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Response of Maize (*Zea mays* L.) to Deficit Irrigation Combined with Reduced Nitrogen Rate is Genotype Dependent

A. M. M. Al-Naggar[1*], M. M. Shafik[1] and R. Y. M. Musa[2]

[1]Department of Agronomy, Faculty of Agriculture, Cairo University, Giza, Egypt.
[2]Department of Agronomy, Faculty of Agriculture, Upper Nile University, Republic of South Sudan.

Authors' contributions

This work was carried out in collaboration among all authors. Author AMMAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MMS and AMMAN managed the literature searches. Author RYMM performed the experimental work and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Deficit irrigation and low nitrogen (N) fertilization rate cause negative effects on maize grain productivity; such effects differ from genotype to genotype. The main objectives of the present investigation were to: (i) identify the drought and low N tolerant genotypes, (ii) estimate the superiority of tolerant to sensitive genotypes and (iii) assess the differential response of maize genotypes to elevated levels of nitrogen. Maize genotypes were evaluated in 2016 and 2017 seasons under three N rates: high; 285.6, medium; 166.6 and low; 47.6 kg N/ha and two irrigation regimes: well-watered (WW) and water stressed (WS) at flowering. A split-split-plot design with three replications was used. Main plots were allotted to irrigation regimes, sub-plots to N rates and sub-sub-plots to genotypes. The tolerant (T) and sensitive (S) genotypes were identified under each stress. Grain yield/plant (GYPP) of T genotypes was significantly ($P \leq 0.01$) superior to as compared to S genotypes by 109.5%, 39.6% and 141.9% under Low-N, drought, and drought combined with low-N, respectively. Superiority of T over S genotypes in GYPP was associated with significant ($P \leq 0.01$) superiority in ears/plant (11.5, 13.15 and 11.99%), 100-kernel weight (38.65,

**Corresponding author: E-mail: medhatalnaggar@gmail.com, ahmedmedhatalnaggar@gmail.com;*

30.46 and 30.99%), kernels/row (22.81, 11.28 and 20.07%), Nitrogen use efficiency (109.49, 39.62 and 141.89%) and shortening in anthesis-silking interval (-44.56, -29.58 and -29.08%), under the three environments, respectively. A significant linear response on elevated levels of nitrogen was shown by 13 genotypes, but a quadratic response was shown by six genotypes. The present study suggested that further investigation should be conducted to identify the optimum N fertilization rate for each newly developed variety of maize.

Keywords: Corn; drought tolerance; low-N tolerance; quadratic regression; linear regression.

1. INTRODUCTION

Maize (*Zea mays* L.) ranks the second after wheat amongst cereal crops grown in Egypt with regard the harvested area and production. According to FAOSTAT database, Egypt (2018) harvested 935,778 hectare of maize and produced about 7.8 million tons of grains, with an average yield of 7.801 t ha^{-1} [1]. It is consumed as food by humans and as a feed for the livestock and poultry. It is also used as basic raw material in numerous industrial products. Maize has high nutritive value as it contains 72% starch, 10 % protein, 4.80% oil, 8.50% fiber, 30% sugar and 1.70% ash [2]. The local of consumption of maize in Egypt is about 16 million tons. So Egypt imports every year *ca* nine million tons of maize grains. Efforts are devoted to increase the production *via* increasing the cultivated area by growing maize in the sandy soils characterized by low water-holding capacity and deficiency in nutrients, particularly nitrogen.

Maize is known to be susceptible to drought stress, which negatively affects yield parameters. Moreover, the expected future shortage in irrigation water necessitates that maize breeders should pay great attention to develop drought-tolerant maize cultivars. Breeding for tolerance to drought is difficult because the genetic mechanism that controls the expression of such tolerance in crop plants is poorly understood and because of the polygenic nature of such a complicated character [3] Selection for increased drought tolerance was associated with a significant reduction in anthesis-silking interval (ASI) and barrenness, and an increase in the number of ears plant^{-1}, stay green and harvest index [4-8].

Several investigators emphasized the role of maize genotypes in drought tolerance. Tolerant genotypes of maize were characterized by having shorter anthesis-silking interval (ASI) [9], more ears/plant [10,11] and greater number of kernels/ear [11]. The presence of genotypic differences in drought tolerance would help plant breeders in initiating successful breeding programs to improve such a complicated character [6,7,11,12].

Breeding for drought tolerance is difficult, because the genetic mechanism that controls the expression of such tolerance in crop plants is poorly understood and because of the polygenic nature of such a complicated character [3,13]. Selection for increased drought tolerance was associated with a significant reduction in anthesis-silking interval (ASI) and barrenness, and an increase in ears/plant, stay green and harvest index [4-6,14,15].

Nitrogen is the most important nutritive element for the production of maize. One of the reasons responsible for low productivity of maize in Egypt is using lower rates of nitrogen fertilizer than that recommended one by the Ministry of Agriculture. Limited availability of N fertilizers and low purchasing power of farmers continued to be an important yield limiting factor in farmer's field. Two other factors could limit the use of N or its availability to hybrids of high N-response. The first factor is related to the high risk of crop failure especially due to drought where N fertilizer rates are often lower than N rates that give maximum yield under optimum conditions [16]. The second factor which decreases the availability of the added nitrogen is the competition of weeds under poor weed control practices. Besides, even under optimum conditions, the problem of fertilizer abuse through over application to get more yield, would cause nitrate leaching which in turn leads to ground water contamination [17].

Breeding for tolerance to Low-N is a difficult task because the genetic mechanisms that control the expression of such tolerance in crop plants is poorly understood and because of the polygenic nature of such a complicated character [3,13]. Such tolerance to Low-N necessitates that plant breeder should improve the nitrogen use efficiency (NUE) under Low-N. Ears/plant and anthesis-silking interval are considered as the most important Low-N adaptive traits [18]. A wide range of researchers have reported the existence of genetic variability in Low-N tolerance [6,19-21].

Hybrid maize breeding programs in Egypt concentrated their activity in the last decades on the improvement of high-yielding hybrids under well irrigation and high soil-N conditions i.e. hybrids of high water and N-responsiveness. Current breeding programs should pay attention to develop hybrid corn of high tolerance to water stress and tolerance to the low soil nitrogen conditions.

To start a successful breeding program for improving low N as well as drought tolerance, available maize germplasm should be screened for productivity, agronomic and physiological performance under Low-N and drought conditions in order to identify the best genotypes that could be used directly or indirectly as suitable sources for developing tolerant hybrids to these stresses. The objectives of the present investigation were: (*i*) to evaluate the effects of stresses resulting from reduced N rate combined with deficit irrigation on maize traits, (*ii*) to identify the drought and low N tolerant genotypes and estimate superiority of tolerant to the sensitive genotypes, and (*iii*) to determine the yield responses of different genotypes on elevated levels of nitrogen.

2. MATERIALS AND METHODS

2.1 Plant Materials

Nineteen maize (*Zea mays* L.) genotypes (9 single crosses, 5 three-way crosses, and 5 open-pollinated populations) obtained from Agricultural Research Center (ARC) (13 genotypes), Hi-Tec Company (3 genotypes), Pioneer-Corteva Company (2 genotypes), Fine Seeds Company (one genotype), were used in this study (Table 1).

2.2 Experimental Procedures

This study was carried out in the two successive growing seasons 2016 and 2017 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). Sowing date was April 24th in the 1st season (2016) and April 30th in the 2nd season (2017). Sowing was done in rows; each row was 4 m long and 0.7 m width. Seeds were over sown in hills 25 cm apart, thereafter (after 21 days from planting and before the 1st irrigation) were thinned to one plant/hill to achieve a plant density of about 24,000 plants/fed. Each experimental plot included two rows (plot size = 5.6 m^2).

2.3 Experimental Design

A split-split-plot design in randomized complete blocks arrangement with three replications was used. Main plots were allotted to two irrigation regimes, *i.e.* well watering (WW) and water stress at flowering (WS). Each main plot was surrounded with an alley (4 m width), to avoid water leaching between plots. Sub-plots were assigned to three nitrogen fertilizer rates, i.e. 47.6, 166.6 and 285.6 kg N/ha, in two equal doses in the form of Urea 46% before 1st and 2nd irrigations and two irrigation regimes, *i.e.*, well-watered (WW) and water stress (WS) as follows: E1: High nitrogen-well watered (HN-WW). E2: High nitrogen-water stress (HN-WS). E3: Medium nitrogen- well watered (MN-WW). E4: Medium nitrogen-water stress (MN-WS). E5: Low nitrogen-well watered (LN-WW). E6: Low nitrogen-water stress (LN-WS). Sub-sub-plots were devoted to nineteen maize genotypes.

2.4 Water Regimes

The following two different water regimes were used: 1-(Well-watered (WW): Full (recommended) irrigation was applied, the second irrigation was given after three weeks and subsequent irrigations were applied every 12 days. 2)-Water stress at flowering (WS): The irrigation regime was just like well watering, but the 4th and 5th irrigations were withheld, resulting in 24 days' water stress just before and during the flowering stage.

2.5 Agricultural Practices

Nitrogen fertilization for each rate was added in two equal doses of Urea 46 % before the first and second irrigation. Triple Superphosphate Fertilizer (46% P_2O_5) at the rate of 70 kg P_2O_5/ha, was added as soil application before sowing during the preparation of the soil for planting. Weed control was performed chemically with Stomp herbicide just after sowing and before the planting irrigation and manually by hoeing twice, the first before the first irrigation (after 21 days from sowing) and the second before the second irrigation (after 33 days from sowing). Pest control was performed when required by spraying plants with Lannate (Methomyl) 90% (manufactured by DuPont, USA) against corn borers. All other agricultural practices were followed according to the recommendations of Agricultural Research Center, Egypt.

Table 1. Designation, origin and grain color of maize genotypes under investigation

Genotype no.	Designation	Origin	Genetic nature	Grain color
1	SC-10	ARC-Egypt	Single cross	White
2	30K8	Pioneer-Corteva	Single cross	White
3	SC-101	Fine seeds, Egypt	Single cross	White
4	SC-131	ARC-Egypt	Single cross	White
5	SC-2031	Hi-tec, Egypt	Single cross	White
6	SC-30 N11	Pioneer-Corteva	Single cross	Yellow
7	SC-168	ARC-Egypt	Single cross	Yellow
8	SC-176	ARC-Egypt	Single cross	Yellow
9	SC-2055	Hi-tec, Egypt	Single cross	Yellow
10	TWC-310	ARC-Egypt	3-ways cross	White
11	TWC-321	ARC-Egypt	3-ways cross	White
12	TWC-1100	Hi-tec, Egypt	3-ways cross	White
13	TWC-352	ARC-Egypt	3-ways cross	Yellow
14	TWC- 360	ARC-Egypt	3-ways cross	Yellow
15	American Early Dent	ARC-Egypt	Population	White
16	Giza-2	ARC-Egypt	Population	White
17	Nubaria-355	ARC-Egypt	Population	White
18	Original Midland	Kensas - USA	Population	Yellow
19	Reid Type Composite	USA	Population	Yellow

[62,12]

2.6 Soil Analysis

Physical and chemical soil analyses of the field experiments were performed at laboratories of Soil and Water Research Institute of ARC, Egypt. The soil type is clay loam (39.48% silt, 35.73% clay, 18.07% fine sand and 6.72% coarse sand as an average of the two seasons). The soil pH (paste extract) was 7.93; the EC was 2.23 dSm^{-1}. Available soil nitrogen in 30 cm depth was analyzed immediately prior to sowing at the laboratories of Water and Environment Unit, Agricultural Research Center, Egypt and found to be 148.0 and 72.6 kg N/ha in 2016 and 2017 seasons, respectively. Available soil nitrogen after adding nitrogen fertilizer was therefore 433.6, 314.6 and 195.6 kg N/ha, in the 1st season and 358.2, 239.2 and 120.2 kg N/ha, in the 2nd season for the 3 N treatments, i.e. HN, MN, and LN, respectively. The available nitrogen to each plant (including soil and added N) was calculated for each environment and found to be 7.59, 5.51 and 3.42 g N/plant in the first season and 6.27, 4.19 and 2.10 g N/plant in the second season, with an average across the two seasons of 6.93, 4.85 and 2.76 g N/plant for the three N treatments, respectively.

2.7 Meteorological Data

The required weather data for the experimental site through the two growing seasons were obtained from Central Lab for Agricultural Climate, Agricultural Research Center at Giza, Governorate, Egypt. Mean temperature in May, June, July and August was 28.9, 33.5, 32.6 and 32.5^{o}C in 2016 season and 29.3, 23.3, 33.5 and 32.5^{o}C in 2017 season. Relative humidity was 38.7, 31.7, 46.3 and 44.3% in 2016 season and 34.0, 23.3, 42.3 and 46.3% in 2017 season. Sunshine duration was 13.4, 13.9, 13.8 and 13.0 hr in 2016 season and 13.4, 13.9, 13.8 and 13.1 hr in 2017 season.

2.8 Morphological Data Recorded

1) Days to 50% silking (DTS), 2) Anthesis-silking interval (ASI) 3) Plant height (PH), 4) Ear height (EH), 5) Chlorophyll concentration index (CCI) by Chlorophyll Concentration Meter, Model CCM-200, USA (available on line at: http://www.apogeeinstruments.co.uk/apogee-instruments-chlorophyll-content-meter-technical-information/), 6) Number of ears plant^{-1} (EPP), 7) Number of rows ear^{-1} (RPE), 8) Number of kernels row^{-1} (KPR), 9) Number of kernels plant^{-1} (KPP), 10) 100-kernel weight (HKW) (g), 11) Grain yield plant^{-1} (GYPP) (g) (adjusted at 15.5% grain moisture), 12) Economic nitrogen use efficiency (NUEe) (g/g) as follows: NUEe = GDM/Ns, where GDM= grain dry matter, Ns = available soil-N/plant, 13) Grain protein content (GPC) in %, 14) Grain starch content (GSC) in % and 15) Grain oil content (GOC) in %. The grain quality traits (GPC, GSC and GOC) were measured in both seasons, on samples taken

from the grain bulk of each maize genotype by using INSTALAB 600 Near Infrared (NIR) Product Analyzer manufactured by DICKEY-john Corporation, Auburn, Illinois, USA.

2.9 Stress Tolerance Index (Sti)

Stress tolerance index is the factor used to differentiate between the genotypes from the tolerance point of view and it is calculated by the equation of Fageria [22] as follows:

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

Where Y1 = trait mean of a genotype at well watering or high/medium nitrogen. AY1 = average trait of all genotypes at well watering or high/medium nitrogen. Y2 = trait mean of a genotype at water stress or Low-N. AY2 = average trait of all genotypes at water stress or Low-N. When STI is ≥ 1, it indicates that the genotype is tolerant (T) to drought or Low-N. If STI is <1, it indicates that the genotype is sensitive (S) to drought or Low-N.

2.10 Biometrical Analysis

Analysis of variance of the split-split-plot design each year was computed on the basis of individual plot observation using the MIXED procedure of MSTAT ®. A combined analysis of variance of the split-split-plot design across the two years was also performed if the homogeneity test was non-significant. Moreover, each of the six environments was analyzed separately as a randomized complete block design (RCBD) for the purpose of determining genetic parameters, i.e. under WW-HN, WW-MN, WW-LN, WS-HN, WS-MN, and WS-LN. Least significant differences (LSD) values at 0.05 and 0.01 probability levels were calculated to compare between means according to [23].

3. RESULTS

3.1 Analysis of Variance

Combined analysis of variance across years (Y) of the split-split plot design for 15 traits of 19 genotypes (G) of maize (9 single crosses + 5 three-way crosses + 5 populations) under two irrigation regimes (I) and three nitrogen (N) levels is presented in Table 2. Mean squares due to years were significant (P ≤ 0.01) for all studied traits, except for ear height (EH), 100-kernel weight (HKW), grain nitrogen content (GN), nitrogen utilization efficiency (NUTE), grain protein content (GPC), grain oil content (GOC) and grain ash content (GAC), indicating significant effect of climatic conditions on 15 out of 22 studied traits.

Mean squares due to genotypes were significant (P ≤ 0.01) for all studied traits, indicating that genotype has an obvious effect on all 15 studied traits. Mean squares due to irrigation and N level were significant (P ≤ 0.01) for all studied traits, except EH, GOC, GAC and Grain fiber content (GFC) for irrigation regime and plant height (PH), EH, GOC, GAC and grain moisture content (GMC) for N level, indicating that irrigation regime and N level have an obvious effect on most studied traits.

Mean squares due to the 1st order interaction, *i.e.,* I×Y, N×Y, I×N, G×Y, G×N and G×I were significant (P ≤ 0.01) for all studied traits, except for HKW, NUTE and GAC for I×Y, 11 traits for N×Y, seven traits for I×N, five traits (PH, EH, HKW, NUE, and NUTE) for G×I and eight traits (DTA, DTS, PH, EH, HKW, NUE, NUTE, GMC) for G×N.

Mean squares due to the 2nd order interaction, *i.e.,* G×I×Y, G×N×Y, and G×I×N were significant (P≤ 0.01 or P≤ 0.05) for all studied traits, except four traits (DTS, PH, HKW and NUE) for G×I×Y, six traits (DTS, ASI, PH, EH, HKW, NUE) for G×N×Y and five traits (DTS, PH, EH, HKW, NUE) for G×I×N. Mean squares due to the 3rd order interaction G×N×I×Y were significant (P ≤ 0.01) for all studied traits, except for six traits (DTA, ASI, PH, EH, HKW, NUE). Coefficient of variation (CV) was generally very low (≤10%) for all studied traits, except for anthesis-silking interval (ASI), grain protein content and grain oil content which was 32.81, 16.69 and 20.67 %, respectively.

Combined analysis of variance across years of a randomized complete blocks design (RCBD) for 15 traits of 19 maize genotypes under each of the six environments (combinations of the two irrigation regimes WW and WS and the three N levels HN, MN, and LN), namely WW-HN, WW-MN, WW-LN, WS-HN, WS-MN, WS-LN, (data not presented). Mean squares due to genotypes under all environments were significant (P≤ 0.01 or P≤ 0.05) for all studied traits, indicating the significance of differences among studied genotypes for all such traits under each of the six environments (a combination of one irrigation regime and one N level).

Mean squares due to seasons were significant (p ≤ 0.05 or 0.01) for most of the cases. Mean squares due to the interaction of genotype × year (G × Y) were significant (p ≤ 0.05 or p ≤ 0.01) for the majority of studied traits under each of the six environments. Plant height, ear height, and 100-kernel weight traits did not show any significant G × Y interaction under all the six environments. DTA and ASI traits showed only one significant G × Y interaction (under WW-HN), two significant G × Y interactions (under WS-HN and WS-MN) and three significant G × Y interactions (under WW-MN, WS-MN, and WS-LN), respectively.

3.2 Effects of Water Stress Combined with Reduced N Level

Drought and low-N are abiotic stresses which have a negative influence on metabolic processes in crop plants, which ultimately manifest itself on the production and quality of agricultural species, including maize. The need for prompt and efficient solutions in this region propelled crops breeding programs to prioritize identification and development of drought and low-N tolerant cultivars. The effect of six combinations between 3 levels of nitrogen (LN, MN and HN) and 2 irrigation treatments (WW and WS) on the studied traits is presented in Table 3. These combinations resulted in six different environments, namely E1 (WW-HN), E2 (WW-MN), E3 (WW-LN), E4 (WS-HN), E5 (WS-MN), and E6 (WS-LN). The last two environments (E5 and E6) represent water stress combined with reduced N levels (medium M and low N). The first environment (E1) represents the control (well watering with the recommended high nitrogen). The highest GYPP was obtained from the E1 environment.

It can be observed that the rigidity of the combinations of the two stresses on GYPP was at maximum (51.52% reduction) under the environment E6 (WS-LN), where both severe stresses (water stress and low nitrogen) existed and moderate in rigidity (40.83 % reduction) under the environment E5 (water stress combined with medium N). The significant (P≤ 0.01) reduction in GYPP due to water stress only exhibited by E4 (24.59%) was less than the significant (P≤ 0.01) reduction in GYPP due to low N only exhibited by E3 (28.56%).

Reductions in grain yield resulted from both stresses (water stress and reduced N levels) were associated with reductions in all yield components (EPP, RPE, KPR, KPP, and 100-KW), PH, EH and CCI. On the contrary, the two severe stresses (water stress and low N) combined in environment E6 caused significant (P≤ 0.01) increases in DTA, ASI, GOC and NUEe. The second severest environment (E5) (water stress and medium N) showed also significant (P≤ 0.01) increases in DTS, ASI and GPC.

3.3 Genotype × Nitrogen Level × Irrigation Regime

Mean grain yield/plant (GYPP) across years under six combinations of 3 N-levels and two irrigation regimes (E1 through E6) for all studied genotypes is presented in Table 4. The highest average GYPP of all studied genotypes was achieved under E1 (WW-HN). but the lowest average GYPP was recorded under the severest environment E6. The best three genotypes in GYPP were in descending order as SC-101>SC-131>SC-30N11 under the optimum environment (E1), SC-101>SC-131>SC-2055 under E2 (WW-MN), SC-101>SC-30K8>SC-10 under the three environments E3 (WW-LN), E5 (WS-MN) and E6 (WS-LN) and SC-30N11>SC-101>SC-30K8 under the environment E4 (WS-HN). On the contrary, the three lowest genotypes in GYPP in ascending order were Reid Type<Midland<TWC-352 under E1, Reid Type <TWC-352<Midland under E2, Reid Type<Midland< A.E.D. under E3, A.E.D<.TWC-352<Midland under E4, Midland<TWC-360< A.E.D. under E5 and Reid Type<Midland<TWC-360 under E6.

In general, the single crosses group had the highest grain yield under all stressed and non-stressed environments, but the lowest yield was recorded by populations group under E2, E3 and E6 and by three-way crosses group under E1, E4 and E5 (Table 3).

3.4 Stress Tolerance Index

Stress tolerance index (STI) values of studied genotypes under the stressed environments are presented in Table 5. According to our scale, when STI is ≥1.0, it indicates that genotype is tolerant (T), and if STI is <1.0, it indicates that genotype is sensitive (S). Based on STI values, the 19 studied maize genotypes were grouped into two categories under each stressed environment, namely tolerant (10, 10, 10, 11 and 8 genotypes) and sensitive (9, 9, 9, 8 and 11genotypes) under WW-MN, WW-LN, WS-HN, WS-MN and WS-LN environments, respectively.

The highest STI under all the stressed environments was exhibited by the single cross SC-101 (G3), except under E4, where the single cross SC-30N11 (G6) had the highest STI. The

Table 2. Combined analysis of variance of split-split plot design across two years for 22 studied traits of 19 maize genotypes evaluated under two irrigation regimes combined with three N fertilizer levels

SOV	DF	Mean squares							
		DTA	ASI	PH	EH	CCI	EPP	RPE	KPR
Year (Y)	1	1355.80**	341.07**	37639.6**	0.041	3614.2**	1.053**	105.27**	1878.6**
Irrigation (I)	1	237.44*	218.96**	23948.7**	1503.2	817.4**	0.22**	16.26**	2701.8**
Y × I	1	1350.18**	244.56**	33245.9**	7675.7**	967.0**	0.423**	15.03**	583.3**
Y × I (Rep.)	8	150.38	22.95	3169.7	1261.8	6.9	0.003	0.59	1.617
Nitrogen (N)	2	107.69**	42.21**	747.5	511.5	1570.8**	0.261**	1.31*	260.3**
Y × N	2	6.09	0.75	1980.7	293.9	1621.7**	0.085**	2.198**	73.76**
I × N	2	7.57	5.56**	1104.7	188.8	42.2**	0.224**	0.51	290.7**
Y × I × N	2	19.08	2.7	1566.3	301.2	79.7**	0.232**	1.29*	14.903**
Y × I × N (Rep.)	16	19.44	1.84	1055.3	1117.7	2.1	0.002	0.3	2.461
Genotype (G)	18	314.03**	39.28**	11564.6**	3852.0**	136.7**	0.20**	55.13**	409.0**
G × Y	18	8.48*	4.45*	480.5	230.5	147.0**	0.05**	1.64**	27.32**
G × I	18	6.13	7.61**	493.5	158.3	66.0**	0.054**	1.42**	40.55**
G × N	36	3.28	3.46*	275.7	101.1	31.1**	0.026**	1.37**	25.067**
G × I × Y	18	7.62*	5.14*	496.7	332.2*	31.4**	0.034**	1.73**	29.105**
G × N × Y	36	2.9	3.04	200.4	122.7	33.0**	0.037**	0.81**	20.850**
G × N × I	36	3.66	3.59*	396.5	142.4	28.2**	0.04**	1.456**	36.527**
G × I × N × Y	36	3.64	2.67	340.8	133.5	43.7**	0.041**	0.87**	34.361**
Error	432	3.22	1.68	266.28	120.5	2.185	0.0027	0.374	2.34
CV%		2.856	32.81	6.739	9.581	9.347	5.26	4.43	3.86
R^2		0.89	0.782	0.7875	0.729	0.9619	0.927	0.896	0.951
SOV	**DF**	**KPP**	**HKW**	**GYPP**	**NUEe**	**GPC**	**GOC**	**GSC**	
Year (Y)	1	2197863.3**	15.23	1179759.3**	175369.3**	12.67	16.6	79.34**	
Irrigation (I)	1	1159009.4**	406.82**	295303.9**	12353.3**	97.06**	0.2	120.40**	
Y × I	1	555097.9**	7.02	107460.0**	3404.2**	70.88**	119.68**	499.50**	
Y × I (Rep.)	8	334.4	54.41	99.2	13.15	15.62	10.08	10.79	
Nitrogen (N)	2	278056.4**	175.33**	86428.6**	43810.6**	67.62**	1.82	76.59**	
Y × N	2	17487.1**	3.05	4960.6**	17526.9**	2.26	2.07*	14.97*	
I × N	2	115643.8**	29.28*	15223.8**	765.5**	12.17**	6.72**	10.85*	
Y × I × N	2	94872.3**	13.7	6717.3**	423.4**	12.01**	6.23**	22.91**	
Y × I × N (Rep.)	16	255.1	11.84	93.9	11.24	1.09	1.33	3.83	

SOV	DF	Mean squares							
		DTA	ASI	PH	EH	CCI	EPP	RPE	KPR
Genotype (G)	18	106655.7**	590.17**	36481.2**	2236.4**	19.26**	9.24**	63.00**	
G × Y	18	40512.7**	20.51*	18710.9**	1333.5**	4.25*	1.06*	4.13	
G × I	18	25813.3**	13.01	6285.7**	352.7	7.19**	1.28**	6.27*	
G × N	36	16002.4**	5.24	6334.3**	547.2	6.06**	1.69**	14.17**	
G × I × Y	18	14075.2**	6.78	3263.8**	182.6	4.33*	2.34**	9.91*	
G × N × Y	36	13560.8**	5.53	2793.5**	335.7	6.52**	1.67**	8.95*	
G × N × I	36	17960.9**	5.92	4756.0**	391.1	4.75*	1.56**	11.08*	
G × I × N × Y	36	13598.7**	4.31	2704.1**	244.3	5.62*	1.41**	10.56*	
Error	432	291.03	6.45	63.865	5.12	1.606	0.396	2.855	
CV%		3.17	7.88	5.42	6.2	16.69	20.67	2.53	
R^2		0.988	0.83	0.992	0.995	0.7393	0.8146	0.7733	

*DTA= Days to 50% anthesis, ASI = anthesis-silking interval, PH = plant height, EH = ear height, CCI= chlorophyll concentration index, EPP = number of ears per plant, RPE = Number of rows per ear, KPR = Number of kernel per row, KPP = number of kernels per plant, HKW = 100-kernel weight, GYPP = grain yield per plant, NUE= nitrogen use efficiency, GPC= grain protein content, GOC= grain oil content, GSC= grain starch content * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

Table 3. Means of studied traits across all genotypes under each of the six environments; E1 (WW-HN), E2 (WW-MN), E3 (WW-LN), E4 (WS-HN), E5 (WS-MN) and E6 (WS-LN) and reduction (Red. %) from E1 to other five environments, across two years

	E1	E2	E3	E4	E5	E6
Trait	WW-HN	WW-MN	WW-LN	WS-HN	WS-MN	WS-LN
DTA	61.9	62.1	62.6	62.8	63.4	64.1
Red. %		-0.32	-1.13	-1.45	-2.42	-3.55*
ASI(day)	2.8	3.2	4.2	4	4.4	5.2
Red. %		-14.29**	-50.00**	-42.86**	-57.14**	-85.71**
PH(cm)	254.1	247.5	242.5	243.1	236.8	228.8
Red. %		2.60	4.57*	4.33*	6.81**	9.96**
EH(cm)	120.1	115.8	112.2	116.6	113.8	109
Red. %		3.58	6.58*	2.91	5.25*	9.24**
CCI (%)	20.45	14.58	12.27	17.31	16.27	14
Red. %		28.70**	40.00**	15.35**	20.44**	31.54**
EPP	1.086	1.013	0.959	1.013	0.94	0.879
Red. %		6.72**	11.69**	6.72**	13.44**	19.06**
RPE	14.45	14	13.68	13.86	13.58	13.25

	E1	E2	E3	E4	E5	E6
Trait	**WW-HN**	**WW-MN**	**WW-LN**	**WS-HN**	**WS-MN**	**WS-LN**
Red. %		5.33**	5.33**	4.08**	6.02**	8.30**
KPR	43.69	42.23	39.54	39.71	37.31	35.99
Red. %		3.34**	9.50**	9.11**	14.60**	17.62**
KPP	626	589.8	520.7	559	489.5	441
Red. %		3.34**	9.50**	9.11**	14.60**	17.62**
100KW(g)	34.08	33.18	32.04	32.43	31.59	30.06
Red. %		2.64	5.99**	4.84**	7.31**	11.80**
GYPP(g)	201.3	170.7	143.8	151.8	119.1	97.6
Red. %		15.20**	28.56**	24.59**	40.83**	51.52**
GPC%	7.52	7.28	6.84	8.43	8.39	7.09
Red. %		3.19	9.04*	-12.10**	-11.57**	5.72
GOC%	2.95	3.09	3.13	2.75	3.07	3.26
Red. %		-4.75	-6.10	6.78	-4.07	-10.51*
GSC%	66.99	67.07	67.78	65.73	66.35	67.24
Red. %		-0.12	-1.18	1.88	0.96	-0.37
NUEg(g/g)	29.05	35.2	52.1	21.9	24.56	35.36
Red. %		-21.17**	-79.35**	24.61**	15.46**	-21.72**

*Negative (-) reduction refer to increase. DTA= Days to 50% anthesis, ASI = anthesis-silking interval, PH = plant height, EH = ear height, CCI= chlorophyll concentration index, EPP = number of ears per plant, RPE = Number of rows per ear, KPR = Number of kernel per row, KPP = number of kernels per plant, HKW = 100-kernel weight, GYPP = grain yield per plant, NUEe= economic nitrogen use efficiency, GPC= grain protein content, GOC= grain oil content, GSC= grain starch content. * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

Table 4. Mean grain yield/plant (g) of each genotype under each of the six environments across two years

		E1	E2	E3	E4	E5	E6
Genotype No.	**Designation**	**WW-HN**	**WW-MN**	**WW-LN**	**WS-HN**	**WS-MN**	**WS-LN**
1	SC-10	213.6	190.7	183.7	165.2	149.6	129.8
2	SC-30K8	199.6	191.3	184.8	194.7	150.7	135.4
3	SC-101	295.7	258.7	229.7	178.6	178.3	157.9
4	SC-131	275.5	199.3	157.4	145.4	131.8	131.5
5	SC-2031	215.7	193.2	161.7	137.2	91.9	89.9
6	SC-30N11	269.2	168.2	143.3	217.6	96.6	96.2
7	SC-168	212.1	177.3	129.9	129.7	115.9	88.3
8	SC-176	187.6	164.2	121.1	169.8	146.6	92.1
9	SC-2055	226.9	220.3	144.0	170.6	142.3	107.6
10	TWC-310	199.6	162.9	148.9	124.8	105.8	96.4
11	TWC-321	180.4	167.0	137.3	169.2	147.8	100.4
12	TWC-1100	145.3	133.9	122.1	140.1	98.3	96.8
13	TWC-352	133.9	128.7	128.5	107.3	86.1	71.5
14	TWC- 360	173.5	153.8	144.9	132.2	78.9	64.6
15	A.E.D.	182.4	108.6	97.7	98.0	80.2	75.5
16	Giza-2	223.7	173.5	171.8	164.9	142.0	75.5
17	Nubaria	202.6	181.2	156.3	173.4	146.1	133.6
18	Midland	131.7	130.8	80.9	122.2	74.0	61.2
19	Reid Type	128.4	127.4	66.4	120.9	89.7	40.6
	LSD_{05}(G)	8.43	8.79	5.98	7.33	13.92	8.72
Aver.		199.8	169.7	143.0	150.6	118.6	97.1
Maximum		295.7	258.7	229.7	217.6	178.3	157.9
Minimum		127.4	108.6	66.4	98	74	40.6
Single crosses		232.9	195.1	162.5	167.6	133.7	114.3
Three-way crosses		166.5	149.3	136.3	134.7	103.4	85.9
Populations		173.6	144.5	114.6	135.9	106.4	77.3
LSD_{05}(G×N)=5.37		LSD_{05}(N×I)=2.24		LSD_{05}(G×N×I)=7.59			
LSD_{01}(G×N)=7.59		LSD_{01}(N×I)=3.32		LSD_{01}(G×N×I)=10.73			

Table 5. Stress tolerance index (STI) of each genotype under sole stress (WW-MN, WW-LN and WS-HN) and combined stresses (WS-MN and WS-LN) across two years

		E2	E3	E4	E5	E6
Genotype No.	**Designation**	**WW-MN**	**WW-LN**	**WS-HN**	**WS-MN**	**WS-LN**
1	SC-10	1.157	1.425	1.172	1.349	1.429
2	SC-30K8	1.126	1.291	1.291	1.270	1.393
3	SC-101	2.256	2.377	1.755	2.226	2.407
4	SC-131	1.619	1.517	1.331	1.533	1.867
5	SC-2031	1.229	1.221	0.983	0.837	1.000
6	SC-30N11	1.335	1.350	1.946	1.098	1.335
7	SC-168	1.109	0.964	0.914	1.038	0.965
8	SC-176	0.908	0.795	1.058	1.161	0.891
9	SC-2055	1.474	1.143	1.286	1.363	1.258
10	TWC-310	0.959	1.040	0.828	0.891	0.992
11	TWC-321	0.888	0.867	1.014	1.126	0.934
12	TWC-1100	0.574	0.621	0.676	0.603	0.725
13	TWC-352	0.508	0.602	0.477	0.487	0.493
14	TWC- 360	0.787	0.880	0.762	0.578	0.578
15	A.E.D.	0.584	0.624	0.594	0.618	0.710
16	Giza-2	1.144	1.345	1.226	1.341	0.871
17	Nubaria	1.082	1.108	1.167	1.250	1.395
18	Midland	0.508	0.373	0.535	0.411	0.415
19	Reid Type	0.482	0.296	0.512	0.482	0.267

2nd highest genotype in STI was the single cross SC-131 (G4) under all the stressed environments, except under E4, where the single cross SC-101 was the highest in STI. The 3rd highest genotype in STI was the single cross SC-10 under E3 and E6, SC-131 under E4, SC-30N11 under E2 and SC-2055 under E5. In general, the three most tolerant genotypes were SC-101 (G2), SC-131 (G4) and SC-10 in descending order; their GYPP was the highest under all stressed and non-stressed environments (Table 5). On the contrary, the most drought and/or low N sensitive genotypes were the open-pollinated populations Reid Type, Midland and the 3-way cross TWC-352 under all stressed environments (Table 4); their grain yield were the lowest (Table 3).

3.5 Superiority of Tolerant (T) to Sensitive (S) Genotypes

Based on grain yield/plant and stress (water stress and/or low N) tolerance index (STI), the most tolerant (T) three genotypes were the single cross hybrids SC-101 (G3), SC-131 (G4), and SC-10 (G1), while the most sensitive (S) genotypes were the populations Reid Type (G19), Midland (G18) and the three-way cross TWC-352 (G13) under all stressed environments. Out of the six studied environments, E1 represented the control, *i.e.* optimum environment (well-watered and high N), E3 represented the solely low N stress environment, E4 represented the solely drought stress environment and E6 represented the two severe stresses together (drought and low N).

Data averaged for each of the two groups (T and S) under all stressed and non-stressed environments (Table 6) indicated that GYPP of tolerant (T) genotypes was significantly ($P \leq 0.01$) superior to the sensitive (S) genotypes by 109.5, 39.6, and 141.9% under low-N stress only (E3), under drought stress only (E4), and under both severe stresses (drought and low-N) (E6), respectively. The significant ($P \leq 0.01$) superiority of stress tolerant genotypes in grain yield under low-N stress only (E3) was much higher than the superiority under drought stress only (E4). Moreover, the significant ($P \leq 0.01$) superiority under the two severe stresses together was much higher than the significant ($P \leq 0.01$) superiority under a sole stress (drought or low N). It is also observed that the stress tolerant genotypes in grain yield were even significantly ($P \leq 0.01$) superior to the sensitive ones under the optimum environment (E1; Well-watered and High-N) by 99.7%.

Significant ($P \leq 0.01$) superiority of tolerant (T) over sensitive (S) genotypes in GYPP under low N (109.5%), under drought (39.6%), and under both severe stresses (141.9%) was associated with significant ($P \leq 0.01$) superiority expressed in higher EPP (11.5, 13.15 and 11.99%), higher 100-KW (38.65, 30.46 and 30.99%), higher KPP (9.21, 5.95 and 22.32%), higher KPR (22.81, 11.28 and 20.07%), shorter ASI (-44.56, -29.58 and -29.08%), higher Nitrogen use efficiency (NUEe) (109.49, 39.62 and 141.89%), higher grain starch content (3.08, 0.90 and 3.91%), respectively but, tolerant genotypes had taller plants (11.53, 12.66 and 16.69%) and higher ear placement than sensitive genotypes (10.95, 16.09 and 18.47%) under low N, drought and both stresses, respectively.

It is observed that the highest superiority of T to S genotypes was shown under the severest environment (WS-LN) for GYPP, KPP, PH, EH, and NUE traits. Moreover, the superiority of T to S genotypes was even shown under the optimum environment (WW-HN) for all studied traits, indicating their superiority in responsiveness to environmental conditions. Hundred kernels weight trait as a grain yield component was the highest contributor in grain yield/plant to superiority of T to S genotypes.

3.6 Grouping Genotypes Based on Stress Tolerance and Grain Yield under Stress

Mean grain yield/plant of studied genotypes under Low-N (WW-LN), water stress (WS-HN) and low N and water stress together (WS-LN), was plotted against stress tolerance index of the same genotypes under WW-LN, WS-HN and WS-LN, respectively (Figs. 1, 2 and 3), which made it possible to distinguish between four groups, namely tolerant high- yielding, tolerant low-yielding, sensitive high-yielding and sensitive low-yielding.

Under low N stress (WW-LN), the genotypes No 3 followed by No. 1, 2, 16, 4, 5, 17, 10, 9 and 6 were classified as the low-N tolerant and high yielding genotypes, *i.e.* they could be considered as the most low-N tolerant and the most responsive genotypes to high-N in this study (Fig. 1). There was no genotype belonging to the group of low-N tolerant and low yielding genotypes under WW-LN. The genotype No. 14

only occupied the group of low-N sensitive and high yielding under WW-LN. The genotypes No 19, 18, 15, 12, 13, 8, 11 and 7 were classified as low-N stress sensitive and low yielding and therefore could be considered sensitive and low yielding (Fig. 1).

Under water stress (WS-HN) (Fig. 2), the genotypes No. 6 followed by 3, 2, 17, 9, 16, 1, 8 and 11 were classified as most drought tolerant and high yielding in this study. On the contrary, genotypes No. 15, 13, 19, 18, 10, 7, 14, 12 and 5 were classified as water stress sensitive and low yielding (Fig. 2). The genotype No. 4 was classified as drought tolerant and low yielding.

Under both stresses, i.e. water stress and low-N stress (WS-HN) (Fig. 3), the genotypes No. 3 followed by 4, 2, 17, 1 and 9 were classified as most drought tolerant, most low-N tolerant and high yielding in this study. On the contrary, genotypes No. 19, 18, 14, 13, 15, 16, 7, 8 and 12 were classified as water stress sensitive, low-N sensitive and low yielding (Fig. 3). The genotype No. 11 was classified as sensitive to both stresses and high yielding. The genotypes No. 6, 10 and 5 were classified as tolerant to both stresses but low yielding.

3.7 Regression of Grain Yield on Elevated Levels of Nitrogen

To further evaluate the relationship between grain yield and N level, linear as well as quadratic responses were graphed for each genotype on the three levels of nitrogen (low, medium and high N) (Figs. 4 and 5).

A significant linear response on elevated levels of nitrogen was shown by 13 genotypes, namely SC-10, SC-30K8, SC-101, SC-131, SC-2031, TWC-310, TWC-1100, TWC- 352, TWC-360, AED, Giza 2, Nubaria and Midland. A quadratic response on elevated levels of nitrogen was shown by six genotypes, namely SC-176, SC-168, SC-2055, SC-30N11, TWC-321 and Reid Type, with optimum nitrogen rate of 100 kg/fed. The highest rate of yield increase per unit nitrogen was exhibited by the single cross SC-30N11 followed by SC-2055, SC-176 and Reid Type; the reason for the high rate of yield increase of these genotypes is their low grain yield under the lowest nitrogen rate (20 kg/fed). On the contrary, the lowest rate of yield increase was shown by SC-10, SC-30K8, and SC-101; the reason for the low rate of yield increase of these genotypes is their high grain yield under the lowest nitrogen rate (20 kg/fed). The response of single crosses group and populations group was curvilinear (quadratic); with optimum N rate of 100 kg/fed but for three way crosses the response was near linear. The rate of yield increase due to increase of a nitrogen unit was the lowest for three-way crosses but the highest rate of yield increase was achieved by the group of single crosses.

4. DISCUSSION

The analysis of variance of split-split plot design indicated significant effect of the three studied factors, irrigation regime (I), N rate (N) and genotype (G) on the studied 15 traits of maize, except ear height and grain oil content for irrigation regime and nitrogen, and plant height for N. Significance of G×I indicated that means of studied traits of genotypes varied with water supply, confirming previous results [6]. Significance of G×N indicated that means of studied traits of genotypes varied with N rate fertilization and the possibility of selection for improved performance under specific soil nitrogen. In this context, [11,21,24-26], confirmed that the performance of maize genotypes varies with N levels for most studied traits. Significance of G×I×Y indicates that genotype performance differ from a combination of year x irrigation treatment to another combination and that the rank of maize genotypes differs from irrigation regime to another, and from one season to another and the possibility of selection for improved performance under a specific water stress for almost all studied traits as proposed by [6,27-29]. Significance of G×I×N indicated that means of studied traits of genotypes varied from a combination of N level and irrigation regime to another combination, confirming previous results [6,11]. Significance of G×N×Y suggests that maize genotypes vary with years and nitrogen supply for such traits, confirming previous results [11,21]. The high G×N×Y variances emphasize the need for multi-environment testing to identify N efficient cultivars with broad adaptation to different levels of N availability.

Significance of G×N×I×Y for all studied traits, except DTA, ASI, PH, EH, HKW and NUE indicated that the rank of maize genotypes differs from a combination of N level, irrigation regime and year to another combination, and the possibility of selection for improved performance under a specific of soil nitrogen combined with a specific irrigation regime and specific season as proposed by [24,28].

Coefficient of variation (CV) was generally very low (<10%) for most of studied traits, indicating the accuracy in implementing the experiment. The exception was ASI, GPC and GOC where CV was 32.81, 16.69 and 20.67 %, respectively. The large CV in this study for ASI is in agreement with results reported by [30,31].

The results of analysis of variance of RCBD under each of the six environments (WW-HN,

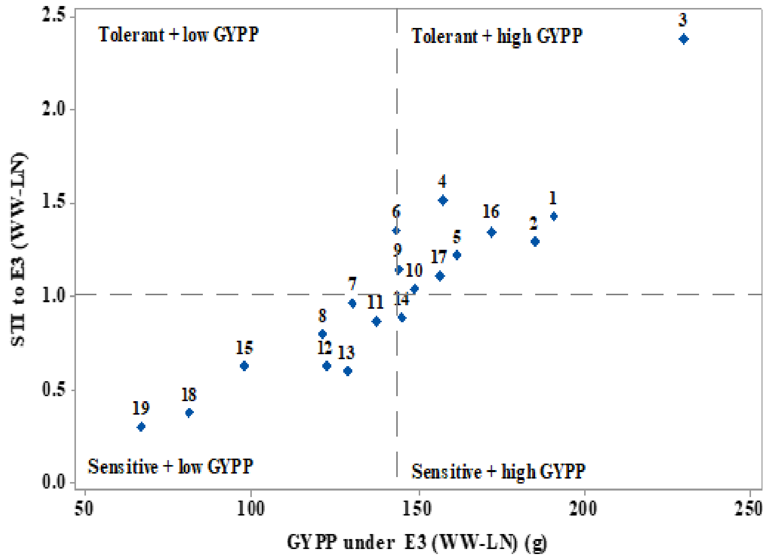


Fig. 1. Relationships between stress tolerance index (STI) of 19 maize genotypes and GYPP under low N stressed environment E3 (WW-LN) combined across 2016 and 2017 seasons. Broken lines represent mean of STI's and GYPP, (numbers from 1 to 19 refer to genotype numbers mentioned in Table 1)

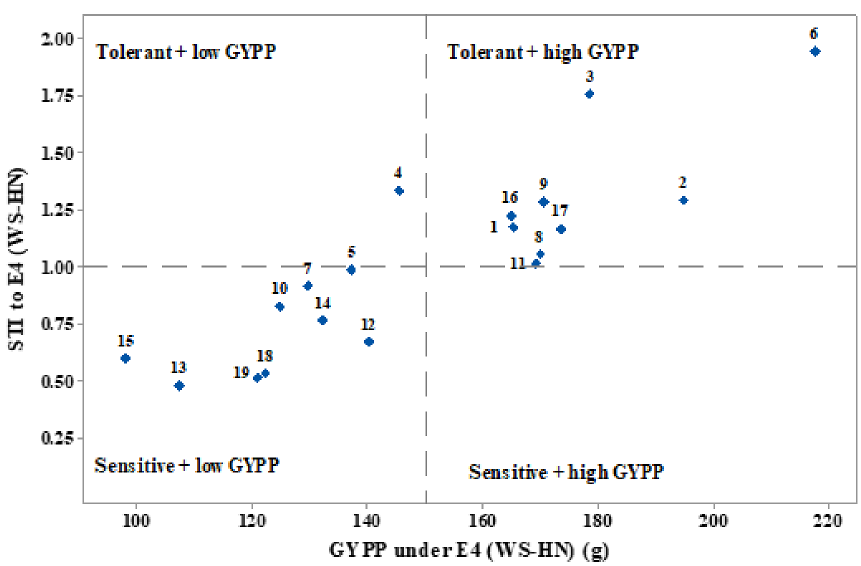


Fig. 2. Relationships between stress tolerance index (STI) of 19 maize genotypes and GYPP under water stressed environment E4 (WS-HN) combined across 2016 and 2017 seasons. Broken lines represent mean of STI's and GYPP, (numbers from 1 to 19 refer to genotype numbers mentioned in Table 1)

Table 6. Superiority (Super %) of the three most tolerant (T) to the three most sensitive (S) genotypes for selected traits under environments E1 (well-watered-high-N), E2 (well-watered-medium-N), E3 (well-watered-low-N), E4 (water stress-high-N), E5 (water stress-medium-N) and E6 (water stress-low-N) combined across 2013 and 2014 seasons

	E1	E2	E3	E4	E5	E6
Genotype	**WW-HN**	**WW-MN**	**WW-LN**	**WS-HN**	**WS-MN**	**WS-LN**
Grain yield/plant (g)						
T	261.6	213.9	192.6	163.1	153.2	139.7
S	131.0	129.3	91.9	116.8	83.3	57.8
Super%	99.7**	65.4**	109.5**	39.6**	84.0**	141.9**
Ears/plant						
T	1.077	0.999	0.998	1.027	0.941	0.881
S	1.015	0.924	0.895	0.907	0.815	0.787
Super %	6.11**	8.15**	11.50**	13.15**	15.37**	11.99**
Kernels/row						
T	46.58	44.93	42.35	43.31	39.87	39.51
S	40.04	38.74	34.49	38.92	35.15	32.90
Super%	16.33**	15.99**	22.81**	11.28**	13.43**	20.07**
Kernels/plant						
T	597.1	575.9	532.7	549.9	516.6	469.3
S	585.7	565.2	487.7	519.0	443.3	383.6
Super %	1.95*	1.91*	9.21**	5.95**	16.53**	22.32**
100-Kernel weight (g)						
T	38.65	37.46	36.60	35.73	36.03	33.63
S	28.09	26.86	26.40	27.39	25.67	25.10
Super %	37.57**	39.46**	38.65**	30.46**	40.35**	33.99**
Anthesis silking interval (day)						
T	1.89	2.11	3.11	3.44	3.84	4.61
S	3.61	3.94	5.61	4.89	5.50	6.50
Super %	-47.69**	-46.41**	-44.56**	-29.58**	-30.24**	-29.08**
Plant height (cm)						
T	256.8	252.2	244.1	251.2	243.1	238.4
S	230.4	223.7	218.8	223.0	214.8	204.3
Super %	11.46**	12.76**	11.53**	12.66**	13.16**	16.69**
Ear height (cm)						

	E1	E2	E3	E4	E5	E6
Genotype	**WW-HN**	**WW-MN**	**WW-LN**	**WS-HN**	**WS-MN**	**WS-LN**
Grain yield/plant (g)						
T	120.4	116.2	113.5	121.0	118.2	114.0
S	110.8	105.3	102.3	104.2	104.8	96.2
Super %	8.67*	10.35*	10.95*	16.09**	12.78**	18.47**
Nitrogen utilization efficiency (NUTEg) (g/g)						
T	92.55	95.36	101.42	78.49	83.50	115.54
S	78.12	85.68	92.43	70.36	74.35	80.21
Super %	18.48**	11.29**	9.73**	11.56**	12.31**	44.04**
Grain starch content %						
T	67.90	67.78	69.04	67.46	68.24	69.32
S	66.90	66.59	66.98	66.86	65.48	66.71
Super %	1.50*	1.79*	3.08**	0.90	4.23**	3.91**

- = decrease

WW-MN, WW-LN, WS-HN, WS-MN, WS-LN) indicated significant genotypic differences in the studied traits under all studied stressed and non-stressed environments. Such results were also recorded by previous investigators in maize [28,29,32,33].

Mean squares due to seasons were significant (p ≤ 0.05 or 0.01) for most of the cases, indicating that climatic conditions of the two seasons of the study were different and had different effects on the studied traits. Significant G × Y interaction for a trait indicates that the rank of genotypes in the first year was different from that in the second year for such traits under a specific environment.

The highest GYPP was obtained from the E1 (a combination of the highest N level and well-watered conditions) which is logic since available nitrogen for each plant was at maximum and the irrigation was optimum across seasons and therefore we considered this environment as the best one for GYPP and the percent change, in different studied traits was calculated relevance to this environment, either in case of increase (-) or decrease (+). Both stresses (nitrogen and drought) were exhibited by E5 and E6 environments; with the severest stress were present in E6, while the environments E2, E3 and E4 exhibit only one stress, but environment E1 exhibits no stress.

Reductions in grain yield (51.52 %) resulted from both stresses (water stress; 24.59% and low N; 28.56%) were associated with reductions in all yield components, PH, EH and CCI. Such reductions were more pronounced in the E6 environment (maximum stresses) followed by E5 and E4. Maximum significant reductions were exhibited by CCI (31.54%) under E6 due to severe stresses of nitrogen and water. The higher concentration of nitrogen might had increased enzymatic activities responsible for translocation of assimilates towards the economic portion [34]. Likewise, optimum irrigation might had improved the leaf longevity that might results in optimum net assimilation rate compared with deficit irrigation [35].

The increased chlorophyll contents of maize leaf in response to increasing levels of nitrogen might be attributed to the impact of nitrogen on leaf growth and leaf area [36]. The same authors reported a high correlation between nitrogen application and leaf chlorophyll contents. It was reported that nitrogen is the structural element of protein and chlorophyll molecule, and its higher concentrations had proved to increase the chloroplast formation and leaf photosynthetic efficiency [37]. Hokmalipour and Darbandi [38] reported that nitrogen fertilization activates the enzymes associated with chlorophyll formation thus results in higher concentration of chlorophyll than control plots. These results are in full accordance with those of Zhao et al. [39]. These results are further supported by Shah et al. [37]. The maximum chlorophyll content in response to optimum irrigation is supported by Liu et al. [40].

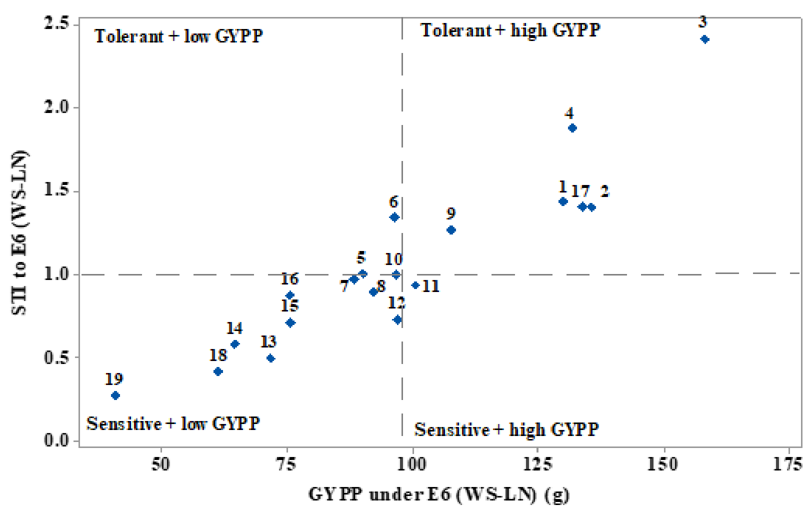
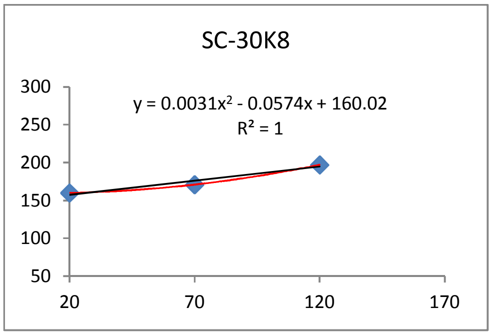
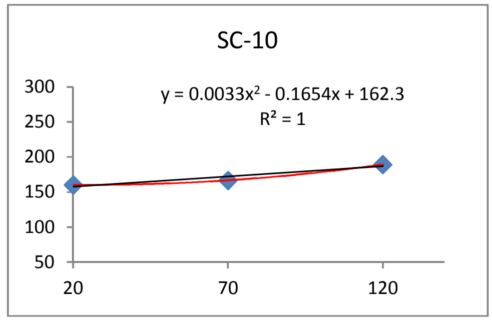
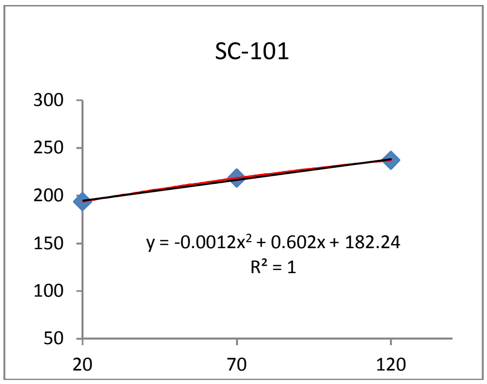
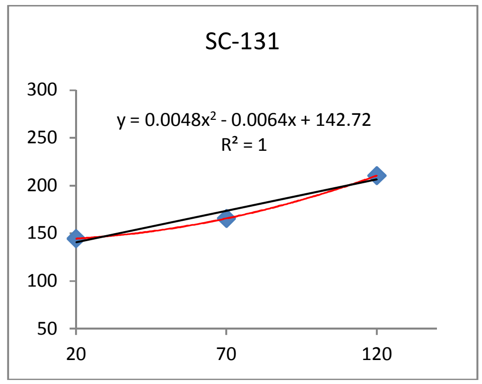


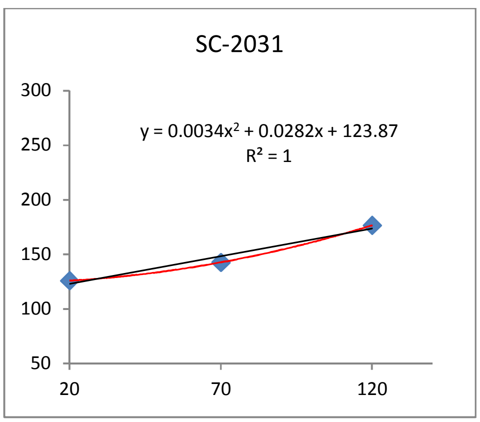
Fig. 3. Relationships between stress tolerance index (STI) of 19 maize genotypes and GYPP under both water stress and low N stress environment E6 (WS-LN) combined across 2016 and 2017 seasons. Broken lines represent mean of STI's and GYPP, (numbers from 1 to 19 refer to genotype numbers mentioned in Table 1)

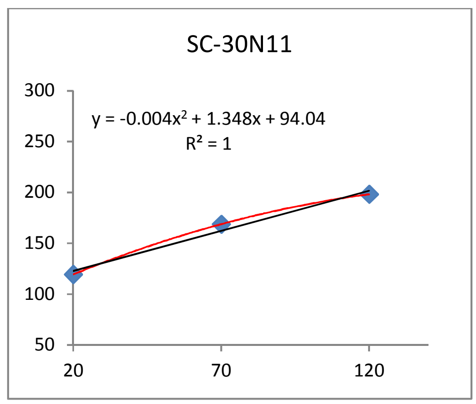

SC-30K8
y = 0.0031x² - 0.0574x + 160.02
R² = 1
300
250
200
150
100
50
20
70
120
170

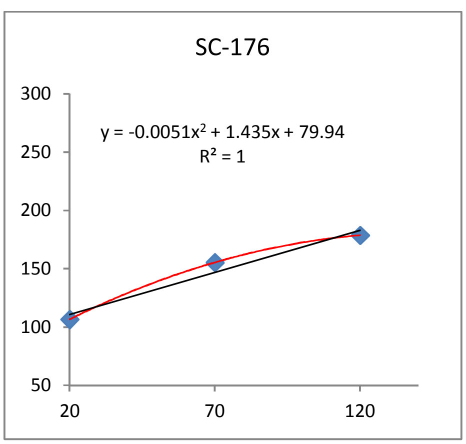

SC-10
y = 0.0033x² - 0.1654x + 162.3
R² = 1
300
250
200
150
100
50
20
70
120

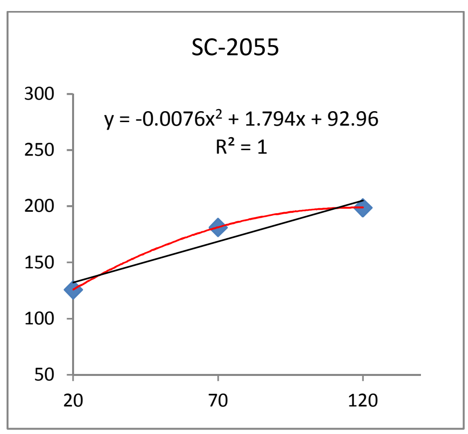

SC-101
y = -0.0012x² + 0.602x + 182.24
R² = 1
300
250
200
150
100
50
20
70
120

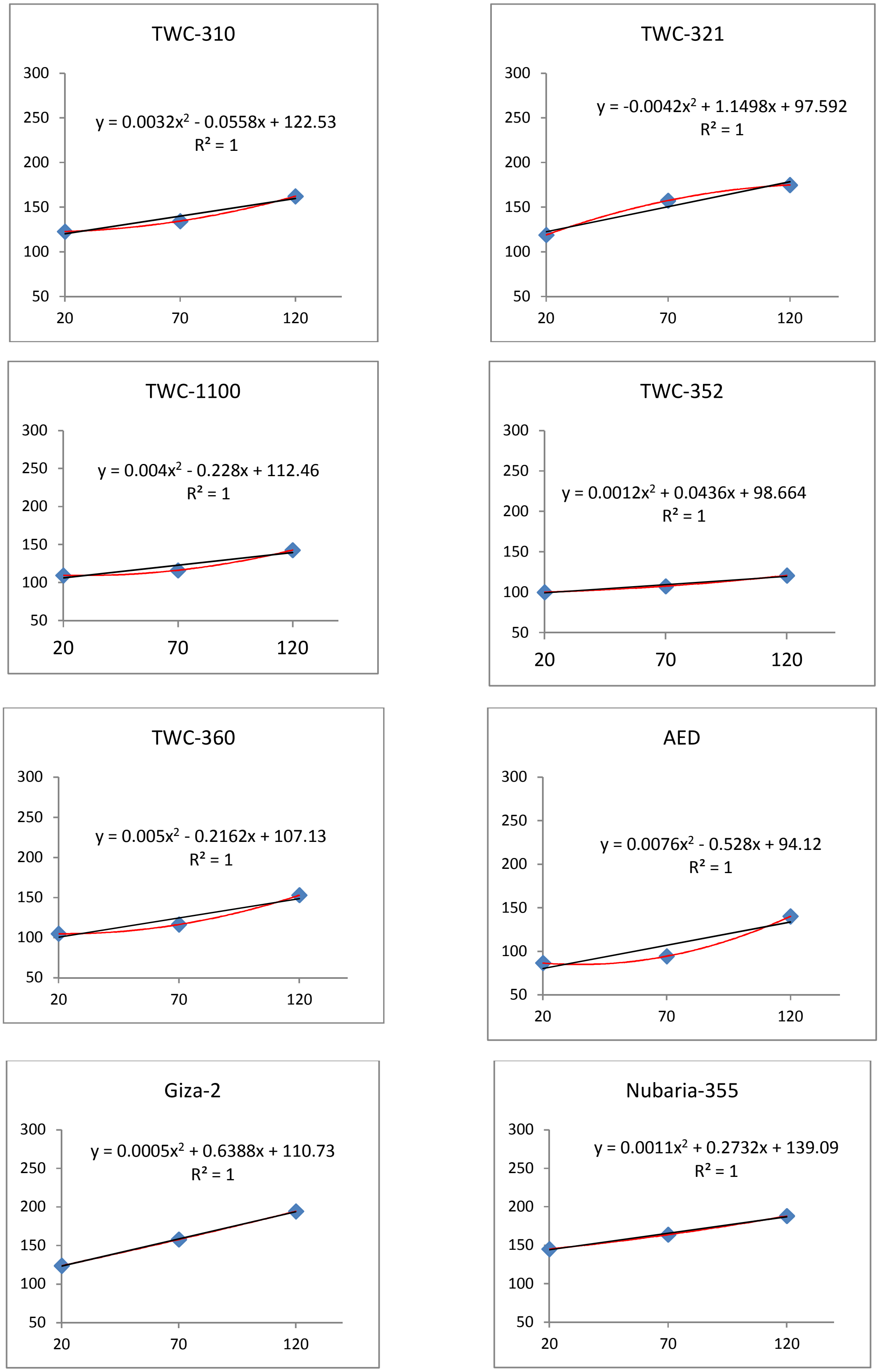

SC-131
y = 0.0048x² - 0.0064x + 142.72
R² = 1
300
250
200
150
100
50
20
70
120


SC-2031
y = 0.0034x² + 0.0282x + 123.87
R² = 1
300
250
200
150
100
50
20
70
120


SC-30N11
y = -0.004x² + 1.348x + 94.04
R² = 1
300
250
200
150
100
50
20
70
120


SC-176
y = -0.0051x² + 1.435x + 79.94
R² = 1
300
250
200
150
100
50
20
70
120


SC-2055
y = -0.0076x² + 1.794x + 92.96
R² = 1
300
250
200
150
100
50
20
70
120


TWC-310
y = 0.0032x² - 0.0558x + 122.53
R² = 1
TWC-321
y = -0.0042x² + 1.1498x + 97.592
R² = 1
TWC-1100
y = 0.004x² - 0.228x + 112.46
R² = 1
TWC-352
y = 0.0012x² + 0.0436x + 98.664
R² = 1
TWC-360
y = 0.005x² - 0.2162x + 107.13
R² = 1
AED
y = 0.0076x² - 0.528x + 94.12
R² = 1
Giza-2
y = 0.0005x² + 0.6388x + 110.73
R² = 1
Nubaria-355
y = 0.0011x² + 0.2732x + 139.09
R² = 1

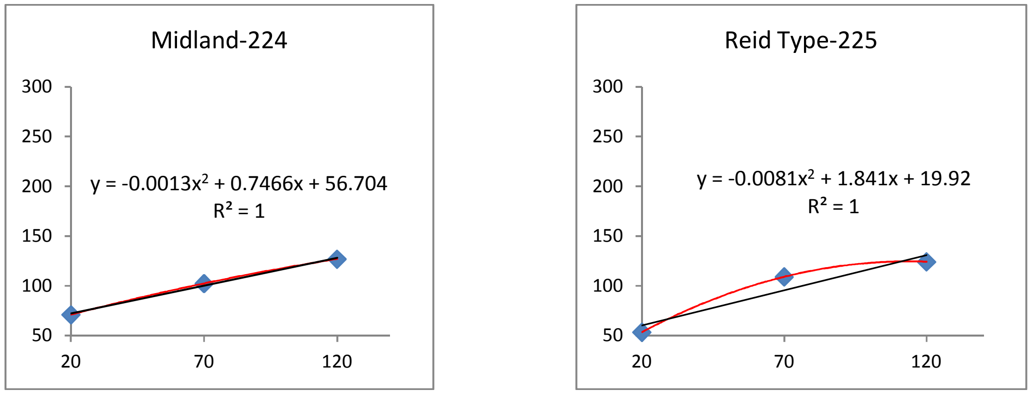


Fig. 4. Linear and quadratic regression of grain yield/plant on the three levels of nitrogen for each of the 19 genotypes across two years

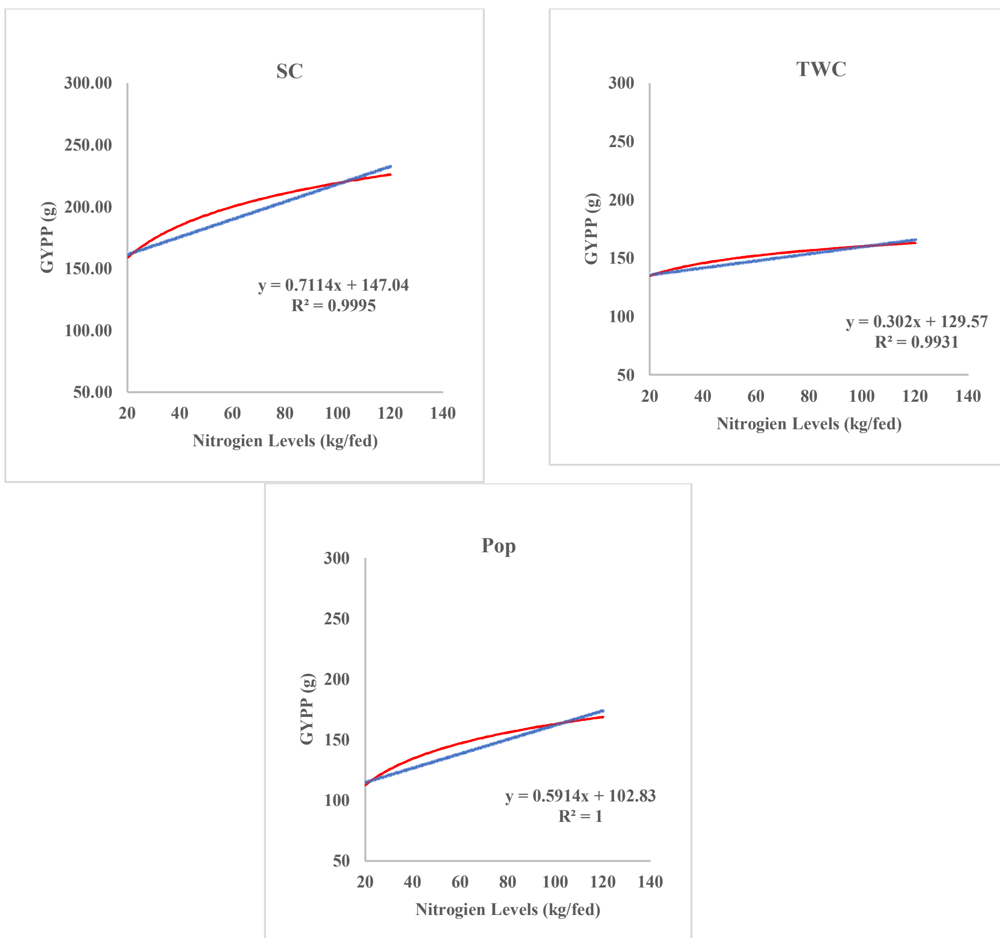


Fig. 5. Linear and quadratic regression of grain yield/plant on the three levels of nitrogen for three groups of genotypes, namely single crosses (SC), three-way crosses (TWC) and populations (Pop) across two years

On the contrary, the water stress combined with low N caused significant ($P \leq 0.01$) increases in DTA, ASI, GOC and NUEe. It is worthy to note that increases in NUEe, grain protein, and oil are favorable, while those in DTA and ASI, are unfavorable. Higher hundred kernels weight, number of kernels/plant and grain yield from plots treated with the highest level of N and optimum irrigation might be attributed to higher chlorophyll contents, that might had increased the photosynthetic efficiency and assimilates production and its availability during grains filling stage [41]. The higher assimilates production through photosynthesis and its efficient partitioning towards the grain might had increased the yield and yield components of maize [42]. These results are fully supported by the results reported by [38] The increase in grain yield with optimum irrigation have also been reported by [42,43]. The optimum irrigation had increased the grain yield in comparison with deficit irrigation which might be explained with proper plant growth and better development due to moisture availability at sensitive stages of phenological development [44]. The increase in grain yield with integrated use of N and optimum irrigation was supported by [42]. Brar and Tiwari [45] also observed the increase in grain yield of maize from integrated use of N and optimum irrigation, and attributed this increase to greater nitrogen use efficiency.

The highest average GYPP of all studied genotypes was achieved under E1 (WW-HN) because of the highest available N/plant and the optimum irrigation but the lowest average GYPP was recorded under the severest environment E6 (the lowest available N/plant and the deficit irrigation).

The change in ranking of the best or the lowest genotypes is due to the genotype × irrigation × Nitrogen level. It is observed that the single cross SC-101 (from Fine Seed Company) ranked the first in all environments, except in E4 environment, where the single cross SC-30N11 (from Pioneer Company) ranked the first. On the contrary, Reid Type population was the lowest yielding in all environments, except in E4 and E5, where the populations A.E.D. and Midland were the lowest, respectively.

In general, the single crosses group had the highest yield under all stressed and non-stressed environments, but the lowest yield was recorded by population group under E2, E3 and E6 and by three-way crosses under E1, E4 and E5 (Table 3). There is good evidence suggesting that hybrids maintain their advantage over open pollinated varieties in both stress and non-stress environments [6, 27, 46-49].

In general, the three most tolerant genotypes to both drought and Low-N in the present study, estimated using the equation suggested by [22] were SC-101 (G2), SC-131 (G4) and SC-10 in descending order; their GYPP was the highest under all stressed and non-stressed environments (Table 6). These genotypes should be recommended to maize breeding programs aiming at improving drought and/or low-N tolerance under corresponding stressed environments. On the other side, the most sensitive (S) genotypes were the populations Reid Type (G19), Midland (G18) and the three-way cross TWC-352 (G13) under all stressed environments.

Grain yield/plant (GYPP) of T genotypes was significantly superior to S genotypes under all stressed environments. Superiority of T over S genotypes in GYPP was associated with significant superiority in ears/plant, 100-kernel weight, kernels/row, nitrogen use efficiency, plant height, ear height and anthesis-silking interval, to sensitive genotypes. Superiority of T to S was also observed under the optimum environment (E1; Well-watered and High-N) by 99.7%, indicating that the best tolerant genotypes are more responsive and have higher yield potential under optimum conditions than the sensitive ones. These results are in agreement with those reported by several investigators [6, 11, 28, 29], who reported superiority of the drought tolerant genotypes to sensitive ones in grain yield and its attributes under drought stress and non-stress conditions at both flowering and grain filling stages. Bonea et al. [49] and Al-Naggar et al. [7] identified the 1000-grain weight as a reliable trait for selection for drought tolerance in maize.

CIMMYT breeders found that maize grain yield under drought was closely related to some secondary traits such as more ears per plant and short ASI [50-52]. These results are in consistency with those reported by [28.29]. Moreover, CIMMYT breeders also found that maize grain yield under low-N was closely related to some secondary traits such as improved N-uptake, high plant nitrate content, large leaf area, high specific leaf-N content, more ears per plant, short ASI and late leaf senescence [50-52]. These results are in consistency with those reported by [6].

Shortening in ASI of tolerant as compared to sensitive genotypes in the present study are desirable and may be considered as important contributors to drought and/or Low-N tolerance. Similar conclusions were reported by [14,15,46, 52,53]. A shortened ASI was considered as an indication of higher flow of assimilates to the developing ears during the early reproductive stage [52].

The present results indicate that the tolerant genotype to drought is also tolerant to Low-N stress, and *vice versa*. So, developing drought tolerant varieties would also be Low-N tolerant and *vice versa*. As an alternative breeding strategy, tolerance to drought has been suggested to improve performance under diverse abiotic stresses including Low- N [11,27,54].

The superiority of modern maize hybrids to the open-pollinated populations was also attributed to synchronization of 50% anthesis with 50% silking [9] and increased prolificacy, *i.e.,* more ears plant^{-1} [55].

It is apparent from the classifications (Figs. 1-3) performed according to [7,11,28,29,56,57] that the genotypes (SC-101) followed by (SC-131), (SC-30K8), (Nubaria), (SC-10), and (SC-2055) were the best genotypes that occupied the first group most of classifications; they are the most efficient, most drought tolerant, the highest yielders in this study under stress and non-stress conditions. On the contrary, the most sensitive to both stresses, lowest yielding and non-responsive genotypes in this study were (Reid Type), (Midland), (TWC-352), (TWC-360), (A.E.D.) and (SC-176).

It may be concluded from this study that the superiority of maize genotypes under drought and/or low N conditions could be a result of superiority in a combination of EPP, 100KW and KPR (grain yield components), low values of ASI (abiotic stress adaptive trait), high value of CCI (physiological trait), high NUE and NUTE values. The present study suggested that further investigation should be conducted to determine the underlying plant mechanisms contributing to the selection of water-efficient and/or N-efficient hybrids of maize.

Whereas a significant linear response estimates an increase in grain yield proportional to a given increase in N, a quadratic response can provide insight as to the optimum nitrogen level for a specific genotype as well as the point at which there is no longer yield gain per plant due to increased N and yield loss per plant may begin to occur [58]. In general, the response of single crosses group and populations group was curvilinear (quadratic); with optimum N rate of 238 kg/ha but for three way crosses the response was near linear. A significant linear response on elevated levels of nitrogen was shown by 13 genotypes with optimum nitrogen rate of 285.6 kg/ha, but a quadratic response was shown by six genotypes with optimum nitrogen rate of 238 kg/ha. The rate of yield increase per unit nitrogen differed from genotype to another. It was the lowest for three-way crosses but the highest rate of yield increase was achieved by the group of single crosses. Differential responses of maize genotypes to water stress combined with Low-N and other abiotic stresses was reported by several investigators [58-62]. Further studies should be conducted to identify the optimum N fertilization rate for each newly developed variety of maize.

5. CONCLUSION

In general, the single crosses had the highest yield under all stressed and non-stressed environments, but the lowest yield was recorded by populations or by three-way crosses. In general, the three most tolerant genotypes to both drought and Low-N in the present study, SC-101, SC-131 and SC-10 in descending order; their GYPP was the highest under all stressed and non-stressed environments. These genotypes should be recommended to maize breeding programs aiming at improving drought and/or Low-N tolerance. The best tolerant genotypes are more responsive and have higher yield potential under optimum conditions than the sensitive ones. Shortening in ASI of tolerant as compared to sensitive genotypes in the present study is desirable and may be considered as important contributor to drought and/or Low-N tolerance. The results concluded that drought tolerant varieties would also be Low-N tolerant and *vice versa*. It may be concluded from this study that the superiority of maize genotypes under drought and/or low N conditions could be a result of superiority in a combination of EPP, 100KW and KPR (grain yield components), low values of ASI (abiotic stress adaptive trait), high value of CCI (physiological trait), high NUE and NUTE values. The present study suggested that further investigation should be conducted to determine the underlying plant mechanisms contributing to the selection of water-efficient and/or N-efficient hybrids of maize and to identify the optimum N fertilization rate for each newly developed variety of maize.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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