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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(2): 395-407. **OPEN ACCESS**

Effects of genotype, plant density and their interaction on maize yield and traits related to plant density tolerance

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The use of elevated plant density and the proper genotype would lead to maximizing maize grain productivity per unit land area. Knowledge about differential responses of maize genotypes to elevated plant densities could be an invaluable aid in maize improvement strategies. The main objective of the present investigation was to assess the effects of elevated plant density (PD), genotype and genotype× PD interaction on maize traits related to PD tolerance. A set of 23 inbred lines of maize, were top-crossed to three testers and 69 testcrosses were produced. 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 PD from 47,600 to 71,400 and 95,200 plants/ha caused a significant reduction in grain yield/plant (GYPP), ears/plant, kernels/plant, rows/ear, 100-kernel weight, leaf angle, chlorophyll concentration index, penetrated light at ear, and a significant increase in plant height, days to anthesis, anthesis silking interval and grain yield/ha (GYPH).Significant differences were observed among inbreds and among testcrosses for most studied traits under all plant densities. Rank of inbreds and crosses differed from one density to another due to genotype × density interaction. Under high PD, the highest GYPH and GYPP were obtained by the inbreds L21, IL15, IL53, L14, Inb176 and IL151 and the testcrosses L28 × Sd7, L21 × Sd7, IL51 × Giza2, IL84 × SC10 and L28 × SC10. These materials could be used in future breeding programs for developing PD tolerant varieties of maize. This study concluded that genotype × plant density interaction had significant effects on all studied traits of maize. The optimum density is genotype dependent and should be identified.

Keywords: Corn, G ×E interaction, Testcrosses, Inbreds, Elevated plant density

INTRODUCTION

Maize (*Zea mays* L.) hybrid varieties currently released in Egypt by the National Maize Breeding Program (NMBP) of the Agricultural Research Center (ARC) are bred and grown at low plant density (ca. 57,000 plants ha^{-1}).Such hybrids cannot withstand elevated plant density; their yields decrease by increasing density because they are not tolerant to plant crowding. Maximum yield per unit area may be obtained by growing maize hybrids that can tolerate high plant density up to 100,000 plants ha^{-1} (Huseyin et al. 2003). 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 (Duvick and Cassman, 1999).

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 (Vega et al. 2001). Maize grain yield of individual plant decreases as the density per unit area increases (Hashemi. 2005). The yield decreases as a response to decreasing light and other environmental resources available to each plant (Widdicombe and Thelen, 2002). Reduction in yield is due mainly to fewer cobs (barrenness) (Bunting, 1973), fewer grains per cob (Tetio-Kagho and Gardner 1988), lower grain weight (Poneleit and Egli, 1979), or a combination of these components (Betran et al. 2003).

Maize genotypes differ in tolerance to high plant density (Maddonni et al. 2001). Liu et al.(1993) reported that maize yield differed significantly at varying plant density levels, owing to differences in genetic potential. There is substantial genetic variation for plant density tolerance (PDT) in maize (Sarlangue et al. 2007). Mansfield and Mumm (2014) reported that in U. S. maize germplasm evaluated for plant density tolerance, a subset of traits including leaf angle, 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}.

In general, significant genotype × stress interaction effects are detected for agronomic and yield characteristics in maize (Oikeh et al. 1998 ; Al-Naggar et al. 2011, 2014, 2015, 2016, 2017). Differential responses of maize genotypes to elevated plant density were reported by Al-Naggar et al.(2014, 2015, 2016, 2017). Knowledge about differential responses of maize genotypes to elevated plant densities could be an invaluable aid in maize improvement strategies. A set of 23 inbred lines of maize, were top-crossed to three testers and 69 testcrosses were produced. Inbreds, testers and testcrosses along with five commercial cultivars were evaluated under three plant densities; namely low, medium and high density (47,600, 71,400 and 95,200 plants/ha, respectively).The objectives of the present investigation were (*i*) to assess the effects of elevated plant density, genotype and the genotype × plant density interaction on maize traits and (*ii*) to identify the potential inbreds and testcrosses for future use in plant breeding programs to improve plant density tolerance.

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 22.5 meters above sea level) in 2015 and 2016 seasons.

Plant material

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. Sixteen of them (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 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).

Making the testcrosses

In 2015 summer season, the 23 inbred lines (females) and the three testers (males) were planted at three sowing dates (May 4th, May 11th and May 18th) 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.

Experimental design and treatments

In 2016 season, a 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 TWC1100). 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 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. 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.

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.

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. (1974).The light intensity in (lux) using Lux-meter apparatus 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:

5. Penetrated light at the base of top-most ear (PL-E) (%): It was 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).

6. 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 (http://www.apogeeinstruments.co.uk/apogee-instruments-chlorophyll-content-meter-technical-information/). It was measured on 5 guarded plants/plot.

7. Number of ears plant^{-1} (EPP): It was estimated by dividing number of ears plot^{-1} on number of plants plot^{-1}.

8. Number of rows ear^{-1} (RPE): Using 10 random ears plot^{-1} at harvest.

9. 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}

.**10. Hundred kernel weight (100KW)** (g): Adjusted at 155g water kg^{-1} grain..

11. 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.

12. 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}.

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 ®

(Littell et al. 1996) 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. (1997)using GENSTAT 10th addition windows software. Least significant difference (LSD) was calculated to test significance of differences between means.

RESULTS AND DISCUSSION

3.a. Analysis of variance

Mean squares due to plant density (D) and genotype (G) for all studied traits were significant (P≤ 0.01) (Table 1), indicating that the elevated plant density has obvious effects on all studied traits and the genotypes differed significantly for all studied traits. Mean squares due to G × D interaction were significant (P ≤ 0.01), suggesting that genotypes behaved differently under different plant density conditions for all studied traits and the possibility of selecting genotypes for improved performance under a specific plant density as proposed by several investigators (Al-Naggar et al. 2014,2015,2016, 2017).

3.b. Effects of elevated plant density

Mean grain yield/plant was significantly (P ≤ 0.01) reduced due to elevating plant density from 47,600plants/ha (LD) to 71,400plants/ha (MD) and 95,200plants/ha (HD) by 23.91 and 38.68%, respectively (Table 2).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%), 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. The reduction was more pronounced at the highest density (95,200 plants/ha) and in kernels/plant and less pronounced in 100-kernel weight and ears/plant, indicating the importance of number of kernels followed by kernel weight and number of ears/plant as measures of tolerance to high-density. This conclusion was previously reported by Vega et al. (2001); Sangoi et al. (2002); Al-Naggar et al. (2011, 2015). It is observed that the 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 investigators on high-density stress in maize (Sarlangue et al. 2007; Al-Naggar et al. 2011, 2015, 2016).

Elevation of plant density from 47,600 to 71,400 and 95,200plants/ha also resulted in significant reductions of leaf angle by 20.69 and 38.17%, chlorophyll concentration index by 9.86 and 20.79%,and penetrated light at top most ear by 44.49and 59.91%, respectively. A significant reduction in leaf angle (erectness) is the result of elevation of plant density in this study, which is in consistency with Edmeades et al. (2000); Tokatlis and Koutroubas (2004); Al-Naggar et al. (2012 a,b, 2015). Reduction in chlorophyll concentration index is likely due to reduction in penetrated sun light in the canopy due to crowding of plants under elevated plant density.

On the contrary, elevated plant densities (71,400 and 95,200plants/ha) caused a significant increase in grain yield/ha (GYPH) by 14.23 and 22.69%, plant height (PH) by 9.73 and18.06%, days to anthesis (DTA) by 4.20 and 8.27 % and anthesis-silking interval (ASI) by 12.33 and 7.81 % as compared with low plant density (47,600plant/ha), respectively.

Typically as plant density increases, plant growth rate during reproductive stages may become reduced (Rossini et al. 2011), leading to delayed pollen shed and silking (Tokatlidis and Koutroubas, 2004). 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 (Kebrom and Brutnell, 2007). As a result, plant height increases (Sangoi et al. 2002). 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 (Monneveux et al. 2005; Al-Naggar et al. 2015, 2016, 2017).

The increase in plant density caused a significant increase in grain yield/ha (GYPH), days to anthesis and anthesis-silking interval (ASI). Widdicombe and Thelen (2002) reported significant increases in grain yield as plant density increased from 56,000 to 90,000 plants ha^{-1}. 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 (Du Plessis and Dijkhuis, 1967) and from 4 to 10 days (Bolanos and Edmeades, 1996). Tokatlis and Koutroubas (2004) 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

Table (1). Significance of mean squares of split plot design for 12 traits of 100 maize genotypes under three plant densities in 2016 season.

SOV	df	Mean squares			
		DTA	ASI	PH	LANG
Density (D)	2	**	**	**	**
Genotype (G)	99	**	**	**	**
G x D	198	**	**	**	**
CV%	-	1.17	19.58	4.71	7.86
		PL-E	CCI	EPP	RPE
Density (D)	2	**	**	**	**
Genotype (G)	99	**	**	**	**
G x D	198	**	**	**	**
CV%		13.87	3.61	2.87	2.81
		KPP	100-KW	GYPP	GYPH
Density (D)	2	**	**	**	**
Genotype (G)	99	**	**	**	**
G x D	198	**	**	**	**
CV%		5.31	2.93	6.71	6.21

DTA = Days to 50% anthesis, ASI = Anthesis-silking interval, PH = Plant height, LANG = Leaf angle, PL-E = Penetrated light at top-most ear, CCI = Chlorophyll concentration index, EPP = ears/ plant, RPE = rows/ear, KPP = kernels/plant, 100-KW = 100-kernel weight, GYPP = grain yield/ plant, GYPH = grain yield/ ha, ** indicate significance at 0.01 probability level.

Table2. Summary of means and changes (Ch%) from 47,600 plants/ha (LD) to 71,400 plants/ha (MD) and 95,200 plants/ha (HD) across all studied maize genotypes (100) 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 (°)			**Penetrated light at top-most ear (%)**			**Chlorophyll concentration index (%)**		
Mean	27.92	22.14	17.26	16.4	9.1	6.57	49.09	44.25	38.88
Ch%		20.69**	38.17**		44.49**	59.91**		9.86**	20.79**
	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	9.73
Ch%		8.07**	13.96**		23.91**	38.68**		-14.23**	22.69**

**** indicate significance at 0.01 probability level.Ch%= 100(LD-MD or HD)/LD.**

Table 3. Means of the highest and lowest inbred line for studied traits under low (LD), medium (MD) and high (HD) plant density in 2016 season.

LD		MD		HD		LD		MD		HD	
Days to 50% anthesis (day)						**Anthesis-silking interval (day)**					
IL84	65.00	**L17**	66.67	**L28**	71.00	**L14**	3.67	**Inb208**	4.00	**L14**	3.33
L21	57.67	**CML104**	61.00	**Inb176**	64.00	**IL53**	2.00	**L18**	2.33	**L53**	2.00
Plant height (cm)						**Leaf angle (°)**					
L17	177.8	**IL84**	192.2	**IL80**	232.2	**IL151**	36.11	**CML104**	29.44	**L14**	25.56
Sk9	111.7	**IL53**	148.3	**Inb176**	175.6	**L17**	21.11	**IL84**	14.89	**L17**	10.56
Penetrated light at top-most ear (%)						**Chlorophyll concentration index (%)**					
L20	40.93	**L20**	14.38	**CML67**	10.62	**IL15**	52.64	**IL15**	47.31	**L18**	40.13
IL84	9.26	**IL84**	7.52	**Inb208**	5.30	**IL53**	35.13	**IL53**	29.73	**CML7**	25.12
Ears per plant						**Rows per ear**					
L28	1.22	**L17**	1.05	**L18**	1.02	**L53**	16.00	**IL15**	13.98	**Inb16**	13.00
Inb213	1.00	**Inb213**	0.90	**CML67**	0.85	**L18**	10.89	**L18**	10.42	**Inb23**	9.00
Kernels per plant						**100-kernel weight (g)**					
IL15	499.6	**IL15**	410.0	**IL15**	329.3	**IL53**	32.20	**IL53**	30.13	**IL53**	24.70
Inb208	245.3	**Inb213**	167.6	**Inb213**	141.1	**Inb14**	18.20	**Inb174**	18.13	**Inb174**	17.00
Grain yield per plant (g)						**Grain yield per hectare (ton)**					
L21	129.9	**L21**	84.5	**L21**	66.5	**L21**	6.00	L21	6.04	**L21**	6.33
Inb208	54.0	**Inb208**	39.1	**Inb208**	29.5	**Inb28**	2.57	**CML104**	2.82	**Inb208**	2.81

Table 4.The highest and lowest testcross for studied traits under low (LD), medium (MD) and high (HD) plant density in 2016 season.

LD		MD		HD		LD		MD		HD	
Days to 50% anthesis (day)						**Anthesis-silking interval (day)**					
IL84×Sd7	62.33	**IL84×Sd7**	65	**IL84×Sd7**	67.33	**Inb20×Sd7**	4.33	**IL171×Sd7**	4	**IL171×SC10**	4.33
L14×Gz2	56.67	**CML67×Sd7**	58	**IL151×Gz2**	60.67	**Inb176×SC10**	1.33	**Inb213×Gz2**	1.67	**IL17×Gz2**	1.67
Plant height (cm)						**Leaf angle (°)**					
IL151×Sd7	275.6	**IL151×Sd7**	289.4	**IL151×Sd7**	310	**IL171×Sd7**	41.33	**Inb174×SC10**	31.67	**IL84×Sd7**	26.33
IL84×Sd7	200	**L53×SC10**	244.4	**Inb176×Gz2**	250.6	**IL80×Gz2**	16.44	**IL80×Gz2**	13.67	**IL80×SC10**	9.78
Penetrated light at top-most ear (%)						**Chlorophyll concentration index (%)**					
L14×Gz2	53.02	**L14×Gz2**	16.04	**IL17×Gz2**	9.2	**L14×SC10**	58.14	**IL53×Gz2**	54.06	**L14×SC10**	49.64
IL84×Sd7	9.43	**IL51×Sd7**	4.99	**L53×Sd7**	3.65	**CML67×SC10**	42.91	**L20×Gz2**	35.84	**L20×Gz2**	29.83
Ears per plant						**Rows per ear**					
L18×SC10	1.27	**L21×SC10**	1.17	**Inb213×SC10**	1.05	**L28×Sd7**	16.22	**L28×Sd7**	16	**L28×Sd7**	15
Inb213×SC10	1	**Inb213×SC10**	1	**L20×SC10**	0.93	**CML6×SC10**	12.67	**IL17×SC10**	12.44	**IL151×Sd7**	11.15
Kernels per plant						**100-kernel weight (g)**					
IL151×Gz2	811.5	**L28×Sd7**	640	**L28×Sd7**	585	**IL51×Gz2**	37.3	**Sk9×Sd7**	34.62	**L28×SC10**	33.6
Inb208×Sd7	474.4	**Inb208×Sd7**	386.7	**Inb208×Sd7**	322	**IL151×Sd7**	25.12	**IL151×Sd7**	24.02	**IL24×Sd7**	21.5
Grain yield per plant (g)						**Grain yield per hectare (ton)**					
IL51×Gz2	278.8	**IL51×Gz2**	206.9	**L28×Sd7**	177.4	**IL51×Gz2**	13.28	**IL51×Gz2**	14.78	**L28×Sd7**	16.9
Inb208×Sd7	123.4	**Inb208×Sd7**	97.2	**Inb20×Sd7**	74.59	**IL84×SC10**	11.56	**L28×SC10**	13.18	**L28×SC10**	15.55

Table 5. Mean grain yield/ha (ton) of the 23 inbred lines and three testers under low (LD), medium (MD) and high (HD) plant density in 2016 season.

	LD	MD	Ch%	HD	Ch%
Inbreds					
L14	4.12	4.58	-11.1**	5.23	-26.7**
L17	3.75	4.64	-23.6**	4.87	-29.7**
L18	3.87	3.89	-0.6	4.06	-4.9
L20	4.77	4.96	-4.0	3.80	20.3**
L21	6.00	6.04	-0.6	6.33	-5.6*
L28	4.63	4.81	-3.8	4.97	-7.2*
L53	4.00	4.00	-0.1	4.35	-8.8*
IL15	5.89	5.98	-1.6	6.12	-3.9*
IL24	4.61	4.67	-1.2	3.96	14.0**
IL51	4.07	5.09	-24.9**	3.86	5.2*
IL53	5.49	5.75	-4.6*	5.78	-5.2*
IL80	4.83	4.88	-0.9	4.88	-0.9
IL84	4.02	4.41	-9.7*	4.55	-13.1**
IL151	4.72	4.79	-1.4	4.98	-5.5*
IL171	4.33	4.42	-2.0	3.90	9.9**
Sk9	3.88	4.96	-27.7**	4.21	-8.3*
CML67	3.86	3.46	10.5**	3.24	16.0**
CML104	2.62	2.82	-7.7*	3.03	-15.9**
Inb174	3.11	3.13	-0.4	3.14	-1.0
Inb176	4.99	5.06	-1.5	5.18	-3.8
Inb208	2.57	2.79	-8.7*	2.81	-9.2*
IL17	3.99	5.30	-32.7**	4.53	-13.6**
Inb213	2.97	2.99	-0.8	3.19	-7.4*
Testers					
Sd7	6.38	6.26	1.78	5.35	16.1**
SC10	9.73	9.77	-0.38	9.91	-1.79
Giza 2	7.85	9.84	-25.3**	9.98	-27.1**

*** and ** indicate significance at 0.05 and 0.01 probability levels, respectively. Ch%= 100(LD-MD or HD)/LD.**

plants m^{-2}. Increased days to silking, days to anthesis and ASI as symptoms of interplant competition were reported by several investigators (Helland, 2012; Al-Naggar et al. 2012b). Several authors indicated that the separation of reproductive organs in maize may also account for this susceptibility to stress at flowering (Haegele et al. 2013). 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 (Monneveux et al. 2005).

3.c. Effects of maize genotype

3.c.1.Effects of inbreds

A wide variation was observed among inbreds for all studied traits under all plant densities. The inbreds showing the highest and lowest mean under low (LD), medium (MD) and high (HD) plant densities for each trait are presented in Table (3).Under LD, the inbred L21 gave the highest grain yield per plant (129.9 g) and per hectare (6.00 ton) while the lowest wasInb208 for GYPP (54.0g) and GYPH (2.5ton). The highest mean for EPP, RPE, KPP and 100-KW under LD was shown by L28, L53, IL15 and IL53, respectively. The lowest mean for EPP, RPE, KPP and 100-

KW under LD was shown by the inbreds Inb213, L18, Inb208 and Inb174, respectively. The earliest inbred in DTA under LD (57.67) was L21, while the latest inbred (65.00) was IL84 with a difference of about 7.33 days. Under LD, the longest ASI (3.67 day) was shown by L14, and the shortest ASI (2.00 day) was shown by IL53. Inbreds showed also great variation in plant height under LD (non-stress); the shortest one was Sk9 (111.7 cm) and the tallest one wasL17 (177.8 cm). Leaf angle of inbreds under non-stress ranged from 21.11^{o} (L17) to 36.11^{o} (IL151). Inbreds ranged for penetrated light at ear position under LD from 9.26% (IL84) to 40.93% (L20), and for chlorophyll concentration index from 35.13% (IL53) to 52.64% (IL15).

3.c.2. Effects of testcrosses

Testcrosses showed a wide variation for all studied traits under all plant densities. The testcrosses showing the highest and lowest means under each plant density for each studied trait are presented in Table (4). Under no stress (LD), the testcross IL51 x Giza2 gave the highest GYPP (278.8 g) and the highest GYPH (13.28 ton), while the lowest in grain yield was Inb208 ×Sd7 for GYPP (123.49 g) and IL84 × SC10 for GYPH (11.56 ton). The testcrosses L18 × SC10, L28 × Sd7, IL151×Giza2 and IL51×Giza2 were the highest in EPP, RPE, KPP and 100-KW, respectively under no density stress. The lowest mean under LD for EPP, RPE, KPP and 100-KW was shown by the testcrosses Inb213 × SC10, Cml67 x SC10, Inb208 × Sd7 and IL151 × Giza2, respectively. Under LD, the earliest testcross in DTA (56.67) was L14×Giza2, while the latest testcross (62.33) was IL84 x Sd7 with a difference of 5.66 days. The longest ASI (4.33 day) under LD was shown by Inb208× Sd7, and the shortest one (2.1 day) was shown by Inb176×SC10. Testcrosses showed also great variation in plant height under LD; the shortest one was IL84×Sd7 (200.0cm) and the tallest one was IL151 x Sd7 (275.6cm). Leaf angle of testcrosses under LD ranged from 16.44^{o} (IL80 × Giza2) to 41.33^{o}(Inb171 × Sd7). Testcrosses under LD ranged for penetrated light at ear position from 9.43% (IL84× Sd7) to 53.02% (L14 × Giza2) and for chlorophyll concentration index from 42.91% (CML67×SC10) to 58.14% (L14 × SC10).In general, the testcrosses were earlier than inbred lines for days to anthesis which reached under high density 3.6 day. Plants of the testcrosses were taller than their inbred parents, indicating the role of heterosis in plant height trait. Variation expressed as a range in PH was obvious among inbred parents and consequently among testcrosses. Penetrated light at top-most ear was higher in inbred parents than testcrosses. The reduction in penetrated light was higher in testcrosses than their inbred parents. This is logic, since the hybrids are more vigorous than their inbred parents due to heterosis phenomenon. Chlorophyll concentration index was higher in testcrosses than inbreds; variation for this trait was higher among inbreds than among hybrids.

3.d. Inbred × plant density interaction

Ranks of inbreds differed from one plant density to another due to the significance of genotype x plant density interaction for all studied traits (Table 1).For grain yield/plant (Table 3), the best inbreds under LD, MD and HD was L21, while the worst inbred was Inb208. The best inbred under all plant densities was IL15 for KPP, IL51 for KPR and IL53 for 100-KW. For EPP, the best inbred was L18 under HD, L17 under MD and L28 under LD (Table 3). On the contrary, the worst inbreds were Inb174 under all plant densities for 100-KW, Inb213 under MD and HD and Inb208 under LD for KPP, inbreds CML104, Inb213 and Inb174 for KPR under LD, MD and HD, respectively, inbreds L18under LD and MD and Inb213 under HD for rows/earand Inb213 under LD and MD and CML67 under HD for ears/plant. The best inbreds under LD, MD and HD were L21, CML104, Inb176 for earliness (DTA), IL53, L18 andL53 for short anthesis-silking interval, Sk9, IL53 and Inb176 for short plant (PH) and L17 for leaf angle, L20,L20 and CML67 for penetrated light at ear, and IL15, IL15 and L18 for chlorophyll concentration index. These inbred lines could be used in the future breeding programs as sources of adaptive traits for plant density tolerance in maize.The highest grain yield/ha under high plant density was obtained by the inbred lines L21, IL15, IL53, L14, Inb176 and IL151 (Table 5). The inbreds L21, IL51 and IL53 occupied the 1st , 2nd and 3rd rank, respectively for GYPH under all plant densities, but L14 ranked 11th , 14th and 4th , Inb176 ranked 4th , 6th and 5th and IL151 ranked 7th , 11th and 6th under LD, MD and HD, respectively. On the contrary, the worst inbreds for GYPH were Inb208, CML104, Inb174, Inb213 and CML67 under all plant densities (LD, MD and HD) conditions. Differential responses of maize inbreds to elevated plant density were mentioned in our previous reports (Al-Naggar et al*.* 2014, 2015, 2016, 2017). For the testers, the inbred Sd7 showed the lowest GYPH under all

Table 6. Mean grain yield/ha (ton) of the testcrosses and the check cultivars under low (LD), medium (MD) and high (HD) plant density in 2016 season.

	Sd7			SC10			Giza 2		
	LD	MD	HD	LD	MD	HD	LD	MD	HD
L14	9.77	10.27	11.93	12.53	12.57	12.73	9.83	12.93	13.60
L17	9.30	10.80	12.27	10.57	10.83	11.80	11.27	12.10	15.10
L18	9.17	11.47	12.97	10.50	10.70	10.83	8.73	10.57	11.47
L20	9.30	10.87	11.60	7.77	8.43	8.90	8.23	9.87	10.87
L21	9.40	11.43	16.63	9.43	11.10	11.60	8.53	10.33	12.87
L28	10.70	13.93	16.90	9.20	13.17	15.53	11.17	11.53	13.87
L53	8.07	8.80	10.20	7.63	9.37	9.97	8.90	8.93	9.17
IL15	9.53	12.10	14.20	9.67	11.23	12.63	8.73	9.63	10.10
IL17	8.13	8.27	8.80	8.30	9.27	9.43	7.80	9.33	10.27
IL24	8.90	9.80	9.67	10.17	11.90	12.23	8.43	9.77	9.97
IL51	9.97	10.70	11.93	11.87	14.50	14.07	13.27	14.77	16.43
IL53	9.90	11.43	11.97	12.40	10.77	12.37	9.03	11.33	12.50
IL80	10.13	10.67	11.47	9.53	10.60	11.23	9.10	12.53	12.10
IL84	8.23	9.77	10.37	11.57	13.20	16.03	8.70	9.90	10.90
IL151	7.50	8.40	8.73	9.30	10.53	11.00	11.40	12.40	13.53
IL171	7.83	9.80	11.23	8.10	9.73	10.67	8.47	10.87	11.60
Sk9	7.20	11.40	12.07	7.83	9.57	9.50	8.37	11.27	11.27
CML67	7.90	11.33	11.33	7.50	8.90	10.13	8.47	9.90	10.17
CML104	7.63	9.30	10.33	7.63	8.27	9.40	8.57	10.87	11.03
Inb174	8.63	10.00	10.43	8.20	9.90	10.70	7.80	9.17	9.77
Inb176	8.77	10.47	10.90	9.00	9.50	9.87	9.87	10.43	11.73
Inb208	5.87	6.93	7.10	8.33	9.40	10.13	8.93	10.20	11.33
Inb213	8.37	8.57	9.90	8.50	9.83	11.07	8.03	9.43	10.20
Average	8.70	10.27	11.43	9.37	10.57	11.40	9.20	10.80	11.73
Checks									
SC2031	8.20				9.90				11.00
TWC1100	9.37				10.60				9.53
SC30K9	8.10				9.70				12.07
SC30N11	7.77				8.03				9.20
SC168	11.50				10.70				10.63
LSD 0.05	D=0.19 G=.51 D*G=2.39								

plant densities; while the single cross SC10 was the highest under LD only and Giza 2 (a synthetic cultivar) was the highest under both MD and HD environments. The superiority in GYPH could be attributed to heterosis for the tester SC10 and adaptation to stress conditions for the tester Giza 2 (a heterozygous and heterogeneous population).

3.e. Testcross x plant density interaction

Ranks of testcrosses differed from one plant density to another due to the significance of genotype x plant density interaction for all studied traits (Table 1). For grain yield/plant and grain yield/ha(Table 4), the best testcross was IL51 x Giza2 under LD and MD and L28 x Sd7 under HD, while the worst testcross was Inb208 x Sd7 under all plant densities. The best testcross under all plant densities was L28 x Sd7 for RPE. The best testcross under LD and MD together was IL51 x Giza2 for GYPP, L14 x Giza2 for PL-E and L80 x Giza2 for LANG. The best testcrosses under MD and HD together were L28 x Sd7 for KPP. On the contrary, the worst testcrosses under all plant densities wereL28 × SC10 for GYPH, Inb208 x Sd7 for GYPP and KPP. Under LD and MD, the best testcross was IL151 × Sd7 for 100-KW and Inb213 x SC10 for EPP.

The best testcrosses under LD, MD and HD were L14×Giza2, CML67×Sd7 and IL151×Giza2 for earliness (DTA), Inb176×SC10, Inb213×Giza2 and IL17×Giza2 for short anthesis-silking interval, IL84×Sd7, L53×SC10 and Inb176×Giza2 for short plant (PH), L14×SC10, IL53×Giza2 and L14×SC10 for chlorophyll concentration index, and L18 × SC10, L21×SC10 and Inb213×SC10 for EPP. The best testcross under HD only was IL17 × Giza2 for PL-E and IL80 x SC10 for LANG.

Mean grain yield/ha under 3 plant density levels for each testcross and check cultivar is presented in Table (6).The highest grain yield/ha and grain yield/plant 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 followed byIL51×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 HD (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.

Significant differences among inbred parents and among testcrosses for GYPH were clearly exhibited under each plant density. The GYPH of testcrosses was 2.15, 2.35 and 2.62 fold higher than that of their inbred parents under low, medium and high plant density, respectively. This increase is due to heterosis in grain yield per unit land area. The increase of GYPH due to elevated plant density was higher for testcrosses (16.04 and 26.68%) than inbreds (6.48 and 3.96%) under medium and high plant density, respectively. This conclusion is in agreement with Has et al. (2008); (Al-Naggar et al. 2012a, 2015), who reported that hybrids were more adapted to high plant density than inbred lines of maize. On the contrary, Monneveux et al. (2005) reported that lines yielded more than open-pollinated varieties and hybrids under high plant population density, probably because of lower vigor and lower competition between plants. Differences in conclusions regarding the effects of high density may be attributed to the differences in the genetic background of the plant materials and/or climatic conditions prevailing through the growing seasons of different studies.

It is worthy to note that some inbreds yielded more GYPH as plant density is increased while others exhibited no increase or even yield loss. Similarly, most of testcrosses yielded more GYPH as plant density is increased, while others exhibited no increase. This conclusion is in agreement with findings of Hashemi et al. (2005); Monneveux et al. (2005). Therefore, the optimum density is genotype dependent and should be identified for each maize genotype.

CONCLUSION

This study concluded that the two factors (genotype and plant density) and their interaction had significant effects on all studied traits of maize. 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. The study concluded that the inbred lines L21, IL15, IL53, L14 and Inb176 and the testcrosses L28×Sd7, L21×Sd7, IL51×Giza2, IL84×SC10 and L28×SC10, were the best in GYPH under elevated plant density and can be offered to future plant breeding programs for improving traits implicated in the expression of plant density tolerance. The optimum density is genotype dependent and should be identified.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors acknowledge Agronomy Department, Faculty of Agriculture, Cairo University and National Research Center (NRC), Egypt for providing technical and financial support for completing this research. Thanks are also given to Prof. Dr. Mazhar Fawzi from Faculty of Agriculture, Cairo University for revising the manuscript and Dr. Abdul-Samad Younis from

NRC for helping in statistical analysis of data.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author Ahmed Medhat M.Al-Naggar designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors Reda A. Shabana and Mosaad S. Hassanein managed the literature searches. Author Ahmed M.A. Metwally managed the experimental process and performed data analyses. All authors read and approved the final manuscript.

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