%), EPP (9.59%), RPE (21.35%), KPR (37.88%), KPP (83.91%), 100-KW (9.88%), *i.e.* in all studied yield component traits. Likewise, under high plant density, the tolerant inbreds showed 3.58% less DTA, 7.69% shorter ASI, 9.25% shorter plant height, 15.69% smaller leaf angle, 16.68% thicker lower stem diameter, 22.89% thicker upper stem diameter, 5.65% smaller leaf area to produce 1g grain (more efficient), 10.35% more penetrated light to ear and 6.81% more chlorophyll concentration index than the sensitive inbreds.

Superiority of T over S testcrosses in GYPH under high density (95,200 plants/ha) was due to their superiority in GYPP (90.83%), EPP (2.26%), RPE (17.46%), KPR (14.87), KPP (37.79%), 100-KW (38.70%), *i.e.* in all studied yield component traits. Moreover, under high plant density, the tolerant testcrosses showed 1.24% less DTA, 4.17% shorter ASI, 6.57% shorter plant height, 9.61% smaller leaf angle, 7.52% thicker lower stem diameter, 30.92% thicker upper stem diameter, 6.25% smaller leaf area to produce 1g grain (more efficient) and 11.84% more penetrated light to ear, than the sensitive testcrosses.

The superiority of modern maize hybrids tolerant to high plant density was also attributed to more leaf erectness [6], synchronization of 50% anthesis with 50% silking [7] and increased prolificacy, i.e. more ears plant^{-1} [8]. A shortened ASI was considered as an indication of higher flow of assimilates to the developing ears during the early reproductive stage under conditions of high density stress [26]. High plant density-tolerant genotypes possess shorter ASI than intolerant ones [27]. Al-Naggar et al. [28] also reported that under high plant density, the tolerant testcrosses showed 314.4% more GYPP, 115.0% more KPP, 48.4% heavier 100-KW, 42.9% more EPP and 63.3 % shorter ASI than sensitive testcrosses. Mansfield and Mumm [14] 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 one gram of grain, kernel rows per ear, kernels plant^{-1}, 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}. These results are consistent with those reported by Al-Naggar et al. [15,16]. Shortening in ASI of tolerant as compared to sensitive inbreds and hybrids in the present study is desirable and may be considered as important contributor to high-density tolerance. Similar conclusions have been reported by several investigators [14,23,26].

Table 3. Superiority (%) of the three most tolerant (T) over the three most sensitive (S) inbreds and the five most tolerant (T) over the five most sensitive (S) testcrosses for studied characters under the high density stressed environment

	Inbreds			Testcrosses		
	T	S	Superiority (%)	T	S	Superiority (%)
DTA	65.89	68.33	-3.58**	63.60	64.40	-1.24*
ASI	2.67	2.89	-7.69*	3.07	3.20	-4.17*
PH(cm)	190.7	210.1	-9.25**	276.67	296.11	-6.57**
LANG(^{o})	15.93	18.89	-15.69**	19.78	18.04	9.61*
SDL(mm)	16.05	13.76	16.68**	19.69	18.32	7.52**
SDU (mm)	10.20	8.30	22.89**	11.09	8.47	30.92**
LA/1g (cm^2)	60.12	63.72	-5.65*	48.88	52.14	-6.25*
PL-E%	8.56	7.76	10.35*	6.13	5.48	11.84*
CCI%	32.90	30.80	6.81**	41.51	41.07	1.07
EPP	1.00	0.91	9.59**	1.00	0.98	2.26*
RPE	12.00	9.89	21.35**	14.05	11.96	17.46**
KPR	23.56	17.08	37.88**	38.62	33.62	14.87**
KPP	283.6	154.2	83.91**	542.89	393.99	37.79**
100-KW (g)	22.77	20.72	9.88**	31.61	22.79	38.70**
GYPP (g)	63.80	31.60	101.9**	171.25	89.74	90.83**
GYPH (t)	6.08	3.01	100. 6**	16.31	8.55	89. 3**

*% Superiority = 100 × [(T – S)/S], * and ** significant at 0.05 and 0.01 probability levels, respectively*

3.4 Differential Response of T×T, T×S and S×S Crosses

Mean performance of traits was averaged across three groups of F_1 testcrosses, *i.e.* T×T, T×S and S×S groups based on parental tolerance to high density stress and presented in Table 4. Number of testcrosses was 16, 15 and 38 for the T×T, T×S and S×S groups, respectively. In general, high density T×T group of testcrosses exhibited better values in most studied traits than high density T×S and S×S groups of testcrosses. The T×T group of testcrosses showed higher means (favorable) for GYPH, GYPP, EPP, RPE, KPR, KPP and 100-KW, SDL, SDU, PL-E and CCI and lower means (favorable) for DTA, ASI, PH, LANG and leaf area to produce 1 g grain than T×S and S×S groups of testcrosses.

Table 4. Means of selected traits across T×T, T×S and S×S groups of F_1 testcrosses under low (LD), medium (MD) and high (HD) plant densities

Trait	LD			MD			HD		
	T×T	T×S	S×S	T×T	T×S	S×S	T×T	T×S	S×S
DTA	58.92	59.61	59.80	61.40	61.70	61.91	63.54	63.79	64.07
ASI	2.63	2.65	2.80	2.94	3.00	3.11	2.98	3.04	3.07
PH(cm)	238.9	244.4	245.6	257.9	262.2	263.5	271.2	278.8	283.6
LANG(^{o})	24.30	28.54	31.70	18.49	22.88	25.36	14.19	17.71	20.63
SDL(mm)	28.01	25.38	22.36	24.48	22.25	19.46	21.55	19.48	16.78
SDU (mm)	18.22	15.24	11.64	15.46	12.51	9.21	13.64	10.34	7.44
LA/1g (cm^2)	31.56	33.28	34.86	41.58	41.87	44.30	50.87	52.98	54.40
PL-E%	20.01	15.51	15.26	9.57	9.17	5.99	6.99	6.35	4.15
CCI%	53.63	50.00	48.88	49.61	45.21	44.17	44.44	40.34	38.76
EPP	1.05	1.03	1.00	1.02	1.00	1.00	1.00	1.00	1.00
RPE	14.87	14.32	14.19	13.95	13.56	13.19	13.06	12.78	12.28
KPR	41.99	41.32	40.18	38.05	37.79	36.99	36.05	35.29	34.53
KPP	656.5	612.1	572.5	541.0	514.3	488.1	470.6	449.6	422.5
100-KW (g)	31.71	30.93	30.41	29.21	28.50	28.24	27.53	26.69	26.54
GYPP (g)	208.4	190.1	174.3	157.9	147.2	138.0	129.5	120.8	112.2
GYPH (t)	9.92	9.05	8.30	11.28	10.31	9.86	12.33	11.30	10.68

T = tolerant, S = sensitive

Table 5. Superiority (%) of T × T and T × S over S × S testcrosses for selected traits under different plant densities

Trait	LD		MD		HD	
	T×T	T×S	T×T	T×S	T×T	T×S
DTA	-1.48*	-0.31	-0.83	-0.34	-0.82	-0.43
ASI	-6.25	-5.39	-5.58	-3.57	-2.85	-0.74
PH	-2.76	-0.50	-2.12	-0.48	-4.35**	-1.68
LANG	-23.36**	-9.99**	-27.09**	-9.79**	-31.23**	-14.17**
SDL	25.23**	13.50**	25.79**	14.32**	28.41**	16.07**
SDU	56.48**	30.84**	67.89**	35.86**	83.30**	38.99**
LA/1g	-9.47**	-4.53	-6.16	-5.50	-6.50*	-2.62
PL-E%	31.12**	1.63	59.71**	52.97*	68.51**	52.98**
CCI	9.73**	2.29	12.31**	2.35*	14.64**	4.07*
EPP	4.59**	3.00**	2.02*	0.38	0.48	0.10
RPE	4.79**	0.94	5.77**	2.76*	6.31**	4.08**
KPR	4.50**	2.83*	2.87*	2.17	4.40**	2.20
KPP	14.67**	6.91**	10.83**	5.35**	11.40**	6.42**
100-KW	4.28**	1.71	3.43**	0.90	3.73**	0.57
GYPP	19.54**	9.07**	14.45**	6.65*	15.41**	7.69**
GYPH	19.50**	9.07**	14.48**	6.65*	15.41**	7.69**

** and ** significant at 0.05 and 0.01 probability levels, respectively*

Superiority of high density T×T and T × S over S×S testcrosses under low (LD), medium (MD) and high (HD) density environments is presented in Table 5. Superiority in most of studied traits was more pronounced under high density (95,200 plants/ha) than under medium (71,400 plants/ha) and low density (47,600 plants/ha). Under high plant density conditions, grain yield/ha of high-density T×T crosses (12.33 ton) was significantly greater than that of S×S (10.68 ton) and T×S (11.30 ton) crosses by 15.41 and 7.69%, respectively. Grain yield per hectare superiority of high-density T×T and T×S over S×S crosses was associated with their superiority in grain yield/plant and most studied yield components.

The T × T and T × S crosses were earlier in DTA, shorter in PH, narrower in LANG (-31.23 and -14.17%), thicker in lower stem diameter (28.41 and 16.07%), thicker in upper stem diameter (83.30 and 38.99%), more penetrating to light at top most ear (68.51 and 52.98%), more CCI (14.64 and 4.07%), and smaller in leaf area to produce 1g grain, *i.e.* more efficient (-6.50 and -2.62%) than S × S crosses, respectively under high-density conditions (95,200 plants/ha) (Table 6). The superiority of T × T and T × S crosses in grain yield and other studied characteristics over S × S crosses under high plant density was also expressed under low and medium plant density (Table 5).

The superiority of modern crosses of maize (tolerant to high plant density) over the old ones in countries grow maize under high plant densities is due to their short stature, erect leaves, prolificacy, synchronization between anthesis and silking [6,14,29]. The present study concluded that to obtain maximum grain yield from a hybrid under elevated plant density, it is better that both the two parents should be tolerant to high plant density. This assures that high plant density stress tolerance trait is quantitative in nature, so the tolerant cross accumulates additive genes of high density tolerance from both parents.

In general, testcrosses classified as high-density tolerant × high-density tolerant crosses in terms of grain yield under high density stress had a better high density adaptive traits such as higher values of all grain yield components, upper stem diameter, penetrated light at ear, chlorophyll concentration index, and lower values of DTA, ASI, PH, LA to produce 1g grain and LANG as compared with high density sensitive × high density sensitive testcrosses. Some investigators reached to a similar conclusion [1,5,6,8,28,30].

3.5 Correlations between Density Tolerance Index and Other Traits

Grain yield per plant or per hectare of inbreds showed very strong and positive association with stress tolerance index (STI) under both MD and HD (Table 6).

Table 6. Rank correlation coefficients between stress tolerance indexes (STI) and all studied traits of inbreds and testcrosses in 2016 season

Trait	Inbreds		Testcrosses	
	MD	HD	MD	HD
DTA	-0.26*	-0.30*	-0.13	-0.10
ASI	-0.16	-0.05	-0.09	-0.004
PH	-0.16	-0.18	0.08	0.01
LANG	0.05	0.001	-0.13	-0.01
SDL	0.13	0.18	0.38**	0.36**
SDU	0.31**	0.41**	0.40**	0.42**
LA 1g	-0.08	0.00	-0.06	-0.07
PL E	0.29*	0.11	0.36**	0.30**
CCI	0.17	0.19	0.41**	0.27**
EPP	0.31**	0.48**	0.28**	0.21**
RPE	0.53**	0.49**	0.50**	0.53**
KPR	0.64**	0.72**	0.57**	0.65**
KPP	0.75**	0.83**	0.79**	0.79**
100-KW	0.46**	0.31*	0.68**	0.67**
GYPP	0.94**	0.93**	0.91**	0.91**
GYPF	0.93**	0.93**	0.92**	0.91**

** and ** significant at 0.05 and 0.01 probability levels, respectively*

Density stress tolerance index of inbreds showed significant and positive correlation coefficients with all grain yield components and SDU; with the strongest one between STI and GYPP, GYPH and KPP and significant and negative association with DTA trait under MD and HD. Significant and positive correlation coefficients were also observed between STI of testcrosses and each of GYPP, GYPH, SDL, SDU, PL-E and CCI under both MD and HD environments, with the strongest one between STI and each of GYPP, GYPH and KPP. These results are in agreement with those reported by other investigators [31-36]. Traits correlated with grain yield across plant densities would highlight traits and categories of traits that may underlie plant density tolerance [14]. Other studies have also found kernel number to be associated with final grain yield under high plant density and other stress conditions [37-39]. The number of kernels per row is determined approximately 1 wk before flowering [40], in contrast to kernel rows per ear, which is determined early in the growing season. The combination of rows per ear and kernels per row may be critical to expression of plant density tolerance. These findings suggest that genotypes with high plant density tolerance may be tolerant of early and midseason stress from high plant-to-plant competition that can trigger changes to ear structure. Therefore, unaltered kernel set (*i.e.*, no significant reduction in rows per ear and/or kernels per row) would allow more kernels per plant, which would support high grain yield under high plant density. Similar conclusions were reported by several investigators [8,26,31,34,35,41,42].

4. CONCLUSION

The present investigation identified the highest density tolerant genotypes under HD which could be offered to future breeding programs to improve maize plant density tolerance (PDT); they were the inbred lines L21, IL15, IL53, Inb176, IL80, L28, IL151 and L14 and the testcrosses IL51 × Giza 2, IL51 × SC10, L14 × SC10, L28 × Sd7, IL53 × SC10 and L28 × SC10, in descending order. It was concluded that tolerant (T) inbreds or testcrosses produced higher grain yield/ha than sensitive ones under high plant density (95,200 plants/ha). The study recommended that to have a tolerant cross, both parents of the cross should be tolerant to density stress. The tolerant genotypes possess adaptive traits to plant density tolerance, namely early DTA, short ASI and plant height, thick stems (lower and upper), narrow leaf angle, small leaf area to produce 1g grain (more photosynthesis efficiency), more penetrated light to ear position, more chlorophyll concentration index, more ears and kernels per plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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