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Curvilinear regression of maize (*Zea mays* L.) grain quality traits on elevated plant density combined with deficit irrigation

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Abstract The main objective of the present investigation was to determine the optimum plant density combined with irrigation regime for obtaining the highest grain protein, oil and starch contents and yields of different maize genotypes. Six inbreds and their 15 diallel hybrids 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 (PD), *i.e.* low (47,600), medium (71,400) and high (95,200) plants/ha in combination with two irrigation regimes, *i.e.* well watering (WW) and water stress (WS) at flowering. Results combined across two seasons revealed that elevated combined stress caused a decrease in GOC and GYPP and an increase in GPC and GSC. The traits GYPH, PYPH, OYPH and SYPH showed a trend of increase from non-stressed to high-density stressed environment followed by a trend of decrease reaching maximum reduction at the most stressed one (WS-high PD). Regression functions revealed that for GYPH, PYPH, OYPH and SYPH, the three highest-yielding hybrids to the elevated plant density combined with deficit irrigation showed a curvilinear response of decrease with an optimum environment of well watering combined with high plant density, except the hybrid L20 x L53, which showed a continuous response of increase. The highest hybrids in GPC and GSC showed near linear regression of decrease, except L18 x L28, which showed a curvilinear regression with optimum environment of the WW-high PD. The highest hybrids in GOC showed a quadratic response of increase with optimum environment of WW-high PD (L53 x Sd7) and WS-low PD (Sk5 x Sd7). The best hybrids for PYPH, OYPH, SYPH and GYPH (L20 x L53, L53 x Sd7 and L53 x Sk5) showed differential response depending on the genetic background of the hybrid and the trait of interest. The optimum environment for obtaining the highest yield/ha for protein, oil or starch was identified for each hybrid.

Keywords Corn grain constituents, Optimum environment, flowering stage drought, High plant density

Introduction

Maize (*Zea mays* L.) 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 [1]. 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 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 [2-6]. 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 [3, 7-11] for oil content and [10, 12-15] 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 [12, 16-18].

Maximization of total production of maize through raising productivity per land unit area could be achieved *via* developing new varieties that can withstand high plant density up to 100,000 plants ha^{-1} [19] along with using the best management, especially irrigation and N fertilization. Average maize grain yield per unit land area in the USA increased dramatically during the second half of the 20th century, due to improvement in crop management practices and greater tolerance of modern hybrids to abiotic stresses such as drought and high plant density stress [20-22]. 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 both plant grain yield and grain quality characteristics, the use of high-density along with well irrigation and fertilization would overcome the negative impacts of such competition and lead to maximizing maize productivity from the same unit area [23-26].

Maize is considered more susceptible than most other cereals to drought stresses at flowering [1]. Grant *et al*. [27] reported that although yields were most severely reduced (70%) by stress coinciding with silking, yields were reduced by 40-54% from stresses occurring in the period 10 to 31 days after mid-silk. Recent studies have shown considerable genetic variation in the response of commercial hybrids to drought stress imposed during reproductive growth [28]. There is good evidence suggesting that hybrids maintain their advantage over open pollinated varieties in both stress and non-stress environments [21, 29, 30]. The presence of genotypic differences in drought tolerance would help plant breeders in initiating successful breeding programs to improve such a complicated character.

In general, significant environment and genotype × environment interaction effects are detected for grain protein and oil contents in maize [5, 6, 12, 13, 16-18, 31, 32] Among the environment factors that influence grain constituents, temperature, availability of water and nitrogen in the soil are the most important [8, 11, 13-18]. Knowledge about genetic diversity and relationships among breeding materials could be an invaluable aid in maize improvement strategies. Studies have documented genetic and phenotypic variability for grain composition traits in maize [32-37]. There are reports on the effects of water stress on the chemical composition of maize grains [38, 39], but little work has been reported about the effect of high density stress (light, water and nutrient stresses) and high density combined with deficit irrigation stress on maize kernel composition of different genotypes. Moreover, there are some reports on the quadratic response of grain yield on the elevated plant density [23-26, 40-42], 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 study: (*i*) the effects of high plant density combined with deficit irrigation on maize grain protein, oil and starch contents and yields and (*ii*) the differential response for such traits of inbreds and hybrids on six environments resulting from the combinations between three plant densities and two irrigation regimes.

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.

Plant material

Based on the results of previous experiments [5], six maize (*Zea mays* L.) inbred lines in the 8thselfed generation (S_8), showing clear differences in performance and general combining ability for grain yield/ha under high plant density, were chosen in this study to be used as parents of diallel crosses. Three of these inbreds (L-20, L-53 and Sk-5) were prolific, of erect leaves and high-yielding and the other three (L-18, L-28 and Sd-7) were of low-yielding under high density.

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 9th selfed generation (S_9 seed).

Evaluation of parents and F_1`s

Field evaluation experiments were carried out at the Agricultural Experiment and Research Station of Faculty of Agriculture, Cairo University, Giza, Egypt in 2013 and 2014 seasons. Each experiment included 15 F_1 crosses, their 6 parents and 2 check cultivars, *i.e.* 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 under two water regimes (well watering; WW and water stress; WS at flowering stage by skipping the 4th and 5th irrigations) and three plant densities, (47,600, 71,400 and 95,200 plants/ha, representing low-, medium- and high-plant density, respectively).

A split-split plot design in randomized complete blocks (RCB) arrangement with three replications was used. Main plots were devoted to water stress (well watering and water stress). Sub-plots were assigned to plant density (D) (low-D, medium-D and high-D). Sub sub-plots were devoted to 23 maize genotypes (6 parents, 15 F_1`s and 2 checks). Each sub 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 1st irrigation) were thinned to one plant/hill to achieve the 3 plant densities, *i.e.* 95,200, 71,400 and 47,600 plants/ha, respectively. Each main plot was surrounded with a wide alley (4 m width) to avoid interference of the two water treatments with irrigation water. Sowing date each season was on May 5 and May 8 in 2013 and 2014 seasons, respectively. The soil analysis of the experimental soil at the experimental site, 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-1are 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°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, in 2013 season. In 2014 season, mean temperature was 26.1, 28.5, 29.1 and 29.9°C, maximum temperature was 38.8, 35.2, 35.6 and 36.4°C 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.

Data recorded

Grain protein content (%) (GPC%). **Grain oil content** (%) (GOC%). **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. **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. **Grain yield per hectare** (GYPH) in ton, by adjusting grain yield/plot to grain yield per hectare. **Protein yield per hectare** (PYPH), by multiplying grain protein content x grain yield per hectare. **Oil yield per hectare** (OYPH), by multiplying grain oil content x grain yield per hectare. **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 [43] 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).

Biometrical analyses

Combined analysis of variance of the split-split plot design in RCB arrangement on the basis of individual plot observation and combined analysis of variance of RCBD for each of the six environments (WW-LD, WW-MD, WW-HD, WS-LD, WS-MD and WS-HD) across the two seasons were performed if the homogeneity test was non-significant using the MIXED procedure of SAS ®[44]. Least significant differences (LSD) were calculated according to Steel *et al*. [45].

Results and Discussion

Analysis of variance

Combined analysis of variance across years (Y) of the split-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 (D) and two irrigation (I) regimes is presented in Table (1). Mean squares due to years were significant or highly significant for all studied traits, except for grain oil content (GOC) and protein yield/ha (PYPH), indicating significant effect of climatic conditions on most studied traits. Mean squares due to irrigation regimes, plant densities and genotypes were significant or highly significant for all studied traits, except grain oil content (GOC) for irrigation regimes and GPC, GOC and GSC for plant densities, indicating that plant density or irrigation regime 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.*, I×Y, D×Y, D×I, G×Y, G×I and G×D were significant ($P \leq 0.05$ or 0.01) for all studied traits, except for 6 traits for I × Y, 3 traits for D×Y and 2 traits for D×I. Mean squares due to the 2nd order interaction, i.e., G×I×Y, G×D×Y and G×D×I were significant or highly significant for all studied traits. However, mean squares due to D×I×Y were insignificant for 5 traits, *i.e.* GSC, GYPH, PYPF, OYPF and SYPF.

Table 1: Combined analysis of variance (% sum of squares) of split-split plot design for studied 23 maize genotypes under two irrigation regimes (I) and three plant densities (D) across 2013 and 2014 years.

SOV	df	% Sum of squares (SS)							
		GPC	GOC	GSC	GYPP	GYPH	PYPH	OYPH	SYPH
Years (Y)	1	3.28*	2.31	4.45**	0.18**	0.14**	0.78**	0.00	0.19**
Irrigation (I)	1	4.71*	4.42	2.81*	8.94**	8.74**	6.06**	10.14**	8.51**
I×Y	1	1.74	0.34	0.00	0.01	0.008*	0.10	0.05	0.011**
Error	8	4.11	12.66	3.29	0.02	0.01	0.20	0.35	0.01
Densities (D)	2	0.07	0.04	0.06	9.54**	7.04**	7.14**	6.47**	7.06**
D×Y	2	0.96**	0.91**	0.69**	0.00	0.01	0.11**	0.05**	0.02
D×I	2	0.56*	0.02	0.03	0.21**	0.27**	0.34**	0.32**	0.25**
D×I×Y	2	0.87**	0.07*	0.29	0.04*	0.02	0.03	0.01	0.03
Error	16	1.10	0.15	0.79	0.08	0.06	0.08	0.05	0.06
Genotypes (G)	22	44.58**	43.80**	32.13**	73.56**	75.45**	74.95**	72.87**	75.6**
G×Y	22	2.11**	4.67**	3.74**	0.18**	0.19**	0.32**	0.44**	0.19**
G×I	22	3.72**	1.72**	3.38**	2.03**	2.15**	1.98**	2.44**	2.16**
G×I×Y	44	2.66**	3.12**	5.85**	2.76**	3.21**	3.49**	3.39**	3.18**
G×D	22	5.73**	4.29**	6.04**	0.17**	0.14**	0.53**	0.37**	0.14**
G×D×Y	44	3.56**	4.10**	5.30**	0.26**	0.24**	0.37**	0.46**	0.24**
G×D×I	44	3.99**	3.58**	7.69**	0.84**	1.06**	1.21**	0.97**	1.09**
G×I×D×Y	44	3.86**	3.87**	9.57**	0.20**	0.17**	0.31**	0.29**	0.18**
Error	528	12.39	9.94	13.90	0.98	1.09	2.00	1.35	1.09
Total SS	827	1301	149.03	695.13	3646883	142950	31706296	5923515	1410000601
CV%		4.9	3.95	0.6	6.04	6.33	8.33	7.53	6.33

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

Mean squares due to the 3rd order interaction G×I×D×Y were significant ($P \leq 0.01$ or 0.05) for all studied traits, indicating that the rank of maize genotypes differ from a combination of irrigation regime, density an year to another and the possibility of selection for improved performance under a specific environment combination of plant density and nitrogen or irrigation level as proposed by several investigators [6, 26, 46-52].

It is observed from Table (1) 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. Comparing irrigation with density effect, it is clear that irrigation variance showed larger contribution to total variance than density variance for 6 traits (GPC, GOC, GSC, GYPH, OYPH and SYPH), indicating that water stress had more effect than elevating plant density on such traits, while density showed larger contribution to total variance than irrigation variance for two traits (GYPP and PYPH), indicating that high plant density had more effect than water stress on latter traits.

Effects of combinations of the two stresses on quality traits

Comparing the five environments (from E2 to E6) with the control non-stressed environment (E1) expressed in change percentages should give a picture of the effects of the elevated stress on the 8 studied traits (Table 2). Elevated combined stress between plant density and irrigation regime from E2 to E6 caused a significant increase in GPC and GSC under environments from E4 to E6, GOC under E3, GYPH, PYPH, OYPH and SYPH under E2 and E3 and PYPH under E6. On the contrary, increased stress from E2 to E6 caused a significant reduction in GYPP under all stressed environment, GOC under E4, E5 and E6, GPC under E2 only, GYPH, PYPH, OYPH and SYPH under E4 and E5 (except PYPH) and OYPH under E6. It can be observed that the rigidity of the stress combinations on GYPP was at maximum (49.71% reduction) under the environment E6 (WS-HD), where both severe stresses (highest plant density and deficit irrigation) existed. The reduction in GYPP due to the effect of water stress (WS) combined with medium plant density (MD) stress (E5) was 37.90%. Significant reductions in GYPP of maize genotypes observed in environments E5 and E6 relative to E1 were due to both drought and high density stresses. On the contrary, GYPH under the environment E6 showed a tendency of increase (1.03%) over that under E1. The same trend of change in GYPH due to both severe stresses (E6) was also shown by PYPH (4.57%) and SYPH (1.55%).

Table 2: Change (%) in studied traits due to elevated plant density combined with deficit irrigation across genotypes and seasons

Trait	E2 WW-MD	E3 WW-HD	E4 WS-LD	E5 WS-MD	E6 WS-HD
GPC (%)	2.72*	1.24	-3.08**	-4.42**	-3.18**
GOC (%)	-0.23	-0.74*	3.91**	3.78**	3.74**
GSC (%)	-0.07	-0.04	-0.42**	-0.46**	-0.53**
GYPP (g)	19.22**	29.98**	25.53**	37.90**	49.71**
GYPH(ton)	-20.59**	-38.48**	25.91**	6.13**	-1.03
PYPH(kg)	-17.37**	-36.80**	23.14**	0.67	-4.57**
OYPH(kg)	-21.92**	-40.07**	28.61**	9.33**	2.55*
SYPH(kg)	-20.76**	-38.43**	25.59**	5.74**	-1.55

Change = 100*(E1–RE)/E1, RE = Respective environment, − = increase, + = decrease, * and ** significant at 0.05 and 0.01 probability levels, respectively.

The effects of stressed environments across all genotypes and seasons, for studied traits on the 6 environments arranged, based on GYPP, from the richest (E1) to the poorest (E6), are illustrated as linear and quadratic regression lines in Fig. (1). The E1 represents the unstressed environment (well watered and low plant density) and will be used hereafter as control environment, E2 represents only medium density stress, E3 represents only high density stress, E4 represents only water stress , E5 represents water stress combined with medium density stress and E6 represents water stress combined with high density stress.

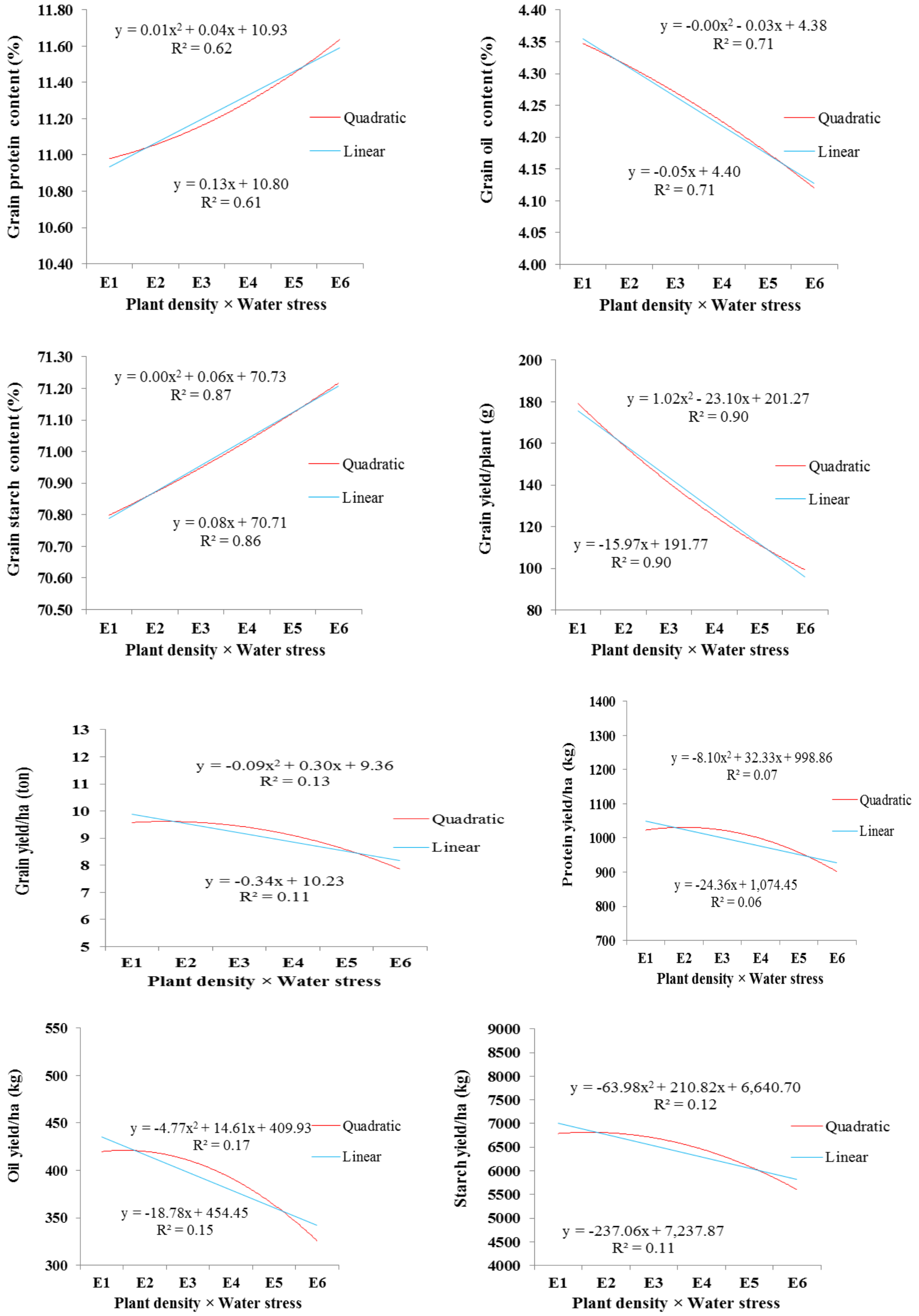

Figure 1: Linear and curvilinear regression of studied grain quality traits on six environment combinations between plant densities and irrigation regimes (E1 thru E6) across two seasons.

By increasing rigidity of stress from E1 to E6, the four traits GOC, GYPP, GPC and GSC showed linear regression; two of them showed a trend of decrease, namely GOC and GYPP, where the optimum environment was E1 and two showed a trend of increase, namely GPC and GSC traits, where the optimum environment was E6. The other four traits GYPH, PYPH, OYPH and SYPH showed quadratic regression on the six environments; they had a trend of increase from E1 to E3 followed by a trend of decrease after E3 to E6. The optimum environment of the latter four traits (grain, protein oil and starch yields) was E3 (well watering and high plant density).

Stress tolerance of inbreds and hybrids

Stress tolerance index (STI) values of studied genotypes estimated using the equation suggested by Fageria [43] under the stressed environments E2 through E6 are presented in Table (3). According to our scale, when STI is ≥1.0, it indicates that genotype is tolerant (T), If STI is < 1, it indicates that genotype is sensitive (S). The highest STI under all five stressed environments was exhibited by the inbred line L53, followed by inbred L20 and then Sk5. These three inbreds had STI value greater than unity under all five studied stresses and therefore could be considered tolerant to water stress, medium and high plant density stress and water stress combined with medium and high density stresses.

On the contrary, the three inbred lines Sd7, L18 and L28 exhibited STI values less than unity under all five stressed environments and therefore could be considered sensitive to water stress, medium and high plant density stress and water stress combined with medium and high density stresses; with the most sensitive one was the inbred Sd7 under E4, E5 and E6 and inbred L18 under E2 and E3 environments. For F_1 crosses, the highest STI value was recorded by the cross L20 x L53 (TxT) under all stressed environments, followed by the cross L53 x Sk5 (TxT) and L53 x Sd7(TxS) under all stresses. On the other hand, the most sensitive crosses under all stressed environments areL18 x L28 (S x S), L53 x L18 (T x S) and Sk5 x Sd7 (T x S).

Table 3: Stress tolerance index (STI) of maize inbreds and hybrids under stressed environments across two seasons.

Genotype	E2 WW-MD	E3 WW-HD	E4 WS-LD	E5 WS-MD	E6 WS-HD
			Inbreds		
L20	2.25	2.12	1.85	1.72	2.24
L53	2.81	2.64	3.39	2.95	3.40
Sk5	1.14	1.14	1.09	1.27	1.02
L18	0.29	0.26	0.49	0.42	0.25
L28	0.36	0.38	0.28	0.37	0.38
Sd7	0.36	0.5	0.22	0.31	0.22
			F_1 crosses		
L20 × L53	1.59	1.46	1.71	1.64	1.70
L20 ×SK5	1.00	1.00	1.00	1.00	1.01
L20 × L18	0.89	0.92	0.92	0.93	0.89
L20 × L28	1.08	1.07	1.07	1.10	1.10
L20 × Sd7	0.99	1.00	1.02	1.01	1.02
L 53 × Sk5	1.33	1.25	1.27	1.27	1.28
L53 × L18	0.70	0.75	0.70	0.70	0.72
L53 × L28	1.22	1.17	1.14	1.17	1.17
L53 × Sd7	1.27	1.21	1.21	1.23	1.21
Sk5 × L18	1.13	1.14	1.10	1.13	1.13
Sk5 × L28	0.94	0.96	0.98	0.97	0.97
Sk5 × Sd7	0.79	0.83	0.78	0.80	0.79
L18 × L28	0.51	0.58	0.54	0.46	0.48
L18 × Sd7	0.83	0.87	0.84	0.86	0.82
L28 × Sd7	1.01	1.04	1.03	1.01	1.02

WW = well watering, WS = water stress, LD = low density, MD = medium density, HD = high density, E = environment.

Superiority of tolerant (T) over sensitive (S) genotypes

To describe the differences between tolerant (T) and sensitive (S) inbreds and hybrids, data of the selected characters were averaged for the two groups of inbreds and hybrids differing in their stress tolerance, namely in grain yield/plant under combined stress of drought and high density (E6) (Table 4).

Table 4: Superiority (%) of the three most tolerant (T) over the three most sensitive (S) inbreds and crosses for selected characters under the most stressed environment (E6) across 2013 and 2014 seasons.

Trait	Inbreds	Crosses
GSC	0.79**	0.44**
GYPP	233.72**	60.25**
GYPH	206.90**	60.25**
PYPH	174.77**	52.73**
OYPH	196.53**	52.87**
SYPH	209.56**	60.96**

% Superiority = 100 × [(T – S)/S].

Data averaged for each of the two groups (T and S) of inbreds and crosses differing in tolerance to combined stress of high density and water stress (E6) indicate that grain yield/ha of combined stress tolerant (T) was greater than combined stress sensitive (S) inbreds and crosses by 206.90 and 60.25%, respectively under combined stress (water stress and 95,200 plants/ha) conditions. Superiority of T over S hybrids in GYPH under combined stress was due to their superiority in GYPP (60.25%) and was associated with superiority in PYPH (52.73%), OYPH (52.87%), SYPF (60.96%) and GSC (0.44%), than sensitive F_1 crosses (Table 4). Superiority of T to S crosses may be attributed to the high water use efficiency of the hybrids and due to heterosis relative to their inbred parents. These results are in agreement with those reported by several investigators [53-55].

Differential response of T×T, T×S and S×S crosses

Mean performance of traits 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 each stress (either high density or water stress) or both stresses together were used to calculate percentage of superiority (Table 5). Number of crosses was 3, 9 and 3 for the T×T, T×S and S×S groups, respectively. In general, T×T crosses had favorable (higher) values for grain yield and its attributes and protein, oil and starch yields than S×S and T×S crosses under combination of both stresses. In general, under the most severe environment (E6) where both severe stresses (water stress and density of 95,200 plants/ha) existed, water stress and high density T×T crosses were the most superior for all studied traits as compared to T x S and S x S crosses (Table 5). The T×S crosses for both stresses came in the second rank for superiority in all traits and the S×S crosses were in the third rank. Under water deficit and high density stresses together (E6), grain yield/ha of water stress and high-D T×T crosses was greater than S×S and T×S by 41.13 and 18.61%, respectively. This indicates that to obtain a tolerant cross to both stresses in the same time, it is preferable that its two parental inbred lines should be tolerant to both stresses. This assures that water stress combined with density stress tolerance traits are quantitative in nature, so the tolerant cross accumulates additive genes of both water stress tolerance and high density tolerance from both parents.

Superiority of water stress and high-D T×T and T×S over S×S crosses in GYPH under water stress and high-D (41.13 and 18.97%, respectively) was due to their superiority in GYPP by 41.13 and 18.99%, PYPH by 32.48 and 15.43%, OYPH by 32.89 and 17.43%, SYPF by 42.53 and 19.88%, , respectively (Table 5).

Table 5: Superiority (%) of T x T and T x S over S x S crosses for selected traits under combination of plant densities and irrigation regimes across two seasons (2013 and 2014).

Trait	WW-LD (E1)		WS-MD (E5)		WS-HD (E6)	
	T ×T	T ×S	T ×T	T ×S	T ×T	T ×S
GYPP	21.66**	10.01**	38.17**	19.08**	41.13**	18.97**
GYPH	21.96**	10.22**	39.19**	17.79**	41.13**	18.99**
PYPH	14.12**	8.49*	28.35**	12.96*	32.48**	15.43*
OYPH	21.91**	9.40**	32.56**	15.39**	32.89**	17.43**
SYPH	22.14**	10.35**	40.66**	18.93**	42.53**	19.88**

% Superiority = 100 × [(T×T) or (T×S) –(S×S)/(S×S)], T = tolerant, S = sensitive, WW = well watering, WS = water stress, LD = low density (20,000 plant/fed), MD = medium density (30,000 plant/fed) and HD = high density (40,000 plant/fed).

Correlations between grain yield and quality traits

Estimates of genetic correlation coefficients between GYPP and other studied traits across the two seasons under the six studied environments (E1 through E6) were calculated across all genotypes and presented in Table (6). Grain yield/plant showed a significant ($P \leq 0.01$), positive and strong association (r_g =>0.99) with grain yield/ha (GYPH) under the six environments. The correlations among grain yield/plant and other studied yield characters, namely, protein yield/ha (PYPH), oil yield/ha (OYPH) and starch yield/ha (SYPH) were very strong, highly significant and positive (r_g = > 0.97). Thus, selection for any one of these yield traits would result in improving the other trait(s), *i.e.* protein, oil and/or starch yield per hectare. These correlations might mainly be attributed to the calculation of these traits, where grain yield/ha is a common component in all these traits, *i.e.* oil yield/ha, protein yield/ha and starch yield/ha.

Table 6: Genotypic correlation coefficients between grain yield/plant and other studied traits across genotypes and seasons

Trait	**E1** **WW-LD**	**E2** **WW-MD**	**E3** **WW-HD**	**E4** **WS-LD**	**E5** **WS-MD**	**E6** **WS-HD**
GYPH	0.99**	.99**	1.00**	0.99**	0.99**	0.99**
GPC	-0.78*	-0.35	-0.64*	-0.69*	-0.13	-0.82*
GOC	-0.20	-0.41	-0.51	-0.11	0.23	0.12
GSC	0.39	0.78*	0.47	0.22	-0.56	0.27
PYPH	0.99**	0.99**	0.99**	0.98**	0.99**	0.98**
OYPH	0.98**	0.99**	0.98**	0.99**	0.97**	0.99**
SYPH	0.99**	0.99**	0.99**	0.99**	0.99**	0.99**

H = high, M = medium, L = low, WW = well watering, WS= water stress, D = density and *and ** indicate that r_g estimate exceeds once and twice its standard error, respectively.

There is a negative and significant correlation between grain yield/plant and grain protein content under E1 (-0.78), E3 (-0.64), E4 (-0.69), and E6 (-0.82).The correlation between GYPP and GOC was negative in all environments, except E5 and E6, but was not significant. An apparent inverse genetic relationship was also reported between grain yield and oil concentration in maize by Simmonds [14] and Feil [15]. It is observed that a positive correlation existed between GYPP and GSC in all environments, except E5, but was not significant, except for E2, which was significant (r_g =0.78).

Regression of quality traits of hybrids on elevated combined stresses

Data were reanalyzed to evaluate responses of the highest three hybrids for each studied trait across elevated level of stress *via* regression technique. For each hybrid, quadratic and linear regression functions were performed for irrigation regime × plant density interaction. The regression functions were used to identify the optimum environment for each trait of the 3 best hybrids. These relationships across years are illustrated in Figs. (2 through 9). For GPC trait (Fig. 2), the hybrids Sk5 x L18 (tolerant) and L18 x Sd7 showed near linear regression of increase across the elevated level of stress, with maximum value at E6, while the hybrid L18 x L28 showed a quadratic regression of increase, with an optimum environment at well watering and high density (E3).

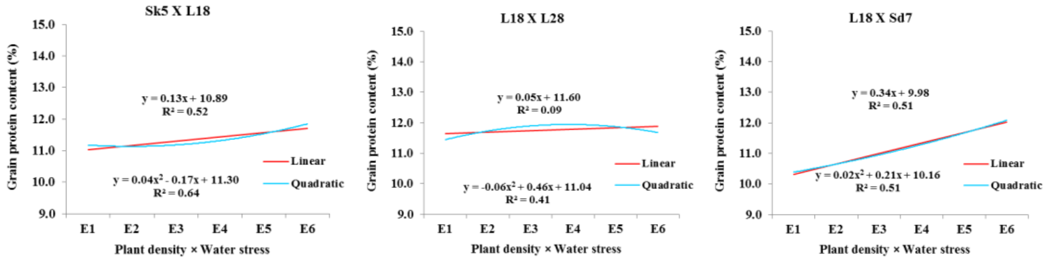

Figure 2: Relationship between grain protein content of the highest three hybrids and elevated plant density combined with water deficit across two years

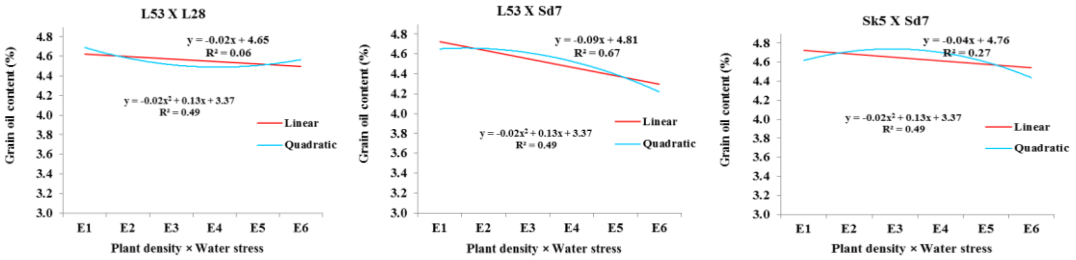

Figure 3: Relationship between grain oil content of the highest three hybrids and elevated plant density combined with water deficit across two years.

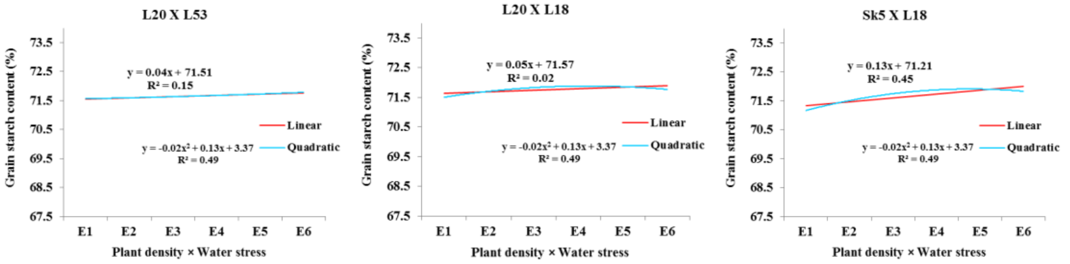

Figure 4: Relationship between grain starch content of the highest three hybrids and elevated plant density combined with water deficit across two years

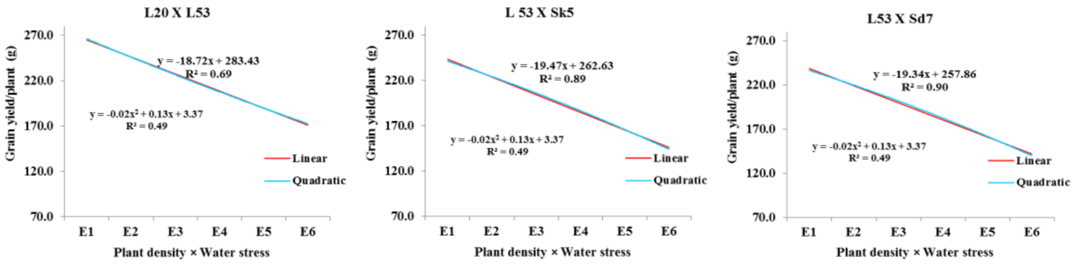

Figure 5: Relationship between grain yield/plant of the highest three hybrids and elevated plant density combined with water deficit across two years

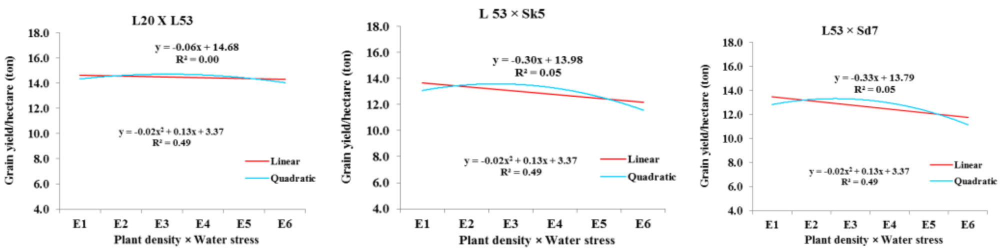

Figure 6: Relationship between grain yield/ha of the highest three hybrids and elevated plant density combined with water deficit across two years

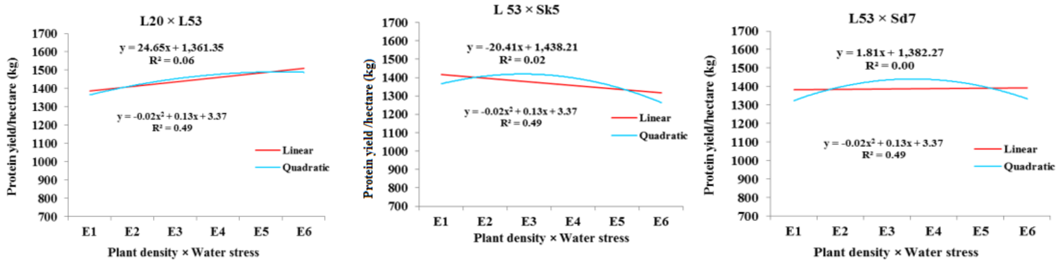

Figure 7: Relationship between protein yield/ha of the highest three hybrids and elevated plant density combined with water deficit across two years.

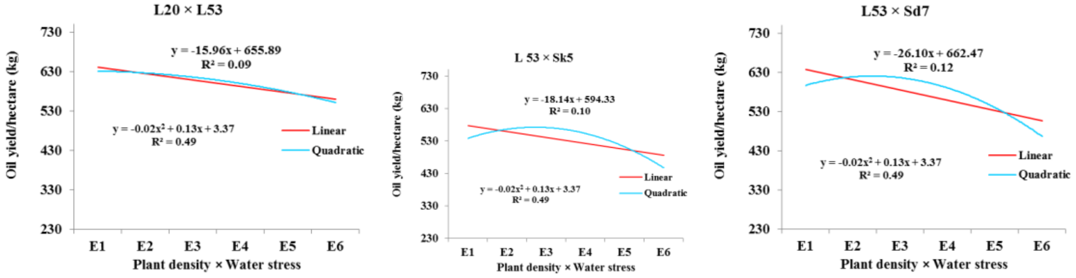

Figure 8: Relationship between oil yield/ha of the highest three hybrids and elevated plant density combined with water deficit across two years

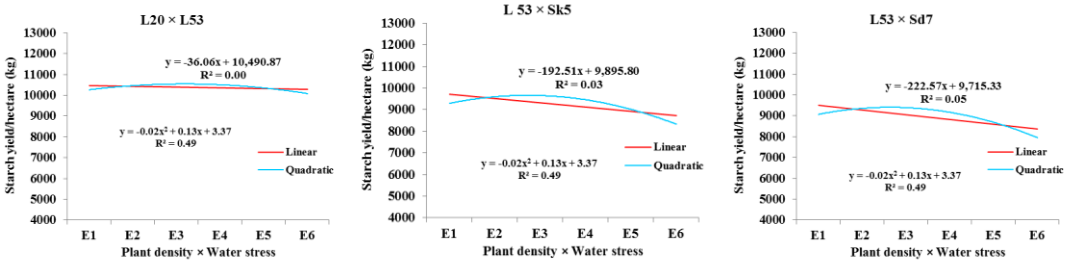

Figure 9: Relationship between starch yield/ha of the highest three hybrids and elevated plant density combined with water deficit across two years.

For GOC trait (Fig. 3), the hybrid L53 x L28 (tolerant) showed near linear regression of decrease due to elevated combined stress, with an optimum environment at E1, but the hybrids L53 x Sd7 (tolerant) and Sk5 x Sd7 showed a quadratic regression of decease, with an optimum environment at E3 (L53 x Sd7) and E4 (Sk5 x Sd7). Regarding GSC trait (Fig. 4), the two hybrids; Sk5 x L18 (tolerant) and L20 x L18 showed a quadratic regression of increase across the environments, with an optimum environment of E3 (L20 x L18) an E4 (Sk5 x L18), while the hybrid L20 x L53 showed near linear regression of increase. For GYPP (Fig. 5), all the best three hybrids showed a linear regression of decrease on the elevated plant density combined with the change from well watering to water stress at flowering stage, so the best environment for GYPP of these hybrids was E1, i.e. well watering combined with low plant density.

With regard to the four yield traits, namely PYPH, OYPH, SYPH and GYPH (Figs. 6-9, respectively), the hybrid L53 x Sk5 showed a quadratic regression of decrease, with an optimum environment of high plant density combined with well watering (E3). The hybrid L53 x Sd7 showed a quadratic regression of decrease for GYPH, OYPH and SYPH; its optimum environment was E3 and a quadratic regression of increase for PYPH with an optimum environment of low plant density combined with water stress at flowering. The hybrid L20 x L53 showed different regression functions with different yield traits; it showed a slight quadratic regression near to linear, but stable regression for GYPH and SYPH traits, with an optimum environment of E3 (GYPH) and between E3 and E4 (SYPH), a quadratic regression of increase for PYPH, with an optimum environment of medium density combined with water stress (E5) and near linear regression of decrease for OYPH, with an optimum environment of well water combined with low plant density (E1).

The differential response of the best hybrids for different quality traits, shown in this study on the elevated stress (elevated plant density coupled with water stress) suggests that the highest yield per unit land area is determined by the genetic background of the hybrid and the trait itself (protein, oil, starch or grain yield/ha). In this context, Shapiro and Wortmann [40] 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 [42] 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). The author 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) and 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. Boomsma *et al.* [41] 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. Al-Naggar *et al.* [26] 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 most tolerant hybrids to both stresses. The differential response of the best hybrids for different quality traits, shown in this study on the elevated stress suggests that the highest yield per unit land area is determined by the genetic background of the hybrid, the plant density, irrigation regime and the trait itself (protein, oil, starch or grain yield/ha).

Conclusion

Although increased plant density combined with water stress at flowering stage in maize caused a decrease in grain yield/plant and grain oil content, it caused an increase in grain protein and starch contents. The increase in plant density could increase grain, protein, oil and starch yields/ha and could reduce the reduction in these traits due to deficit irrigation at flowering stage. This study could develop some hybrids of much higher grain, protein, oil and starch yields/ha than the best check in the experiment under high plant density (95,200 plants/ha). These materials could be offered to plant breeding programs for improving high density tolerance and/or drought tolerance at flowering stage. The differential response of the best hybrids for different quality traits, shown in this study on the elevated stress suggests that the highest yield per unit land area is determined by the genetic background of the hybrid, the plant density, irrigation regime and the trait itself (protein, oil, starch or grain yield/ha). Further studies should be conducted to enhance our understanding of these relationships.

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