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The Effect of Increasing Plant Density on Performance and Heterobeltiosis in Maize Testcrosses among 23 Inbreds and Three Testers

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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 ASMY and AMAM managed the experimental process and performed data analyses. All authors read and approved the final manuscript.

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ABSTRACT

Information on the heterosis of maize germplasm is essential in maximizing the effectiveness of hybrid development. The objectives of the present study were to assess the effects of elevated plant density on mean performance and heterobeltiosis and to identify the best testcrosses in these parameters. A set of 23 inbred lines of maize, were top-crossed to three testers to produce 69 testcrosses. Inbreds, testers, testcrosses and five commercial cultivars were evaluated in the field under three plant densities using a split plot design with three replications. Elevating plant density from 47,600 (low density; LD) to 71,400 (medium density; MD) and 95,200 (high density; HD) plants/ha caused a significant reduction in grain yield/plant (GYPP), and its components, leaf angle, penetrated light at ear and chlorophyll concentration index (CCI) and significant increase in grain yield/ha (GYPH), days to anthesis, anthesis silking interval, and plant height. Increasing the

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plant density increased average heterobeltiosis in eight traits, namely GYPH, GYPP, 100 kernel weight, rows/ear, tassel branch number, tassel dry weight and CCI. The best testcrosses in heterobeltiosis for GYPH (L28 x Sd7 and L21 x Sd7 under HD and IL51 x Giza2, IL84 x SC10 and L28 x SC10 under HD and MD) were also the best crosses in *per se* performance for GYPH under respective environments. These crosses could be useful in future breeding programs for improving plant density tolerance in maize.

Keywords: Useful heterosis; line x tester; corn; adaptive traits; plant density tolerance.

1. INTRODUCTION

Egyptian maize (*Zea mays* L.) hybrids are bred and grown under low plant density, but are subject to yield losses when grown under high density because they are not tolerant to this stress [1]. Maximum grain yield per land unit area may be obtained by growing maize hybrids that can withstand high plant density up to 100,000 plants/ha [2]. In the USA, average maize grain yield per land unit area increased dramatically during the second half of the 20th century, due to greater tolerance of modern hybrids to high plant [3]. Introducing high density adaptive traits to Egyptian cultivars is important to enable these cultivars to produce higher grain yield than present cultivars. Mansfield and Mumm [4] reported that in US maize germplasm evaluated for plant density tolerance, a subset of traits including leaf angle, kernel rows per ear, kernels per plant, 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}.

Maize grain yield of individual plant decreases as the density per unit area increases [5]. The yield decreases as a response to decreasing light and other environmental resources available to each plant [6]. Reduction in yield is due mainly to fewer cobs (barrenness) [7], fewer grains per cob [8], lower grain weight [9], or a combination of decrease in these components [10]. Maize genotypes differ in tolerance to high plant density [11]. Liu et al. [12] reported that maize yield differed significantly at varying plant density levels, owing to differences in genetic potential. There is a substantial genetic variation for plant density tolerance (PDT) in maize [13].

Heterosis of adaptive traits to high density should be studied; such information is scarce. It is a prerequisite for developing economically viable hybrid maize varieties. Information on the heterotic patterns of maize germplasm is essential in maximizing the effectiveness of hybrid development [14]. Heterosis is the genetic expression of the superiority of a hybrid compared to its parents [15]. This phenomenon manifests in increased size, or other parameters resulting from the increase in heterozygosity in the F_1 generation of crosses between inbred [16] and is associated with higher stress tolerance [17]. In general, based on parents used, two major types of estimation of heterosis are reported in literature: (1) Mid-parent or average heterosis, which is the increased vigor of the F_1 over the mean of two parents. (2) High-parent or better parent heterosis, which is the increased vigor of the F_1 over the better parent [17,18]. The term heterobeltiosis has been suggested to describe the increased performance of the hybrid over the better parent [19]. Heterosis is also modified by the interaction between genotypes and environment [16,20]. Since inbreds are more sensitive to environmental differences, some traits have been found to be more variable among inbreds than among hybrids [21]. Similarly, Betran et al. [10] reported extremely high expression of heterosis in maize under stress, especially under severe drought stress because of the poor performance of inbred lines under these conditions. Knowledge about heterosis of maize traits in diverse plant density environments is essential for plant breeding programs. The objectives of this study were: (i) to assess the effects of plant density stress on the mean performance of maize inbreds and testcrosses, (ii) to estimate heterobeltiosis percentages under elevated plant density and (iii) to identify the best testcrosses in *per se* performance and heterobeltiosis estimates.

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 growing seasons.

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 female parents in this study. Sixteen of those lines (IL15, IL17, IL24, IL51, IL53, IL80, IL84, IL151, IL171, Sk9, CML67, CML104, Inb174, Inb176, Inb208 and Inb213) were obtained from Agricultural Research Center, Ministry of Agriculture, Egypt and seven (L14, L17, L18, L20, L21, L28 and L53) were obtained from Agronomy Department, Faculty of Agriculture, Cairo University, Egypt. Three testers of different genetic backgrounds were used as male parents 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 crossed to three testers and 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 sufficient quantities of seeds for the evaluation experiment in the next season.

2.3 Experimental Design and Treatments

In 2016 growing season, 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 randomized complete blocks arrangement with three replications was used. The main plots were allotted to three plant densities 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 20th 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 per hill and plants were thinned to one plant per hill before the first irrigation to achieve the plant densities 95,200, 71,400 and 47,600 plants/ha, respectively [1]. Nitrogen fertilizer was added 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 for corn borer was performed when required by spraying plants with Lannate (Methomyl) 90% (manufactured by DuPont, USA).

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 (400), Potassium (398), DTPA-extractable Zn (4.34), DTPA-extractable Mn (9.08) and DTPA-extractable Fe (10.14). Meteorological data in the 2016 growing season were obtained from Agro-meteorological Station at Giza, Egypt. For months of May, June, July and August, mean temperatures were 27.87, 29.49, 28.47 and 30.33℃, maximum temperatures were 35.7, 35.97, 34.93 and 37.07℃ 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 per plot.
2. **Anthesis-silking interval (ASI)** (day) [Number of days between 50% silking and 50% anthesis], it was measured on all plants per plot.
3. **Plant height (PH)** (cm) measured on 10 guarded plants per plot from ground to the point of flag leaf insertion.
4. **Leaf angle** (LANG) (^{o}) 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. [22] .
5. **Lower stem diameter (SDL)** (mm) measured with caliper from 10 guarded plants/plot as 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) measured with caliper from 10 guarded plants/plot as stem diameter on third internode below flag leaf.
7. **Leaf area to produce 1 g of grain (LA/1G g)** (cm^2) measured as leaf area per plot /grams of grains per plot.
   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.
8. **Penetrated light at the base of top-most ear (PL-E)** (%) calculated from 10 guarded plants/plot 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)** (%) measured by Chlorophyll Concentration Meter, Model CCM-200, USA 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. **Tassel fresh weight (TFW)** (g) measured on 5 guarded plants per plot^{-}.
11. **Tassel dry weight (TDW)** (g) measured on the same fresh tassels after oven drying to a constant weight.
12. **Tassel branch number (TBN)** measured as number of branches on 5 guarded plants per plot. Traits No. 10, 11 and 12 were measured according to Mansfield and Mumm [4].
13. **Number of ears per plant (EPP)** estimated by dividing number of ears per plot on number of plants per plot.
14. **Number of rows per ear (RPE)** using 10 random ears per plot at harvest.
15. **Number of kernels per plant (KPP)** calculated by multiplying number of ears per plant by number of rows per ear by number of kernels per row.
16. **Hundred kernel weight (100KW)** (g) adjusted at 155 g water kg^{-1} grain.
17. **Grain yield per plant (GYPP)** (g) estimated by dividing the grain yield per plot (adjusted at 15.5% grain moisture) on number of plants per plot at harvest.
18. **Grain yield per hectare (GYPH)** (ton) estimated by adjusting grain yield per plot at 15.5% grain moisture to grain yield ha^{-1}.

2.6 Biometrical Analyses

Analysis of variance of the split-plot design was performed on the basis of individual plot observation using the MIXED procedure of SAS ® [23]. The data collected from each plant density were subjected to the standard analysis of variance of randomized complete blocks design according to Steel et al.[24]using GENSTAT Ver.10 windows software. Least significant difference (LSD) was calculated to test significance of differences between means. Heterobeltiosis of analyzed traits was calculated as percentage of F_1 hybrid value relative to the better-parent (BP) values as follows: Heterobeltiosis (%) = $100[(\overline{F}_1-\overline{BP})/\overline{BP}]$; where: $\overline{F}_1$= mean of an F_1 cross and $\overline{BP}$= mean of the better parent of this cross. The significance of heterobeltiosis was determined as the least significant differences (LSD) at 0.05 and 0.01 levels of probability according to Steel et al. [24] using the following formula: $LSD_{0.05} = t_{0.05}(edf) \times SE$, $LSD_{0.01} = t_{0.01}(edf) \times SE$; where: *edf*= the error degrees of freedom, SE**=** the standard error.SE for heterobeltiosis **=**$(2MS_e/r)^{1/2}$ where:$t_{0.05}$ and $t_{0.01}$ are the tabulated values of '*t*' for the error degrees of freedom at 0.05 and 0.01 levels of probability, respectively. MS_e: The mean squares of the experimental error from the analysis of variance Table, *r*: Number of replications.

3. RESULTS

3.1 Analysis of Variance

Analysis of variance of a randomized complete blocks design for studied traits of all maize genotypes was performed for each of the three environments (low, medium and high density) (data not presented). Mean squares due to genotypes were significant (P≤ 0.01) under all three densities for all studied traits. Mean squares due to 69 testcrosses were significant (P≤ 0.01) under each of the three densities for all studied traits, indicating the significance of differences among studied testcrosses in all cases. Mean squares due to 26 parents were significant (P≤ 0.01) under all three densities for all studied traits, indicating that parents used in this study possess different genetic background for all studied traits and these parents (female and male) are valid for line × tester analysis. Mean squares due to parents vs. F_1 testcrosses were significant (P≤ 0.01) for all studied traits under all three environments, except for ASI under medium density and leaf angle and penetrated light at ear under low density, suggesting the presence of significant heterosis for 51 out of 54 studied cases.

3.2 Effects of Elevated Plant Density on Mean Performance

The effects of elevating plant density on the means of studied traits across all genotypes are presented in Table (1). The environment LD represents the non-stressed one (47,600 plants/ha), while MD and HD represent elevated plant density (stressed) environments (71,400 and 95,200 plants/ha, respectively).Mean grain yield/plant was significantly (P ≤ 0.01) reduced due to elevating plant density from 47,600 plants/ha (Env1) to 71,400plants/ha(Env2) and 95,200plants/ha(Env3) by 23.91 and 38.68%, respectively (Table 1).

Mean grain yield/ha under 3 plant density levels for each testcross and check cultivar is presented in Table (2).The highest grain yield/ha (GYPH) was obtained by the testcross L28×Sd7 followed by L21×Sd7, IL51×Giza2, IL84×SC10 and L28×SC10 under high plant density, and IL51×Giza2, IL51×SC10, L28×SC10, IL84×SC10 and L28×SC10 under medium plant density. Under low plant density, the best testcrosses for GYPH were IL51×Gz2, L14×SC10, IL53×SC10, IL51×SC10 and IL84×SC10 in descending order.

On the contrary, the lowest GYPH was shown by the testcross Inb208×Sd7 under all plant densities, followed by IL151×Sd7, IL17×Sd7, L20×SC10 and L53×Giza2 under high plant density, CML104×SC10, IL17×Sd7, IL151×Sd7and L20×SC10 under medium plant density and Sk9×Sd7, IL151×Sd7, CML104×SC10 and L53×SC10 under low plant density. The increase in GYPH of these crosses under MD and HD over that under LD could be attributed to the elevation of plant density. The best GYPH in this experiment was obtained under high density and the best crosses in this environment were L28×Sd7 (16.90 ton), L21×Sd7 (16.64 ton), IL51×Giza2 (16.42 ton), IL84×SC10 (16.04 ton) and L28×SC10 (15.55 ton) with a significant superiority over SC30K9 (the best check under HD in this experiment) (12.07 ton) by 40.0, 37.9, 36.1.5, 32.9 and 28.9%, respectively. Some hybrids in this experiment showed significant superiority over the best check in the medium and low density environments; these superiorities reached 30.1% over SC168 under LD for the cross IL51 × Giza2 and 7.5% over SC168 under MD for the same cross.

3.3 Effects of Elevated Plant Density on Heterobeltiosis

Estimates of better parent heterosis (heterobeltiosis) across all testcrosses, minimum and maximum values and number of crosses showing significant favorable heterobeltiosis for all studied traits under the three environments are presented in Table 3. Favorable heterobeltiosis in the studied crosses was considered negative for DTA, ASI, PH, LANG and LA/1gG and positive for the remaining studied traits under all plant densities. In general, the highest average significant and positive (favorable) heterobeltiosis was shown by grain yield per plant (16.67, 27.38 and 47.32%) and reached to maximum in some crosses to 69.08, 122.64 and 215.85% under LD, MD and HD, respectively. The traits ASI, PH, LANG and PL-E under all environments, LA/1gG and EPP under LD and HD, SDU under MD and HD and ROE under LD showed on average unfavorable heterobeltiosis. However, some crosses showed significant favorable heterobeltiosis in all studied traits, except PH trait. Maximum number of testcrosses showing significant favorable heterobeltiosis was observed in GYPP and GYPH (35, 44 and 48) followed by 100KW (37, 35 and 49), KPP (28, 36 and 36), CCI (22, 34 and 29) and SDL (29, 31 and 28) under LD, MD and HD, respectively.

In general, HD environment, where density stress was maximum, exhibited the largest average heterobeltiosis and the largest number of crosses showing significant favorable heterobeltiosis for 9 out of 18 studied traits (GYPP, GYPH, 100-KW, RPE, PL-E, LANG, SDU, DTA and ASI). For grain yield and 100KW, the most severely stressed environment (HD) showed the highest maximum heterobeltiosis. On the contrary, the LD environment (the non-stressed environment) showed the lowest average favorable heterobeltiosis for most studied yield traits (Table 3). The largest significant favorable heterobeltiosis for GYPH in this study (215.9%) was shown by the testcross (L28 × Sd7) under HD environment (Table 4). This cross also showed the highest significant and favorable heterobeltiosis for grain yield under MD (122.64%).

Table 1.Summary of means and changes (Ch%) from low density (LD) (47,600 plants/ha) to medium density (MD) (71,400 plants/ha) and high density (HD) (95,200 plants/ha) across all studied maize genotypes in 2016 season

Statistic	LD	MD	HD	LD	MD	HD	LD	MD	HD
	Days to 50% anthesis			Anthesis-silking interval (day)			Plant height (cm)		
Mean	59.85	62.36	64.8	2.73	3.07	2.94	219.4	240.76	259.02
Ch%		-4.20**	-8.27**		-12.33**	-7.81**		-9.73**	-18.06**
	Leaf angle (°)			Lower stem diameter (mm)			Upper stem diameter (mm)		
Mean	27.92	22.14	17.26	24.62	21.33	18.46	14.86	12.2	10.2
Ch%		20.69**	38.17**		13.34**	25.03**		17.93**	31.37**
	Leaf area produce 1 g grain (cm^2)			Penetrated light at top-most ear (%)			Chlorophyll concentration index (%)		
Mean	34.93	44.85	54.66	16.4	9.1	6.57	49.09	44.25	38.88
Ch%		-28.40**	-56.49**		44.49**	59.91**		9.86**	20.79**
	Tassel fresh weight (g)			Tassel dry weight (g)			Tassel branch number		
Mean	28.85	22.33	17.27	14.98	12.26	10.05	23	20.35	17.85
Ch%		22.59**	40.14**		18.14**	32.93**		11.50**	22.36**
	Ears/plant			Rows/ear			Kernels/plant		
Mean	1.04	1.0	0.99	14.13	13.22	12.27	554.21	458.01	392.98
Ch%		3.50**	5.02**		6.45**	13.15**		17.36**	29.09**
	100- kernel weight (g)			Grain yield/plant (g)			Grain yield/ha (ton)		
Mean	29.47	27.09	25.36	166.65	126.81	102.2	7.93	9.06	9.71
Ch%		8.07**	13.96**		23.91**	38.68**		-14.23**	-22.69**

Change = 100(LD–MD or HD)/LD,* and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

Table 2. Mean grain yield/ha (ton) of the testcrosses and check cultivars under low (LD), medium (MD) and high (HD) plant density in 2016 season

	Entry number	Sd7			Entry number	SC10			Entry number	Giza2		
		LD	MD	HD		LD	MD	HD		LD	MD	HD
					Testcrosses							
L14	**1**	9.77	10.27	11.93	**24**	12.53	12.57	12.73	**47**	9.83	12.93	13.6
L17	**2**	9.3	10.8	12.27	**25**	10.57	10.83	11.8	**48**	11.27	12.1	15.1
L18	**3**	9.17	11.47	12.97	**26**	10.5	10.7	10.83	**49**	8.73	10.57	11.47
L20	**4**	9.3	10.87	11.6	**27**	7.77	8.43	8.9	**50**	8.23	9.87	10.87
L21	**5**	9.4	11.43	16.63	**28**	9.43	11.1	11.6	**51**	8.53	10.33	12.87
L28	**6**	10.7	13.93	16.9	**29**	9.2	13.17	15.53	**52**	11.17	11.53	13.87

	Entry number	Sd7			Entry number	SC10			Entry number	Giza2		
		LD	**MD**	**HD**		**LD**	**MD**	**HD**		**LD**	**MD**	**HD**
L53	**7**	8.07	8.8	10.2	**30**	7.63	9.37	9.97	**53**	8.9	8.93	9.17
IL15	**8**	9.53	12.1	14.2	**31**	9.67	11.23	12.63	**54**	8.73	9.63	10.1
IL17	**9**	8.13	8.27	8.8	**32**	8.3	9.27	9.43	**55**	7.8	9.33	10.27
IL24	**10**	8.9	9.8	9.67	**33**	10.17	11.9	12.23	**56**	8.43	9.77	9.97
IL51	**11**	9.97	10.7	11.93	**34**	11.87	14.5	14.07	**57**	13.27	14.77	16.43
IL53	**12**	9.9	11.43	11.97	**35**	12.4	10.77	12.37	**58**	9.03	11.33	12.5
IL80	**13**	10.13	10.67	11.47	**36**	9.53	10.6	11.23	**59**	9.1	12.53	12.1
IL84	**14**	8.23	9.77	10.37	**37**	11.57	13.2	16.03	**60**	8.7	9.9	10.9
IL151	**15**	7.5	8.4	8.73	**38**	9.3	10.53	11	**61**	11.4	12.4	13.53
IL171	**16**	7.83	9.8	11.23	**39**	8.1	9.73	10.67	**62**	8.47	10.87	11.6
Sk9	**17**	7.2	11.4	12.07	**40**	7.83	9.57	9.5	**63**	8.37	11.27	11.27
CML67	**18**	7.9	11.33	11.33	**41**	7.5	8.9	10.13	**64**	8.47	9.9	10.17
CML104	**19**	7.63	9.3	10.33	**42**	7.63	8.27	9.4	**65**	8.57	10.87	11.03
Inb174	**20**	8.63	10	10.43	**43**	8.2	9.9	10.7	**66**	7.8	9.17	9.77
Inb176	**21**	8.77	10.47	10.9	**44**	9	9.5	9.87	**67**	9.87	10.43	11.73
Inb208	**22**	5.87	6.93	7.1	**45**	8.33	9.4	10.13	**68**	8.93	10.2	11.33
Inb213	**23**	8.37	8.57	9.9	**46**	8.5	9.83	11.07	**69**	8.03	9.43	10.2
Average		8.7	10.27	11.43		9.37	10.57	11.4	**70**	9.2	10.8	11.73
Checks												
SC2031			8.2				9.9				11	
TWC1100			9.37				10.6				9.53	
SC30K9			8.1				9.7				12.07	
SC30N11			7.77				8.03				9.2	
SC168			11.5				10.7				10.63	
LSD 0.05		D=0.19 G=0.51 D*G=2.39										

Table 3. Estimates of average (Aver), minimum (Min) and maximum (Max) heterobeltiosis and number (No.) of testcrosses showing significant favorable heterobeltiosis for studied traits under low (LD), medium (MD) and high (HD) plant density

	LD	MD	HD	LD	MD	HD	LD	MD	HD
		DTA			**ASI**			**PH**	
Average	0.12	-0.76	-0.46	21.60	7.12	17.19	70.09	50.14	41.14
Max	5.68	3.83	6.42	160.00	50.00	66.67	128.36	85.77	67.07
Min	-5.43	-7.45	-6.53	-50.00	-44.44	-44.44	27.86	28.41	15.78
No.	16	20	26	3	2	2	0	0	0
		LANG			**SDL**			**SDU**	
Average	21.83	15.24	21.00	7.25	4.26	8.37	1.35	-0.24	-4.11
Max	81.46	99.25	125.71	34.51	25.51	29.54	46.26	36.88	40.48
Min	-26.00	-32.79	-34.81	-16.06	-25.16	-28.57	-37.99	-32.84	-35.02
No.	1	9	15	29	31	28	19	22	27
		LA/1gG			**PL-E**			**CCI**	
Average	13.88	-1.33	1.18	-1.46	-22.61	-28.35	2.47	4.43	4.94
Max	111.00	69.19	59.25	307.09	80.70	40.07	13.88	30.73	59.25
Min	-50.02	-42.52	-40.16	-79.66	-68.75	-77.91	-17.14	-22.41	-29.00
No.	14	21	18	8	3	3	22	34	29
		TFW			**TDW**			**TBN**	
Average	32.32	29.21	27.21	24.43	24.92	28.21	19.25	22.68	33.72
Max	234.41	214.68	187.15	179.11	140.09	136.83	53.23	91.42	164.47
Min	-38.66	-48.32	-44.66	-23.49	-41.98	-48.80	-18.01	-16.09	-34.94
No.	3	13	9	3	7	8	2	7	5
		EPP			**RPE**			**KPP**	
Average	-6.10	0.15	-0.54	-1.60	2.05	7.80	5.81	15.24	26.57
Max	17.18	17.00	5.26	15.87	24.14	50.00	41.62	77.34	132.23
Min	-18.18	-4.55	-6.67	-15.28	-8.90	-8.61	-18.60	-10.53	-8.56
No.	6	4	1	8	18	33	28	36	34
		100-KW			**GYPP**			**GYPF**	
Average	7.89	7.99	13.60	16.67	27.38	47.32	16.66	27.39	47.32
Max	36.63	39.02	41.47	69.08	122.64	215.85	69.08	122.64	215.85
Min	-11.04	-11.75	-9.44	-22.80	-15.53	-10.01	-22.80	-15.53	-10.01
No.	37	35	49	35	44	48	35	44	48

Table 4. Estimates of heterobeltiosis (%) for grain yield/ha of testcrosses under low (LD), Medium (MD) and high (HD) plant density conditions

	LD			MD			HD		
	Sd7	**SC10**	**Giza2**	**Sd7**	**SC10**	**Giza2**	**Sd7**	**SC10**	**Giza2**
L14	53.36**	28.65**	25.27**	64.11**	28.68**	31.42**	123.3**	28.44**	36.19**
L17	46.00**	8.49	43.40**	72.66**	11.04**	23.00**	129.6**	19.01**	51.30**
L18	43.64**	7.81	11.13	83.10**	9.59**	7.40*	142.3**	9.43	14.84**
L20	45.63**	-20.17**	4.70	73.44**	-13.59**	0.39	116.6**	-10.01	8.80
L21	47.39**	-2.97	8.58	82.73**	13.50**	5.20	162.6**	17.24**	28.92**
L28	67.96**	-5.46	42.24**	122.64**	34.91**	17.15**	215.9**	56.96**	39.02**
L53	26.28**	-21.72	13.20**	40.73**	-4.12	-9.23**	90.62**	0.75	-8.26
IL15	49.69**	-0.51	11.10	93.16**	14.92**	-2.09	132.2**	27.52**	1.28
IL17	27.75**	-14.80**	-0.75	32.09**	-5.07	-5.27	64.70**	-4.62	3.03
IL24	39.83**	4.37	7.27	56.55**	21.81**	-0.75	80.51**	23.42**	-0.13
IL51	56.07**	21.80**	69.08**	71.11**	48.50**	50.24**	123.4**	41.99**	64.59**
IL53	55.16**	27.41**	15.21**	82.58**	10.13**	15.35**	107.0**	24.87**	25.16**
IL80	58.81**	-2.21	15.76**	70.52**	8.54**	27.30**	114.61**	13.26**	21.34**
IL84	29.19**	18.79**	11.02	55.77**	35.09**	0.58	94.0**	61.90**	9.11
IL151	17.84*	-4.43	45.26**	34.24**	7.73**	25.95**	63.5**	10.96*	35.57**
IL171	22.90**	-16.89**	7.89	56.41**	-0.23	10.55**	109.7**	7.84	16.26**
Sk9	12.95	-19.65**	6.48	81.93**	-1.95	14.40**	125.9**	-4.17	13.04**
CML67	23.81**	-22.80**	7.74	81.16**	-9.01**	0.71	112.0**	2.37	1.74
CML104	19.98**	-21.52**	9.13	48.60**	-15.53**	10.31**	92.99**	-5.03	10.53*
Inb174	35.59**	-15.73**	-0.60	59.56**	1.39	-6.83	94.93**	8.11	-2.06
Inb176	37.52**	-7.48	25.58**	67.35**	-2.76	5.93	103.6**	-0.34	17.59**
Inb208	-7.87	-14.43**	13.81*	10.90	-3.87	3.61	32.78**	2.29	13.55**
Inb213	31.28**	-12.75**	2.33	36.97**	0.77	-4.25	85.12**	11.63*	2.14
Average	36.55	-3.75	17.17	64.27	8.28	9.61	109.47	14.95	17.54

*HD = High density, MD = Medium density, LD = Low density. *and** indicate significant at 0.05 and 0.01 probability level, respectively*

The average heterobeltiosis of testcrosses varied according to the common parent (tester) and plant density (Table 4). In general, the testcrosses of Sd7 (as a common tester) with 23 inbreds showed higher heterobeltiosis than those of SC10 tester and Giza 2 tester under all plant densities. The single cross (inbred × inbred) heterobeltiosis is higher than that of 3-way cross (inbred × single cross) and/or that of inbred × synthetic variety cross. The highest average heterobeltiosis (109.47%) was shown across the 23 testcrosses between the 23 inbreds and the inbred tester Sd7 under high plant density followed by the average heterobeltiosis of same testcrosses under MD (64.27%) and LD (36.55%).

4. DISCUSSION

Maize grain yield of individual plant decreases as the density per unit area increases [5]. The yield decreases as a response to decreasing light and other environmental resources available to each plant [6]. The reduction in GYPP was associated with reductions in all yield components, namely ears/plant (3.50 and 5.02%), kernels/plant (17.36 and 29.09%), kernels/row (8.80 and 15.02%), rows/ear (6.45 and 13.15%) and 100-kernel weight (8.07 and 13.96%)at plant density of 71,400 and 95,200 plants/ha, respectively as compared with 47,600 plants/ha, indicating the importance of number of kernels followed by kernel weight and number of ears per plant as measures of tolerance to high-density. This was previously reported by several investigators [1,25-27]. Reduction in number of kernels/plant was 2.15 and 2.08 fold greater than reduction in 100-kernel weight under elevated plant density (71,400 and 95,200 plants/ha, respectively), which is consistent with previous investigations on high-density stress in maize [13,27-30].

Several studies have investigated the impact of increased plant density on yield and yield components. Lashkari et al. [31] reported that kernels per row, kernels per plant, and ear diameter decreased as plant density increased. Sangoi et al. [26] reported reduced kernels per plant and a 16% decrease in kernel weight as plant density increased from 25,000 (10,000) to 100,000 plants ha^{-1}. Likewise, Hashemi et al. [5] reported that kernels per row and kernel weight both decreased as plant density increased, suggesting a complex interaction between the sink and assimilate supply. Gambin et al. [32] reported drought as well as plant density greatly influence kernel growth rates and final kernel weights. Maddonni et al. [33] reported reduction in kernel size as plant density increased. The reductions in yield components are logic and could be attributed to the increase in competition between plants at higher densities for sun light, nutrients and water as previously reported by several investigators [1,29,30,34-37]

Elevation of plant density from the low density (47,600 plants/ha) to 71,400 and 95,200 plants/ha also resulted in significant reductions of LANG (20.69 and 38.17%, respectively). A significant reduction in leaf angle (as angle decreases, erectness increases) was the result of elevation of plant density in this study, which is in consistency with [1,38,39]. Stem diameter was reduced due to competition among increased number of plants per unit area to 71,400 and 95,200 plants/ha by 17.93 and 31.37%, respectively for the upper stem diameter (SDU) and 13.34 and 25.03%, respectively for the lower stem diameter (SDL) (Table 1).

The increase in plant density caused a significant reduction in chlorophyll concentration index by 9.86% under 47,600 plants/ha and 20.79% under 95,200 plants/ha. This is likely due to reduction in penetrated sun light in the canopy due to crowding of plants under elevated plant density. It also caused subjective reduction in percentage of penetrated light around plant which reached 44.49 and 59.91% at top most ear under MD and HD, respectively.

All three studied tassel traits showed reduction due to increasing plant density from 47,600 to 71,400 and 95,200 plants/ha. Reduction was more pronounced in tassel fresh weight (reduction was 22.59 and 40.14%, respectively). The importance of tassel traits as adaptive traits to high plant density tolerance was reported by some investigators [4].

On the contrary, higher plant densities (71,400 and 95,200 plants/ha) caused a significant increase in grain yield/ha (GYPH) compared to the low-density (47,600 plants/ha) by 14.23 and 22.69%, respectively (Table 1). Widdicombe and Thelen [40] reported significant increases in grain yield as plant density increased from 56,000 (22,000) to 90,000 plants ha^{-1} (36,000 plants/acre).Leaf area to produce 1 g of grain, a photosynthetic efficiency trait, showed an increase as a result of elevating plant density to 71,400 and 47,600 plants/ha by 28.40 and 56.49%, respectively. Increase in leaf area to produce 1 g of grain due to elevated plant density suggests a reduction in photosynthetic efficiency of the leaves of ear and those located above ear leaf, which might be attributed to the reduction in penetrated light because of crowding of plants under the medium and high plant density conditions.

Moreover, higher plant density (71,400 and 95,200 plants/ha) caused a significant increase in plant height (PH) by 9.73 and 18.06%, days to anthesis (DTA) by 4.20 and 8.27%, and anthesis-silking interval (ASI) by 12.33 and 7.81% as compared to low plant density (47,600 plants/ha), respectively. Typically as plant density increases, plant growth rate during reproductive stages may become reduced [32,41,42], leading to delayed pollen shed and silking [38]. As plants intercept red light, far-red light is reflected creating a far-red light enriched environment. This leads to shade avoidance response causing plants to partition more assimilates towards vegetative growth instead of reproductive growth [43]. As a result, plant height increases and stem diameter decreases [26]. Elongation of plant stalks exhibited in this study due to elevating the plant densities could be attributed to lower sun light level and greater competition between plants for sun light. This conclusion was previously reported by other investigators [1,29,30,36,37, 44,45].

In general, the elongation of ASI due to high plant density, in this study was less than that reported by other investigators. Such ASI elongation ranged from 0 to 28 days [46] and from 4 to 10 days [47]. Tokatlis and Koutroubas[38] reported that the time gap between pollen shedding and silking increased from 0 to 9 days by increased plant density from 5 plant m^{-2} to 20 plants m^{-2}. Increased days to anthesis and ASI as symptoms of interplant competition were reported by several investigators [27,48]. These traits are also considered as indicative of barrenness or high-density intolerance [35,36,49]. Several authors indicated that the separation of reproductive organs in maize may also account for this susceptibility to stress at flowering [50]. Delayed silking under conditions of high-density or drought is related to less assimilates being partitioned to growing ears around anthesis, which results in lower ear growth rates, increased ear abortion, and more barren plants [51]. When assimilate supply is limited under stress, it is usually preferentially distributed to the stem and tassel at the expense of ear nutrition, leading to poor pollination and partial or complete failure of seed set. This occurs with practically all kinds of stress, including drought, low soil N and P, excess moisture, low soil pH, iron deficiency and high population density [45]. The term heterobeltiosis has been suggested to describe the increased performance of the hybrid over the better parent [40]. The reason for getting the highest average heterobeltiosis estimates under HD environment could be attributed to the large reduction in grain yield and its components of the parents compared to that of F_1 crosses due to severe stress of high plant density in this environment. It was observed that the magnitude of heterobeltiosis increased by increasing plant density for 8 traits (GYPH, GYPP, 100KW, KPP, RPE, TBN, TDW and CCI). These results are in agreement with those of Weidong and Tollenaar [40], who reported that increasing plant density from 4 to 12 plants m^{-2} resulted in increased heterosis for grain yield of maize. The average heterobeltiosis in GYPH again increased as plant density increased; this is because the yield reduction due to density stress on inbred parents is higher than that on F_1 testcrosses. This conclusion was previously reported by Al-Naggar et al. [35-37,52,53]. It is worthy to note that the best testcrosses in heterobeltiosis for grain yield/ha L28 × Sd7 and L21 × Sd7 (single crosses) under HD and IL51 × Giza 2 (inbred × synthetic), IL84 × SC10 and L28 × SC10 (3-way crosses) under HD and MD (Table 4) were the best crosses in *per se* performance for GYPH under respective density stressed environments (Table 2). Those crosses could therefore be recommended for commercial application under elevated plant density conditions after performing necessary stability evaluation under more locations and years or as good genetic material for maize breeding programs.

5. CONCLUSIONS

Analysis of variance in this study indicated that variance components due to parents vs. F_1 testcrosses were significant ($P \leq 0.01$) for most studied traits under the three density environments, suggesting the presence of significant heterosis for most studied cases. Increasing plant density caused significant reduction in GYPP, EPP, RPE, KPP, 100-KW, LANG, PL-E and CCI and significant increase in GYPH, DTA, ASI, and PH. We conclude that the inbred lines L21, IL15, IL53, L14, Inb176 and the testcrosses L28×Sd7, L21×Sd7, IL51×Giza2, IL84×SC10, L28×SC10, which showed the best GYPH under elevated plant density, can be offered to future plant breeding programs for improving plant density tolerance. The results showed that increasing the plant density increased average heterobeltiosis in grain yield and most of its components.The best testcrosses in heterobeltiosis for grain yield/ha L28 x Sd7 and L21 x Sd7 under HD and IL51 x Giza2, IL84 x SC10 and L28 x SC10 under HD and MD were the best crosses in *per se* performance for GYPH under respective density stressed environments. Those crosses could therefore be recommended for commercial application under elevated plant density conditions and as good genetic material for maize breeding programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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