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Correlations and heritability for root architecture traits of maize under water stress at flowering and grain filling

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Root system architecture is important for plant productivity under drought stress. Strong associations between root traits and drought tolerance, high heritability and high genetic advance for such traits would allow plant breeder to use such traits as selection criteria for selecting drought tolerant genotype(s). The objectives of the present investigation were: (i) to elucidate the relationships between the drought tolerance index (DTI) and root traits of 22 maize genotypes and (ii) to estimate the heritability (h^2_b) and genetic advance (GA) of such traits, in order to determine the root selection criteria for DTI. A two-year experiment was carried out using a split plot experiment with three replications. The main plots were devoted to irrigation regimes, *i.e.* well watering at flowering (WWF), and at grain filling (WWG), water stress at flowering (WSF) and at grain filling (WSG), and sub plots to maize genotypes. Based on the correlation (r) analysis between studied traits and DTI or GYPP under drought at WSF and WSG and their corresponding estimates of broad-sense heritability (h^2_b) and genetic advance from selection (GA), it is evident that the best selection criteria for drought tolerance in our study are: crown roots number, crown roots branching (CB), crown root length, root circumference (RC) and root dry weight under WSG, RC under WSF and CB and RC under WWG, since they showed high (r) values, high (h^2_b) and high GA estimates under the respective environments. Results suggested that selection for root traits would be more effective when practiced at later stages (grain filling) of plant growth than at earlier stages (flowering).

Keywords: Maize, Brace roots, Crown roots, Drought tolerance index, Flowering, Grain filling

INTRODUCTION

Egypt ranks the fifth in the world with respect of average productivity of maize after USA, France, Germany and Italy (FAOSTAT, 2018), though the local production of maize is not sufficient to satisfy the local consumption and Egypt imports annually about six million tons of maize grains. Efforts in Egypt are devoted to extend the acreage of maize in the desert and to raise its yield per area unit in order to reach self-sufficiency. Growing maize in the sandy soils of low water-holding capacity would expose maize plants to water stress, which could result in obtaining low grain yields under such conditions. Moreover, the predicted future shortage in irrigation water in Egypt necessitates that maize breeders should pay more effort to develop drought tolerant maize hybrids that could give high grain yield under water-stress conditions. Maize is very sensitive to water stress during the flowering and grain-filling periods (Bai et al. 2006). However, Witt et al., (2012) reported that the most critical period for yield production goes approximately from 2 weeks before flowering time until 2 weeks after flowering time. Root system architecture is important for plant productivity under drought stress (Lynch, 1995). Plants avoid dehydration by increasing water uptake in the soil profile and adapt to the chemical and physical soil constraints, particularly under drought conditions, thanks to the morphological plasticity of their root system (Lynch 2007). The importance of a deep and vigorous root system for maintaining yield under drought stress has been reported in maize (Hund et al., 2011). Drought tolerant genotypes generally increase the photosynthates allocation for root elongation under drought stress (Rauf and Sadaqat, 2008). Genetic variation for this trait has been shown in maize (Rauf et al., 2009). Root architecture is difficult to evaluate directly in soil. Several high-throughput procedures to measure root systems have been reported. At the flowering stage, roots have been measured in the field (Kato et al., 2006), in soil boxes (Araki et al. 2000) and in soil columns (Hund et al., 2009; Zhu et al., 2010). Growing plants in columns or boxes, filled with soil or artificial substrate, can help to reduce sampling efforts compared to field studies and allows growth under controlled conditions. However, the excavation of roots and measurement of root traits in these systems remains labor-intensive and does not allow for high throughput. In the field, roots and shoots are exposed to very different environmental conditions, which affect the root development (Hund, 2010). Visual scoring using a defined rating system has been employed for high throughput phenotyping of shoot traits. A high throughput method that utilizes visual scoring of the numbers, angles and branching density of brace and crown roots has not yet been used for the investigation of root architecture. Trachsel et al., (2011) presented a method to visually score 10 root architectural traits of the root crown of an adult maize plant in the field in a few minutes. According to them, visual measurement of the root crown required 2 min per sample irrespective of the environment. The visual evaluation of root architecture will be a valuable tool in tailoring crop root systems to specific environments. The root ideotype should however be defined after a detailed understanding of the factors that limit the availability of soil moisture and the metabolic cost needed to develop and maintain a vigorous root system (Tuberosa, 2014). Recurrent selection for increased yield in drought-stressed tropical maize was associated with a decrease in root mass (Bolaños et al., 1993). The effects of root size and architecture on yield also depend on the distribution of soil moisture and the competition for water resources within the plant community (King et al., 2009). The study of correlation is regarded as an important step in breeding programs of maize since the information obtained is useful estimating the correlated response to directional selection for the formulation of selection indices. The estimation of the heritability is a very useful parameter for breeders because it allows one to predict the possibility of success with the selection, as it reflects the proportion of phenotypic variation that can be inherited; in other words, the heritability coefficient measures the reliability of the phenotypic value as an indicator of genotypic value (Vasconcelos et al., 2012). Heritability estimates facilitate the choice of methods and characters used in the initial and advanced phases of improvement programs, thereby allowing the study of mechanisms, genetic values and variability for one character (Cruz et al., 2012). The estimations of high coefficients of heritability are associated with a greater genetic variability, greater selective accuracy (Cargnelutti Filho et al., 2009) and greater possibilities for success in selecting genotypes with higher productivity of grain.

The objectives of the present investigation were:

(i) to elucidate the relationships between the drought tolerance index (DTI) or grain yield/plant (GYPP) and root traits of available maize germplasm and (ii) to estimate the heritability and genetic advance of these traits, in order to determine the selection criteria for DTI or GYPP under drought stress conditions at flowering and grain filling stages.

MATERIALS AND METHODS

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).

Plant materials

Seeds of 22 maize (*Zea mays* L.) genotypes (10 single crosses, 5 three-way crosses and 7 open-pollinated populations) obtained from Agricultural Research Center (ARC) (13 genotypes), Hi-Tec Company (3 genotypes), DuPont Pioneer Company (3 genotypes), Fine Seeds Company (one genotype), Egaseed Company (one genotype), and Watania Company (one genotype) were used in this study (Table 1).

Experimental procedures

Sowing date was April 24th in the 1st season (2016) and April 30ht 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 24,000 plants/fed. Each experimental plot included two rows (plot size = 5.6 m^2).

Experimental design

A split-plot design in randomized complete block (RCB) arrangement with three replications was used. Main plots were allotted to three irrigation regimes, *i.e.* well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG). Each main plot was surrounded with an alley (4m width), to avoid water leaching between plots. Sub plots were devoted to twenty-two maize genotypes.

Water regimes

1. Well watering (WW): Irrigation was applied by flooding, the second irrigation was given after three weeks and subsequent irrigations were applied every 12 days.
2. Water stress flowering (WSF): 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 flowering stage.
3. Water stress grain filling (WSG): The irrigation regime was just like well watering, but the 6th and 7th irrigations were withheld, resulting in 24 days water stress during grain filling stage.

Agricultural practices

All other agricultural practices were followed according to the recommendations of ARC, Egypt. Nitrogen fertilization at the rate of 120 kg N/fed 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 30 kg P_2O_5/fed, was added as soil application before sowing during 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.

Soil analysis

Physical and chemical soil analyses of the field experiments were performed at laboratories of Soil and Water Research Institute of ARC, Egypt. Across the two seasons, soil type was clay loam: Silt (36.4%), clay (35.3%), fine sand (22.8%) and coarse sand (5.5%), pH (7.92), EC (1.66 dSm^{-1}), SP (62.5), $CaCO_3$(7.7 %), Soil bulk density (1.2 g cm^{-3}), HCO_3 (0.71 mEqu/l), Cl (13.37 mEqu/l), SO_4 (0.92 mEqu/l), Ca^{++} (4.7 mEqu/l), Mg^{++}(2.2 mEqu/l), Na^+ (8.0 mEqu/l), K^+ (0.1 mEqu/l), N, P, K, Zn, Mn and Fe (371, 0.4, 398, 4.34, 9.08 and 10.14 mg/kg, respectively).

Data recorded

1.Grain yield plant^{-1} (GYPP) (g):

It was estimated by dividing the grain yield plot^{-1} (adjusted at 15.5% grain moisture) on number of plants plot^{-1} at harvest.

At the end of each water stress treatment (80 and 100 days from emergence for WSF and WSG, respectively) and just after irrigation, three plant roots from each experimental plot were excavated by removing a soil cylinder of 40 cm diameter and a depth of 40 cm with plant base as the horizontal centre of the soil cylinder. Excavation was carried out using standard shovels. The excavated root crowns were shaken briefly to remove a large fraction of the soil adhering to the root crown. Most of the remaining soil was then removed by soaking the root crown in running water. In a third step remaining soil particles were removed from the root crown by vigorous rinsing at low pressure. The clean roots were measured or visually scored (Fig. 1) for the following traits:

2.Number of above-ground whorls occupied with brace roots (BW).
3.Number of brace roots (BN).
4.Angle of 1st arm of the brace roots originating from whorl 1 (BA) (score).
5.Branching density of brace roots (BB) (score).
6.Number of crown roots (CN) (score).
7.Crown roots angle (CA) (score).
8.Branching density of crown roots (CB) (score).

Traits BA, BB, CN, CA and CB were assigned values from one to nine according to Trachsel et al., (2011), where one indicates shallow root angles (10°), low root numbers and a low branching density and nine indicates steep root angles (90°), high numbers and a high branching density (Fig.1).

Table1. Designation, origin and grain color of maize genotypes under investigation.

Genotype No.	Designation	Origin	Genetic nature	Grain colour
1	Hi-Tec-2031	Hi-Tec, Egypt	Single cross	White
2	P-30K09	DuPont Pioneer, Egypt	Single cross	White
3	Fine-1005	Fine Seeds, Egypt	Single cross	White
4	Egaseed-77	Egaseed Co., Egypt	Single cross	White
5	SC-10	ARC, Egypt	Single cross	White
6	SC-128	ARC, Egypt	Single cross	White
7	Hi Tec- 2066	Hi-Tec, Egypt	Single cross	Yellow
8	P-3444	DuPont Pioneer, Egypt	Single cross	Yellow
9	SC-166	ARC, Egypt	Single cross	Yellow
10	P-32D99	DuPont Pioneer, Egypt	Single cross	Yellow
11	Hi Tec 1100	Hi-Tec, Egypt	Three-way cross	White
12	Watania 11	Watania Co., Egypt	Three-way cross	White
13	TWC-324	ARC, Egypt	Three-way cross	White
14	TWC-360	ARC, Egypt	Three-way cross	Yellow
15	TWC-352	ARC, Egypt	Three-way cross	Yellow
16	Giza Baladi	ARC, Egypt	Population	White
17	Population-45	ARC, Egypt	Population	Yellow
18	Nubaria	ARC, Egypt	Population	Yellow
19	Nebraska Midland	USA	Composite	Yellow
20	Midland Cunningham	Eldorado, Kansas, USA	Population	Yellow
21	Golden Republic	Beltsville, Kansas, USA	Population	Yellow
22	Sweepstakes 5303	USA	Population	Yellow

9.Crown root length (CRL).

The root length, measured as the distance between the last node to the end tip of the root.

10.Root circumference (RC).

RC was measured from maximum root system width.

11.Root (crown and brace) dry weight (RDW).

The measured root was first spread out in the sun for partial drying and then put in an oven for total drying at 40°C for 24 hours. After drying the roots were weighed using an electronic scale.

Drought tolerance index (DTI):

Drought tolerance index is the factor used to differentiate between the genotypes from tolerance point of view and it is calculated by the equation of Fageria (1992) as follows: DTI = (Y1/AY1) X (Y2/AY2), Where, Y1 = trait mean of a genotype at well watering. AY1 = average trait of all genotypes at well watering. Y2 = trait mean of a genotype at water stress. AY2 = average trait of all genotypes at water stress. When DTI is ≥ 1, it indicates that genotype is tolerant (T) to drought. If DTI is <1, it indicates that genotype is sensitive (S) to drought.

Biometrical analyses

Analysis of variance of the split-split plot design in RCB arrangement was performed on the basis of individual plot observation using the MIXED procedure of MSTAT ®. Combined analysis of variance across the two growing seasons was also performed if the homogeneity test was non-significant. Moreover, combined analysis for each environment separately across seasons was performed as randomized complete block design. Least significant difference (LSD) values were calculated to test the significance of differences between means according to Steel et al., (1997). Expected mean squares at separate and across seasons under each irrigation regime were estimated from ANOVA table according to Hallauer et al. (2010). Genotypic (σ^2_g), genotype x season (σ^2_{gs}), error (σ^2_e) and phenotypic (σ^2_{ph}) variances were computed as follows: $\sigma^2_g = (M_3 - M_2)/sr$, $\delta^2_{gs} = (M_2 - M_1)/r$, $\sigma^2_{ph} = \sigma^2_g + \sigma^2_{gs}/r + (\sigma^2_e/rs)$. Where r = number of replications, g = number of genotypes and s= number of seasons.

Heritability in the broad sense

Heritability in the broad sense (h^2_b %) for a trait in a separate environment was estimated according to Singh and Narayanan (2000) using the following formula: $h^2_b\ \% = 100 \times (\sigma^2_g / \delta^2_{ph})$ Where: σ^2_g = genetic variance, and δ^2_{ph}= phenotypic variance.

Expected genetic advance from selection

Expected genetic advance from selection for all studied traits as a percent of the mean was calculated according to Singh and Narayanan (2000) as follows: GA (%) = $(100\ K\ h^2_b\ \sigma_{ph})/\bar{x}$, Where: $\bar{x}$ = 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).

Figure. 1. Images of brace roots angle (BA), brace roots branching density (BB), crown roots number (CN), crown roots angle (CA) and crown roots branching (CB) displayed were scored with 1, 3, 5, 7 and 9.

Rank correlation coefficients

Spearman's rank correlation coefficients calculated among studied root and grain yieldtraits under studied environments. It was computed by using SPSS 17 computer software and the significance of the rank correlation coefficient was tested according to Steel et al., (1997).

RESULTS

Analysis of variance

Combined analysis of variance across seasons (S) of the split-split plot design (Table 2) indicated that mean squares due to seasons were significant ($P \leq 0.05$ or 0.01) for brace root whorls (BW), brace root angle (BA), crown root angle (CA), crown root branching (CB), and grain yield/plant (GYPP). Mean squares due to irrigation regime were significant ($P \leq 0.05$ or 0.01) for crown root number (CN), CB, root circumference (RC), root dry weight (RDW), and GYPP. Mean squares due to genotype were significant ($P \leq 0.01$) for all studied root and grain yield traits. Mean squares due to the 1st order interaction were significant ($P \leq 0.05$ or 0.01) for four traits (BN, RC and RDW) due to T×S, for six traits (BB, CN, CB, RDW and GYPP) due to G×S and one trait (GYPP) due to G×T. Mean squares due to the 2nd order interaction, *i.e.* G×S×T, were significant ($P \leq 0.01$) for three traits, namely BB and GYPP (Table 2). Combined analysis of variance of a randomized complete blocks design (RCBD) (data not presented) under four environments, *i.e.* well watering at flowering (WWF), well watering at grain filling (WWG), water stress at flowering (WSF) and water stress at grain filling (WSG) across two seasons indicated that mean squares due to genotypes under all environments were significant ($P \leq 0.05$ or 0.01) for 35 out of 46 studied cases (76.1%).

Drought tolerance index

Drought tolerance index (DTI) values of studied genotypes under the stressed environments WSF and WSG are presented in Table (3). According to our scale, when DTI is ≥1.0, it indicates that genotype is tolerant (T), if DTI is 1.0, it indicates that genotype is moderately tolerant (MT) and if DTI is <1.0, it indicates that genotype is sensitive (S). Based on DTI values, the 22 studied maize genotypes were grouped into three categories under water stress at flowering, namely tolerant (10 genotypes), moderately tolerant (two genotypes) and sensitive (10 genotypes) (Table 3). Under water stress conditions at grain filling, number of tolerant (T), and sensitive (S) genotypes were 11, and 11, respectively.

The highest DTI under both the two stressed environments (WSF and WSG) was exhibited by the genotype No. 8 (P-3444). The 2nd and 3rd highest genotypes in DTI were SC-128 and Egaseed-77 under WSF and SC-128 and SC-10 under WSG. For productivity (grain yield/plant) under WSF, the genotype Egaseed-77 ranked 1st , but P-3444 and SC-128 ranked 3rd. Under WSG, P-3444, SC-128 and SC-10 ranked 1st, 2nd and 3rd, for productivity as well as drought tolerance index. On the contrary, the most drought sensitive genotypes were the open-pollinated populations Sweepstakes 5303,Golden Republic and Nebraska Midland under both water stress environments (WSF and WSG); their grain yield was the lowest.

Superiority of drought tolerant (T) to sensitive (S) genotypes

Based on grain yield/plant and drought tolerance index (DTI) the best three genotypes were the single cross hybrids P-3444, SC-128 and Egaseed-77 under WSF and P-3444, SC-128 and SC-10 under WSG, while the most drought sensitive and lowest yielding genotypes were the populations Sweeps takes, Golden Republic and Nebraska Midland under both water stress environments (WSF and WSG). Data averaged for each of the two groups (T and S) under WSF and under WSG indicated that GYPP of drought tolerant (T) was greater than that of the sensitive (S) genotypes by 189.0 and 131.3 % under drought at flowering (WSF) and grain filling (WSG), respectively (Table 4).

Significant superiority of drought tolerant (T) over sensitive (S) genotypes in GYPP under drought at flowering and grain filling was associated with significant superiority in higher CN (76.7 and 45.2%), CB (42.6 and 84.4%), higher CRL (11.3 and 25.4 %), higher RC (25.4 and 23.6%) and higher RDW (86.7 and 126.3%), respectively.

Correlations between drought tolerance and root traits

Drought tolerance index had a strong significant ($p \leq 0.01$) and positive correlation with grain yield/plant (r= 0.912** and 0.941**) under WSF and WSG conditions, respectively (Table 5).

Table 2. Mean squares from combined analysis of variance of split-split plot design for studied root traits of 22 maize genotypes under four irrigation regimes (T) across 2016 and 2017 years.

Variance source	Mean squares					
	BW	BN	BA	BB	CN	CA
Season (S)	5.32*	487.8	33.5**	5.5	0.4	103.2**
Treatment (T)	2.78	2139.6**	3.2	12.9	32.5*	5.4
T x S	4.9*	615.6	3.3	15.1	4.3	10.4
Genotype (G)	2.91**	1014.5**	6.1**	16.6**	12.3**	9**
G x S	0.218	85.9	2.2	10.8**	4*	1.7
G x T	0.449	146.8	1.5	3.7	2.5	1.6
G x S x T	0.362	122.6	1.2	5.2*	2.3	1.1
	CB	CRL	RC	RDW	GYPP	
Season (S)	28.2**	243.5	107.5	94.5	26041.5*	
Treatment (T)	26**	115.7	618.1**	1336.5**	47158.4**	
T x S	3.8	201.9	232.9*	1278.1**	3864.3	
Genotype (G)	13.1**	59.4**	263.2**	955.5**	12428.3**	
G x S	4.7**	13.6	26.9	234.1**	3439.6**	
G x T	2.5	17.2	26.7	132.9	1335.8**	
G x S x T	1.8	23.1	32.2	142.4	1383.5**	

BW = Number of above-ground whorls occupied with brace roots, BN = Number of brace roots, BA = Brace root angle, BB = Branching density of brace roots, CN = Number of crown roots, CA = Crown roots angle, CB = Branching density of crown roots, CRL = Crown root length, RC = Root circumference, RDW = Roots dry weight, GYPP = Grain yield/plant, * and ** indicate significance at 0.05 and 0.01 probability levels, respectively.

Table 3. Drought tolerance index (DTI) of each genotype under WSF and WSG environments.

Genotype No.	Designation	WSF	WSG	Genotype No.	Designation	WSF	WSG
1	Hi-Tec-2031	1.3	1.6	**12**	Watania -11	1.2	1.2
2	P-30K09	1.0	1.2	**13**	TWC-324	1.7	1.7
3	Fine 1005	1.0	1.3	**14**	TWC-360	0.7	0.6
4	Egaseed-77	2.4	1.6	**15**	TWC-352	0.6	0.4
5	SC-10	1.8	1.8	**16**	Giza Baladi	0.4	0.5
6	SC-128	2.5	2.2	**17**	Population-45	0.4	0.5
7	Hi-Tec-2066	1.4	0.9	**18**	Nubaria	0.5	0.6
8	P-3444	3.0	3.4	**19**	Nebraska Midland	0.3	0.3
9	SC-166	1.4	1.4	**20**	Midland Cunningham	0.4	0.4
10	P-32D99	1.3	1.4	**21**	Golden Republic	0.3	0.3
11	Hi-Tec-1100	0.9	0.9	**22**	Sweepstakes 5303	0.1	0.2

Table 4. Superiority (Sup.%) of the three most tolerant (T) to the three most sensitive (S) genotypes for selected characters under the stressed environments WSF and WSG, combined across 2016 and 2017 seasons.

Trait	WSF			WSG		
	T	S	Sup. %	T	S	Sup. %
Grain yield/plant	147.3	51.0	189.0**	158.1	68.3	131.3**
Crown root number	4.2	2.4	76.7**	3.4	2.3	45.2*
Crown root branching	5.4	3.8	42.6*	4.6	2.5	84.4**
Crown root length	25.6	22.9	11.3*	23.3	18.6	25.4*
Root circumference	35.6	28.4	25.4**	32.6	26.4	23.6*
Root dry weight	20.1	10.7	86.7*	33.1	14.6	126.3**

*** and ** indicate significance at 0.05 and 0.01 probability levels, respectively.**

Table 5. Correlation coefficients between drought tolerance index (DTI) and means of studied traits of all genotypes under water stress at flowering (WSF) and at grain filling (WSG) across seasons.

Trait	WSF	WSG
Grain yield/plant	.912**	.941**
Number of brace root whorls	-.598**	-.288
Brace root Number	-.250	-.231
Brace root angle	-.183	-.193
Brace root Branching	.169	.506*
Crown root number	.469*	.320
Crown root angle	-.319	-.203
Crown root branching	.381	.489*
Crown root length	.693**	.561**
Root circumference	.440*	.499*
Root dry weight	.410*	.592**

*** and ** indicate significance at 0.05 and 0.01 probability levels, respectively.**

Drought tolerance had a significant and positive correlation coefficient, with crown root length (r = 0.693** and 0.561**), root circumference (0.440* and 0.499*) crown root dry weight (r = 0.410* and 0.592**) under WSF and WSG conditions, respectively. Moreover, drought tolerance index had a significant and negative correlation coefficient with brace root whorls; BW (-0.598**) and a significant and positive correlation coefficient with brace root branching; BB (0.506*) and crown root branching (0.489*) under WSG.

Correlations between grain yield and root traits

Estimates of rank correlation coefficients among grain yield/plant and all studied root traits across the two seasons under well watering, water stress at flowering (WSF) and grain filling (WSG) were calculated across all genotypes and presented in Table 6. Under well watering, grain yield/plant had a significant ($p \leq 0.01$) and positive association with the root dry weight (RDW) (0.42), root circumference (RC) (0.43), crown root length (0.26), crown root branching (CB) (0.27), number of crown roots (CN) (0.23) and brace root branching (BB) (0.34).

Data in Table (6) showed that under WSF, grain yield/plant was significantly ($P \leq 0.01$) and positively correlated with each of RC (0.33) and CN (0.27). Under water stress at grain filling (WSG), grain yield/plant had a significant and positive correlation ($p \leq 0.01$ or $p \leq 0.05$) with CRL (0.33), CB (0.25), RDW (r=0.23), BB (0.18) and RC (r=0.17).

Heritability

For root traits (Table 7), broad-sense heritability (h^2_b) ranged from 0.00 % for SDU, BB, CB and CRL under WWF and BB under WWG to 89.15% for CCI under WWG and 8349 % for SDL under WWF. In general, the estimates of h^2_b for root traits ranged from low to medium in magnitude. The lowest h^2_b estimates (< 40 %) were expressed by BN, BA, CN and CA under WWF, BA and CRL under WWG and BA, CN and CRL under WSF.

It is also obvious from the results of root traits that h^2_b estimates were generally the lowest under full irrigation. On the contrary, h^2_b estimates were generally the highest under water stress environments (RDW, CRL, CB, CN, CA, BA under WSG and BB, RC under WSF). However, under well watering (WWG) two traits showed the highest h^2_b estimates (BW and BN). High heritability (> 50 %) was exhibited by nine traits at WSG (all root traits except BB), five traits at WSF (BW, BN, CB, RC and RDW), four traits at WWG (BW, BN, CB and RC) and two traits at WWF (BW and DRW).

Genetic advance

The magnitude of expected genetic advance (GA) from direct selection for root traits (Table 7) was the lowest under well-watered environment for 8 root traits (BW, BN, BB, CN, CA, CB, CRL and RC under WWF and BB under WWG), but was the lowest under water stressed environments for two traits (BA and RDW) under WSF.

Table 6. Correlation coefficients between grain yield/plant and each of studied root traits of maize under well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) across two years.

Environment	BW	BN	BA	BB	CN	CA	CB	CRL	RC	RDW
WW	-0.2	-0.07	-0.09	0.34**	0.23**	-0.14	0.27**	0.26**	0.43**	0.42**
WSF	-0.07	0.01	-0.2	0.13	0.27**	-0.03	0.08	-0.03	0.33**	0.13
WSG	-0.14	-0.12	-0.02	0.18*	0.21**	-0.08	0.25**	0.33**	0.17*	0.23**

* and ** indicate significance at 0.05 and 0.01 probability levels, respectively. GYPP = grain yield per plant, BW= Number of above-ground whorls occupied with brace roots, BN= Number of brace roots, BA= Angle of 1st arm of the brace roots originating from whorl 1, BB= Branching density of brace roots, CN= Number of crown roots, CA= Crown roots angle, CB= Branching density of crown roots, CRL= Crown root length, RC= Root circumference, RDW= Roots dry weight

Table 7. Heritability in the broad sense (h^2_b) and genetic advance (GA) from selection for root traits of maize genotypes evaluated under well-watered at flowering (WWF), well water at grain filling (WWG), water stress at flowering (WSF) and water stress at grain filling (WSG) across two years.

Trait	WWF		WWG		WSF		WSG	
	h^2_b	GA%	h^2_b	GA%	h^2_b	GA%	h^2_b	GA%
Number of brace root whorls	50.0	12.8	68.7	27.3	59.0	13.4	66.6	22.2
Brace root Number	32.9	10.8	71.7	38.9	69.1	18.6	68.5	27.6
Brace root angle	32.0	6.4	30.7	6.3	38.5	4.7	62.5	12.3
Brace root Branching	0.0	0.0	0.0	0.0	47.1	17.3	41.5	14.6
Crown root number	36.7	17.7	41.8	18.5	38.6	22.0	65.4	33.7
Crown root angle	39.4	7.6	48.1	11.7	47.6	8.7	70.8	17.2
Crown root length	0.0	0.0	29.0	5.0	8.6	1.0	62.9	13.1
Root circumference	41.1	7.8	65.6	13.3	78.3	17.1	75.0	15.1
Root dry weight	59.2	32.4	40.5	29.9	53.5	25.2	62.9	35.1

The magnitude of GA from direct selection was the highest under water stressed environments (BA, CN, CA, CB, CRL and RDW under WSG and BB and RC under WSF). High GA estimate (> 12 %) was exhibited by all studied root traits under WSG, seven traits under WSF (all traits except BA, CA and CRL), seven traits under WWG (all traits except BA, BB and CRL) and four traits under WWF (BA, BB, CA, CB, CRL and RC).

DISCUSSION

In the present investigation, mean squares due to the studied 22 genotypes were significant (P ≤ 0.01) for all root traits and GYPP, indicating that genotype has an obvious effect on all such traits. The role of maize genotype in root traits is in accordance with those reported by Rauf et al., (2009) and Trachsel et al., (2011). Also, mean squares due to irrigation regime were significant for CN, CB, RC, RDW, and GYPP, indicating that drought stress has a significant effect on such root and grain yield traits. The effect of deficit irrigation on root architecture traits of maize was previously reported by several investigators (Lynch, 2007, Hund et al., 2011 and Tuberosa, 2014). The results of ANOVA also indicated that differences between the two seasons of study had a significant effect on some traits, namely BW, BA, CA, CB and GYPP. A root system architecture specifically adapted to the prevailing soil conditions might be advantageous (Lynch 1995). After the onset of drought, water is often found in deeper soil layers. Deeper soil layers are predominantly reached by maize genotypes forming a sparsely branched axile root system (Hund et al., 2009). In order to improve plant performance breeders need to select genotypes with a root architecture adapted to the conditions of the target environment. Significance of interaction mean squares for some root traits and GYPP indicated that for such traits, the rank of maize genotypes differ from irrigation regime to another, and from one year to another and the possibility of selection for improved root and grain yield under a specific water stressed environment (El-Ganayni et al., 2000; Al-Naggar et al*.,* 2009, 2011, 2016, 2017). Based on DTI estimates calculated by using the equation of Fageria (1992), the three genotypes P-3444, SC-128 and Egaseed-77 under WSF and P-3444, SC-128 and

SC-10 under WSG were found the most tolerant genotypes in descending order under the respective water stress environment. These genotypes should be recommended to maize breeding programs aiming at improving drought tolerance under corresponding drought stressed environments. The significant superiority of tolerant (T) to sensitive groups of genotypes was exhibited in GYPP, CN, CB, CRL, RC and RDW. Superiority was more pronounced for GYPP, CN and RC under WSF, but for CB, CRL and RDW under WSG. The perfect association between DTI and GYPP under both WSF and WSG indicates that grain yield was the best indicator of drought tolerance in this experiment. This result is in complete agreement with that reported by Al-Naggar et al. (2016). Rank correlation indicates that drought tolerant genotypes under both WSF and WSG conditions were characterized by high GYPP, deep crown root, large root circumference and heavy root dry weight. Moreover, drought tolerant genotypes are characterized by low number of whorls carrying brace roots and high number of crown roots under water stress at flowering (WSF) and characterized by high brace root branching and crown root branching under water stress at grain filling (WSG). These traits could be considered as selection criteria for drought tolerance in maize if they proved high heritability and high predicted genetic advance from selection. This conclusion is in accordance with other investigators (Bolaños and Edmeades, 1996; Banziger et al., 2002, El-Ganayni et al., 2000; Al-Naggar et al., 2000, 2011, 2016, 2017 for yield traits) and Rauf and Sadaqat, 2008, Rauf et al., (2009), Trachsel et al., 2011; Hund et al. ,2011,Lynch, 2013 Zhu et al., 2010; Saengwilai et al., 2014; Burton et al., 2014 Chimungu et al., 2015) for root traits. Root system performance has a major impact on the economics of commercial maize production, given its influence on yield under drought conditions (Bänziger et al., 2002). The importance of a deep and vigorous root system for maintaining yield under drought stress has been reported in maize (Hund et al., 2011). Drought tolerant genotypes generally increase the photosynthates allocation for root elongation under drought stress (Rauf and Sadaqat, 2008). Rauf et al., (2009) noted an increase in main root length of sunflower under stress conditions and a decrease in lateral root growth and total root biomass. In wheat, root mass in lower soil profile was noted to be positively correlated with yield (Lopes and Reynolds 2010). Results on rank correlation between GYPP and studied root traits indicated that selection for large values of CRL, CB, RDW, BB and RC traits under WSG would result in getting higher grain yield. Results also indicated the importance of two traits (RC and CN) as selection criteria for improving grain yield under water stress at flowering. Heavier root weight, larger root circumference, longer crown root length, branching and number and more brace root branching were associated with higher GYPP under WW. These root traits could be considered good selection criteria for high grain yield under WW, if they proved high heritability and high genetic advance from selection. The lowest h^2_b estimates expressed by BN, BA, CN and CA under WWF, BA and CRL under WWG and BA, CN and CRL under WSF, indicated that the genetic variance was the smallest component of phenotypic variances, and that environment was of great effect on the performance of these root traits. Low heritability estimates for these traits, could be attributed to the very small magnitude of genotypic variance as reported by Al-Naggar et al. (2008, 2012 and 2017). In general, the lowest h^2_b estimates were exhibited under full irrigation (WW), but the highest estimates were shown under water stress environments. (Blum, 1988, Laffitte and Edmeades (1994) and Al-Naggar et al., 2008, 2009, 2010, 2015, 2017). However, some researchers reported a decrease in heritability under stressed environments (Shabana et al., 1980, Atlin and Frey, 1990 and Banziger et al., 2002). These results indicated that predicted selection gain would be higher if selection was practiced under WSG for high values of BA, CN, CA, CB CRL and RDW under WSG and BB and RC under WSF and BW, BN, under WWG. It is worthy to mention that direct selection under the water-stressed environments would ensure the preservation of alleles of drought tolerance, while direct selection under full irrigation regime would take advantage of the high heritability (Banzinger et al., 2002; Al-Naggar et al., 2004&2012). Ageing of maize plant; expressed in change from WWF to WWG caused an obvious increase in the magnitude of heritability and genetic advance from selection in 8 out of 10 root traits, namely BW, BN, BA, CN, CA, CB CRL and RC. This suggests that selection for such traits would be more effective when practiced at later stages of plant growth than at earlier stages. Change from WSF to WSG caused an obvious increase in the magnitude of heritability and genetic advance from selection in 8 out of 10 root traits, namely BW, BN, BA, CN, CA, CB, CRL and RDW. These also ascertain that selection for such traits would be more effective when practiced at WSG than at WSF. Based on the correlation (r) analysis between studied traits and DTI and GYPP under drought at flowering (WSF) and grain filling (WSG) and their corresponding estimates of broad-sense heritability (h^2_b) and genetic advance from selection (GA), it is evident that, among studied root traits, the best secondary traits (selection criteria) for drought tolerance in our study are: CN, CB, CRL, RC and RDW under WSG, RC under WSF and CB and RC under WWG, since they showed high (r) values, high (h^2_b) estimates and high GA estimates under the respective environments.

CONCLUSION

Results suggested that selection for the root traits BW, BN, BA, CN, CA, CB CRL and RC would be more effective when practiced at later stages of plant growth than at earlier stages. Based on the correlation (r) analysis between studied traits and DTI and GYPP under drought at flowering (WSF) and grain filling (WSG) 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 (root selection criteria) for drought tolerance in our study are: CN, CB, CRL, RC and RDW under WSG, RC under WSF and CB and RC under WWG, since they showed high (r) values, high (h^2_b) estimates and high GA estimates under the respective environments. Results suggested that selection for root traits would be more effective when practiced at later stages (WSG) of plant growth than at earlier stages (WSF).

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CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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