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Quadratic Regression of Grain Protein, Oil and Starch Contents and Yields of Different Maize (*Zea mays* L.) Genotypes on Elevated Plant Density

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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 MMMA and MAA managed the literature searches. Author ASMY managed the experimental process and performed data analysis. All authors read and approved the final manuscript.

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ABSTRACT

The main objective of the present investigation was to identify the optimum plant density for the best performance of grain protein, oil and starch contents and yields of different maize genotypes *via* quadratic regression functions. Diallel crosses among diverse maize inbreds were evaluated in the field for grain protein (GPC), oil (GOC) and starch (GSC) contents, grain (GYPH), protein (PYPH), oil (OYPH), and starch (SYPH) yield per hectare under three plant densities, *i.e.*47,600, 71,400 and 95,200 plants/ha, using a split plot design with 3 replications in two growing seasons. Results combined across seasons revealed that elevated density from 47,600 to 95,200 plants/ha caused a significant reduction in GYPP, GPC and a significant increase in GYPH, PYPH, OYPH, SYPH and GOC. Regression functions revealed that for GYPH, PYPH, OYPH and SYPH, the response of the four groups of genotypes (tolerant and sensitive inbreds and hybrids) to the elevated plant density showed a quadratic response of increase in hybrids and near-linear response of increase in inbreds, but the response of increase was stronger for tolerant than sensitive groups. A quadratic response of increase was observed for GPC of sensitive hybrids,

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GOC of sensitive and tolerant hybrids and GSC of tolerant inbreds and hybrids. On the contrary, a quadratic response of decrease was observed for GPC of tolerant inbreds and hybrids, GOC of tolerant and sensitive inbreds, GSC of sensitive hybrids and GYPP of the four groups.

Keywords: Grain composition; regression functions; optimum plant density; density tolerant maize stress tolerance.

1. INTRODUCTION

Although high plant density results in interplant competition (especially for light, water and nutrients), which affects vegetative and reproductive growth of maize (*Zea mays* L.) causing reduction in both plant grain yield and grain quality characteristics, the use of high-density would overcome the negative impacts of such competition and lead to maximizing maize productivity from the same unit area [1-5].

Globally, maize contributes 15% (representing more than 50 million ton) of the protein and 20% of the calories derived from food crops in the world's diet [6]. In many developing countries in Latin America Africa and Asia, maize is the stable food and sometimes the only source of protein in diet. Because maize is a relevant food source, the quantification of the grain constituents with a nutritional role is important for the best exploitation of the different genotypes. Specifically, different industries have different requirements of maize for their particular use. The wet milling industry would like soft starch, and low protein content, while hard starch is required for dry milling and for mass production. The feed industry would gain value from maize with increased energy content, *i.e.* maize with higher oil content, and from increased protein content and a better amino acid balance.

Grain quality is an important objective in corn breeding [7-11]. In corn grain, a typical hybrid cultivar contains approximately 4% oil, 9% protein, 73% starch, and 14% other constituents (mostly fiber). The existence of satisfactory genetic variability is the first prerequisite for successful selection for a given trait. The information on genetic variability of the chemical structure of maize grain is abundant, and studies are numerous [10,12-17] for oil content and [14,15,18-21] for protein content), but breeding progress has been limited by an apparent inverse genetic relationship between maize grain yield and each of oil and protein concentration [21-24].

In general, significant environment and genotype × environment interaction effects are detected for grain protein and oil contents in maize [9-11, 21,24-26]. Among the environment factors that influence grain constituents, temperature, availability of water and nitrogen in the soil are the most important [18-27]. Knowledge about genetic diversity and deferential response of genotypes to abiotic stresses could be an invaluable aid in maize improvement strategies. Studies have documented genetic and phenotypic variability for grain composition traits in maize [28-34]. There are reports on the effects of water stress on the chemical composition of maize grains [35,36], but little work has been reported about the effect of high density stress (light, water and nutrient stresses) on maize kernel composition in different genotypes of maize. Moreover, there are some reports on the quadratic response of grain yield on the elevated plant density [3-5,37-39], but to the best of our knowledge, the work on response of grain composition traits on the elevated density is very scarce. Therefore, the objectives of the present investigation were to: (*i*) study the effects of high plant density on maize grain protein, oil and starch contents and yields (*ii*) determine the role of genotype and the interaction of genotype x density in the response of these characters to elevated plant density and (*iii*) identify the optimum plant density for the best performance of grain quality traits of different maize genotypes *via* quadratic regression functions.

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 2012, 2013 and 2014 seasons.

2.1 Plant Material

Based on the results of previous experiments [11], six maize (*Zea mays* L.) inbred lines in the 8^{th}selfed generation (S_8), showing clear differences in performance and general combining ability for grain yield/hectare under high plant density, were chosen in this study to be used as parents of diallel crosses (Table 1).

2.2 Making F_1 Diallel Crosses

In 2012 season, all possible diallel crosses (except reciprocals) were made among the six parents, so seeds of 15 direct F_1 crosses were obtained. Seeds of the 6 parents were also increased by selfing in the same season (2012) to obtain enough seeds of the inbreds in the 9^{th}selfed generation (S_9 seed).

2.3 Evaluation of Parents and F_1`s

Two field evaluation experiments were carried out in 2013 and 2014 seasons. Each experiment included 15 F_1 crosses, their 6 parents and 2 check cultivars, namely SC 130(white), obtained from the Agricultural Research Center (ARC) and SC 2055(yellow) obtained from Hi-Tech Company-Egypt. Evaluation in each season was carried out three plant densities, namely low-, medium- and high-plant density (D) (47,600, 71,400 and 95,200 plants/ha, respectively).A split plot design in randomized complete blocks (RCB) arrangement with three replications was used. Main plots were devoted to plant density (high-D, medium-D and low-D). Sub plots were devoted to 23 maize genotypes (6 parents, 15 F_1`s and 2 checks). Each sub plot consisted of one ridge of 4 m long and 0.7 m width, *i.e.* the experimental plot area was 2.8 m^2. Seeds were sown in hills at 15, 20 and 30 cm apart, thereafter (before the 1^{st} irrigation) were thinned to one plant/hill to achieve the 3 plant densities, *i.e.* 47,600, 71,400 and 95,200 plants/ha, respectively. Sowing date was on May 5 and May 8 in 2013 and 2014 seasons, respectively.

The soil analysis of the experimental soil as an average of the two growing seasons 2013 and 2014, indicated that the soil is clay loam (4.00% coarse sand, 30.90% fine sand, 31.20% silt, and 33.90% clay), the pH (paste extract) is 7.73, the EC is 1.91 dSm-1, soil bulk density is 1.2 g cm-3, calcium carbonate is 3.47%, organic matter is 2.09%, the available nutrient in mg kg-1 are Nitrogen (34.20), Phosphorous (8.86), Potassium (242), hot water extractable B (0.49), DTPA - extractable Zn (0.52), DTPA - extractable Mn (0.75) and DTPA - extractable Fe (3.17). Meteorological variables in the 2013 and 2014 growing seasons 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℃, maximum temperature was 35.7, 35.97, 34.93 and 37.07℃ and relative humidity was 47.0, 53.0, 60.33 and 60.67%, respectively, in 2013 season. In 2014 season, mean temperature was 26.1, 28.5, 29.1 and 29.9℃, maximum temperature was 38.8, 35.2, 35.6 and 36.4℃ and relative humidity was 32.8, 35.2, 35.6 and 36.4%, respectively. Precipitation was nil in all months of maize growing season for both seasons. All other agricultural practices were followed according to the recommendations of ARC, Egypt. Sibbing was carried out in each entry for the purpose of determining the grain contents of protein, oil and starch.

2.4 Data Recorded

1-Grain yield per plant (GYPP in g) estimated by dividing the grain yield per plot (adjusted at 15.5% grain moisture) on number of plants/plot at harvest. 2-Grain yield per hectare (GYPH) in ton, by adjusting grain yield/plot to grain yield per

Table 1. Designation, origin and most important traits of 6 inbred lines (L) used for making diallel crosses of this study

Inbred designation	Origin	Institution (country)	Prolificacy	Productivity under high density	Leaf angle
L20-Y	SC 30N11	Pion. Int.Co.	Prolific	High	Erect
L53-W	SC 30K8	Pion. Int.Co.	Prolific	High	Erect
Sk5-W	Teplacinco # 5 (Tep-5)	ARC-Egypt	Prolific	High	Erect
L18-Y	SC 30N11	Pion. Int.Co.	Prolific	Low	Wide
L28-Y	Pop. 59	ARC-Thailand	Non-Prolific	Low	Wide
Sd 7-W	A.E.D. (old local OPV)	ARC-Egypt	Non-Prolific	Low	Erect

ARC = Agricultural Research Center, Pion. Int. Co. = Pioneer International Company in Egypt, SC = Single cross, W = White grains, Y = Yellow grains, A.E.D. = American Early Dent, Pop = Population hectare.3-Grain protein content (%) (GPC%).4-Grain oil content (%) (GOC%). 5-Grain starch content (%) (GSC%). Grain protein content (%),grain oil content (%) and grain starch content (%) were determined using the non-destructive grain analyzer, Model Infratec TM 1241 Grain Analyzer, ISW 5.00 valid from S/N 12414500, 1002 5017/Rev.1, manufactured by Foss Analytical AB, Hoganas, Sweden.6- Protein yield per hectare (PYPH), by multiplying grain protein content x grain yield per hectare.7- Oil yield per hectare(OYPH), by multiplying grain oil content x grain yield per hectare.8- Starch yield per hectare(SYPH), by multiplying grain starch content x grain yield per hectare. Stress tolerance index (STI) modified from equation suggested by Fageria [40] was used to classify genotypes for tolerance to high density stress. The formula used is as follows: STI= (Y_1/AY_1) X (Y_2/AY_2) Where, Y_1 = grain yield mean of a genotype at non-stress.AY_1 = average yield of all genotypes at non-stress.Y_2 = grain yield mean of a genotype at stress.AY_2 = average yield of all genotypes at stress When STI is ≥ 1.0, it indicates that genotype is tolerant (T), If STI is < 1, it indicates that genotype is sensitive (S).

2.5 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® [41]. Combined analysis of variance across the two seasons was also performed if the homogeneity test was non-significant. Moreover, data of each environment were separately analyzed across seasons as randomized complete block design using GENSTAT 10th addition windows software. Least significant differences (LSD) were calculated to test the significance of differences between means according to Steel et al. [42].

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Combined analysis of variance across years (Y) of the split plot design for the studied 23 genotypes (G) of maize (6 inbreds +15 F_1's + 2 check commercial single-cross hybrids) under three plant densities is presented in Table 2. Mean squares due to years were significant or highly significant for all studied traits, except for grain protein content, grain oil content and oil yield/ha, indicating significant effect of climatic conditions on most studied traits. Mean squares due to plant densities and genotypes were significant or highly significant for all studied traits, except grain oil content (GOC), grain protein content (GPC) and grain starch content (GSC) for densities, indicating that plant density has a significant effect on most studied traits and that genotype has an obvious and significant effect on all studied traits. Mean squares due to the 1st order interaction, *i.e.* G×Y, and G×D for all studied traits and D×Y for 3 traits (GYPP, GYPH and SYPH were significant ($P \leq 0.05$ or 0.01). Mean squares due to the 2nd order interaction, *i.e.* G×D×Y were highly significant for all studied traits, indicating that the rank of maize genotypes differ from a combination of density and year to another and the possibility of selection for improved performance under a specific plant density as proposed by several investigators [2,11,43-49].

It is observed from Table 2 that variance due to genotypes was the largest contributor to the total variance in this experiment for all studied traits, as measured by percentage of sum of squares to total sum of squares.

Combined analysis of variance of randomized complete block design under each environment across two seasons (data not presented) showed that mean squares due to parents and crosses under all environments were highly significant for all studied traits, indicating the significance of differences among studied parents and among F_1diallel crosses in all cases. Mean squares due to parents *vs.* F_1 crosses were also highly significant for all studied traits under all 3 environments (densities), suggesting the presence of significant average heterosis for all studied traits. Mean squares due to the interactions parents × years (P×Y) and crosses × years (F_1×Y) were significant or highly significant for all studied traits under all environments, except, GYPF under 47,600 plants/ha for P × Y and F_1 × Y, GPC under 71,400 plants/ha for F_1 × Y, GOC under 95,200 plants/ha for P × Y, GSC under 47,600 plants/ha and 95,200 plants/ha for P x Y, PYPF under 47,600 plants/ha and 71,400 plants/ha for P × Y and 47,600 plants/ha for F_1 × Y, OYPF under 47,600 plants/ha and 71,400 plants/ha for P × Y and SYPF under 47,600 plants/ha for P × Y and F_1 × Y. Mean squares due to parents *vs.* crosses × years were significant or highly significant in 15 out of 24 cases, indicating that heterosis differ from season to season in these cases. Among genotypes components under all three environments (24 cases), the largest contributor to total variance was parents vs. F_1's (heterosis) variance for 16 cases, followed by F_1 crosses (6 cases) and parents (2 cases).

3.2 Effect of Elevated Plant Density

The effects of elevated plant density on the means of studied traits across all genotypes and across the two years are presented in Table 3. Mean grain yield/plant was significantly ($P \leq 0.01$) reduced due to elevating plant density from 47,600 plants/ha to 71,400 plants/ha and 95,200 plants/ha, by 19.22 and 29.98%, respectively.

The reduction in grain yield/plant is logic and could be attributed to the increase in competition between plants at higher densities for light, nutrients and water. This conclusion was previously reported by several investigators [1,3,50-57]. Elevation of plant density from the low density (47,600 plants/ha) to 71,400 and 95,200 plants/ha also resulted in a slight reduction in grain protein content (2.72 and 1.24 % at 71,400 and 95,200 plants/ha, respectively).

Table 2. Combined analysis of variance across 2013 and 2014 years (% sum of squares) of split plot design for studied 23 maize genotypes under three plant densities

SOV	df	% Sum of squares (SS)			
		GPC%	**GOC%**	**GSC%**	**GYPP**
Years (Y)	1	0.24	0.93	4.37**	0.26**
Densities (D)	2	0.98	0.1	0.05	11.70**
Y x D	2	0.19	0.6	1.77	0.06**
Error (a)	12	4.49	8.32	4.27	0.07
Genotypes (G)	22	59.07**	45.33**	29.98**	82.00**
Y x G	22	4.00**	17.69**	16.40**	2.89**
D x G	44	7.85**	6.66**	12.39**	1.92**
Y x D x G	44	9.80**	9.81**	15.32**	0.53**
Error (b)	264	13.37	10.55	15.45	0.59
Total SS	**413**	**651.92**	**70.56**	**348.42**	**1888599**
		GYPH	**PYPH**	**OYPH**	**SYPH**
Years (Y)	1	0.21**	0.34**	0.05	0.29**
Densities (D)	2	9.38**	9.90**	8.90**	9.33**
Y x D	2	0.06*	0.03	0.06	0.09**
Error (a)	12	0.06	0.21	0.4	0.08
Genotypes (G)	22	83.55**	80.83**	79.97**	83.75**
Y x G	22	2.82**	3.61**	5.64**	2.51**
D x G	44	2.87**	3.03**	3.06**	2.86**
Y x D x G	44	0.58**	0.78**	1.09**	0.60**
Error (b)	264	0.48	1.28	0.83	0.5
Total SS	**413**	8228	82719242	17729391	4148226057

*GPC = grain protein content percentage, GOC = grain oil content percentage, GSC = grain starch content percentage, GYPP = grain yield per plant, GYPH = grain yield per hectare, PYPH = protein yield per hectare, OYPH = oil yield per hectare, SYPH = starch yield per hectare, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

Table 3. Change (%) in studied traits from low to medium and high density combined across all studied genotypes and across 2013 and 2014 seasons

Trait	71,400 plants/ha	95,200 plants/ha	Trait	71,400 plants/ha	95,200 plants/ha
GPC%	-2.72*	-1.24*	GYPH	+20.59**	+38.48**
GOC%	0.23	+0.74*	PYPH	+17.37**	+36.80**
GSC%	0.07	0.04	OYPH	+21.92**	+40.07**
GYPP	-19.22**	-29.98**	SYPH	+20.76**	+38.43**

*GPC = grain protein content percentage, GOC = grain oil content percentage, GSC = grain starch content percentage, GYPP = grain yield per plant, GYPH = grain yield per hectare, PYPH = protein yield per hectare, OYPH = oil yield per hectare, SYPH = starch yield per hectare, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively,, - = decrease. + = increase*

On the contrary, higher plant densities (71,400 and 95,200 plants/ha) caused a significant increase in grain yield/ha (GYPH) compared with the low-density by 20.59 and 38.48%, respectively and a slight but significant increase in grain oil content by 0.74% under high density (95,200 plants/ha) only. Increasing plant density from 47,600 plants/ha to 71,400 and 92,400 plants/ha caused also a significant increase in grain protein yield/ha (PYPH) by 17.37 and 36.80%, oil yield/ha (OYPH) by 21.92 and 40.07% and starch yield/ha (SYPH) by 20.76 and 38.43%, respectively. It seems that the increase in protein, oil and/or starch yield/ha as a result of increasing plant density is due mainly to the increase of grain yield/ha, since the percentage of protein, oil and/or starch content in maize grain changed very slightly and mostly non-significantly from one plant density to another. Although high plant density results in interplant competition (especially for light, water and nutrients), which affects vegetative and reproductive growth of maize causing reduction in grain yield/plant and some grain quality characteristics (GPC in the present experiment), the use of high-density would overcome such negative impacts of competition and lead to maximizing grain, protein, oil and starch yields from the same unit area (GYPH, PYPH, OYPH and SYPH) in this experiment. This conclusion is in agreement with that reported by several investigators [1,3,57].

3.3 Effect of Genotype

Means of studied traits of 6 inbred parents, 15 F_1 crosses and 2 checks under low- (47,600 plants/ha), medium- (71,400 plants/ha) and high- (95,200 plants/ha) densities combined across two years are presented in Table 4.

In general, the F_1 hybrids were lower in grain protein content than inbred lines under the three plant densities. This result is in agreement with that reported by Al-Naggar et al. [9,36]. On the other hand, F_1 hybrids showed higher means than inbreds for GYPP, GOC, GSC, GYPH, PYPH, OYPH and SYPH under all densities, indicating that heterozygotes exhibit better (more favorable) values for most studied traits than homozygotes, which is logic and could be attributed to heterosis phenomenon.

For grain protein content (GPC), the inbreds showed remarkable variability. Three inbreds (L18, L28 and Sk5) exhibited the highest percentage (14.38, 13.35 and 12.82%, respectively), while the lowest GPC (9.30%) was recorded by the inbred Sd7, all under medium density. For the F_1 crosses, variability in GPC was much less than in inbreds; *i.e.* from 9.5% for L20 × L53 to 11.58% for L18 × L28 under medium density. The cross L18 × L28 recorded the highest GPC, while the cross L20 × L53 recorded the lowest percentage under the three plant densities. Out of 15 crosses, 9 crosses showed the highest GPC under low density, four under medium density and two under high density, assuring that in general, there is a tendency of reduction of grain protein percentage due to elevated plant density in most studied genotypes.

For grain oil content, the range of variability was between 3.68% for Sk5 under high density to 4.55% for L28 under low density for inbreds and from 4.03% (Sk5 × L18) to 4.87% (L18 × L28 and L53 × L28) under medium density for crosses. The range of variability in grain oil content in the present study is similar to that found in the literature for normal maize, which was between 3.5 and 4.5% [58]. In another study on the genetic variation for oil content in maize with normal endosperm, Mittelmann [59] found values between 3.77 and 5.10%. The F1 crosses were generally higher than their parental inbred in grain oil content under the three densities, suggesting the superiority of heterozygotes to homozygotes in maize grain oil content. Similar conclusion was reported by previous investigators [11,60-63]). Heterosis for grain oil content of maize was also reported by several investigators [60-66]). The variability for grain starch content ranged from 69.03% (L28) under low density to 72.23% (L20) under high density for inbreds and from 69.82% (L18 **×** L28) under medium density to 71.67% (L20 **×** L53) under low density for F_1 crosses.

3.4 Genotype × Plant Density Interaction

In general, GYPP of three inbreds, *viz.* L53, L20 and Sk5 was higher than that of the other three inbreds (L18, L28 and Sd7) under all densities. Reduction due to elevated plant density was the highest in the inbred L18 under high-density (57.0%), and the lowest in inbred L20 under medium density (12.9%). The highest GYPP of all inbreds was achieved under low density, where competition between plants is at minimum. The effect of the first order interaction (G × D) was clearly shown by the F_1 crosses, where the rank of crosses was changed from one plant density to another, especially when comparing poor with good environments.

Table 4. Means of studied grain yield and quality traits of each inbred and cross under three plant densities and change (Ch %) from low density across two seasons

Genotypes	Grain yield/plant(g)					Grain yield/ha(ton)				
	Low-D	Med-D	Ch%	High-D	Ch%	Low-D	Med-D	Ch%	High-D	Ch%
Inbred parents (P)										
L20	106.58	92.85	12.9**	71.48	32.9**	4.95	6.41	-29.5**	6.64	-34.1**
L53	132.05	93.69	29.1**	71.7	45.7**	6.13	6.47	-5.5**	6.66	-8.6**
Sk5	77.56	64.94	16.3**	52.97	31.7**	3.6	4.48	-24.5**	4.92	-36.6**
L18	46.69	27.23	41.7**	20.07	57.0**	2.16	1.85	14.5**	1.86	13.9**
L28	44.37	35.38	20.3**	30.45	31.4**	2.06	2.44	-18.5**	2.83	-37.3**
Sd7	55.1	29.14	47.1**	32.87	40.3**	2.01	2.5	-24.1**	3.05	-51.7**
Average	77.06	57.2	25.8**	46.59	39.5**	3.49	4.03	-15.5**	4.33	-24.1**
Crosses (C)										
L20 × L53	277.36	238.19	14.12**	191.55	30.94**	12.88	16.45	-27.71**	17.05	-32.42**
L20 ×SK5	221.68	182.28	17.77**	153.06	30.95**	10.22	12.59	-23.19**	14.21	-39.11**
L20 × L18	219.17	193.75	11.60**	178.07	18.75**	10.15	13.38	-31.77**	16.04	-58.00**
L20 × L28	232.77	186.52	19.87**	156.26	32.87**	10.81	12.88	-19.17**	14.51	-34.26**
L20 × Sd7	226.7	182.42	19.53**	159.88	29.47**	10.53	12.6	-19.67**	14.85	-41.05**
L 53 × Sk5	245.53	224.51	8.56**	184.72	24.77**	11.4	15.5	-35.99**	16.47	-44.48**
L53 × L18	197.48	147.69	25.21**	138.34	29.95**	8.99	10.2	-13.38**	12.85	-42.82**
L53 × L28	237.53	168.89	28.89**	165.7	30.24**	11.03	11.66	-5.75**	14.99	-35.90**
L53 × Sd7	240.96	219.13	9.06**	181.95	24.49**	11.19	15.13	-35.24**	16.3	-45.74**
Sk5 × L18	234.83	197.02	16.10**	165.1	29.69**	10.9	13.6	-24.77**	15.18	-39.20**
Sk5 × L28	223.2	201.32	9.80**	167.12	25.12**	10.34	13.9	-34.41**	15.45	-49.40**
Sk5 × Sd7	207.22	157.58	23.96**	145.21	29.92**	9.58	10.88	-13.59**	13.48	-40.77**
L18 × L28	171.09	124.38	27.30**	122.94	28.14**	7.91	8.59	-8.60**	11.42	-44.37**
L18 × Sd7	213.29	161.79	24.15**	148.59	30.34**	9.88	11.17	-13.08**	13.8	-39.66**
L28 × Sd7	227.64	183.46	19.41**	165.78	27.18**	10.49	12.67	-20.75**	14.67	-39.84**
Average	225.1	184.6	18.0**	161.62	28.20**	10.42	12.75	21.80**	14.75	-41.55**
Checks										
S.C 130	229.77	168.42	26.7**	146.68	36.2**	10.67	11.76	-10.2**	13.59	-27.4**
S.C 2055	215.4	179.91	16.5**	149.05	30.8**	10	12.58	-25.8**	13.87	-38.7**
LSD 0.05	D =0.04, G =0.11 , G×D=0.19					D =0.002, G =0.01 , G×D=0.01				

Genotype	GPC(%)			GOC(%)			GSC(%)		
	Low-D	Medium-D	High-D	Low-D	Medium-D	High-D	Low-D	Medium-D	High-D
Inbred parents (P)									
L20	10.97	10.63	11.65	4.23	3.9	3.82	71	72.17	72.23
L53	11.82	10.97	11.47	4.15	4.2	4.13	70.48	71.17	70.87
Sk5	12.8	12.82	12.8	3.48	3.52	3.68	71.25	70.97	70.7
L18	13.52	14.38	13.43	4.03	4.15	4.05	70.35	69.48	71.02
L28	12.88	13.35	12.85	4.55	4.28	4.48	69.93	68.87	69.92
Sd7	12.57	9.3	11.38	4.4	4.28	4.28	70.75	70.85	71.28
Average (P)	12.43	11.91	12.26	4.14	4.06	4.07	70.63	70.59	71
Crosses (C)									
L20 X L53	9.73	9.5	9.57	4.38	4.32	4.22	71.67	71.48	71.52
L20 XSK5	10.55	10.33	10.28	4.8	4.68	4.4	70.12	70.33	70.87
L20 X L18	10.95	10.47	10.55	4.05	4.17	4.25	71.63	71.53	71.37
L20 X L28	10.63	10.7	10.5	4.38	4.4	4.65	71.15	70.85	70.52
L20 X Sd7	10.33	11.4	10.63	4.5	4.27	4.37	70.97	70.68	70.63
L 53 X Sk5	10.58	10.3	10.3	4.12	4.2	4.1	70.8	71.13	71.63
L53 X L18	10.57	10.47	10.7	4.27	4.3	4.35	70.75	70.92	70.53
L53 X L28	10.63	10.37	10.58	4.53	4.87	4.53	70.77	70.55	71.02
L53 X Sd7	10.5	10.28	10.8	4.57	4.77	4.67	70.87	70.55	70.4
Sk5 X L18	11.35	10.87	11.03	4.1	4.05	4.03	71.13	71.75	71.35
Sk5 X L28	11.42	10.68	10.58	4.4	4.17	4.5	70.4	71.08	70.58
Sk5 X Sd7	10.83	11	10.63	4.68	4.58	4.78	70	70.2	70.17
L18 X L28	11.57	11.58	11.65	4.45	4.87	4.6	70.72	69.82	70.55
L18 X Sd7	10.85	10.05	10.52	4.42	4.42	4.58	71.07	71.12	70.48
L28 X Sd7	10.67	10.2	10.48	4.32	4.58	4.57	70.77	71.22	70.17
Average (C)	10.74	10.55	10.59	4.4	4.44	4.44	70.85	70.88	70.79
Checks									
SC 130	10.22	9.92	10.17	3.95	3.93	4.07	71.32	71.97	71.35
SC 2055	10.3	9.7	10.5	4.5	4.6	4.88	70.92	71.3	70.3
LSD 0.05	D =0.03, G =0.08 , G×D=0.14			D =0.01, G =0.03 , G×D=0.05			D =0.02, G =0.06 , G×D=0.11		

Genotype	PYPH (kg)			OYPH (kg)			SYPH (kg)		
	Low-D	Medium-D	High-D	Low-D	Medium-D	High-D	Low-D	Medium-D	High-D
Inbred parents (P)									
L20	541.8	680.6	771.7	209.5	249.7	253.1	3513	4627	4801
L53	734.6	705.7	765.5	252.3	271.5	275.9	4319	4610	4718
Sk5	461.7	577.5	633.5	125.8	156.8	180.1	2566	3181	3481
L18	294.5	264.8	251.3	86.7	76.6	75.2	1523	1285	1322
L28	265.2	325.4	363.4	93.4	104.7	126.5	1440	1681	1976
Sd7	257.4	225.2	349.9	86.5	104.7	129.1	1423	1770	2179
Average	425.9	463.2	522.5	142.4	160.7	173.3	2464	2859	3080
Crosses (C)									
L20 X L53	1253.5	1561.9	1632.9	563.5	710.2	718.5	9230	11756	12195
L20 XSK5	1081.7	1294.7	1467.3	491.7	598.5	626.9	7149	8829	10061
L20 X L18	1111.1	1404.6	1693.1	411.5	558.1	681.5	7273	9565	11450
L20 X L28	1149	1373.2	1522.9	473.9	569.5	674.7	7689	9121	10233
L20 X Sd7	1087.8	1436	1578.4	473.4	537.5	648.1	7470	8903	10487
L 53 X Sk5	1206.4	1596.8	1695.1	469.4	651.1	674.6	8072	11027	11802
L53 X L18	950.4	1067.9	1374.3	384.1	438.3	557.9	6363	7233	9065
L53 X L28	1172.7	1208.9	1583.8	500.2	567.6	680.1	7804	8227	10647
L53 X Sd7	1174.7	1556	1759.6	510.8	721.1	759.3	7928	10674	11482
Sk5 X L18	1237.4	1478.8	1675.5	447.2	551.1	611.8	7755	9761	10829
Sk5 X L28	1180	1484.7	1634.2	455.1	579.1	694.5	7281	9880	10909
Sk5 X Sd7	1037.6	1196.5	1431.4	448.4	498.7	643.5	6705	7638	9467
L18 X L28	915.3	994.8	1330.3	351.7	418	525.3	5592	5996	8053
L18 X Sd7	1071.8	1122.6	1451.6	436.3	493.6	632.1	7022	7945	9726
L28 X Sd7	1116.4	1292.3	1540.5	462.7	599.7	682.6	7405	8999	10278
Average	1116.4	1338	1558	458.7	566.1	654.1	7382	9037	10445
Checks									
SC 130	1090	1161.7	1381.2	421.2	462	552.7	7609	8468	9698
SC 2055	1031.2	1225.3	1455.4	451.2	579.8	676.7	7092	8966	9750
LSD 0.05	D =0.62, G =1.90 , G×D=3.29			D =0.32, G =0.98 , G×D=1.69			D =1.70, G =5.18 , G×D=8.98		

*D= Density, G = Genotype, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively. GPC= Grain protein content, GOC= Grain oil content, GSC= Grain starch content, GYPP= Grain yield per plant, PYPH= Protein yield/ha, OYPH= Oil yield/ha, SYPH= Starch yield/ha, GYPH= Grain yield/ha*

The second highest GYPP of studied crosses was obtained under the medium plant density. The highest GYPP in this experiment (277.36 g) was obtained from the cross L20 × L53 under low-density followed by the crosses L53 × Sk5 (245.53 g), L53 × Sd7 (240.96 g) and L53 × L28 (237.53 g) under the same density. These crosses could therefore be considered responsive to the good environment. The highest GYPP under the most severe stress (high density, *i.e.* 95,400 plants/ha) was obtained by the crosses L20 × L53 (191.55 g), L53 × Sk5 (184.72 g), L53 × Sd7 (181.95 g) and L20 × L18 (178.07 g); these crosses were considered tolerant to high density stress. The three crosses L20 × L53, L53 × Sk5 and L53 × Sd7 were tolerant to high density stress and responsive to low density. Some F_1 crosses showed significant superiority in GYPP over the best check in this experiment, namely the crosses L20 × L53, L53 × Sk5, L53 × Sd7 and L53 × L28 under low density.

The rank of inbred parents for GYPH was approximately similar under all the three densities, indicating less effect of interaction between inbreds and plant density on GYPH. The percent reduction in GYPH due to density stress relative to low-density was smaller for the inbred lines L20, L28 and L53 than the inbreds L18, Sk5 and Sd7 in low-performing ones, which could be attributed to the higher potential yield of the first group of lines than the second one, under good environmental conditions. Regarding GYPH of the F_1 crosses, the rank varied from one plant density level to another, indicating that for GYPH the interaction between genotype and plant density plays a role its expression.

Comparing with the non-stressed environment (low density), all 15 F_1 crosses showed an increase in their GYPH ranging from 5.75 to 35.99% under medium density and from 32.42 to 58.0% under high density. The increase in GYPH of these crosses under medium and high density over that under low density could be attributed to the elevation of plant density. This indicates that the increase of GYPH due to the increase in plant density could compensate the reduction in GYPP due to competition among plants and even this could happen in some crosses if they have more tolerance to high density stress. The best GYPH in this experiment was obtained under high density and the best crosses in this environment were L20 × L53 (17.05 ton), L53 × Sk5 (16.47 ton), L53 × Sd7 (16.30 ton) and L20 × L18 (16.04 ton), with a significant superiority over SC 2055 (the best check under this environment) by 22.92, 18.74, 17.52 and 15.72%, respectively. The increase in GYPH due to high plant density was accompanied with increases in PYPH, OYPH and SYPH. The crosses L20 **×** L53, L53 **×** Sk5, L53 **×** Sd7 and L20 **×** L18 out-yielded the best check in this experiment (SC2055) under high density by 24.9, 20.9, 17.7 and 17.4% for SYPH, 12.2, 16.55, 20.9 and 16.3% for PYPH and 6.2, 0.0, 12.2 and 0.7% for OYPH, respectively.

3.5 Stress Tolerance of Inbreds and Hybrids

Stress tolerance index (STI) values of studied genotypes estimated using the equation suggested by Fageria [40] under the stressed environments medium and high density (Table 6) indicated that the highest STI under stressed

Table 5. Stress tolerance index (STI) of maize inbreds and hybrids under medium and high density stress

Genotype	Med- density	High-density	Genotype	Med-density	High-density
			Inbreds		
L20	2.25	2.12	L18	0.29	0.26
L53	2.81	2.64	L28	0.36	0.38
Sk5	1.14	1.14	Sd7	0.36	0.50
			F_1 crosses		
L20 × L53	1.59	1.46	L53 × Sd7	1.27	1.21
L20 ×SK5	1.00	1.00	Sk5 × L18	1.13	1.14
L20 × L18	0.89	0.92	Sk5 × L28	0.94	0.96
L20 × L28	1.08	1.07	Sk5 × Sd7	0.79	0.83
L20 × Sd7	0.99	1.00	L18 × L28	0.51	0.58
L 53 × Sk5	1.33	1.25	L18 × Sd7	0.83	0.87
L53 × L18	0.70	0.75	L28 × Sd7	1.01	1.04
L53 × L28	1.22	1.17			

environments was exhibited by the inbred line L53, followed by inbred L20 and then Sk5. On the contrary, the three inbred lines Sd7, L18 and L28 exhibited STI values less than unity under the two stressed environments and therefore could be considered sensitive to elevated plant density stress; with the most sensitive one was the inbred inbred L18.

For F_1 crosses, the highest STI value was recorded by the cross L20 x L53 (T×T), followed by the cross L53 × Sk5 (T×T) and L53 × Sd7 (T×S) under the two stressed environments. On the other hand, the most sensitive crosses under both stressed environments are L18 x L28 (S × S), L53 × L18 (T × S) and Sk5 x Sd7 (T × S). It is observed that all three T × T crosses (L20 × L53, L20 × Sk5 and L53 × Sk5) were tolerant under each stress, indicating hybrid accumulation of effects of stress tolerance genes from its two parents. Among the three S × S crosses, two (L18 × L28 and L18 × Sd7) were sensitive and one (L28 × Sd7) was tolerant to elevated density stress. The stress tolerance exhibited in the latter S × S hybrid could be attributed to epistasis effects. Among the nine T × S crosses, five (L20 × L28, L20 × Sd7, L53 × L28, L53 × Sd7 and Sk5 × L18) were tolerant, while four (L20 × L18, L53 × L18, Sk5 × L28 and Sk5 × Sd7) were sensitive under each environment. The tolerance of the first five T x S crosses indicated accumulating of more genes of dominance effects of tolerance over sensitivity, while the tolerance of the latter four T × S crosses suggested accumulating less number of dominant tolerance genes.

3.6 Superiority of Tolerant (T) Over Sensitive (S) Genotypes

Data averaged for each of the two groups (T and S) of inbreds and crosses differing in tolerance to high density indicate that grain yield/ha of high density tolerant (T) was greater than that of the sensitive (S) inbreds and crosses by 135.21 and 32.00%, respectively under high density (95,400 plants/ha) conditions. Superiority of high-density tolerant (T) over sensitive (S) inbreds in GYPH under high density was due to their superiority in GYPP (135.21%) (Table 6). Likewise, under high plant density, the tolerant inbreds showed 0.75% more GSC, 125.05% more PYPH, 114.35% more OYPH and 137.35% more SYPH than the sensitive inbreds. Superiority of T over S hybrids in GYPH under high density (95,400 plants/ha) was due to their superiority in GYPH (37.32%), PYPH (23.01%), OYPH (24.65%), SYPH (33.46%), than sensitive F_1 crosses. Al-Naggar et al. [11] also reported that under high plant density, the tolerant testcrosses showed 314.4% more GYPP than sensitive testcrosses.

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

Mean performance of traits were averaged across three groups of F_1 crosses, *i.e.,* T×T, T×S and S×S groups based on grain yield per plant of their parental lines under stress and non-stress conditions, *i.e.,* parental tolerance to high density stress. Number of crosses was 3, 9 and 3 for the T×T, T×S and S×S groups, respectively. In general, high density T×T group of crosses exhibited better values in most studied traits than high density T×S and S×S groups of crosses. Superiority of high density T×T and T × S over S × S crosses (Table 7) was more pronounced under medium density (71,400 plants/ha) than under high (95,400 plants/ha) and low density (47,600 plants/ha).

Under high plant density conditions, grain yield/ha superiority of high-density T×T (19.65%) and T × S (11.68%) over S×S crosses was associated with their superiority in grain yield/plant by 21.04 and 11.11%, PYPH by 10.94 and 9.94%, OYPH by 9.79 and 7.88%, SYPH by 21.39 and 12 34, respectively. The superiority of T × T and T × S crosses in grain yield and other studied characters over S × S crosses under high plant density was also expressed under low and medium plant density (Table 7). This study concluded that to obtain maximum grain yield from a hybrid under elevated plant density, it is better that both of its two parents to be tolerant to high plant density. This assures that high plant density stress tolerance trait is quantitative in nature, so the tolerant cross accumulates additive genes of high density tolerance from both parents [5].

3.8 Regression of Grain Quality and Yield Traits on Elevated Density

Data were re-analyzed to evaluate responses of studied traits of inbreds and hybrids across varying plant densities *via* regression technique. The quadratic regression function between averages across two years for each studied trait of the 4 groups: tolerant 3 inbreds (L20, L53 and Sk5), sensitive 3 inbreds (L18, L28 and Sd7), the highest tolerant 3 F_1's (L20 × L53, L53 × Sk5 and L53 × Sd7) and the most sensitive 3 F_1's (L18 × L28, L53 × L18 and Sk5 × Sd7) and plant densities was performed (Fig. 1). The regression functions were used to identify which plant density provides optimum value for each studied trait of each group of genotypes.

For grain protein content (GPC), all groups, except the tolerant hybrids showed a quadratic response of decrease from the low to medium density and then an increase towards the high density, with an optimum density of 47,600 plants/ha for sensitive inbreds and 95,200 plants/ha for the tolerant inbred and hybrid groups. While, GPC for the sensitive hybrids group showed a quadratic response of slight increase towards elevated plant density, with an optimum density of 71,400 plants/ha (medium).

For grain oil content (GOC), the tolerant group of hybrids showed a strong quadratic response of increase towards elevated plant density, with an optimum density of 71,400 plants/ha). However, the sensitive group of hybrids exhibited a curvilinear response of higher increase than the tolerant hybrid group, with an optimum density in-between 71,400 and 95,200 plants/ha, i.e.*ca* 83,800 plants/ha. The tolerant and sensitive inbred groups showed a quadratic response of decrease by elevated density, with an optimum density of 47,600 plants/ha; the response of decrease was stronger in sensitive than tolerant group of inbreds

For grain starch content, the tolerant group of inbreds showed a strong quadratic response of increase towards elevated plant density, with an optimum density in-between 71,400 and 95,200 plants/ha. The tolerant inbreds group showed a quadratic response of decrease by elevated density, with an optimum density of 71,400 plants/ha. For the sensitive inbred and hybrid groups, they showed a quadratic response of decrease from low to medium density followed by quadratic response of increase up towards the high density, with an optimum density of 71,400 plants/ha.

For grain yield/plant (GYPP), all groups of tolerant and sensitive inbred and hybrids showed a general response of decrease by elevated plant density. The regression response of decrease in GYPP due to the increase in plant density was in curvilinear shape for sensitive inbreds and hybrids, but was very close to linear and linear regression of decrease for tolerant hybrids and tolerant inbreds, respectively.

Table 6. Superiority (%) of the most tolerant inbreds (3) and most tolerant hybrids (3) over the most sensitive inbreds (3) and most sensitive hybrids (3) for selected characters under high plant density (95,400 plants/ha) conditions across two seasons

Trait	Inbreds			Crosses		
	T	S	% Superiority	T	S	% Superiority
GSC%	71.27	70.74	0.75**	71.18	70.42	1.09**
GYPP (g)	65.38	27.8	135.21**	186.07	135.5	37.32**
GYPH (ton)	6.07	2.58	135.21**	16.60	12.58	32.00**
PYPH (kg)	723.3	321.4	125.05**	1695.2	1378.1	23.01**
OYPH (kg)	236.2	110.2	114.35**	717.2	575.3	24.65**
SYPH (kg)	4331.7	1825.0	137.35**	11821.7	8858.1	33.46**

% Superiority = 100 × [(T – S)/S]

Table 7. Superiority (%) of T × T and T × S over S × S crosses for selected traits under different plant densities across two seasons (2013 and 2014)

Trait	Low density		Medium density		High density	
	T ×T	T ×S	T ×T	T ×S	T ×T	T ×S
GYPP	21.66**	10.01**	37.33**	17.41**	21.04**	11.11**
GYPH	21.96**	10.22**	37.31**	17.42**	19.65**	11.68**
PYPH	14.12**	8.49*	30.61**	19.28**	10.94**	9.94**
OYPH	21.91**	9.40**	29.68**	11.14**	9.79**	7.88**
SYPH	22.14**	10.35**	37.80**	17.68**	21.39**	12.34**

% Superiority = 100 × [(T×T) or (T×S) –(S×S)/(S×S)], T = tolerant, S = sensitive, LD = low density (47,600 plants/ha), MD = medium density (71,400 plants/ha) and HD = high density (95,400 plants/ha)

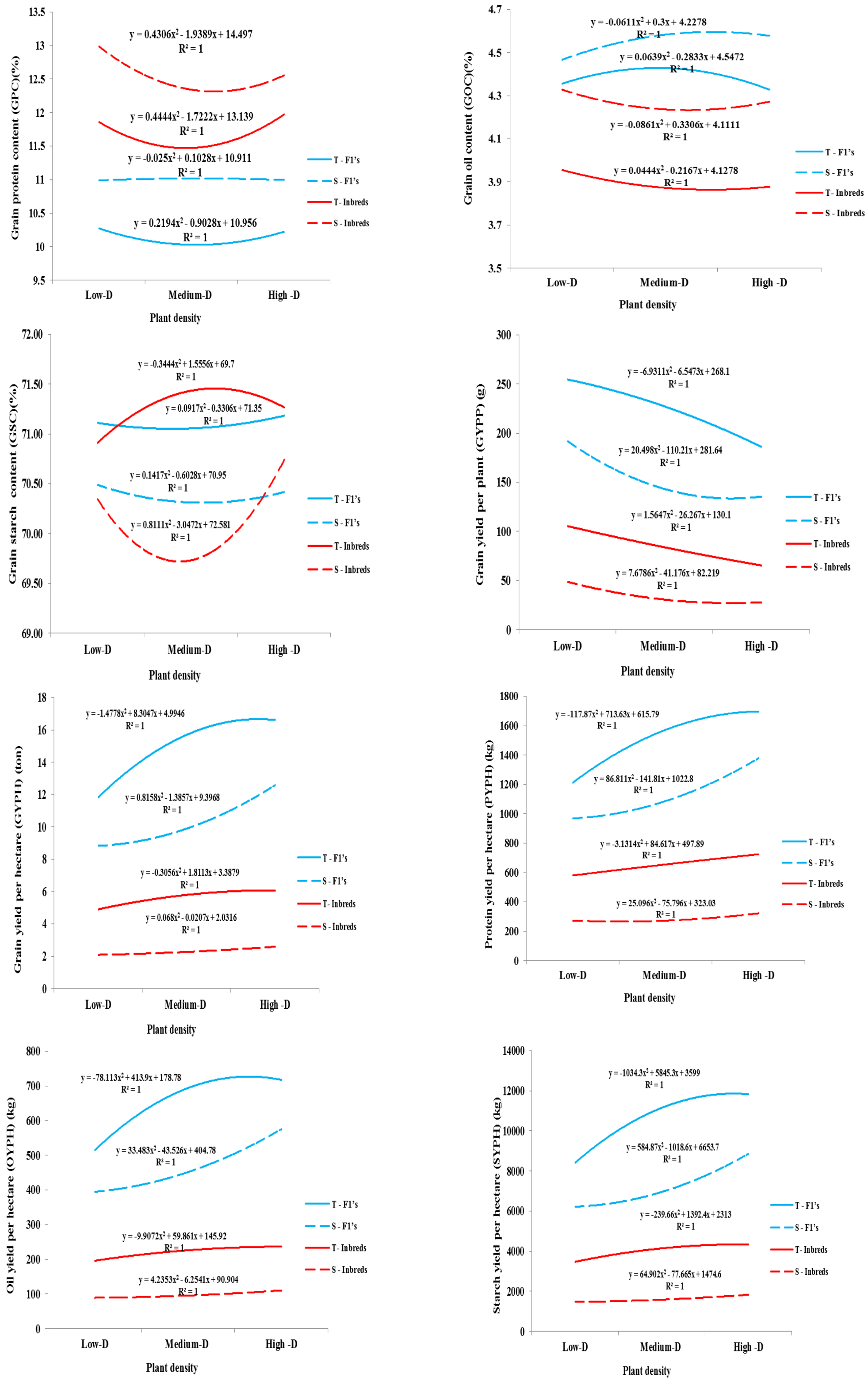

Fig. 1. Regression of studied grain composition and yield traits of inbred parents and F_1crosseson elevated plant density across two seasons

For GYPH, PYPH, OYPH and SYPH, the response of each group of the four groups of genotypes (tolerant inbreds, sensitive inbreds, tolerant F_1's and sensitive 3 F_1's) to the elevated plant density was similar. They showed a quadratic response of increase in hybrids and near linear response of increase in inbreds for both tolerant and sensitive groups, but the response of increase was stronger for tolerant than sensitive groups. The regression functions indicated that the optimum density these four traits was in-between 71,400 and 95,200 plants/ha (i.e.*ca* 83,800 plants/ha) for the tolerant hybrid group and high density (95,200 plants/ha) for the other three groups (tolerant inbreds, sensitive inbred and sensitive hybrids).

In this context, Shapiro and Wortmann [36] 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. Clark [39] mentioned that there was a curvilinear increase until yield plateau at the middle density (83,980 plants/ ha). He concluded that no support was found for the idea that increasing corn yield requires increases in plant density above rates typically used. Boomsma et al. [67] reported that under large ranges of plant density (54,000-104,000 plants/ha), higher densities required more N. Al-Naggar et al. [5] found that the tolerant inbreds to high density (L17, L18 and L53) showed a quadratic regression function, with an optimum density of 95,200 plants/ha. While, the sensitive inbreds L54, L29 and L55 showed a weak quadratic regression very close to linear response, with an optimum density of 47,600 plants/ha. The grain yield/ha across years and across all groups of F_1 crosses showed a quadratic regression function under the three plant densities, with an optimum density of 95,200 plants/ha. The most responsive crosses to elevated plant density were belonging to the groups efficient-responsive and efficient-non responsive. Their and our results advance our understanding that the complexities of genotype interaction with plant density will require additional work.

4. CONCLUSION

The use of high-density along with density tolerant genotypes would overcome negative impacts of competition and lead to maximizing grain, protein, oil and starch yields from the same unit area in this experiment. The response of the four groups of genotypes (tolerant and sensitive inbreds and hybrids) to the elevated plant density showed a quadratic response of increase in hybrids and near-linear response of increase in inbreds, but the response of increase was stronger for tolerant than sensitive groups. The optimum density for studied traits was 83,800 plants/ha for the tolerant hybrid group and 95,200 plants/ha for the other three groups. A quadratic response of increase was observed for GPC of sensitive hybrids, GOC of sensitive and tolerant hybrids and GSC of tolerant inbreds and hybrids, with an optimum density of 83,800plants/ha. On the contrary, a quadratic response of decrease was observed for GPC of tolerant inbreds and hybrids, GOC of tolerant and sensitive inbreds, GSC of sensitive hybrids and GYPP of the four groups, with an optimum density of 47,600 plants/ha.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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