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Research Article

Slopes of linear and quadratic relationships between maize (*Zea mays* L.) crop yield of inbreds and testcrosses on elevated plant density

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Abstract Development of density tolerant maize hybrids and knowing their optimum plant density are prerequisite for maximizing grain productivity from unit land area. The objectives of this study were to evaluate linear and quadratic relationships between grain yield response and plant density among genetically diverse inbreds and testcrosses and to determine their optimum plant density for maximizing the grain yield per unit area. Twenty three inbreds and their 69 testcrosses with three testers were evaluated under three plant densities; low (LD), medium (MD), and high (HD) density (47,600, 71,400 and 95,200 plants/ha, respectively). Out of 23 inbreds, seven were classified as density efficient and responsive (E-R), four as efficient and non-responsive (E-NR), three as inefficient and responsive (I-R) and nine as inefficient and non-responsive (I-NR). Out of 69 testcrosses, 23, 4, 4 and 38 were E-R, E-NR, I-R and I-NR, respectively. The inbreds E-R showed linear regression of slight increase and quadratic regression showed optimum density of 83,300 plants/ha. The inbreds E-NR exhibited linear increase with optimum density of 92,820 plants/ha. Inbreds I-R and I-NR showed linear regression of decrease with optimum density of 71,400 and 80,920 plants/ha, respectively. The testcross groups E-R and E-NR, exhibited linear regression of increase very close to quadratic regression with optimum density of 95,200 plants/ha (HD). Testcross groups I-R and I-NR showed linear regression with slight increase or no change and optimum density of 47,600 plants/ha (LD). Inbreds and testcrosses of groups E-R and E-NR showed high and significant slopes of linear regression, so they carry alleles of plant density tolerance and can help in future breeding programs for improving maize.

Keywords Regression, Corn, Unit area productivity, Optimum plant density

Introduction

According to FAOSTAT (2014) [1], Egypt ranks sixth in the world with respect to average productivity (ca 8 tons ha^{-1}) after Germany, France, Canada, Italy and USA, where average yield for these countries reached > 12 ton ha^{-1}. Hybrid varieties currently released in Egypt by the National Maize Breeding Program (NMBP) are bred and grown at low plant density (ca. 57,000 plants ha^{-1}), *i.e.* much less than that used in such developed countries, because Egyptian hybrids cannot withstand higher plant densities, due to their tallness, one-eared, decumbent leaf and large-size type plants. Growing commercial varieties of maize in Egypt, released locally by NMBP, at high plant density (HPD) causes a drastic reduction in grain yield per plant and sometimes a decrease in grain yield per land unit area. On the contrary, modern maize hybrids in USA and developed countries are characterized with high yielding ability from unit area under HPD, due to their high-density adaptive traits, such as early silking, short anthesis silking interval (ASI), less barren stalks and prolificacy [2]. Radenovic *et al.* (2007) [3] pointed out that maize genotypes with erect leaves are very desirable for increasing the population density due to better light interception. This may be one of the important reasons of getting lower maize productivity in Egypt from unit land area than that in the developed countries, which use high-density tolerant varieties. One of the potential methods to maximize maize productivity per unit land area in Egypt is through growing density tolerant hybrids under the optimum plant density for each hybrid.

There is substantial genetic variation for plant density tolerance (PDT) in maize [4]. Some hybrids yield more as plant density is increased while others exhibit no increase or even yield loss [5-8]. The presence of genotypic differences in plant density tolerance would help plant breeders in initiating successful breeding programs to improve such a complicated character. Liu *et al.* (2004) [9] reported that maize yield differed significantly at varying plant density levels, owing to differences in genetic potential. Differential responses of maize genotypes to high plant density was reported by some investigators [10-15]. There are some reports on the quadratic response of grain yield on the elevated plant density [13-14, 16-20]. Knowledge about differential responses of maize genotypes to elevated plant densities and the optimum density for each genotype could be an invaluable aid in maize improvement strategies. The objectives of the present investigation were: (i) to identify the plant density efficient and responsive inbreds and hybrids, (ii) to evaluate linear and quadratic relationships between grain yield response and plant density among genetically diverse inbreds and hybrids and (iii) to determine the optimum plant density for maximizing the grain yield per unit area of studied inbreds and testcrosses.

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.

Plant materials: Twenty three diverse 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 in 2015 season 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). Serial numbers and names of inbreds and testcrosses are presented in Table (1).

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 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. 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.

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. Number of ears plant^{-1} (EPP): It was estimated by dividing number of ears plot^{-1} on number of plants plot^{-1}. **2. Number of rows ear^{-1} (RPE)**: Using 10 random ears plot^{-1} at harvest. **3. 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}. **4. Hundred kernel weight (100KW)** (g): Adjusted at 155g water kg^{-1} grain. **5. 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. **6. 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 in RCB arrangement was performed on the basis of individual plot observation using the MIXED procedure of SAS ® [21]. 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.* (1997) [22]. Linear regression of grain yield on plant density was performed for each individual inbred and hybrid to assess yield responses to increased plant density, evaluating both linear and quadratic responses across the three plant densities.

Results and Discussion

a. Analysis of variance

Mean squares due to plant density (D) and genotypes (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.

Table 1: Significance of mean squares of split plot design for six traits of studied maize genotypes under three plant densities in 2016 season

SOV	df	Mean squares					
		EPP	RPE	KPP	100-KW	GYPP	GYPH
Density (D)	2	**	**	**	**	**	**
Genotype (G)	99	**	**	**	**	**	**
G x D	198	**	**	**	**	**	**
CV%		2.87	2.81	5.31	2.93	6.71	6.21

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.

Mean squares due to G × D interaction were significant (P ≤ 0.01), suggesting that genotypes behaved differently under different plant densities for all studied traits and the possibility of selecting genotypes for improved performance under a specific plant density as proposed by previous studies [23-26].

b. Mean performance under elevated plant density

Mean grain yield/plant was significantly (P ≤ 0.01) reduced due to elevating plant density from 47,600 plants/ha (LD) to 71,400 plants/ha (MD) and 95,200 plants/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) [27], Sangoi *et al.* (2002) [28] and Al-Naggar *et al.* [10-14]. 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 [4, 10-17].

Table 2: 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
		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.**

On the contrary, the increase in plant density caused a significant increase in grain yield/ha (GYPH). Widdicombe and Thelen (2002) [29] reported significant increases in grain yield as plant density increased from 56,000 to 90,000 plants ha^{-1}. The highest grain yield/ha (GYPH) under high plant density was obtained by the inbred lines (females) L21, IL15, IL53, L14, Inb176 and IL151 (Table 4). 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 [10-17].

Table 3: Mean grain yield/ha (ton) of the inbreds and testers under low (LD), medium (MD) and high (HD) plant density in 2016 season

Serial number	Inbred	LD	MD	HD	Serial number	Inbred	LD	MD	HD
1	**L14**	4.12	4.58	5.23	**15**	**IL151**	4.72	4.79	4.98
2	**L17**	3.75	4.64	4.87	**16**	**IL171**	4.33	4.42	3.9
3	**L18**	3.87	3.89	4.06	**17**	**Sk9**	3.88	4.96	4.21
4	**L20**	4.77	4.96	3.8	**18**	**CML67**	3.86	3.46	3.24
5	**L21**	6	6.04	6.33	**19**	**CML104**	2.62	2.82	3.03
6	**L28**	4.63	4.81	4.97	**20**	**Inb174**	3.11	3.13	3.14
7	**L53**	4	4	4.35	**21**	**Inb176**	4.99	5.06	5.18
8	**IL15**	5.89	5.98	6.12	**22**	**Inb208**	2.57	2.79	2.81
9	**IL17**	3.99	5.3	4.53	**23**	**Inb213**	2.97	2.99	3.19
10	**IL24**	4.61	4.67	3.96		**Mean**	4.22	4.5	4.39
11	**IL51**	4.07	5.09	3.86			**Testers**		
12	**IL53**	5.49	5.75	5.78	**1**	**Sd7**	6.38	6.26	5.35
13	**IL80**	4.83	4.88	4.88	**2**	**SC10**	9.73	9.77	9.91
14	**IL84**	4.02	4.41	4.55	**3**	**Giza2**	7.85	9.84	9.98
	LSD 0.05				D=0.19G=0.51D*G=2.39				

For the testers, the inbred Sd7 showed the lowest GYPH under all plant densities; while the single cross SC10 showed the highest GYPH under LD only and Giza2 (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 (heterozygous and heterogeneous population).

Mean grain yield/ha under 3 plant density levels for each testcross and check cultivar is presented in Table (4). 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.

Table 4: Mean grain yield/ha (ton) of the testcrosses and check cultivars under low (LD), medium (MD) and high (HD) plant density in 2016 season

	Serial number	**Sd7**			**Serial number**	**SC10**			**Serial 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
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					

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.

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 [30] and Al-Naggar *et al.*, 2012a [25] and 2015 [10], who reported that hybrids were more adapted to high plant density than inbred lines of maize. On the contrary, Monneveux *et al.* (2005) [8] 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 concluded that some inbreds yielded more GYPH as plant density is increased while others exhibited no increase or even yield loss. Moreover, 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) [7] and Monneveux *et al.* (2005) [8]. Therefore, the optimum density is genotype dependent and should be identified for each maize genotype.

b. Grouping inbreds and testcrosses

Mean grain yield per hectare of 23 inbreds and 69 testcrosses under high density (HD) was plotted against same trait of the same genotypes under LD (Figs. 1 and 2) where numbers from 1 to 23 refer to inbred names and numbers from 1 to 69 refer to testcross names (Tables 3 and 4), which made it possible to distinguish between efficient and inefficient genotypes on the basis of above-average and below-average grain yield under high-D and responsive and non-responsive genotypes on the basis of above-average and below-average grain yield under low-D, according to Sattelmacher *et al.* (1994) [31], Worku *et al.* (2007) [32] and Al-Naggar *et al.* (2015) [10].

According to Fageria and Baligar (1994 and 1997a and b) [33-35] genotypes (progenies) belonging to the 1st group "efficient and responsive" (above all) and 2nd group "efficient and non-responsive" (to a lesser extent) appear to be the most desirable materials for breeding programs that deal with adaptation to high density stress.

According to efficiency under high density and responsiveness to low density, studied inbreds and testcrosses were classified into four groups, *i.e.* density efficient and responsive, density efficient and non-responsive, density non-efficient and responsive and density non-efficient and non-responsive based on GYPF trait. The seven inbreds [No.5 (L21), No.8 (IL15), No.12 (IL53), No.21 (Inb176), No.13 (IL80), No.6 (L28) and No.15 (IL151)] were classified as density efficient and responsive, while four inbreds [No.1 (L14), No.2 (L17), No. 14 (IL84) and No. 9 (IL17)] were classified as density efficient and non-responsive (Fig.1). The three inbreds [No.4 (L20), No.10 (IL24) and No.6 (L28)] were classified as inefficient and responsive. The rest of inbreds (9) were classified as inefficient and non-responsive [No. 22, 19, 23, 20, 18, 11, 3, 17 and 7].

Number of the F_1 testcrosses that belong to the group efficient and responsive (E-R) was 23 and included crosses No. 6 (L28 × Sd7), No.5 (L21 x Sd7), No. 57 (IL51 x Giza2), No. 37 (IL 84 × SC10), No.29 (L28 x SC10) and No. 48 (L17 × Giza2), that have the highest GYPF under high-D and Low-D, *i.e.* they could be considered as the most density efficient and the most responsive genotypes in this study (Fig. 2). The number of crosses that belong to the group density efficient and non-responsive (E-NR) was 4 and included No. 51 (L21 x Giza2), No.17 (Sk9 x Sd7), No. 62 (IL171 x Giza2), and No.58 (IL53 x Giza2). The number of crosses that belong to inefficient and responsive group (I-R) was 4 and included No. 26 (L18 x SC10), No.36 (IL80 x SC10), No.38 (IL151 x SC10) and No.13 (IL80 x Sd7). The rest of testcrosses (38 crosses) belonged to the inefficient and non-responsive (I-NR) group and included crosses No. 22 (Inb208 x Sd7), No. 15 (IL151 x Sd7) and No. 27 (L20 x SC10).

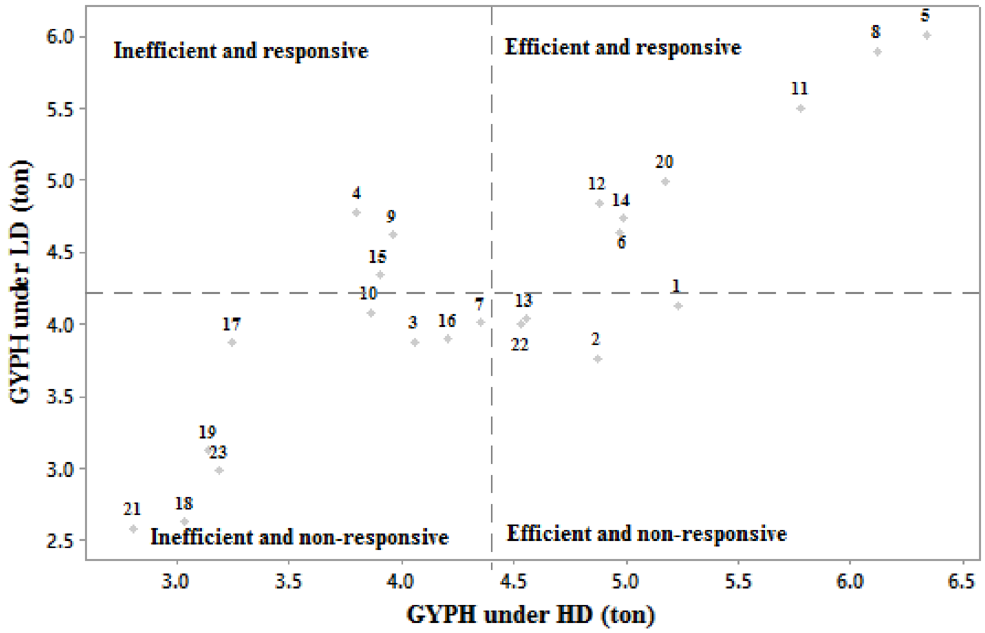


Figure 1: Relationships between GYPF of 23 parental inbreds under high- (HD) and low- (LD) density. Broken lines represent mean of GYPF (numbers from 1 to 23 refer to parental inbred names).

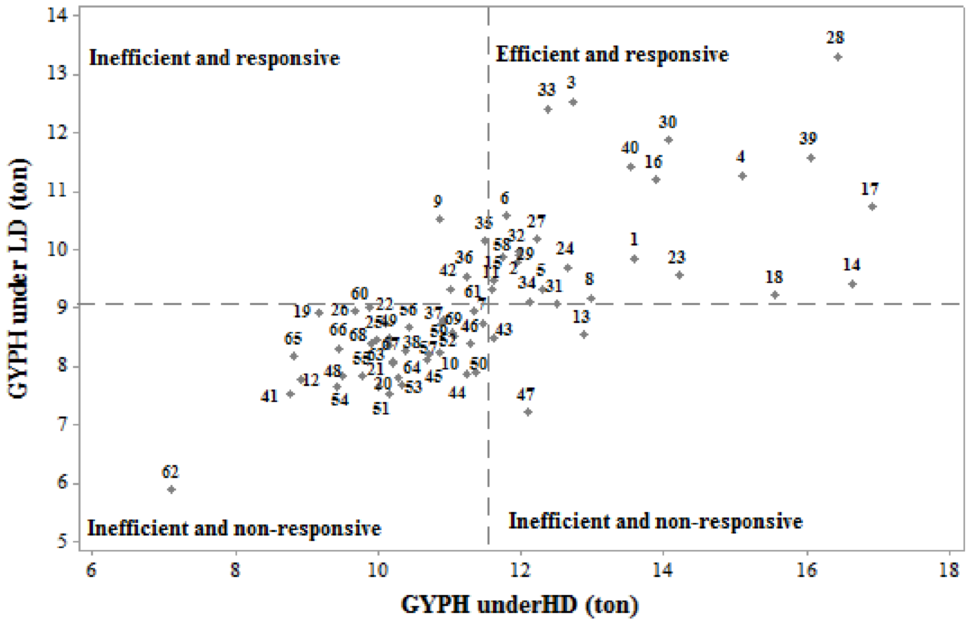


Figure 2: Relationships between GYPF of 69 testcrosses under high- (HD) and low- (LD) density. Broken lines represent mean of GYPH (numbers from 1 to 69 refer to testcross names).

c. Regression of grain yield on elevated levels of plant density

To further evaluate the relationship between grain yield and plant density, linear as well as quadratic responses were graphed for each of selected inbreds and testcrosses (Figs. 9 through 11). Whereas a significant linear response estimates an increase in grain yield proportional to a given increase in plant density, a quadratic response can provide insight as to the optimum plant density range for a specific inbred or hybrid as well as the point at which there is no longer yield gain per unit area due to increased plant density and yield loss per unit area may begin to occur [36].

c.1. Relationship of grain yield and plant density among inbreds

The relationships between grain yield/ha of the groups of inbreds and plant densities are illustrated in Figs. (3 through 6). For the first group of inbreds (efficient and responsive), which includes seven inbreds, it is obvious that the quadratic response is close to the linear response, with a tendency of slight increase in most inbreds and nearly no change in IL80 and Inb176. It is clear that this group of inbreds showed an optimum GYPF at plant density around 83,300 plants/ha.

For the second group of inbreds (efficient and non-responsive), which includes four inbreds (L14, L17, IL84 and IL17), it is clear that the quadratic regression on plant density is very close to linear regression of increase (Fig. 4) in three inbreds (L14, L17 and IL84) with an optimum plant density close to the high density (92,820 plants/ha). The inbred IL17 showed a quadratic response with an optimum density of about 78,540 plants/ha.

For the third group of inbreds (inefficient and responsive) (Fig. 5), which includes three inbreds (L20, IL24 and L28), the linear response of decrease was obvious in inbreds L20 and IL24, with an optimum density of about 64,260 plants/ha, but the linear regression of increase was close to quadratic regression for the inbred L28, with an optimum plant density of about 80,920 plants/ha.

For the fourth group of inbreds (inefficient and nonresponsive), which includes nine inbreds (Fig. 6), two inbreds (CML67 and IL51) showed linear regression of decrease, but the rest of inbreds in this group showed a linear regression of slight increase. The quadratic regression of the inbreds of this group indicated an optimum density around 71,400 plants/ha.

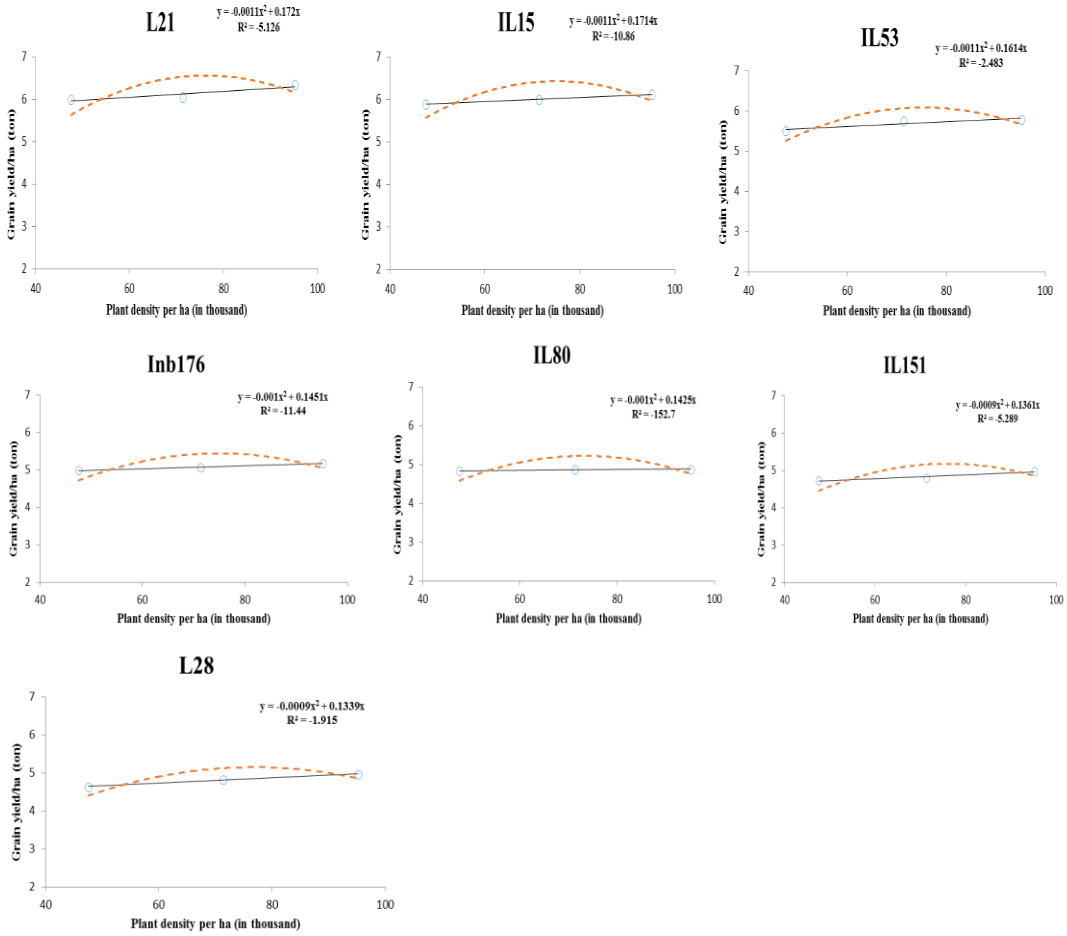


Figure 3: Regression of GYPF of seven efficient-responsive (E-R) inbreds on elevated plant density

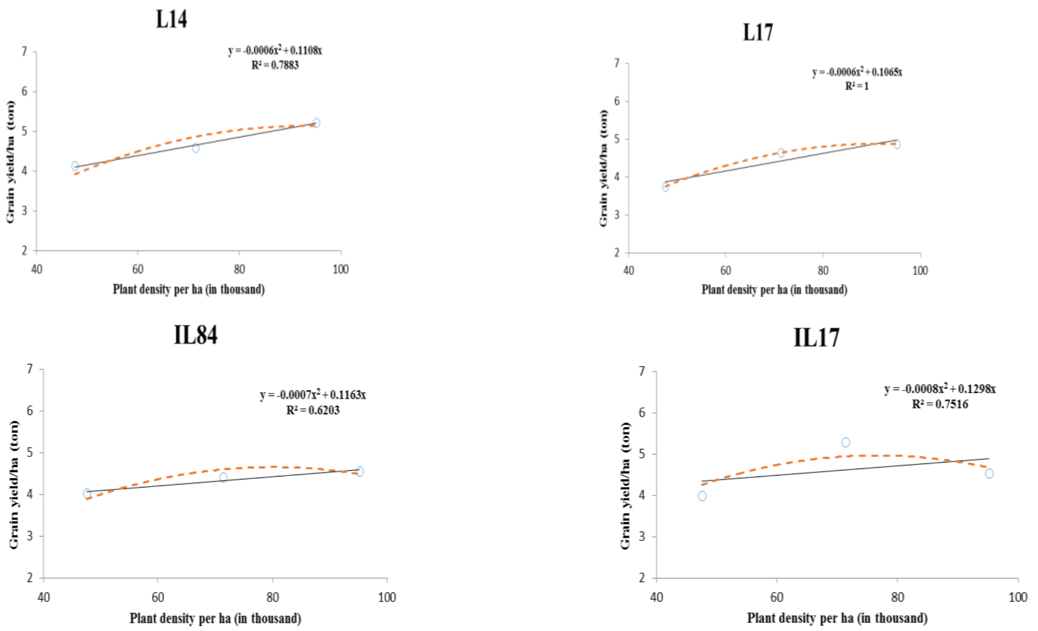


Figure 4: Regression of GYPF of four efficient-nonresponsive (E-NR) inbreds on plant density

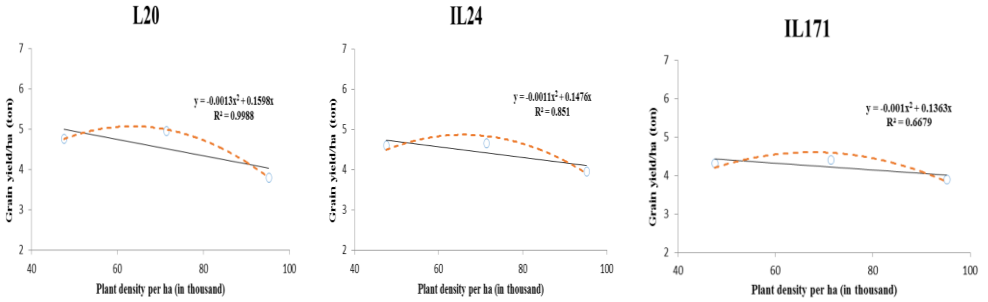
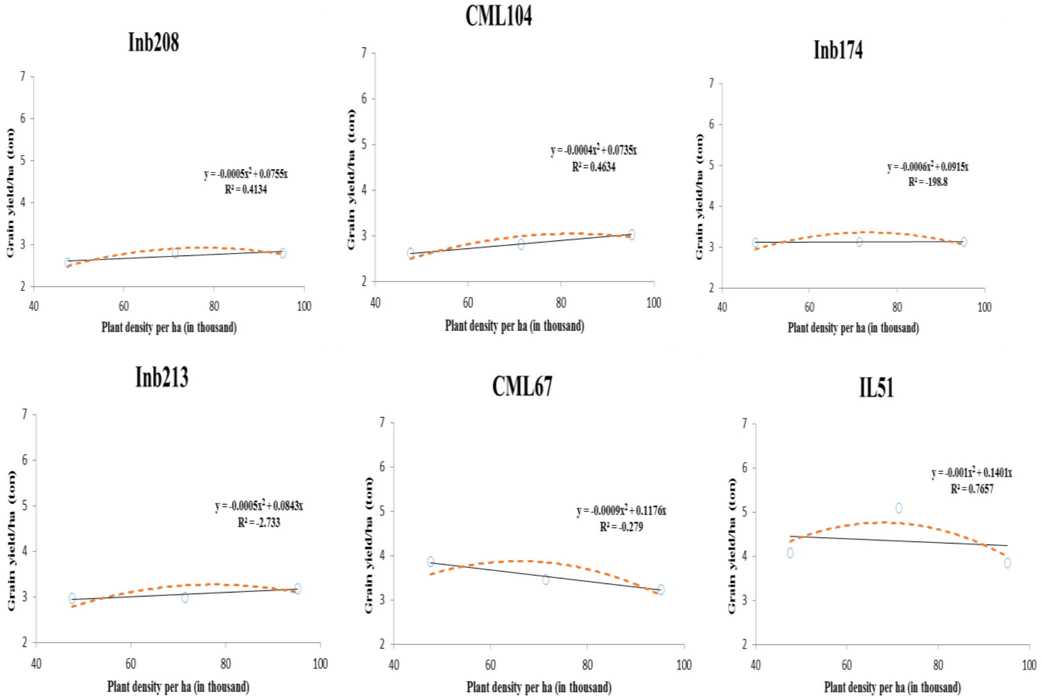


Figure 5: Regression of GYPF of three inefficient-responsive (I-R) inbreds on plant density



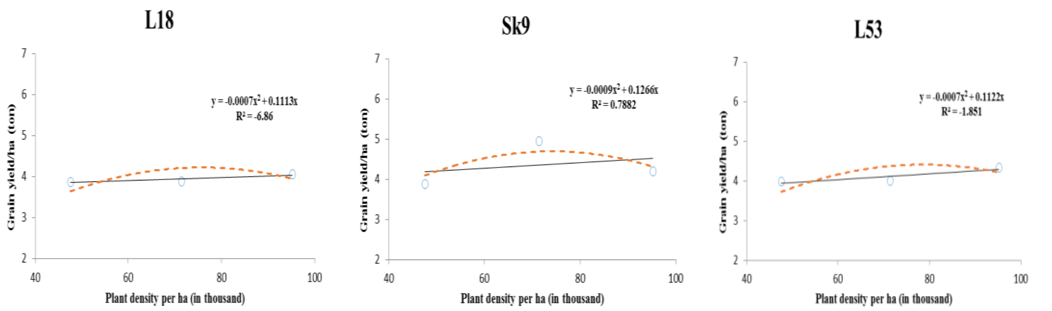


Figure 6: Regression of GYPF of nine inefficient-nonresponsive (I-NR) inbreds on plant density

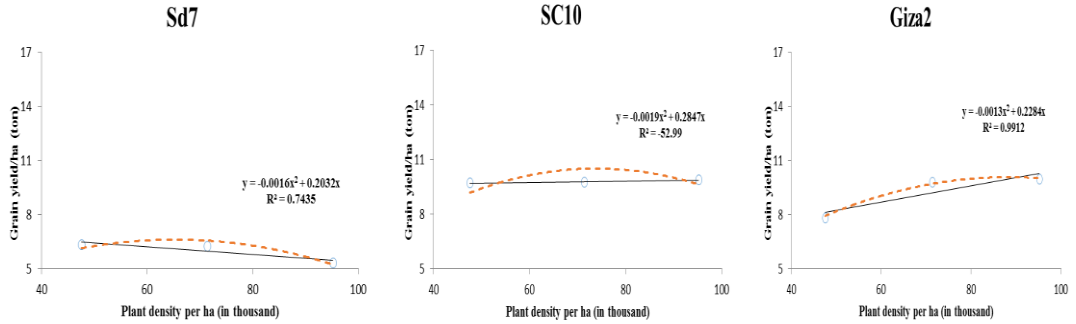


Figure 7: Regression of GYPF of three testers on plant density

For the tester (male) parents (Fig. 7), Sd7, SC10 and Giza 2, the linear regression of increase was obvious in Giza 2, where GYPF increased by increasing plant density, while that of SC10 showed no increase and no decrease, *i.e.* no change by increasing plant density. On the contrary, the inbred tester Sd7 showed a linear regression of increase in GYPF by increasing plant density. The quadratic response indicated that the optimum density was 71,400 plants/ha for Sd7, 78,540 plants/ha for SC10 and 89,250 plants/ha for Giza 2. This means that out of the three, testers only Giza 2 tester parent possess alleles of PDT.

The inbred lines used in this study could be grouped into three classes. The first class showing a significant increase in GYPH due to increasing plant density (especially L14, L17 and IL84), i.e. showing substantial tolerance to plant densities up to 95,200 plants/ha based on grain yield performance, probably due to the existence of alleles of PDT. The second class includes those showing a significant decrease in GYPF due to increasing plant density (such as CML67, IL51, L20, IL24 and IL80). The third class includes those showing neither significant increase nor significant decrease in GYPF (such as Inb174 and Inb176). It seems that the inbreds of the last two classes do not possess alleles of PDT. Moreover, with regard to optimum plant density, the 23 inbreds could be classified into three groups. The first group (high density as an optimum density) such as L14, L17 and IL84. The second group (medium density as an optimum density) such as IL17, IL51 and Sk9. The third group (low density as an optimum density) such as L20 and IL24. It is therefore concluded that some inbreds yielded more GYPH as plant density is increased while others exhibited no increase or even yield loss. This conclusion is in agreement with findings of Hashemi *et al*. (2005) [7], Monneveux *et al*. (2005) [8] and Mansfield and Mumm (2013) [36]. Therefore, the optimum density is genotype dependent and should be identified for each maize genotype.

c.2. Relationship of grain yield and plant density among testcrosses

For each of the 69 test hybrids, grain yield was regressed on plant density to evaluate each genotype's capacity to produce more grain as the number of plants per hectare was increased. A positive slope (b_i) expressing the linear relationship would signal the ability to achieve higher yields with higher plant densities, with significant and higher b_i values, suggesting the presence of favorable alleles for PDT. Forty hybrids exhibited a significant or highly significant positive change in grain yield as plant density increased (Table 5). In addition, one hybrid exhibited a highly significant negative change in grain yield as plant density increased. And 28 hybrids exhibited no significant response of grain yield across plant densities. Mean b_i's were calculated across hybrid combinations for each inbred (Table 5). Nine inbreds exhibited mean slopes >1, that is, L14, L17, L18, L21, L28, IL15, IL51, IL80 and IL84, which represent five of the ten parents of five top performing hybrids at high density. Notably, the five highest yielding hybrids at high plant density had one of these inbreds as a parent. The other three parents exhibited mean slopes in the 0.90 to 1.01 range. Notably, Giza 2 (tester) exhibited a mean slope of 1.01 and high performance for grain yield at high density (Table 4).

Table 5: Slopes (bi) reflecting the linear relationship between grain yield and plant density for hybrid combination and mean slopes across all hybrid combinations for each inbred and each tester. Slopes were tested for difference from zero

	Sd7	**SC10**	**Giza2**	**Mean**
L14	1.02**	1.18**	1.17**	1.12
L17	1.04**	1.04**	1.23**	1.10
L18	1.08**	1.00**	0.98*	1.02
L20	1.01**	0.79	0.93	0.91
L21	1.24**	1.02**	1.03**	1.10
L28	1.35**	1.24**	1.16**	1.25
L53	0.86	0.86	0.84	0.85
IL15	1.16**	1.07**	0.90	1.04
IL17	0.79	0.85	0.88	0.84
IL24	0.89	1.09**	0.89	0.96
IL51	1.03**	1.28**	1.41**	1.24
IL53	1.05**	-1.10**	1.06**	0.34
IL80	1.02**	0.99*	1.08**	1.03
IL84	0.90	1.31**	0.94	1.05
IL151	0.78	0.97*	1.18**	0.98
IL171	0.93	0.91	0.99*	0.94
Sk9	1.00**	0.85	0.99*	0.95
CML67	0.98*	0.85	0.90	0.91
CML104	0.87	0.8.0	0.97*	0.88
Inb174	0.92	0.92	0.85	0.90
Inb176	0.96*	0.89	1.01**	0.95
Inb208	0.63	0.88	0.97*	0.83
Inb213	0.85	0.94	0.88	0.89
Mean	0.97	0.90	1.01	

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

The relationship between plant density and GYPF of the studied groups of F_1 crosses is illustrated in Figs. (8 through 11). Because the number of testcrosses is high, representative hybrids from each group were illustrated separately. In general, the group efficient-responsive (E-R) showed that the curvilinear regression was very close to the linear regression in all the seven testcrosses. This was more pronounced in L21 x Sd7 followed by L28 x Sd7 and L28 x SC10 and less pronounced in IL51 X Giza2. The linear response of these crosses of group E-R was positive and in the direction of increase by increasing plant density. The quadratic regression in the testcrosses of E-R group, showed an optimum density of 95,200 plants/ha for all testcrosses, except for the testcross IL51 x Giza2 which was 90,440 plants/ha. Most of the crosses of the group E-R possess alleles of plant density tolerance and are therefore tolerant to elevated plant density.

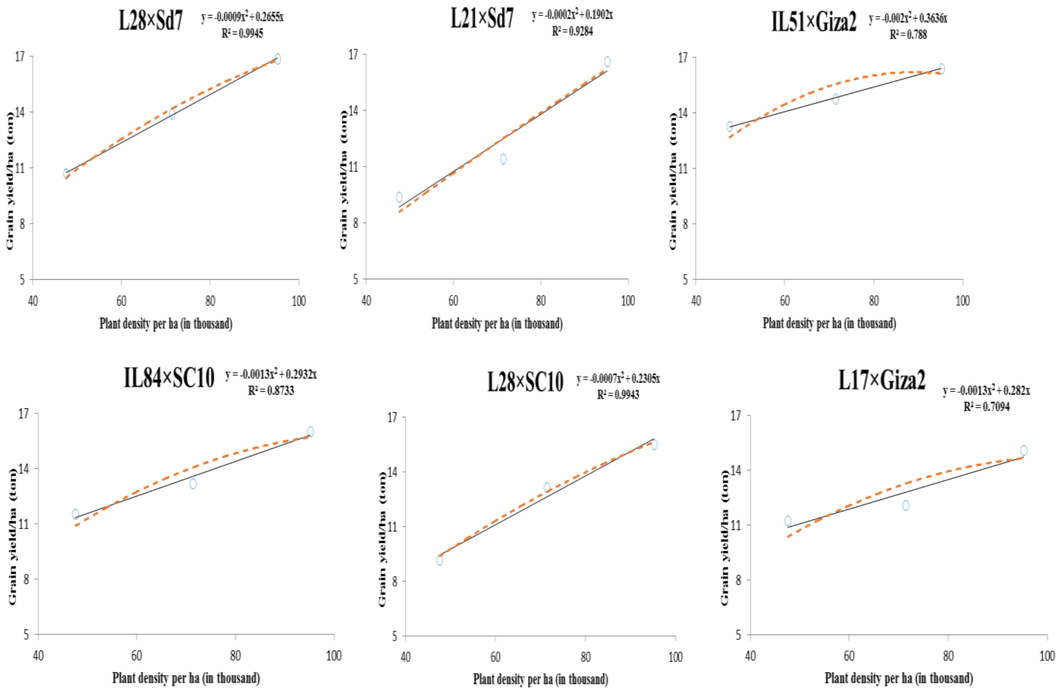


Figure 8: Quadratic and linear regression of GYPF of six efficient-responsive (E-R) testcrosses on elevated plant density

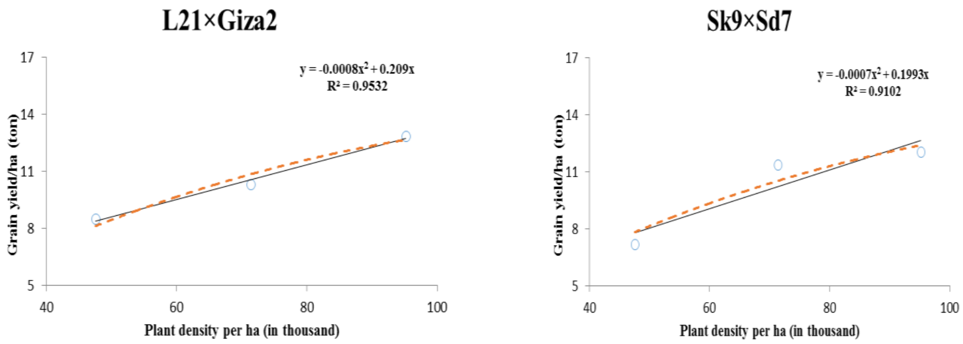


Figure 9: Quadratic and linear regression of GYPF of two efficient-nonresponsive (E-NR) testcrosses on elevated plant density

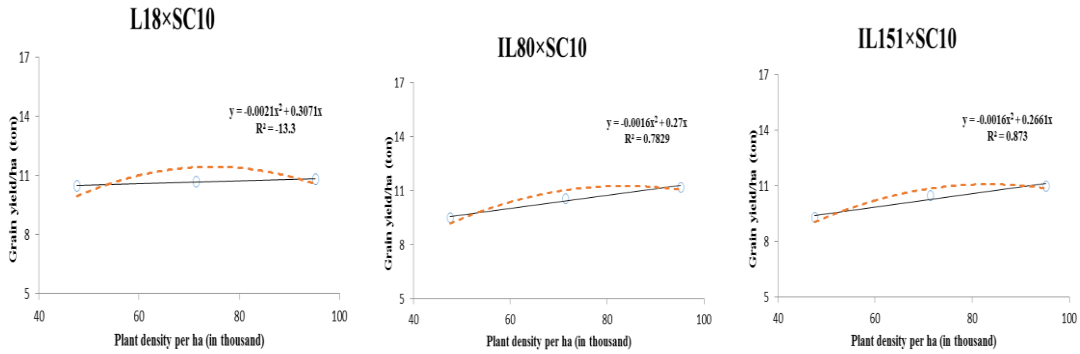


Figure 10: Quadratic and linear regression of GYPF of three inefficient-responsive (I-R) testcrosses on elevated plant density

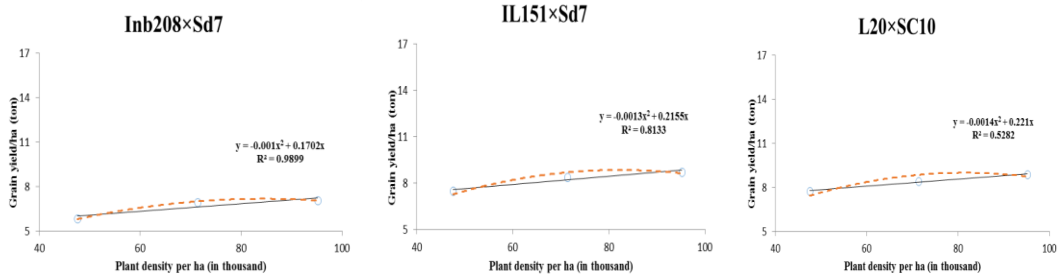


Figure 11: Quadratic and linear regression of GYPF of three inefficient-nonresponsive (I-NR) testcross on elevated plant density

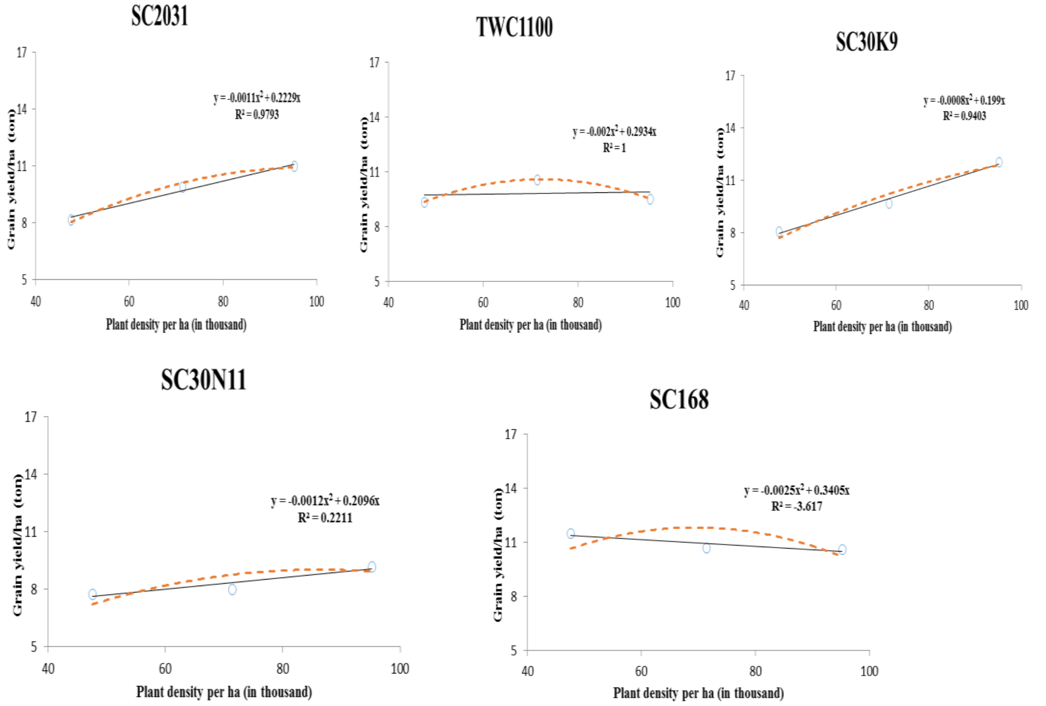


Figure 12: Quadratic and linear regression of GYPF of five check cultivars on elevated plant density

For the group efficient non-responsive (E-NR), the test crosses L21 x Giza2 and Sk9 x Sd7 exhibited linear regression of increase very close to quadratic regression with an optimum density of 95,200 plants/ha. For the group inefficient-responsive (I-R), the testcrosses L14 x SC10 and IL51 x SC10 showed weak curvilinear regression very close to linear regression with no significant change from one density to another; the optimum density for these two crosses was low density (47,600 plants/ha). For the representative of inefficient-non-responsive (I-NR) group, the cross Inb208 x Sd7 showed near linear regression, with slight increase in GYPF due to increase in plant density; the optimum density was close to 88,060 plants/ha. Again, like in inbreds, it could be concluded that some testcrosses yielded more GYPH as plant density is increased while others exhibited no increase. Therefore, the optimum density should be identified for each maize testcross.

For the check cultivars (Fig. 12), the linear response of increase was very strong for SC30K9 followed by SC2031 and SC30N11. Linear response of no change by increasing plant density was exhibited by the check cultivar TWC1100. On the contrary, the linear response of the check cultivar SC168 was in the negative direction, i.e. GYPF decreased by increasing plant density. The optimum density was LD (47,600 plants/ha) for SC 168, MD (71,400 plants/ha) for TWC 1100 and HD (95,200 plants/ha) for SC 2031, SC 30K9 and SC 30 N11.

In this context, Shapiro and Wortmann (2006) [37] reported that the corn grain yield typically exhibits a quadratic response to plant density with a near-linear increase across a range of low densities, a gradually decreasing rate of yield increase relative to density increase and finally a yield plateau at some relatively high plant density. Most recently, Clark (2013) [38] mentioned that there was little yield response to N rates above 90 kg N/ha at the low and high densities, as there was a curvilinear increase until yield plateau at the low density (8.1 Mg/ha at 133 kg N/ha) and the high density (5.9 Mg/ha at 102 kg N/ha). He added that response to N was greatest at the middle density (83,980 plants/ ha), as there was a quadratic response with maximum yield at 188 kg N/ha (8.7 Mg/ha). He found that across the low-stress environments, the lowest density (44,460 plants/ha) responded little to N rates above 90 kg N/ha, while there was greater response to N rates at the middle density (13.5 Mg/ha at 162 kg N/ha) and the high density (13.4 Mg/ha at 174 kg N/ha). He concluded that no support was found for the idea that increasing corn yield requires increases in both plant density and N rate above rates typically used. A recent Indiana study [39] showed that under large ranges of plant density (54,000-104,000 plants/ha) and N rate (0-330 kg N/ha), higher densities required more N. This seems logic, given the prevailing belief that high yields require more plants, and that more plants require more N. There is substantial genetic variation for plant density tolerance (PDT) in maize [4]. Some hybrids yield more as plant density is increased while others exhibit no increase or even yield loss [5-8]. The relationship between plant density and grain yield was assessed by Mansfield and Mumm (2013) [36] for each hybrid, with a wide range of responses observed. They reported that five hybrids showed substantial tolerance to plant densities ≥116,000 plants/ha based on grain yield performance. Understanding the complexities of hybrid interaction with plant density will require additional work.

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