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Secondary Traits for Maize (*Zea mays* L.) Tolerance to Drought Combined with Reduced N Fertilization Rate

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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

The secondary trait for a given abiotic stress tolerance, should be of strong correlation (r) with grain yield, high heritability (h^2_b) and high genetic advance (GA) under stressed conditions. The main objective of the present investigation was to identify secondary trait(s) for drought and/or low-N tolerance in maize genotypes. A two-year experiment was conducted, using a split-split-plot design. Main plots were allotted to two irrigation regimes, *i.e.* well watering (WW) and water stress at flowering (WS), sub-plots to three N fertilizer rates, i.e. low (LN), medium (MN) and high (HN) and sub-sub-plots to nineteen maize genotypes. Analysis of variance of randomized complete blocks design (RCBD) was also performed under each of the six environments (WW-HM, WW-MN, WW-LN, WS-HN, WS-MN and WS-LN). Tolerance to drought and/or low-N was strongly correlated with grain yield/plant (GYPP) under stressed environments. GYPP had high (h^2_b) and (GA); thus it is considered the best indicator of drought, low N or both stresses tolerance. The best secondary traits are high 100-kernel weight (100-KW), ears/plant (EPP), kernels/row (KPR), and short

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anthesis-silking interval (ASI) for low-N tolerance, high EPP, 100-KW, plant height (PH) and short ASI for drought tolerance, high 100-KW, EPP, KPR, PH and short ASI, for tolerance to drought combined with low N, and high 100-KW, rows/ear (RPE) and KPR under optimum conditions (WW-HN), since they show high (r), high (h^2_b) and high (GA) estimates under the respective environments. Under low-N and/or drought, future research should focus on the incorporation of secondary traits such as EPP, KPR, 100-KW, PH, ASI in the selection programs along with the grain yield trait.

Keywords: Selection criteria; drought tolerance; low-N tolerance; correlations; heritability; genetic advance.

1. INTRODUCTION

Maize (*Zea mays* L.) in Egypt is used as human food and animal feed and ranks second to wheat among cereal crops. Egypt in 2018 grew 935778 hectares producing 7.3 million tons of grains, with an average yield of 7.8-ton ha^{-1} [1]. To satisfy the local consumption (16 million tons), Egypt imports annually about 9 million tons of maize grains. To increase local maize production, Egypt extends the acreage of maize in the deserts, where sandy soil is characterized by low water-holding capacity and low in nutrients, particularly nitrogen, the most important nutritive element for the production of maize. Most Egyptian farmers use low-N fertilizer rates because of high price ratio between fertilizer and grain. Limited availability of N fertilizers and low purchasing power of farmers continued to be an important yield limiting factor in farmer's field. This expose maize plants to drought and/or low-N stresses. Exposing maize plants to such stresses results in a huge reduction in grain yields [2-6] for drought and [7-10] for low-N. This necessitates that maize breeders should pay great attention to develop drought-tolerant and low-N tolerant maize cultivars that could give high grain yield under such stressed conditions.

Maize breeders need information about interrelationships, heritability and expected selection gain under stressed and non-stressed environment to start an accurate breeding program for developing tolerant varieties to low-N and/or drought stress conditions. One approach to increasing the efficiency of selection in a stressed environment relies on the use of correlated secondary traits [11]. The secondary trait for a given abiotic stress (drought or low N) tolerance, should be of strong association with grain yield, of high heritability, high genetic advance under stressed conditions and is easy to measure [12].

Significant correlations under drought stress were found between maize grain yield and each of number of barren plants [13,14], anthesis-silking interval (ASI), ears per plant, stay green [15-17] grain filling period, leaf rolling, leaf senescence and number of kernels $plant^{-1}$ [18-21]. These investigators suggested that such traits could be used as indicators of drought tolerance in maize.

Moreover, grain yield of maize under low-N was associated with reduced ASI and stay green rates and increased ears per plant and kernels weight [2,12,21,22] Grain yield of maize under low-N was also related to ears per plant, ASI, leaf senescence [23,24], number of grains per plant and grain specific weight, which may also be indicative of performance under low-N, leaf N concentration and leaf dry matter content [23,25]. Moreover, the number of ears per plant was the most effective secondary selection criterion under low-N, followed by leaf senescence [26-28].

In the literature, there are two contrasting conclusions, based on results regarding heritability and predicted genetic advance (GA) from selection under a certain environment. A group of researchers found that heritability and GA from selection are higher under normal than stressed conditions [29-33]. However, other investigators reported that heritability and expected GA are higher under stressed than non-stressed, and that selection should be practiced in the target (stressed) environment to obtain higher genetic advance [11,33,34].

The objectives of the present investigation were: (i) to identify secondary traits for drought and/or low-N tolerance in maize hybrids and populations to be used in screening programs for selecting the tolerant genotypes and (ii) to identify the best selection environment for such stresses tolerance.

2. MATERIALS AND METHODS

2.1 Plant Materials

Nineteen maize (*Zea mays* L.) genotypes were used in this study, namely nine single cross Egyptian varieties (SC-10, SC-131, SC-168 and SC-176 from Agricultural Research Center ; ARC, Egypt, SC-30K8 and SC-30N11, from Pioneer-Corteva Agriscience, SC-2031 and SC-2055 from Hi-Tec Company and SC-101 from Fine Seeds Company), five three-way cross Egyptian varieties (TWC-310, TWC-321, TWC-352, TWC-360 from ARC, Egypt, and TWC-1100 from Hi-Tec Company) and five open-pollinated populations from ARC, Egypt (American Early Dent; AED, Giza 2 Synthetic and Nubaria-355 Synthetic of Egyptian origin, and Original Midland and Reid Type Composite of USA origin). The grain color is white for 11 genotypes (SC-10, 30K8, SC-101, SC-131, SC-2031, TWC-310, TWC-321, TWC-1100, AED, Giza-2 and Nubaria) and yellow for the rest of genotypes.

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 30^{ht} 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 ARC, Egypt.

2.6 Data Recorded

1)-Days to 50% tasselling (DTA). 2)-Days to 50% silking (DTS). 3)-Anthesis-silking interval (ASI). 4)-Plant height (PH). 5)-Ear height (EH). 6)-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/). 7)-Number of ears plant^{-1} (EPP). 8)-Number of rows ear^{-1} (RPE). 9)-Number of kernels row^{-1} (KPR). 10)-Number of kernels plant^{-1} (KPP). 11)-100-kernel weight (HKW) (g). 12)-Grain yield plant^{-1} (GYPP) (g): (adjusted at 15.5% grain moisture). Stress tolerance index

(STI) is calculated by the equation of Fageria [35] 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.

2.7 Biometrical Analysis

A combined analysis of variance of the split-split-plot design across the two years was performed if the homogeneity test was non-significant using the MIXED procedure of MSTAT ®. Moreover, each of the six environments was analyzed separately as a randomized complete block design (RCBD) across two years for the purpose of determining genetic parameters, i.e. under WW-HN, WW-MN, WW-LN, WS-HN, WS-MN, and WS-LN. LSD values were calculated to test the significance of differences between means according to [36]. Coefficients of Pearson correlations between attributes and their significance were calculated according to [36] by using SPSS 20 computer software. Expected mean squares across seasons under each environment (combinations of two irrigation regimes with three nitrogen levels) were estimated from the ANOVA table (Table 1) according to [37].

Table 1. Analysis of variance and Expected Mean Squares (EMS) across years

SV	df	MS	EMS
Year (Y)	1	-	-
R(Y)	8	-	-
Genotype (G)	18	M_3	$\delta^2_e + r\,\delta^2_{gy} + ry\,\delta^2_g$
G x Y	18	M_2	$\delta^2_e + r\,\delta^2_{gy}$
Error	68	M_1	δ^2_e

Genotypic (σ^2_g), phenotypic (σ^2_{ph}), genotype x year (σ^2_{gy}) and error (σ^2_e) variances were computed as follows: $\delta^2_{ge} = (M_2\text{-}M_1)/r$, $\sigma^2_g = (M_3 - M_2)\,/yr$ $\sigma^2_{ph} = \sigma^2_g + \sigma^2_{gy}/r + (\sigma^2_e\,/ry)$. where r = number of replications, g= number of genotypes and y= number of years. Heritability in the broad sense (h^2_b %) for a trait in a separate environment was estimated according to [38] using the following formula: h^2_b % = 100 × (σ^2_g / δ^2_{ph}), where: σ^2_g = genetic variance, and δ^2_{ph}= phenotypic variance. Expected genetic advance from selection for all studied traits as a percent of the mean was calculated according to [38] as follows: GA (%) = (100 K h^2_b σ_{ph})/ $\overline{x}$, where: $\overline{x}$ = General mean, σ_{ph} = Square root of the denominator of the appropriate heritability, h^2_b = The applied heritability, K = Selection differential (K = 1.76, for 10% selection intensity, used in this study).

3. RESULTS

3.1 Analysis of Variance

Combined analysis of variance across years (Y) of the split-split plot design for 12 traits of 19 genotypes (G) of maize under two irrigation regimes (I) and three nitrogen (N) levels was performed (Table 2). Mean squares due to genotypes were significant (P ≤ 0.01) for all studied traits. Mean squares due to irrigation and N level were significant (P ≤ 0.01) for all studied traits, except EH, for irrigation regime and PH, EH, for N level. 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 100-KW for I×Y, PH, EH and 100-KW for G×I and DTA, DTS, PH, EH and 100-KW 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 DTS, PH, 100-KW for G×I×Y, DTA, DTS, ASI, PH, EH, 100-KW for G×N×Y and DTA, DTS, PH, EH, 100-KW 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, DTS, ASI, PH, EH, 100-KW).

Combined analysis of variance across years of RCBD for 21 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, is presented in (Table 3).

Mean squares due to genotypes under all environments were significant (P≤ 0.01 or P≤ 0.05) for all studied traits. Mean squares due to the interaction of genotype × year (G × Y) were significant (p≤0.05 or p≤0.01) for some of studied traits, especially GYYP, KPP, KPR, EPP and CCI under all the six environments.

Table 2. Combined analysis of variance of split-split plot design across two years for 12 studied traits of 19 maize genotypes evaluated under two irrigation regimes combined with three N fertilizer levels

SOV	DF	Mean squares					
		DTA	**DTS**	**ASI**	**PH**	**EH**	**CCI**
Year (Y)	1	1355.80**	3142.04**	341.07**	37639.6**	0.041	3614.2**
Irrigation (I)	1	237.44*	949.76*	218.96**	23948.7**	1503.2	817.4**
Y × I	1	1350.18**	2808.47**	244.56**	33245.9**	7675.7**	967.0**
Y × I (Rep.)	8	150.38	278.12	22.95	3169.7	1261.8	6.9
Nitrogen (N)	2	107.69**	244.66**	42.21**	747.5	511.5	1570.8**
Y × N	2	6.09	6.52	0.75	1980.7	293.9	1621.7**
I × N	2	7.57	1.06	5.56**	1104.7	188.8	42.2**
Y × I × N	2	19.08	32.24	2.7	1566.3	301.2	79.7**
Y × I × N (Rep.)	16	19.44	26.29	1.84	1055.3	1117.7	2.1
Genotype (G)	18	314.03**	292.31**	39.28**	11564.6**	3852.0**	136.7**
G × Y	18	8.48*	11.95**	4.45*	480.5	230.5	147.0**
G × I	18	6.13	13.15**	7.61**	493.5	158.3	66.0**
G × N	36	3.28	4.65	3.46*	275.7	101.1	31.1**
G × I × Y	18	7.62*	8.45	5.14*	496.7	332.2*	31.4**
G × N × Y	36	2.9	5.85	3.04	200.4	122.7	33.0**
G × N × I	36	3.66	7.37	3.59*	396.5	142.4	28.2**
G × I × N × Y	36	3.64	5.46	2.67	340.8	133.5	43.7**
Error	432	3.22	3.9	1.68	266.28	120.5	2.185
CV%		2.856	2.96	32.81	6.739	9.581	9.347
R^2		0.89	0.9089	0.782	0.7875	0.729	0.9619
SOV	**DF**	**EPP**	**RPE**	**KPR**	**KPP**	**100-KW**	**GYPP**
Year (Y)	1	1.053**	105.27**	1878.6**	2197863.3**	15.23	1179759.3**
Irrigation (I)	1	0.22**	16.26**	2701.8**	1159009.4**	406.82**	295303.9**
Y × I	1	0.423**	15.03**	583.3**	555097.9**	7.02	107460.0**
Y × I (Rep.)	8	0.003	0.59	1.617	334.4	54.41	99.2
Nitrogen (N)	2	0.261**	1.31*	260.3**	278056.4**	175.33**	86428.6**
Y × N	2	0.085**	2.198**	73.76**	17487.1**	3.05	4960.6**
I × N	2	0.224**	0.51	290.7**	115643.8**	29.28*	15223.8**
Y × I × N	2	0.232**	1.29*	14.903**	94872.3**	13.7	6717.3**
Y × I × N (Rep.)	16	0.002	0.3	2.461	255.1	11.84	93.9
Genotype (G)	18	0.20**	55.13**	409.0**	106655.7**	590.17**	36481.2**
G × Y	18	0.05**	1.64**	27.32**	40512.7**	20.51*	18710.9**
G × I	18	0.054**	1.42**	40.55**	25813.3**	13.01	6285.7**
G × N	36	0.026**	1.37**	25.067**	16002.4**	5.24	6334.3**
G × I × Y	18	0.034**	1.73**	29.105**	14075.2**	6.78	3263.8**
G × N × Y	36	0.037**	0.81**	20.850**	13560.8**	5.53	2793.5**
G × N × I	36	0.04**	1.456**	36.527**	17960.9**	5.92	4756.0**
G × I × N × Y	36	0.041**	0.87**	34.361**	13598.7**	4.31	2704.1**
Error	432	0.0027	0.374	2.34	291.03	6.45	63.865
CV%		5.26	4.43	3.86	3.17	7.88	5.42
R^2		0.927	0.896	0.951	0.988	0.83	0.992

*DTA= Days to 50% anthesis, DTS = days to 50% silking, 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, 100-KW = 100-kernel weight, GYPP = grain yield per plant, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

3.2 Trait Interrelationships

Estimates of Pearson's correlation coefficients (r) between pairs of studied traits across all genotypes, all stressed and non-stressed environments and across two years are given in (Table 4). In general, across all stressed and non-stressed environments and across years, the grain yield/plant exhibited a significant (P≤0.05 or 0.01) and positive correlation coefficient with KPP (0.621), KPR (0.521), EPP (0.462), 100-KW (0.406), PH (0.406) and EH (0.164).

Table 3. Combined analysis of variance across years of RCBD for studied traits under each of the six environments (Combinations of two water regimes and three N levels)

SOV	DF	Mean squares					
		WW-HN	WW-MN	WW-LN	WS-HN	WS-MN	WS-LN
				Days to anthesis			
Geno. (G)	18	44.67**	44.35**	52.12**	66.64**	59.27**	67.01**
G x Y	18	2.90*	2.34	4.8	5.3	5.02	8.8
error	68	1.4	1.72	3.74	3.13	4.58	4.77
				Days to silking			
Geno. (G)	18	41.54**	48.24**	44.80**	74.17**	66.98**	53.76**
G x Y	18	2.67	4.61	6.69	9.54*	11.15*	8.35
error	68	1.54	2.21	4.68	3.96	5.49	5.52
				Anthesis silking interval			
Geno. (G)	18	4.38**	11.10**	22.11**	8.01**	8.99**	6.40**
G x Y	18	1.25	3.54*	1.19	4.3	5.91*	4.83*
error	68	1	1.23	1.98	2.23	1.86	1.78
				Plant height			
Geno. (G)	18	2272.9**	2762.3**	2034.8**	2094.7**	2198.6**	2039.5**
G x Y	18	198.9	230.6	269.5	282.6	437.5	640.3
error	68	208.4	276.3	254.7	216.6	316.2	325.5
				Ear height			
Geno. (G)	18	632.9**	834.4**	923.0**	704.8**	762.9**	639.3**
G x Y	18	188.7	148.1	185.5	221.8	179.5	151.5
error	68	163.3	114.6	133.3	117.9	109.9	83.9
				Chlorophyll concentration index			
Geno. (G)	18	14.29**	22.26**	10.84**	161.39**	60.39**	52.29**
G x Y	18	33.78**	32.10**	16.28**	122.75**	80.47**	46.54**
error	68	1.9	1.14	1.07	4.89	2.4	1.71
				Ears/plant			
Geno. (G)	18	0.030**	0.048**	0.054**	0.131**	0.085**	0.038**
G x Y	18	0.014**	0.023**	0.035**	0.086**	0.062**	0.022**
error	68	0.003	0.002	0.002	0.004	0.003	0.002
				Rows/ear			
Geno. (G)	18	10.75**	9.62**	12.66**	8.69**	10.46**	10.02**
G x Y	18	0.60*	0.80**	0.66*	1.14	1.36*	2.16**
error	68	0.31	0.23	0.28	0.63	0.46	0.33
				Kernels/row			
Geno. (G)	18	73.77**	76.76**	158.56**	100.61**	90.49**	72.58**
G x Y	18	14.52**	18.01**	35.51**	41.57**	40.41**	16.83**
error	68	2.37	1.8	2.62	1.81	2.82	2.64
				Kernels/plant			
Geno. (G)	18	32667**	38292**	38620**	39444**	32531**	18842**
G x Y	18	11658**	12895**	26968**	21356**	25048**	10979**
error	68	248.8	353	148.7	579.6	193.3	222.8
				100-Kernel weight			
Geno. (G)	18	122.52**	129.79**	105.73**	100.62**	88.30**	78.52**
G x Y	18	5.35	6.51	7.9	8.19	9.09	9.94
error	68	4.63	4.3	4.78	4.92	9.46	10.65
				Grain yield/plant			
Geno. (G)	18	18189.0**	9154.5**	10874.2**	8768.6**	12798.0**	5163.3**
G x Y	18	2702.4**	3425.3**	3456.5**	7317.7**	11642.4**	4425.4**
error	68	53.6	58.3	27	40.6	146.2	57.5

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

Table 4. Pearson's correlation coefficients (r) among pairs of studied traits of 19 maize genotypes across two irrigation regimes, three N levels and two years (N=684)

Trait	GYPP	DTA	DTS	ASI	PH	EH	CCI	EPP	RPE	KPR	KPP
DTA	-0.286**										
DTS	-0.454**	0.903**									
ASI	-0.497**	0.172*	0.571**								
PH	0.406**	-0.087	-0.257**	-0.412**							
EH	0.164*	0.173*	0.049	-0.208**	0.655**						
CCI	-0.163*	0.286**	0.324**	0.209**	-0.158*	0.033					
EPP	0.462**	-0.037	-0.174*	-0.326**	0.321**	0.218**	0.072				
RPE	0.039	-0.338**	-0.261**	0.048	-0.186*	-0.196*	-0.168*	-0.057			
KPR	0.521**	-0.252**	-0.394**	-0.433**	0.347**	0.127*	-0.234**	0.145*	-0.118*		
KPP	0.621**	-0.331**	-0.464**	-0.438**	0.299**	0.098	-0.183*	0.666**	0.369**	0.651**	
100KW	0.406**	0.076	-0.067	-0.299**	0.41**	0.294**	0.081	0.255**	-0.55**	0.359**	0.106*

*DTA= Days to 50% anthesis, DTS = days to 50% silking, 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, 100-KW = 100-kernel weight, GYPP = grain yield per plant, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

On the contrary, the grain yield/plant exhibited a significant (P≤0.05 or 0.01) and negative correlation coefficient with ASI (-0.497), DTA (-0.286), DTS (-0.454), CCI (-0.163). DTA showed the strongest positive correlation (r= 0.903) with DTS but the later had a significant (P≤ 0.01) and positive correlation with ASI (0.571). The trait ASI had a significant and negative correlation coefficient with most of yield components, plant height and ear height. Plant height and ear height are strongly and positively inter-correlated (0.655). Number of kernels/plant had strong positive correlation with each of ears/plant (0.666) and KPR (0.651).

3.3 Correlations between STI or GYPP and Studied Traits under Each Stress

Stress tolerance index had a strong significant (p≤ 0.01) and positive correlation coefficient with GYPP (0.948, 0.925, 0.879, 0.899 and 0.928) under the five stressed environments WW-MN, WW-LN, WS-HD, WS-MN and WS-LN, respectively (Table 5). So, STI, and grain yield/plant had a strong positive correlation coefficient with 100-KW under all stressed environments, EPP under four environments (WW-LN, WS-HD, WS-MN and WS-LN), KPP under two environments (WS-MN and WS-LN), KPR under three environments (WW-LN, WS-MN and WS-LN), and plant height under two environments (WS-HN and WS-LN). On the contrary, STI, and grain yield/plant had a significant (P≤ 0,05 or 0.01) and negative correlation coefficient with ASI under all stressed environments (Table 5).

3.4 Heritability and Genetic Advance

Estimates of heritability in the broad sense (h^2_b) and genetic advance (GA) from selection based on 10 % selection intensity for agronomic and grain yield traits under well-watered high N (WW-HN), well-watered medium N (WW-MN), well-watered low N (WW-LN), water stress high N (WS-HN), water stress medium N (WS-HN), and water stress low N (WS-LN), are presented in Table 6. Estimates of h^2_b ranged from 55.5 % for CCI under E1 (WW-HN) to 98.0 % for 100-KW under the same environment (E1). The largest h^2_b estimates (> 90.0 %) were shown by DTA, DTS, PH, EH, RPE, KPR, and 100-KW traits under all the six environments. The h^2_b estimates of the traits EPP, KPP ranged from > 80.0 % to < 90.0%. The h^2_b estimates were variable from environment to another environment for the remaining traits, i.e. from > 75 % to > 95 % for the traits GYPP and ASI, and from 55.2 % to 79.5 % for CCI.

The environments that showed the highest estimates of heritability were the optimum non-stressed one E1 (WW-HN) for 5 out of 12 traits, namely DTS, PH, KPR, 100-KW, GYPP, followed by the environment E3 (WW-LN) for two traits, namely ASI, RPE, the environment E2 (WW-MN) for four traits, namely DTA, EH, EPP and KPP and the environment E4 (WS-HN) for only one trait (CCI).

Table 5. Pearson correlation coefficients between each of stress tolerance index (STI) or grain yield/plant (GYPP) and studied traits under the five stressed environments well-watered medium N (WW-MN), well-watered low N (WW-LN), water stress high N (WS-HN), water stress medium N (WS-MN) and water stress low N (WS-LN)

Trait	E2 WW-MN		E3 WW-LN		E4 WS-HN		E5 WS-MN		E6 WS-LN	
	STI	GYPP	STI	GYPP	STI	GYPP	STI	GYPP	STI	GYPP
DTA	0.046	0.045	0.127	0.194	0.158	0.178	-0.127	-0.244	-0.009	0.066
DTS	-0.163	-0.250	-0.211	-0.249	0.053	0.022	-0.291	-0.362	-0.147	-0.077
ASI	-0.441*	-0.621**	-0.510**	-0.674**	-0.350*	-0.384*	-0.554**	-0.620**	-0.403*	-0.460*
PH	0.352	0.294	0.245	0.275	0.488*	0.466*	0.362	0.274	0.408*	0.343*
EH	0.189	0.111	0.171	0.181	0.501**	0.383	0.194	0.005	0.407*	0.287
CCI	0.289	0.323	0.563**	0.373	0.294	0.326	-0.005	-0.141	-0.088	-0.191
EPP	0.364	0.363	0.504*	0.560**	0.594**	0.518**	0.500**	0.442*	0.487*	0.450*
KPR	0.377	0.530	0.451*	0.624**	-0.01	-0.004	0.404*	0.433*	0.386*	0.558**
KPP	0.116	0.283	0.127	0.313	0.325	0.35	0.450*	0.513**	0.440*	0.520**
100-KW	0.684**	0.657**	0.768**	0.744**	0.585**	0.430*	0.649**	0.492*	0.714**	0.672**
GYPP	0.948**	-	0.925**	-	0.879**	-	0.899**	-	0.928**	-

*DTA= Days to 50% anthesis, DTS = days to 50% silking, 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, 100-KW = 100-kernel weight, GYPP = grain yield per plant, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively*

Table 6. Heritability in broad sense (h^2_b %) and genetic advance from selection (GA%) for studied traits under each of the studied environments

Parameter	E1 (WW-HN)	E2 (WW-MN)	E3 (WW-LN)	E4 (WS-HN)	E5 (WS-MN)	E6 (WS-LN)
	Days to anthesis (d)					
h^2_b %	97.4	97.7	95.9	96.7	96.1	94.7
GA %	18.8	18.7	19.9	22.5	20.9	21.9
	Days to silking (d)					
h^2_b %	97.3	96.2	93.7	95.1	93.5	93.6
GA %	17.3	18.4	17.1	22.1	20.5	18
	Anthesis silking interval (d)					
h^2_b %	88.2	88.9	96.8	81.6	79.8	77.0
GA %	123.6	172.8	193.9	112.5	107.1	75.2
	Plant height (cm)					
h^2_b %	95.7	95.7	93.9	94.1	91.7	88.4
GA %	32.3	36.6	31.7	32.2	33.4	32.7
	Ear height (cm)					
h^2_b %	87.5	92.4	91.7	88.3	90.7	90.8
GA %	34.5	42.2	45.6	37.7	40.7	38.9
	Chlorophyll concentration index (%)					
h^2_b %	55.2	67.1	65.9	79.5	68.9	76.8
GA %	24.2	46.7	38.3	115.1	69.8	79.7
	Ears/plant					
h^2_b %	85.3	85.7	81.8	81.7	80.1	83.2
GA %	25.9	35.2	38.6	56.8	48.8	35.6
	Rows/ear					
h^2_b %	97.7	96.9	97.9	94.7	95.2	92.8
GA %	39.5	38.4	45.3	36.4	40.9	40.5
	Kernels/row					
h^2_b %	93.4	92.4	92.8	87.7	86.7	92.3
GA %	33.4	35.1	54.0	41.6	41.8	40.0
	Kernels/plant					
h^2_b %	89.3	89.8	81.1	84.5	79.5	83.6
GA %	48.0	55.3	59.8	57.5	57.8	50.1
	100-Kernel weight (g)					
h^2_b %	98.0	97.8	96.9	96.6	95.0	93.9
GA %	56.6	59.8	55.6	53.5	51.0	50.3
	Grain yield/plant (g)					
h^2_b %	95.2	88.8	90.4	78.2	76.6	77.7
GA %	115.1	93	121.3	96	146.3	114.2

On the contrary, most of the lowest estimates of heritability were shown by the severest stressed environments, namely E6 (WS-LN) for six traits (DTA, DTS, ASI, PH, RPE and 100-KW), E5 (WS-MN) for four traits (EPP, KPR, KPP, GYPP), and E1 for only one trait (EH).

The magnitude of expected genetic advance as a percent from the mean (GA %) from direct selection (Table 5) ranged from 17.1 % for DTS under E3 to 193.9 % for ASI under E3. The studied traits were classified into three groups based on the GA magnitude; the first group achieved > 100 % GA (ASI, CCI, GYPP), the second group achieved from 40 to 70 % GA (PH, EH, EPP, RPE, KPR, KPP, 100-KW) and the third group achieved <22.5 % GA (DTA and DTS). The best environment that showed the highest estimates of genetic advance from selection were E5 for GYPP, E4 for 4 traits (DTA, DTS, CCI, EPP), E3 for 5 traits (ASI, EH, RPE, KPR and KPP), and E2 for PH and 100-KW.

4. DISCUSSION

Analysis of variance of the split-split plot design indicates that genotype (G), irrigation regime (I) and N level have an obvious effect on most studied traits. Significance of G×I indicated that means of studied traits of genotypes varied with water supply, and the possibility of selection for improved performance under specific water stress conditions, confirming previous results [4,16,39, 40]. 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 as proposed by several investigators [4,8-10,41, 42] Significance of G×I×N indicated that means of studied traits of genotypes varied from a combination of N level with irrigation regime to another combination, confirming previous results [10,43]. Moreover, analysis of variance of the RCBD indicated the significance of differences among studied genotypes for all such traits under each of the six environments. Such genotypic differences in studied traits under all studied stressed and non-stressed environments were also recorded by previous investigators in maize [5,6,10, 44-47].

One approach to increasing the efficiency of selection in a stressed environment relies on the use of correlated secondary traits [11]. Correlations between grain yield, yield components, and agronomic traits may be of value in determining useful criteria for low-N and/or drought tolerance.

The yield component of the strongest positive correlation with grain yield is number of kernels/plant. Yield in this study was significantly correlated with grain number per plant and ears per plant as observed by [17,23,48]. The strong relationships between grain yield and number of kernels/plant (the product of EPP × rows/ear × kernels/row) are in harmony with other reports [47-49].

The strong relationships between grain yield and all yield components under water stress and non-stress conditions are in harmony with other reports [47-50]. Grain yield/plant showed a significant and positive correlation with plant height in this study, indicating that taller genotypes are of high yielding. This conclusion is in agreement with others [47,51]. In contrast, [52-54] reported that shorter genotypes are higher yielding than taller genotypes under both stressed and non-stressed environments.

The strongest negative correlation with grain yield is anthesis-silking interval (Table 4). In this respect, [23,55] observed a significant negative correlation between ASI and grain yield (r_g = -0.81). When plants are subjected to nitrogen deficiency, genotypes for which ASI does not increase would have a more efficient nitrogen metabolism or a physiology leading to greater yield at low-N input [55]. An increased ASI (or asynchronous flowering) has usually been associated with reduction in grain yield [2,4,16, 56-58].

Similar results on the positive relationship between the grain yield and grain weight under drought stress are expected as reported in previous studies. The 1000-grain weight was identified as a reliable trait for selecting for drought tolerance in maize [59].

The selection criterion for a given abiotic stress (drought or low N) tolerance index (STI) or for grain yield/plant, should be of strong association with STI or GYPP and of high heritability under stressed conditions. Data (Table 5) indicated that grain yield is the best indicator of drought, low N or both stresses tolerance in this experiment. So, STI, and grain yield/plant had a strong positive correlation coefficient with the yield components 100-KW, EPP, KPP, KPR and PH, and negative correlation coefficient with ASI, under severest stressed environments.

The correlation analysis presented in (Table 5) suggests that the selection criteria of low N tolerance represented in WW-LN environment (E3) are high GYPP, high 100-kernels weight, high EPP, high KPR and low ASI. The selection criteria of drought tolerance represented in WS-HN environment (E4) are high GYPP, high EPP, high 100-KW, tall plant, and low ASI. The selection criteria of tolerance to both stresses (drought tolerance and low N) represented in WS-LN environment (E6) are high GYPP, high 100-KW, high EPP, high number of kernels/plant, tall plant and low ASI. These traits could be considered as selection criteria for drought or/and low N tolerance in maize if they proved high heritability and high predicted genetic advance from selection. This conclusion is in accordance with other investigators [2,22,43, 60,61]. It is well known that one of the best indicators of plant tolerance to water stress during flowering and tolerance to low N is the anthesis-silk interval (ASI), as ASI shows a strong negative correlation with grain yield under these stressed conditions [2, 62].

In general, the estimates of h^2_b for most studied traits ranged from medium to very high in magnitude. The low estimates of heritability of some traits under some environments indicate that environmental variance and/or genetic × environment variance had great effect on the performance of these traits. Low heritability estimates for these traits, could also be attributed to the small magnitude of genotypic variance relative to that of the phenotypic variance as suggested by [4,5,47,63]. It is also obvious from these results that h^2_b estimates were generally the highest under full irrigation as compared to those under drought stress for 20 out of 21 studied traits, but under medium N for five traits, low-N for seven traits and under water stress only for one trait (CCI). So the best selection environment according to our results should be well watered combined with high N for 5 traits (DTS, PH, KPR, 100-KW, GYPP), medium N for 4 traits (DTA, EH, EPP and KPP) and low N for two traits (ASI, RPE). Only for CCI trait, the best selection environment was the water stressed one combined with high N.

On the contrary, most of the lowest estimates of heritability were shown by the severest stressed environments (E5 and E6). Similar to these results, some researchers found a decrease in heritability under stressed environments [22,30-32,64] This is similar to what happened in the present study for 5 out of 12 studied traits, namely DTS, PH, KPR, 100-KW and GYPP, and to some extent under WW-MN environment for 4 traits, namely DTA, EH, EPP and KPP. Others reported that genetic variance and consequently heritability was increased in stressful environments [5,9,10, 23,34]. This is similar to that happened in the present study for 3 out of 12 traits, namely ASI, RPE and CCI.

The studied traits were classified into three groups based on the magnitude of expected genetic advance as a percent from the mean; the first group achieved > 100% GA (ASI, CCI and GYPP), the second group achieved from 40 to 70% GA (PH, EH, EPP, RPE, KPR, KPP and 100-KW, and the third group achieved <22.5% GA (DTA and DTS).

It is interested to mention that the best environments that achieved the highest estimates of heritability for the majority of traits were the first three well-watered environments (E1, E3 and E2), while those environments that achieved the highest estimates of genetic advance were the three environments E5 (WS-MN), E4 (WS-HN) and E3 (WW-LN) in descending order.

In the literature, there are two contrasting conclusions, based on results regarding heritability and predicted genetic advance (GA) from selection under stress and non-stress environments. Many researchers found that heritability and GA from selection for grain yield is higher under non-stress than those under stress [22,30-32]. However, other investigators reported that heritability and expected GA for the same trait is higher under stress than non-stress, and that selection should be practiced in the target environment to obtain higher genetic advance [9, 11,33,34]. It is worthy to mention that direct selection under the stressed environments would ensure the preservation of alleles of stress tolerance [8,16,30,47,65,66], while direct selection under optimum conditions would take advantage of the high heritability [11,63,67].

Traits showing high heritability along with high genetic advance from selection suggest that such traits are controlled with additive genetic variance and predict that selection for improving these traits would be highly efficient. In the present study, these traits are GYPP, 100-KW, KPR, KPP, RPE, EPP, ASI, PH and EH. Based on the correlation (r) analysis between studied traits and STI or GYPP under five stressed environments and their corresponding estimates of broad-sense heritability (h^2_b) and genetic advance from selection (GA), it is evident that the best secondary traits (selection criteria) in our study are: high GYPP, high 100-kernels weight, high EPP, high KPR, low ASI for low-N tolerance under E3 (WW—LN), high GYPP, high EPP, high 100-kernels weight, tall plant, and low ASI for drought tolerance under E4 (WS-HN) and high GYPP, high 100-kernels weight, high EPP, high number of kernels/plant, tall plant and low ASI, for tolerance to both stresses (drought tolerance and low N) represented in WS-LN environment (E6), since they show high (r) values, high (h^2_b) estimates and high GA estimates under the respective environments. Under optimum conditions, i.e. well watering and high N conditions, GYPP, ASI, KPP, 100KW, NUE, RPE and KPR traits showed high (r) values, high (h^2_b) estimates and high GA estimates and therefore could be considered selection criteria for GYPP under non-stressed environment.

Selection for improved performance under low-N and/or drought based on grain yield alone has often been considered efficient, but the use of secondary traits of adaptive value whose genetic variability increased under low-N and/or drought can increase selection efficiency [2]. Plant breeders have advocated the judicious incorporation of secondary traits within breeding programs [11, 68]. Results of the present study suggest that to maximize the genetic gain from selection, for improving grain yield, under low-N and/or drought, future research should focus on the incorporation of secondary traits such as EPP, KPP, 100-KW, PH and ASI traits in the selection programs along with the grain yield trait. In this aspect, the secondary traits proposed for high productivity were also KPP, EPP, 100-KW, and ASI under low nitrogen stress [4,12,22-24, 34,43] and ASI, EPP and KPP under drought stress [5,6,13,16-21].

5. CONCLUSIONS

Data of the present study proved that the secondary traits for drought and/or low-N tolerance, could be identified if they have strong association with GYPP, high heritability and high genetic advance from selection under such stressed conditions. Data indicated that grain yield is the best indicator of drought, low N or both stresses tolerance in this experiment. Results concluded that the best secondary traits were high 100-KW, EPP, KPR, and short ASI for low-N tolerance, high EPP, 100-KW, PH and short ASI for drought tolerance, high 100-KW, EPP, KPR, PH and short ASI, for tolerance to drought combined with low N, and high 100-KW, RPE and KPR for no-stress (optimum) conditions (WW-HN). So, it is concluded that to maximize the genetic gain from selection, for improving grain yield, under low-N and/or drought, future research should focus on the incorporation of secondary traits such as EPP, KPP, 100-KW, PH and ASI, in the selection programs along with the grain yield trait.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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