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# Research Article Maximizing Maize Grain, Protein, Oil and Starch Yields by Using High Plant Density and Stress Tolerant Genotype

<sup>1</sup>Younis, A.S.M., <sup>2</sup>A.M.M. Al-Naggar, <sup>1</sup>B.A. Bakry and <sup>3</sup>S.M.A. Nassar

# **Abstract**

**Background and Objective:** Enhancing yield of protein, oil and carbohydrate of maize grain (*Zea mays* L.) can be achieved either by increasing grain contents of these constituents or by increasing grain yield per land unit area. The main aim of the current investigation was to determine the effects of elevated plant density and genotype on corn grain protein, oil and carbohydrate contents and yields. **Materials and Methods:** A two year experiment was conducted in the field. The experimental design was a split plot with three replications. The main plots were devoted to 3 plant densities, i.e., Low Density (LD, 47,600 plants  $ha^{-1}$ ), Medium Density (MD, 71,400 plants  $ha^{-1}$ ) and High Density (HD, 95,200 plants  $ha^{-1}$ ) and sub plots to 17 genotypes. **Results:** The HD did not significantly affect grain protein, oil and starch contents but caused a significant increase of 27.57% for grain yield  $ha^{-1}$  (GYPH), 26.34% for protein yield  $ha^{-1}$  (PYPH), 27.57% for oil yield  $ha^{-1}$  (OYPH) and 28.15% for starch yield  $ha^{-1}$  (SYPH). The highest yields  $ha^{-1}$  of grain, protein, oil and starch were recorded under HD and the lowest under LD. The harpha (SYPH) and SYPH (14.94 t), PYPH (1.42 t), OYPH (0.582 t) and SYPH (10.67 t). **Conclusion:** The use of HD would overcome the negative impacts of interplant competition and lead to maximizing GYPH, PYPH, OYPH and SYPH, such maximization was more pronounced by the highest HD-tolerant genotypes.

Key words: Quality traits, plant density, HD-tolerant genotypes, interplant competition, density tolerance index, grain chemical composition

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Corresponding Author: B.A. Bakry, Department of Field Crops Research, Agricultural and Biological Research Division, National Research Centre, 33 El-Bohooth st., Dokki, P.O. Box 12622, Cairo, Egypt

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**Competing Interest:** The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

<sup>&</sup>lt;sup>1</sup>Department of Field Crops Research, Agricultural and Biological Research Division, National Research Centre, 33 El-Bohooth st., Dokki, P.O. Box 12622, Cairo, Egypt

<sup>&</sup>lt;sup>2</sup>Department of Agronomy, Faculty of Agriculture, Cairo University, Giza, Egypt

<sup>&</sup>lt;sup>3</sup>Plant Breeding Unit, Department of Genetic Resources, DRC, El-Matarya, Cairo, Egypt

### **INTRODUCTION**

Maximizing protein, oil and carbohydrate yields of maize grain (*Zea mays* L.) can be achieved either by increasing grain contents (percentages) of these constituents or by increasing grain yield per land unit area. Both of them are important breeding objectives for improving grain quality traits<sup>1,2</sup> and improving maize grain yield per land unit area *via* improving high density tolerance<sup>3-5,6</sup>. Tan and Morrison<sup>6</sup> reported that corn grain of a maize hybrid normally contains about 4% oil, 9% protein, 73% starch and 14% other constituents, the oil is concentrated in the germ, while carbohydrate and protein are concentrated in the endosperm. It is used in some countries as a human food. The feed industry needs maize with higher oil and protein contents and balanced amino acids.

At present, Egyptian maize varieties are grown under low plant population density of about 5.7 plants m<sup>-2</sup>, i.e., about half of the density used in USA. Egyptian maize varieties cannot give higher yield under elevated plant densities. This might be one of the reasons that the yield from land unit area cultivated by maize in Egypt is lower than that in USA. One of the potential technologies to maximize corn crop is *via* increasing plant density up to 10 plants m<sup>-2</sup> along with using maize dense tolerant hybrids<sup>3</sup>. Previous studies<sup>3,7-9</sup> reported that mean grain yield of corn per unit land area in the USA increased markedly during the last 50 years, because of the improvement occurred in crop agricultural practices and breeding for tolerance to high plant density.

Breeding dense tolerant cultivars of maize in Egypt could be considered as an important objective to maximize grain, protein, oil and starch yields from land unit area. Competition between dense plants results in a reduction in plant grain yield, grain protein and oil contents<sup>4,5</sup>. However, several investigators<sup>9,10</sup> reported that the use of elevated plant density along with dense tolerant genotypes would compensate the negative effects of such competition and result in maximizing protein, oil and grain yields per hectare.

In the literature, several reports were published on the existence of genotypic variation in maize grain quality traits, which is a prerequisite for starting a successful breeding program, e.g., 3,11-16 (for oil content) and 13-17 (for protein content). However, Simmonds 18, Feil 19 and Al-Naggar *et al.* 20 reported that breeding progress has been limited by a negative correlation between maize grain yield and each of oil and protein content.

The effects of genotype and its interaction with environment are significant for maize grain protein and oil contents, few<sup>3,20,21-24</sup> carried out the research on the effects of

drought stress on the quality traits of maize grain but studies on the effects of elevated plant density stress on such traits are scarce.

The aims of the current investigation were to assess the effects of increased plant density and maize genotype on grain protein, oil and carbohydrate contents and yields and identify the genotypes characterized by HD tolerance and high grain, protein, oil and carbohydrates yield ha<sup>-1</sup>.

#### **MATERIALS AND METHODS**

**Genetic materials:** Six genetically diverse maize inbred lines (*Zea mays* L.) (Table 1) were used in this study as parents of diallel crosses. In 2016 growing season, a half diallel crossing design was performed among the 6 parents and seeds of 15 F<sub>1</sub> crosses were obtained and used in this investigation along with the two checks SC 2055-yellow (obtained from Hi-Tech Company-Egypt) and SC 130-white (obtained from the ARC, Egypt).

**Experimental site:** This study was carried out in the field of the Agric. Experimental and Res. Station of the Fac. of Agric., Cairo University, Giza (31°13'E longitude, 30° 02'N latitude and an altitude of 22.50 m asl), during the maize growing seasons 2016, 2017 and 2018.

**Field experiments:** Evaluation experiments in the field were implemented in two growing seasons (2017 and 2018). The experimental design was a split plot with three replications. The 3 plant densities, namely low (LD), medium (MD) and high (HD) were arranged in the main plots, while the 17 genotypes were arranged in the sub plots. Each experimental unit consisted of two rows of 400 cm long and 70 cm width. Therefore, the experimental plot area was 5.6 m². The seeds were sown in hills at distances of 15, 20 and 30 cm along the rows, thereafter, were thinned to one plant/hill before the 1st irrigation, to achieve the three plant densities 95,200, 71,400 and 47,600 plants ha<sup>-1</sup>, respectively. The planting date was on 5th and 8th of May 2017 and 2018 seasons, respectively. The recommendations of Agric. Res. Center of Egypt were followed for all other agricultural practices.

**Soil analysis:** Physical and chemical soil analyses of the experimental site (Table 2) were performed at laboratories of Soil and Water Research Institute of ARC, Egypt.

**Meteorological data:** The weather data for the experimental site during the two growing seasons of maize were obtained

Table 1: Name, pedigree and some important traits of 6 inbred lines used in this study

Entry designation	Pedigree/origin	Institution (country)	Grain color	Prolificacy	Leaf angle
Sd-7 (P1)	American early dent	ARC-Egypt	W	Non-prolific	Erect
CML-104 (P2)	CIMMYT	Mexico	Υ	Unknown	Erect
Inb-17 (P3)	G 268 Jellicarse (from Recurrent selection)	ARC-Egypt	W	Non-prolific	Wide
Inb-171 (P4)	Rg-37 G.S. (PI221866×307A)(SC-14)]	ARC-Egypt	Υ	Prolific	Erect
Inb-92 (P5)	Rg-49 G.S. (Beida × 307) (SC-14)	ARC-Egypt	W	Prolific	Erect
Inb-24 (P6)	G 336 Loc. Bred (H-309 1969)	Mexico	W	Prolific	Wide

ARC: Agricultural research center, Sd-7: Local inbred line (Sids-7), CML: CIMMYT, SC: Single cross, W: White grains and Y: Yellow grains and Inb: Inbred line

Table 2: Soil analysis of the experimental site at Giza in the two growing seasons

	Physical	Physical analysis Chemical analysis											
			Fine	 Coarse		pH paste	EC		CaCO <sub>3</sub>	Soil bulk		anions (m	
Seasons	Silt (%)	Clay (%)	sand (%)	sand (%)	Soil type	extract	$dSm^{-1}$	SP	(%)	density g cm <sup>-3</sup>	HCO <sub>3</sub>	Cl	$SO_4$
2017	42.6	36.1	13.4	7.9	Clay loam	7.95	2.8	61.5	4.8	1.15	8.00	12.7	7.25
2018	36.4	35.3	22.8	5.5	Clay loam	7.92	1.66	62.5	7.7	1.20	0.71	13.3	0.92
			ons (mEqu L	,			e nutrients (						
Seasons		Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	N	Р		K	Zn	Mn		Fe
2017		12.0	7.7	8.1	0.19	182	8.86		409	6.55	10.1	2	15.2
2018		4.7	2.2	8.0	0.10	371	6.35		398	4.34	9.0	18	10.1

Source: Central Lab for Soil Analysis, Agricultural Research Center, Cairo, Egypt

Table 3: Meteorological data during the two growing seasons of the experiment

	remperature	remperature									
Month	Maximum (°C)	Minimum (°C)	Average (°C)	Relative humidity (%)	Wind speed (m sec <sup>-1</sup> )	Sunshine duration (h)					
2017											
May	34.6	19.4	29.3	34.0	2.0	13.4					
June	36.7	16.0	23.3	23.3	2.0	13.9					
July	38.2	24.5	33.5	42.3	1.6	13.8					
August	37.1	24.6	32.5	46.3	2.0	13.1					
2018											
May	34.6	19.1	28.9	38.7	3.4	13.4					
June	38.6	22.5	33.5	31.7	2.0	13.9					
July	36.6	24.3	32.6	46.3	2.1	13.8					
August	37.2	23.8	32.5	44.3	3.5	13.0					

Source: Central Lab for Agricultural Climate, Agricultural Research Center, Giza Governorate, Egypt

from the Central Lab for Agricultural Climate, ARC at Giza, Egypt (Table 3). Rainfall was nil during all months of maize growing seasons.

#### Data recording:

- Ears Per Plant (EPP) calculated by dividing number of ears on number of plants per plot
- Rows Per Ear (RPE) using a sample of 10 random ears plot<sup>-1</sup>
- Kernels Per Row (KPR) using a sample of 10 random ears plot<sup>-1</sup>
- 100-kernel weight (100KW) (g) using shelled grains of each plot and adjusted at 15.5% grain moisture
- Grain Protein Content (GPC%), 6: Grain Oil Content (GOC%), 7: Grain Starch Content (GSC%) And 8: Grain

yield plant<sup>-1</sup> (GYPP) using a sample of 10 random plants plot<sup>-1</sup> at 15.5% grain moisture

- Grain Yield Per Hectare (GYPH) by adjusting grain yield plot<sup>-1</sup> to grain yield per hectare
- Protein Yield Per Hectare (PYPH) by multiplying GPC by GYPH
- Oil Yield Per Hectare (OYPH) by multiplying GOC by GYPH
- Starch Yield Per Hectare (SYPH) by multiplying GSC by grain yield GYPH

GPC, GOC and GSC traits were estimated using a non-destructive grain analyzer, Model Infratec TM 1241, ISW 5.00, manufactured by Foss Analytical AB, Hoganas, Sweden. Density Tolerance Index (DTI) modified from equation suggested by Fageria<sup>25</sup> was used to classify genotypes for tolerance to elevated density. The formula used is as follows:

DTI = (Y1/AY1) X (Y2/AY2)

#### **RESULTS**

#### Where:

Y1 = Grain yield mean of a genotype at non-stress (low density)

AY1 = Average yield of all genotypes at non-stress (low density)

Y2 = Grain yield mean of a genotype at stress (medium or high density)

AY2 = Average yield of all genotypes at stress (medium or high density)

when, DTI is  $\geq$ 1.25, it indicates that genotype is Highly Tolerant (HT), if DTI is  $\geq$ 1.0 and <1.25, it indicates that genotype is Moderately Tolerant (MT), if DTI is <1.0 and  $\geq$ 0.75, it indicates that genotype is Moderately Sensitive (MS), If DTI is <0.75, it indicates that genotype is Very Sensitive (VS).

**Biometrical analysis:** Combined analysis of variance of the split-plot design in RCB arrangement on the basis of individual plot observation and combined analysis of variance of RCBD for each of the three environments (LD, MD, HD) across the two seasons were performed if the homogeneity test was non-significant using the MIXED procedure of SAS® Littell *et al.*<sup>26</sup> Least Significant Differences (LSD) were calculated according to Steel *et al.*<sup>27</sup>.

**Analysis of variance:** Combined analysis of variance across years (2017 and 2018) for studied corn grain quality and yield traits of 17 maize genotypes (15 diallel  $F_1$  crosses and two checks) under three plant densities using a split plot design is presented in (Table 4). Variances due to years were significant (p $\le$ 0.01) for all studied traits, except for grain starch content. Variances due to plant density and maize genotype were significant (p $\le$ 0.01) for all studied traits, except grain oil content, grain protein content and grain starch content for densities.

The variances due to D $\times$ Y, G $\times$ Y and G $\times$ D interactions for all traits under investigation were significant (p $\leq$ 0.01), except grain starch content for D $\times$ Y. The variance due to G $\times$ D $\times$ Y interaction was significant (p $\leq$ 0.01) for all studied characteristics.

The combined analysis of variance of RCBD under each plant density across seasons (data not presented) showed that variances due to genotypes under all densities were significant ( $p \le 0.01$ ) for all studied characteristics.

**Effect of elevated plant density:** Mean grain yield plant<sup>-1</sup> was significantly (p $\leq$ 0.01) reduced due to increasing plant density from 47,600-71,400 and 95,200 plants ha<sup>-1</sup>, by 21.56 and 41.38%, respectively (Table 5). The reduction in grain yield plant<sup>-1</sup> due to the increase in plant density to 71,400

Table 4: Combined analysis of variance of split-plot design for grain quality and yield traits of 17 maize genotypes (G) under three plant densities (D) across 2017 and 2018 years (Y)

		Mean squares	Mean squares							
Source of variance	df	Grain yield plant <sup>-1</sup>	Ears plant <sup>–1</sup>	Rows/ear	Kernels/row	100-kernel weight	Grain oil content (%)			
Years (Y)	1	74581**	0.01**	1.36**	1.32**	390**	0.66**			
Densities (D)	2	144408**	3.87**	58.03**	1112**	859**	0.006			
$D \times Y$	2	29.42**	0.032**	0.23**	35.05**	5.45**	0.22**			
Error a	12	0 0.48	0.0003	0.012	0.132	0.02	0.151			
Genotypes (G)	16	7752**	0.090**	16.97**	221.1**	136**	2.44**			
$G{ imes}Y$	16	379.6**	0.026**	0.89**	23.46**	12.48**	0.20**			
$G{ imes}D$	32	865.0**	0.029**	0.44**	8.90**	3.37**	0.08**			
$G \times D \times Y$	32	489.8**	0.023**	0.47**	5.36**	6.19**	0.06**			
Error b	192	3.02	0.001	0.009	0.09	0.08	0.009			
CV (%)		1.21	2.29	0.66	0.71	0.94	2.15			
		Grain protein	Grain starch	Oil	Protein	Starch	Grain			
	df	content (%)	content (%)			yield ha <sup>-1</sup>				
Years (Y)	1	4.35**	0.30	0.19**	0.99**	73.95**	143.08**			
Densities (D)	2	0.84	1.79	0.32**	1.83**	81.07**	158.47**			
$D \times Y$	2	3.56**	2.43	0.03**	0.08**	5.50**	10.46**			
Error a	12	0.33	1.66	0.002	0.003754	0.04	0.02			
Genotypes (G)	16	10.47**	14.92**	0.07**	0.27**	14.57**	28.89**			
$G{ imes}Y$	16	1.05**	1.18**	0.01**	0.06**	2.63**	5.20**			
$G{ imes}D$	32	0.57**	0.84**	0.01**	0.05**	2.04**	3.93**			
$G \times D \times Y$	32	0.61**	0.92**	0.01**	0.04**	1.65**	3.29**			
Error b	192	0.27	0.14	0.0001	0.003	0.02	0.03			
CV (%)		4.99	0.52	2.65	5.17	1.84	1.75			

G: Genotypes, D: Plant densities, Y: Years (Y), ha: Hectare and \*,\*\*Indicate significance at 0.05 and 0.01 probability levels, respectively

Table 5: Means of studied traits and change (%) from low (47,600 plants ha<sup>-1</sup>) to medium (71,400 plants ha<sup>-1</sup>) and high (95,200 plants ha<sup>-1</sup>) density combined across all studied genotypes and across 2017 and 2018 seasons

Trait	47,600 plants ha <sup>-1</sup>	71,400 plants ha <sup>-1</sup>	Change (%)	95,200 plants ha <sup>-1</sup>	Change (%)
Grain yield plant <sup>-1</sup> (g)	181.39	142.29	21.56**	106.32	41.38**
Ears plant <sup>-1</sup>	1.20	0.96	19.51**	0.81	32.31**
Rows/ear	15.49	14.67	5.31**	13.99	9.73**
Kernels/row	46.05	41.98	8.84**	39.48	14.26**
100-Kernel weight	33.12	29.78	10.09**	27.33	17.46**
Grain oil content (%)	4.40	4.39	0.22	4.40	0.00
Grain protein content (%)	10.45	10.24	2.08	10.42	0.37
Grain starch content (%)	71.17	71.43	-0.37	71.33	-0.22
Oil yield (t ha <sup>-1</sup> )	0.39	0.43	-10.26**	0.50	-27.57**
Protein yield (t ha <sup>-1</sup> )	0.93	0.99	-6.73**	1.17	-26.34**
Starch yield (t ha <sup>-1</sup> )	6.36	7.09	-11.47**	8.15	-28.15**
Grain yield (t ha <sup>-1</sup> )	8.94	9.78	-9.48**	11.39	-27.45**

t: Ton, ha: Hectare, -: Decrease, +: Increases, \*, \*\*Indicate significance at 0.05 and 0.01 probability levels, respectively

and 95,200 plants  $ha^{-1}$  was associated with significant (p $\leq$ 0.01) reductions in all yield components, i.e., ears plant<sup>-1</sup> (by 19.51 and 32.31%), rows/ear (by 5.31 and 9.73%), kernels/row (by 8.84 and 14.26%) and 100-kernel weight (by 10.09 and 17.46%), respectively.

In contrast, compared with the low-density, medium and high plant densities caused a significant increase in oil yield  $ha^{-1}$  by 6.73 and 26.34%, protein yield  $ha^{-1}$  by 6.73 and 26.34%, starch yield  $ha^{-1}$  by 11.47 and 28.15% and grain yield  $ha^{-1}$  by 9.48 and 27.45%, respectively.

Effect of maize genotype: Ranges (difference between maximum and minimum mean values) of the studied 17 genotypes combined across the three plant densities varied significantly for all studied 12 traits (Table 6). Means of genotypes across all plant densities varied from 99.86 g ( $2 \times 4$ ) to 171.91 g (1 $\times$ 5) for grain yield plant<sup>-1</sup>, from 0.915 (5 $\times$ 6) to 1.087 (2×6) for number of ears plant<sup>-1</sup>, from 13.01 (1×2) to 16.53 (4 $\times$ 6) for number of rows/ear, from 36.34 (2 $\times$ 4) to  $48.16 (1 \times 5)$  for number of kernels/row, from 25.65 g (2 × 4) to 34.59 g (1 $\times$ 5) for 100- kernel weight, from 3.91% (3 $\times$ 5) to 5.12 (1 $\times$ 2) for grain oil content, from 9.13% (5 $\times$ 6) to 11.84  $(2\times4)$  for grain protein content, from 69.38  $(1\times2)$  to 72.81  $(5\times6)$  for grain starch content, from 0.352 t  $(2\times4)$  to 0.582 t  $(1\times5)$  for oil yield ha<sup>-1</sup>, from 0.844 t  $(2\times6)$  to 1.214 t  $(1\times5)$  for protein yield ha<sup>-1</sup>, from 5.10 t (2×4) to 9.10 t (1×5) for starch yield ha<sup>-1</sup> and from 7.24 t (2×4) to 12.81 t (1×5) for grain yield  $ha^{-1}$ .

**Superiority of tolerant (T) over sensitive (S) genotypes:** Data of selected traits averaged for the two groups of genotypes differing in their Density Tolerance Index (DTI) (tolerant, T and sensitive, S) based on grain yield plant<sup>-1</sup> under high plant density are presented in Table 7.

Data in Table 7 indicated that grain yield plant<sup>-1</sup> of Tolerant (T) was higher than that of the Sensitive (S) hybrids by

27.94 and 32.95%, under 70,400 and 95,400 plants ha<sup>-1</sup>, respectively. Superiority of tolerant (T) to sensitive (S) hybrids in grain yield ha<sup>-1</sup> under HD was due to their superiority in grain yield ha<sup>-1</sup> (31.1 and 32.95%) under MD and HD, respectively. Moreover, under MD and HD, the T hybrids showed 27.14 and 28.20% more oil yield ha<sup>-1</sup>, 30.28 and 28.97% more protein yield ha<sup>-1</sup> and 30.66 and 32.01% more starch yield ha<sup>-1</sup> than the S hybrids, respectively (Table 7).

**Grouping genotypes based on drought tolerance and yields ha**<sup>-1</sup>: Density Tolerance Index (DTI) across years of the studied 17 genotypes under High Density (HD) stress was plotted against each of grain yield ha<sup>-1</sup> (GYPH), protein yield ha<sup>-1</sup> (PYPH), oil yield ha<sup>-1</sup> (OYPH) and starch yield ha<sup>-1</sup> under high density, of the same genotypes (Fig. 1), which made it possible to distinguish 16 groups (a combination between four groups of DTI, namely:

HT = Highly tolerant

MT = Moderately tolerant

MS = Moderately sensitive

VS = Very sensitive and four groups of yields  $ha^{-1}$ , namely

VHY = Very high yield

AAY = Above-average yield

BAY = Below-average yield

VLY = Very low yield

i.e., VHY-HT, AAY-HT, BAY-HT, VLY-HT, VHY-MT, AAY-MT, BAY-MT, VLY-MT, VHY-MS, AAY-MS, BAY-MS, VLY-MS, VHY-VS, AAY-VS, BAY-VS and VLY-VS.

The relation between Density Tolerance Index (DTI) and grain yield  $ha^{-1}$  (Fig. 1a) proved that the genotypes No.11 (3×5) and No.16 (the check SC 2055 from Hi-Tech Company) belong to the group VHY-HT "very high grain yield and highly tolerant", genotype No. 2 belong to the group AAY-HT "above-average grain yield and highly tolerant" and

# Asian J. Plant Sci., 20 (1): 91-101, 2021

Table 6: Maximum and Minimum values of studied grain quality and yield traits of the  $F_1$  crosses and checks single cross 2055 (SC 2055) and single cross 130 (SC 130) under 3 plant densities and their combined across two seasons

Parameter	47,600 plants ha <sup>-1</sup>	71,400 plants ha <sup>-1</sup>	95,200 plants ha <sup>-1</sup>	Combined
Grain yield plant <sup>-1</sup> (g)	, ,	· ·	· ·	
Maximum	238.08 (1×5)	175.62 (3×5)	123.49 (1×5)	171.91 (1×5)
Minimum	129.05 (2×4)	95.60 (2×4)	74.92 (2×4)	99.86 (2×4)
SC 2055	225.39	168.13	147.82	180.45
SC 130	184.66	190.95	120.55	165.39
LSD <sub>0.05</sub>	3.07	2.02	2.79	1.14
Ears plant <sup>-1</sup>	5.07	2.02	2.73	1.17
	1 46 (1 × 5)	1.00 (2 × 4)	0.90 (2×6)	1 007 (2 × 6)
Maximum	1.46 (1×5)	1.09 (3×4)	0.89 (2×6)	1.087 (2×6)
Minimum	1.05 (5×6)	0.92 (3×6)	0.74 (2×4)	0.915 (5×6)
SC 2055	1.43	1.05	0.90	1.128
SC 130	1.22	0.88	0.72	0.94
LSD <sub>0.05</sub>	0.04	0.01	0.02	0.02
Rows/ear				
Maximum	17.26 (4×6)	16.41 (5×6)	16.06 (4×6)	16.53 (4×6)
Minimum	13.79 (1×3)	12.51 (1×2)	12.39 (1×3)	13.01 (1×2)
SC 2055	15.91	15.23	13.88	15.01
SC 130	15.58	15.10	14.33	15.00
LSD <sub>0.05</sub>	0.15	0.15	0.15	0.06
Kernels/row				
Maximum	51.33 (1×5)	47.20 (1×5)	45.96 (1×5)	48.16 (1×5)
Minimum	40.84 (2×4)	35.48 (2×6)	32.47 (2×4)	36.34 (2×4)
SC 2055	50.81	46.29	42.47	46.52
SC 130	46.58	43.94	40.96	43.83
LSD <sub>0.05</sub>	0.38	0.34	0.61	0.20
100-kernel weight (g)	0.50	0.5 1	0.01	0.20
	38.18 (1×5)	24 02 (1 × E)	31.76 (1×5)	34.59 (1×5)
Maximum		34.93 (1×5)	* *	
Minimum	28.74 (4×6)	24.60 (2×4)	22.13 (2×4)	25.65 (2×4)
SC 2055	31.19	28.39	27.14	28.90
SC 130	35.86	33.36	30.94	33.39
LSD <sub>0.05</sub>	0.46	0.46	0.38	0.19
Grain oil content (%)				
Maximum	5.13 (1×2)	5.17 (1×2)	5.05 (1×2)	5.12 (1×2)
Minimum	3.80 (3×5)	3.90 (3×5)	3.97 (3×6)	3.91 (3×5)
SC 2055	4.50	4.60	4.88	4.66
SC 130	3.95	3.93	4.07	3.98
LSD <sub>0.05</sub>	0.16	0.12	0.16	0.06
Grain protein content (%)				
Maximum	11.73 (2×4)	12.00 (2×4)	11.80 (2×4)	11.84 (2×4)
Minimum	9.23 (5×6)	9.12 (5×6)	9.05 (5×6)	9.13 (5×6)
SC 2055	10.30	9.70	10.50	10.17
SC 130	10.22	9.92	10.17	10.10
LSD <sub>0.05</sub>	0.35	0.15	0.15	0.21
Grain starch content (%)				
Maximum	72.77 (5×6)	72.83 (5×6)	72.82 (5×6)	72.81 (5×6)
Minimum	69.55 (1×2)	69.47 (1×2)	69.13 (1×2)	69.38 (1×2)
SC 2055	70.92	71.3	70.3	70.84
SC 130	71.32	71.97	71.35	71.54
LSD <sub>0.05</sub>	0.43	0.43	0.4	0.25
Oil yield ha <sup>-1</sup> (t)				
Maximum	0.45 (1×2)	0.51 (1×2)	0.65 (1×5)	$0.582(1 \times 5)$
Minimum	0.32 (2×4)	0.33 (2×4)	0.36 (4×6)	$0.352(2\times4)$
SC 2055	0.45	0.59	0.61	0.55
SC 130	0.36	0.41	0.52	0.43
LSD <sub>0.05</sub>	0.13	0.03	0.03	0.01
Protein yield ha <sup>-1</sup> (t)				
Maximum	1.12 (1×3)	1.15 (3×5)	1.42 (1×5)	1.214 (1×5)
Minimum	0.72 (2×6)	0.71 (2×6)	0.80 (4×6)	0.844 (2×6)
SC 2055	1.04	1.22	1.32	1.19
SC 130	0.91	1.02	1.26	1.06
LSD <sub>0.05</sub>	0.02	0.02	0.02	0.04
· U.U.D				

Table 6: Continue

Parameter	47,600 plants ha <sup>-1</sup>	71,400 plants ha <sup>-1</sup>	95,200 plants ha <sup>-1</sup>	Combined
Starch yield ha <sup>-1</sup> (t)				
Maximum	8.07 (1×5)	8.56 (1×5)	10.67 (1×5)	9.10 (1×5)
Minimum	4.53 (2×4)	4.68 (2×4)	6.10 (2×4)	5.10 (2×4)
SC 2055	7.09	9.19	8.96	8.42
SC 130	6.43	7.53	8.88	7.61
LSD <sub>0.05</sub>	0.02	0.13	0.13	0.10
Grain yield ha <sup>-1</sup> (t)				
Maximum	11.51 (1×5)	12.01 (1×5)	14.93 (1×5)	12.81 (1×5)
Minimum	6.44 (2×4)	6.66 (2×4)	7.84 (4×6)	$7.24(2 \times 4)$

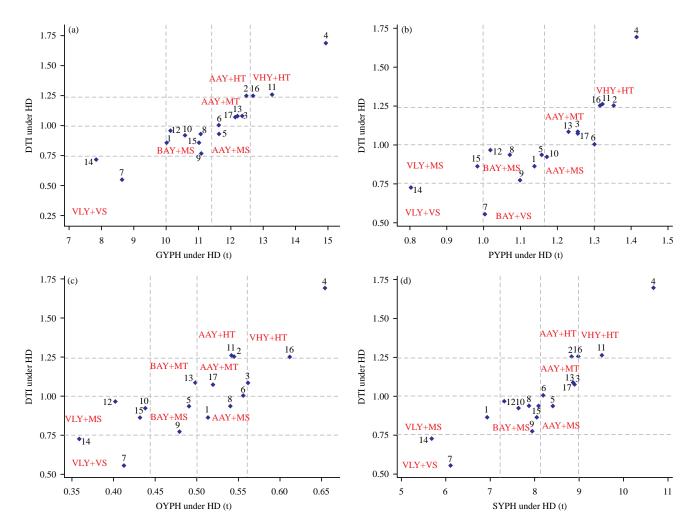


Fig. 1(a-d): Relationship between density tolerance index (DTI) and means

(a) grain yield ha<sup>-1</sup> (GYPH), (b) protein yield ha<sup>-1</sup> (PYPH), (c) oil yield ha<sup>-1</sup> (OYPH) and (d) starch yield ha<sup>-1</sup> (SYPH) in tons under high plant density (HD) across two seasons. Numbers from 1-17 refer to 15 F1 hybrid names and 2 checks (Table 3). VHY: Very high yield, AAY: Above-average yield, BAY: Below-average yield, VLY: very low yield, HT: Highly tolerant, MT: Moderately tolerant, MS: Moderately sensitive and VS: Very sensitive

genotypes No. 3 (1×4), 13 (4×5), 17 (the check SC 130 from ARC, Egypt) and 6 (2×3) belong to the group AAY-MT" above-average grain yield and moderately tolerant". On the contrary, for the relation between density tolerance index (DTI) and grain yield ha $^{-1}$ , genotypes No. 7 (2×4) and 14 (4×6) belong to the group VLY-VS "very low grain yield and very sensitive".

For the relation between Density Tolerance Index (DTI) and protein yield  $ha^{-1}$  (Fig. 1b), the genotypes No. 2 (1×3), No. 11 (3×5) and No. 16 (the check SC 2055 from Hi-Tech Company) proved to belong to the group VHY-HT "very high protein yield and highly tolerant", genotypes No. 3 (1×4), 6 (2×3), 13 (4×5) and 17 (the check SC 130) belong to the

Table 7: Superiority (%) of the three most tolerant (T) to the three most sensitive (S) hybrids for selected traits under 70,400 and 95,200 plants ha<sup>-1</sup> across 2017 and 2018 seasons

	70,400 plant	s ha <sup>-1</sup>		95,200 plants ha <sup>-1</sup>			
Trait	T	S	 Superiority (%)	T	S	Superiority (%)	
Grain yield plant <sup>-1</sup>	161.40	116.30	27.94**	119.85	80.36	32.95**	
Kernels/row	44.76	38.83	13.25**	43.61	34.64	20.55**	
100-kernel weight	33.78	28.55	15.49**	30.68	24.12	21.38**	
Oil yield ha <sup>-1</sup> (t)	0.48	0.35	27.14**	0.58	0.42	28.20**	
Protein yield ha-1 (t)	1.14	0.79	30.28**	1.36	0.97	28.97**	
Starch yield ha <sup>-1</sup> (t)	8.16	5.66	30.66**	9.66	6.57	32.01**	
Grain yield ha-1 (t)	11.42	7.87	31.10**	13.56	9.18	32.27**	

T: Tolerant, S: Sensitive, t: Ton, ha: Hectare, Superiority (%): 100×[(T-S)/S], \*,\*\*Significant at 0.05 and 0.01 probability levels

group AAY-MT "above-average protein yield and highly tolerant" but genotype No. 14 ( $4\times6$ ) belong to the group VLY-VS "very low protein yield and very sensitive".

Regarding the relation between Density Tolerance Index (DTI) and oil yield ha $^{-1}$  (Fig. 1c), the genotypes No. 4 (1×5) and No. 16 (the check SC 2055 from Hi-Tech) proved to belong to the group VHY-HT "very high oil yield and highly tolerant", genotypes No. 2 (3×5) and 11 belong to the group AAY-HT "above-average oil yield and highly tolerant", genotypes No. 3 (1×4), 17 (the check SC 130 from ARC, Egypt) and No. 6 (2×3) belong to the group AAY-MT" above-average oil yield and moderately tolerant" but genotype No. 14 (4×6) and No. 7 (2×4) belong to the group VLY-VS "very low protein yield and very sensitive".

With respect of the relation between Density Tolerance Index (DTI) and starch yield  $ha^{-1}$  (Fig. 1d), the genotypes No. 4 (1×5) and No. 11 (3×5) proved to belong to the group VHY-HT "very high starch yield and highly tolerant", genotypes No. 2 (3×5) and 16 (the check SC 2055 from Hi-Tech Company) proved to belong to the group AAY-HT "above-average starch yield and highly tolerant", genotypes No. 3 (1×4), 17 (the check SC 130 from ARC, Egypt), No. 13 (4×5) and 6 (2×3) belong to the group AAY-MT" above-average oil yield and moderately tolerant" but genotype No. 14 (4×6) and No. 7 (2×4) belong to the group VLY-VS "very low oil yield and very sensitive".

# **DISCUSSION**

Oil, protein and starch yields of maize grain per land unit area would be maximized either by increasing their percentages (contents) in the grain or by increasing the yield of grains per land unit area. The present research aimed at increasing the yield of oil, protein and starch per unit area via increasing the yield of grains per unit area. This could be achieved though using elevated plant density along with using the adaptable genotype to high plant density.

Analysis of variance (ANOVA) of the split plot design indicated that variances due to year, plant density and genotype were significant for all studied traits, except grain starch content for year and grain protein content, grain oil content and grain starch content for plant density, suggesting significant effect of climatic conditions, plant densities and maize genotypes on all studied characteristics, except grain starch content trait for years and grain protein content, grain oil content and grain starch content for plant densities. Current study results are in agreement with other studies 18,28. Mean squares due to first and second order interaction were significant for all studied traits, except grain starch content for D×Y, suggesting that the rank of maize genotypes differ density to another and from a density-year combination to another and the possibility of selection for improved performance under a specific plant density-year combination as proposed by several investigators<sup>3-5,30-34</sup>. Moreover, ANOVA of RCBD suggested that maize hybrids differed significantly under each of the three studied environments for all traits under investigation. The existence of genotypic variation in maize grain quality traits was reported by several<sup>3,11,16</sup> for grain oil content and 13-15 for grain starch content 17 and for grain protein content.

The reduction in grain yield plant<sup>-1</sup> due to elevated plant density is logic because of the competition between plants at high densities for light, nutrients and water requirements. This conclusion was also reported in several investigations<sup>4-5,10,35-41</sup>. Raising plant density from 47,600-71,400 and 95,200 plants ha<sup>-1</sup> caused reductions in all yield components. Ears plant<sup>-1</sup> trait was the most affected trait followed by 100-kernel weight and kernels/row but rows/ear was the least affected trait. This indicates that prolificacy, kernel size and number are important traits for breeding tolerant varieties to high density. However, increasing plant density from low to medium and high did not result in any significant change in all grain quality traits (grain oil content, grain protein content and grain starch content). On the other hand, increasing plant density from 47,600-95,200 plants ha<sup>-1</sup>

caused a significant increase in grain yield ha<sup>-1</sup>, protein yield ha<sup>-1</sup>, oil yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup> by 27.45, 26.34, 26.34 and 28.15 %, respectively. It seems that the increase in protein, oil and/or starch yield ha<sup>-1</sup> as a result of increasing plant density is due mainly to the increase of grain yield ha<sup>-1</sup>, since the percentage of protein, oil and/or starch content in maize grain did not change significantly from one plant density to another. Although high plant density in our experiment resulted in reduction in grain yield plant<sup>-1</sup>, the use of high-density would overcome the negative effects of competition and led to maximizing grain yield ha<sup>-1</sup>, protein yield ha<sup>-1</sup>, oil yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup> in this experiment. This conclusion is in agreement with that reported by several investigations<sup>4,5,9-10</sup>.

The range of means of the studied 17 genotypes combined across the three plant densities was wide for all studied traits, except for ears plant<sup>-1</sup>, grain protein content, grain oil content and grain starch content, which was narrow. The best  $F_1$  in this study  $1 \times 5$  (Sd7 $\times$ Inb 92), across all 3 environments, excelled significantly the best check SC 2055 by 14.17 % for grain yield ha<sup>-1</sup>, 1.85 % for protein yield ha<sup>-1</sup>, 6.00% for oil yield ha<sup>-1</sup> and 8.08% for starch yield ha<sup>-1</sup>. 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%"42. In another study Mittelmann et al.42 on the genetic variation for oil content in maize with normal endosperm, the author reported values between 3.77 and 5.10%. Several studies<sup>3,13</sup> Misevic and Alexander<sup>43</sup> reported the existence of genotypic differences and the prospect of selection for maize oil content. Similarly, the range of variability in protein content in the present study is in the range found in the literature for normal maize. Moreover, several investigators investigations<sup>4,5,13,14,20</sup> reported the existence of genetic variability for protein content in maize. The existence of genetic variability for grain protein content, grain oil content, grain starch content, protein yield ha<sup>-1</sup>, oil yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup> in this study indicates that these traits of maize grain could be improved by classical breeding programs.

The use of HD would overcome the negative impacts of interplant competition and lead to maximizing grain yield  $ha^{-1}$ , protein yield  $ha^{-1}$ , oil yield  $ha^{-1}$  and starch yield  $ha^{-1}$ , such maximization was more pronounced by some genotypes, such genotypes would be tolerant to elevated plant density. In general, the cross  $(1\times5)$  under the high plant density environment (HD) recorded the highest means of grain yield traits (grain yield plant<sup>-1</sup>, grain yield  $ha^{-1}$ , protein yield  $ha^{-1}$  and oil yield  $ha^{-1}$ ) and the highest means of the grain yield components (ears plant<sup>-1</sup>, rows/ear, kernels/row and

100 kernels weight). However, the highest means of grain quality traits (grain protein content, grain oil content and grain starch content) were recorded by different crosses ( $2\times4$ ,  $1\times2$  and  $5\times6$ , respectively), under the medium density environment (MD). Significant environment and genotype×environment interaction effects are in general detected for protein content<sup>21,22</sup> and East and Jones<sup>17</sup> and Oikeh *et al.*<sup>23</sup> reported that "among the environment factors that influence protein content, temperature and availability of water and nitrogen in the soil are the most important".

Density Tolerance Index (DTI) estimated using the equation suggested by Fageria<sup>25</sup> indicated that the hybrid  $1\times5$  (Sd7 $\times$ Inb 92) is the highest density tolerant one but the most sensitive one was the hybrid  $2\times4$  (CML104 $\times$ Inb 171). Superiority of T to S F<sub>1</sub> crosses in grain yield ha<sup>-1</sup> under MD and HD was due to their superiority in grain yield plant<sup>-1</sup>, protein yield ha<sup>-1</sup>, oil yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup>. Also, Al-Naggar *et al.*<sup>4</sup> reported that under high plant density, the tolerant testcrosses showed 314.4% more grain yield plant<sup>-1</sup> than sensitive test crosses.

Grouping genotypes based on drought tolerance and yields ha<sup>-1</sup> was performed according to<sup>44,45</sup> with some modifications. According to Fageria and Baligar<sup>46,47</sup> genotypes (progenies) belonging to the 1st group VHY-HT "very high yield and highly tolerant" (above all), the group AAY-HT "above-average yield and highly tolerant", the group AAY-MT "above-average yield and moderately tolerant" and the group VHY-MT "very high yield and moderately tolerant" (to a lesser extent) appear to be the most desirable materials for breeding programs that deal with adaptation to high density stress". The results in Fig 1 indicated that the genotype No.4 (1 $\times$ 5) was the most tolerant and the highest in grain yield ha<sup>-1</sup>, protein yield ha<sup>-1</sup>, oil yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup>, i.e., it belongs to a group that include "very high yield of grain, protein, oil and starch and highly tolerant", the genotype No.11 ( $3 \times 5$ ) was the most tolerant and the highest in grain yield ha<sup>-1</sup>, protein yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup>, i.e., it belongs to a group that include "very high yield of grain, protein and starch and highly tolerant", the genotype No. 16 (SC 2055) was the most tolerant and the highest in grain yield ha<sup>-1</sup>, protein yield ha<sup>-1</sup> and oil yield ha<sup>-1</sup>, i.e., it belongs to a group that include "very high yield of grain, protein and oil and highly tolerant", the genotype No. 2  $(1\times3)$  was the most tolerant and the highest in protein yield ha<sup>-1</sup> and had above-average oil, starch and grain yield. These genotypes (No. 4, 11, 16 and 2) could be recommended to future breeding programs aimed at improving grain, protein, oil and starch yields and tolerance to high density stress.

### **CONCLUSION**

Results concluded that elevated plant density resulted in significant reductions in grain yield plant<sup>-1</sup> and the grain quality traits content. However, the use of high-density could overcome such reduction and led to maximize grain, oil, protein and starch yields per hectare, such maximization was more pronounced by the highly tolerant hybrids to Both Medium (MD) and High Density (HD). Using HD Tolerant (T) hybrids was superior to Sensitive (S) hybrids under highdensity environment by 32.27% (grain yield ha<sup>-1</sup>), 28.20% (oil yield ha<sup>-1</sup>), 28.97% (protein yield ha<sup>-1</sup>) and 32.01% (starch yield ha<sup>-1</sup>). The  $F_1$  cross 1×5 (Sd 7×Inb 92) showed the maximum value for oil yield ha<sup>-1</sup>, protein yield ha<sup>-1</sup> and starch yield ha<sup>-1</sup> under HD environment followed by the F₁ cross  $3\times5$  (Inb  $17\times$ Inb 92) and the  $F_1$  cross  $1\times3$  (Sd7 $\times$ Inb 17). These hybrids should be given more attention in future breeding programs aiming at development of maize hybrids of high yields of oil, protein and/or starch per land unit area and high tolerance to high density in order to maximize yield ha<sup>-1</sup> from such grain quality traits.

#### SIGNIFICANCE STATEMENT

"This study discovered the role of HD-tolerant genotype that can be beneficial for maximizing yield per land unit area of grain, oil, protein and starch when using high plant density. This study will help the researchers to uncover the critical areas of negative effects of high plant density in reducing grain yield per plant due to interplant competition that many researchers were not able to explore. Thus a new theory on maximizing yield of grain and important quality traits may be arrived at".

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