# Putative mechanisms of drought tolerance in maize (Zea mays L.) via root system architecture traits

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#### ABSTRACT

Identifying maize genotypes with favorable root architecture traits for drought tolerance is prerequisite for initiating a successful breeding program for developing high yielding and drought tolerant varieties of maize. The objectives of the present investigation were: (i) to identify drought tolerant genotypes of maize at flowering and grain filling, (ii) to elucidate the relationships between the drought tolerance and root architecture traits and (iii) to identify the putative mechanisms of drought tolerance via root system traits. A two-year experiment was carried out using a split plot experiment with three replications. The main plots were devoted to three3 irrigation regimes, i.e. well watering (WW), water stress at flowering (WSF) and at grain filling (WSG), and sub plots to 22 maize cultivars and populations. Drought tolerance index (DTI) had strong and positive associations with crown root length (CRL), root circumference (RC) and root dry weight (DRW) under both WSF and WSG, a negative correlation with brace root whorls (BW), and positive correlations with crown root number (CN) under WSF and brace root branching (BB) and crown root branching (CB) under WSG. These root traits could be considered as putative mechanisms of drought tolerance. The cultivars Pioneer-3444, SC-128, Egaseed-77, SC-10 and TWC-324 showed the most drought tolerant and the highest yielding in a descending order; each had a number of such drought tolerance mechanisms. Further investigation should be conducted to determine the underlying root mechanisms contributing to the selection of water-efficient hybrids of maize.

Key words: Corn, Crown and Brace roots, Correlations, Drought tolerance index.

### INTRODUCTION

Maize (*Zea mays* L.) in Egypt is mainly used for poultry industry and animal feed. For acreage and production, it ranks second to wheat among cereal crops in Egypt. It is grown as a summer season crop and well irrigated by water coming from Nile River and its branches and canals. Current maize hybrids cultivated in Egypt are selected under well irrigation and therefore are subject to yield losses when grown under water deficit. The amount of water available for irrigation is reducing, especially at the ends of canals and due to expanding maize cultivation into the deserts, where sandy soils are of low water holding capacity. In order to stabilize maize production in Egypt, there is a need to develop drought tolerant maize hybrids.

Maize is very sensitive to water stress during the flowering and grain-filling periods (Bai *et al.* 2006) [1]. However, Witt *et al.* (2012) [2] reported that the most critical period for yield production goes approximately from 2 weeks before flowering time until 2 weeks after flowering time. Developing maize varieties that are tolerant to drought is, therefore considered

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critical for increasing the maize production. Several investigations have been undertaken across the years to improve drought tolerance in breeding programs. Edmeades *et al.* (1993) [3] reported that germplasm developed from drought tolerant source populations performed significantly better under drought stress compared to conventional populations.

Root system architecture traits are important for plant productivity under drought stress\* (Lynch1995) [4]. Plants avoid dehydration by increasing water uptake in the soil profile and adapt to the chemical and physical soil constraints, particularly under drought conditions, *via* the morphological plasticity of their root system (Lynch 2007) [5]. The importance of a deep and vigorous root system for maintaining yield under drought stress has been reported in maize by Hund *et al.* (2011) [6]. Rauf and Sadaqat (2008) [7] stated that "drought tolerant genotypes generally increase the photosynthates allocation for root elongation under drought stress". Rauf *et al.* (2009) [8] reported that genetic variation for root elongation has been shown in maize. The effects of root architecture and size on maize yield also depend on the distribution of soil moisture and the competition for water resources within the plant community (King *et al.* 2009) [9].

Trait interrelationships in particular determine the degree of association among traits and how they may increase selection efficiency. It is useful if indirect selection for root traits gives greater response to selection for grain yield trait than direct selection for the same trait. The main criterion for drought tolerance selection is the association of each root trait with grain yield under stress conditions [9, 10] (King *et al.* 2009 and Trachsel *et al.*, 2011).

To start a successful breeding program for improving drought tolerance, available maize germplasm should be screened for related traits to drought tolerance; e.g. root architecture traits under deficit irrigation to identify the best ones for further use in extracting the best parental inbred lines for developing drought tolerant hybrids. The objectives of the present investigation were to: (i) characterize 22 maize genotypes for root architecture traits and tolerance to deficit irrigation at flowering and grain filling stages in order to identify drought tolerant ones, (ii) elucidate the relationships between the drought tolerance and root traits and (iii) identify the putative mechanisms of drought tolerance *via* root system architecture.

## 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,

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Giza, Egypt (30° 02'N latitude and 31° 13'E longitude with an altitude of 22.50 meters above sea 68 69

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lines.

Plant materials

Twenty two maize (Zea mays L.) genotypes were used, namely 15 Egyptian cultivars (10 single 71 crosses and 5 three-way crosses) and 7 open-pollinated populations (Table 1). These materials 72 73 were kindly provided by Hi-Tec Company (Hi-Tec-2031, Hi Tec-2066, Hi Tec 1100), DuPont Pioneer Company (P-30K09, P-3444, P-32D99), Fine Seeds Company (Fine-1005), Egaseed 74 75 Company (Egaseed-77), Wataniya Company (Watania 11) and Agricultural Research Center-Egypt (the rest of genotypes). These genotypes were chosen to represent the available germplasm 76 in Egypt and some of them could be considered sources for extracting drought tolerant inbred 77

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**Table 1.**—Designation, origin and grain color of studied maize genotypes.

Genotype No.	Designation	Origin	Genetic nature	Grain colour
1	Hi-Tec-2031	Hi-Tec, Egypt	Single cross	White
2	P-30K09	DuPont Pioneer	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	Single cross	Yellow
9	SC-166	ARC, Egypt	Single cross	Yellow
10	P-32D99	DuPont Pioneer	Single cross	Yellow
11	Hi-Tec 1100	Hi-Tec, Egypt	3-way cross	White
12	Watania 11	Watania Co., Egypt	3-way cross	White
13	TWC-324	ARC, Egypt	3-way cross	White
14	TWC-360	ARC, Egypt	3-way cross	Yellow
15	TWC-352	ARC, Egypt	3-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

ARC = Agricultural Research Center, SC = Single cross, TWC = Three-way cross

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#### **Experimental procedures**

- 84 Sowing date was April 24<sup>th</sup> in the 1<sup>st</sup> season (2016) and April 30<sup>ht</sup> in the 2<sup>nd</sup> season (2017).
- 85 Sowing was done in rows; each row was 4 m long and 0.7 m width. Seeds were over sown in
- 86 hills 25 cm apart, thereafter (after 21 days from planting and before the first irrigation) were
- 87 thinned to one plant/hill to achieve a plant density of 24,000 plants/fed. Each experimental plot
- included two rows (plot size =  $5.6 \text{ m}^2$ ).

#### 89 Experimental design

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

#### 95 Water regimes

- 96 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 4<sup>th</sup>
- 99 and 5<sup>th</sup> irrigations were withheld, resulting in 24 days water stress just before and during
- 100 flowering stage.

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- 3. Water stress grain filling (WSG): The irrigation regime was just like well watering, but the
- 102 6<sup>th</sup> and 7<sup>th</sup> 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<sub>2</sub>O<sub>5</sub>) at the rate of
- 30 kg P<sub>2</sub>O<sub>5</sub>/fed, was added as soil application before sowing during preparation of the soil for
- 108 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
- 110 (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)
- 112 90% (manufactured by DuPont, USA) against corn borers.

#### Soil analysis 113 Physical and chemical soil analyses of the field experiments were performed at laboratories of 114 Soil and Water Research Institute of ARC, Egypt. Across the two seasons, soil type was clay 115 loam: Silt (36.4%), clay (35.3%), fine sand (22.8%) and coarse sand (5.5%), pH (7.92), EC (1.66 116 dSm<sup>-1</sup>), SP (62.5), CaCO<sub>3</sub>(7.7 %), Soil bulk density (1.2 g cm<sup>-3</sup>), HCO<sub>3</sub> (0.71 mEqu/l), Cl (13.37 117 mEqu/l), SO<sub>4</sub> (0.92mEqu/l), Ca<sup>++</sup> (4.7mEqu/l), Mg<sup>++</sup> (2.2mEqu/l), Na<sup>+</sup> (8.0mEqu/l), K<sup>+</sup> 118 (0.1mEqu/l), N, P, K, Zn, Mn and Fe (371, 0.4, 398, 4.34, 9.08 and 10.14 mg/kg, respectively). 119 Data recorded: 120 Formatted: Space After: 0 pt Grain yield plant (GYPP) (g): It was estimated by dividing the grain yield plot (adjusted at 15.5% grain Formatted: Indent: Left: -0.25" 121**1.** moisture) on number of plants plot<sup>-1</sup> at harvest. 122 Grain yield ha<sup>-1</sup> (GYPH) (ton): It was estimated by adjusting grain yield plot<sup>-1</sup> at 15.5% grain 123**2.** moisture to grain yield ha<sup>-1</sup> (ton). 124 **Root traits:** 125 At the end of each water stress treatment (80 and 100 days from emergence for WSF and 126 127 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 128 the horizontal centre of the soil cylinder. Excavation was carried out using standard shovels. The 129 excavated root crowns were shaken briefly to remove a large fraction of the soil adhering to the 130 root crown. Most of the remaining soil was then removed by soaking the root crown in running 131 132 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 133 following traits: 134 Number of above-ground whorls occupied with brace roots (BW). Formatted: Indent: Left: -0.25" 135**3** Number of brace roots (BN). 1364 1375 Angle of 1st arm of the brace roots originating from whorl 1 (BA) (score). 1386 Branching density of brace roots (BB) (score). 139**7** Number of crown roots (CN) (score).

Traits from No. 5 to No. 9 were assigned values from one to nine according to Trachsel et

al. (2011) [10], where one indicates shallow root angles (10°), low root numbers and a

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Crown roots angle (CA) (score).

Branching density of crown roots (CB) (score).

low branching density and nine indicates steep root angles (90°), high numbers and a 144 high branching density (Fig.1). 145 14610. Crown root length (CRL) (cm). The root length, measured as the distance between the last node to the end tip of the root. 147 . Root circumference (RC) (cm). RC was measured from maximum root system width. 148**1** Root (crown and brace) dry weight (RDW) (g). The measured root was first spread out in the 149**1** sun for partial drying and then put in an oven for total drying at 40°C for 24 hours. After drying 150 the roots were weighed using an electronic scale. 151 **Drought tolerance index (DTI):** 152 Formatted: Right: 0" Drought tolerance index is the factor used to differentiate between the genotypes from tolerance 153 point of view and it is calculated by the equation of Fageria (1992) [11] as follows: 154 DTI = (Y1/AY1) X (Y2/AY2)155 Formatted: Indent: Left: 0", Right: 0" Where, Y1 = trait mean of a genotype at well watering. AY1 = average trait of all genotypes at 156 well watering. Y2 = trait mean of a genotype at water stress. AY2 = average trait of all 157 genotypes at water stress. When DTI is  $\geq 1$ , it indicates that genotype is tolerant (T) to drought. 158 If DTI is <1, it indicates that genotype is sensitive (S) to drought. 159 160Biometrical analyses Formatted: Indent: Left: -0.25" Analysis of variance of the split-split plot design in RCB arrangement was performed on the Formatted: Space After: 0 pt 161 basis of individual plot observation using the MIXED procedure of MSTAT ®. Combined 162 163 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 164 seasons was performed as randomized complete block design. Least significant difference (LSD) 165 values were calculated to test the significance of differences between means according to Steel et 166

al. (1997) [12].

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Fig-ure 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

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Simple correlation coefficients were calculated between pairs of studied traits under wellwatering (WW), water stress (WS), severe water stress (SWS) and combined across all irrigation treatments according to Singh and Narayanan (2000) [13]. Spearman's rank correlation coefficients calculated among studied root traits and other studied traits under studied environments. It was

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computed by using SPSS 17 computer software and the significance of the rank correlation coefficient was tested according to Steel et al. (1997) [12].

## RESULTS AND DISCUSSION

#### 3.1. 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 ( $P \le 0.05$  or  $P \le 0.01$ ) for six out of studied 12 traits, namely brace root whorls (BW), brace root angle (BA), crown root angle (CA), crown root branching (CB), grain yield/plant and grain yield/ha. Mean squares due to irrigation regime were significant ( $P \le 0.05$  or  $P \le 0.01$ ) for six out of studied 12 traits, namely crown root number (CN), CB, root circumference (RC) and root dry weight (RDW), GYPP and GYPH. Mean squares due to genotype were significant ( $P \le 0.01$ ) for all studied root and grain yield traits.

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Table -2: Mean squares from combined analysis of variance across 2016 and 2017 years for studied roottraits of 22 maize genotypes under four irrigation regimes.

Variance Source			Mean Squa	ares		4//
[	BW	BN	BA	BB	CN	CA 🛂
Season (S)	5.32*	487.8	33.5**	5.5	0.4	103.2**//
Irrigation regime(I)	2.78	2139.6**	3.2	12.9	32.5*	5.4 🛂
IxS	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** 🎷 /
GxS	0.218	85.9	2.2	10.8**	4*	1.7 🛂
GxI	0.449	146.8	1.5	3.7	2.5	1.6 📲
GxSxI	0.362	122.6	1.2	5.2*	2.3	1.1 🕶
	СВ	CRL	RC	RDW	GYPP	GYPH_/
Season (S)	28.2**	243.5	107.5	94.5	26041.5*	124.7**
Irrigation regime(I)	26**	115.7	618.1**	1336.5**	47158.4**	2041.1**
IxS	3.8	201.9	232.9*	1278.1**	3864.3	225.5**
Genotype (G)	13.1**	59.4**	263.2**	955.5**	12428.3**	707.3**
GxS	4.7**	13.6	26.9	234.1**	3439.6**	46.4**
GxI	2.5	17.2	26.7	132.9	1335.8**	34.8**
GxSxI	1.8	23.1	32.2	142.4	1383.5**	19.6**

193 BW= Number of above-ground whorls occupied with brace roots, BN= Number of brace roots, BA= Brace roots angle, BB= Branching density of brace roots, CN= Number of crown roots, CA=Crown roots angle, CB=Branching

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density of crown roots, CRL= Crown root length, RC=Root circumference, RDW= Roots dry weight, GYPP= Grain yield/plant, GYPH= grain yield/ha, \* and \*\* indicate significance at 0.05 and 0.01 probability levels, respectively.

Mean squares due to the 1<sup>st</sup> order interaction were significant ( $P \le 0.05$  or 0.01) for four traits (BN, RC, RDW and GYPH) due to I×S, for six traits (BB, CN, CB, RDW, GYPP and GYPH) due to G×S and two traits (GYPP and GYPH) due to G× I. Mean squares due to the 2<sup>nd</sup> order interaction, *i.e.* G×S× I, were significant ( $P \le 0.01$ )—for three traits, namely BB, GYPP and GYPH (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 ( $P \le 0.05$  or 0.01) for 35 out of 46 studied cases (76.1%).

Root system architecture is important for plant productivity under drought stress-conditions [4] (Lynch, 1995). In order to improve plant performance, breeders need to select genotypes with a root architecture adapted to the conditions of the target environment. Results of the present study indicated that climatic conditions had a significant effect on BW, BA, CA, CB, GYPP and GYPH and that irrigation regime had a significant effect on CN, CB, RC, RDW, GYPP and GYPH. Moreover, genotype had an obvious effect on all studied traits. The role of maize genotype is in accordance with the finding of Trachsel et al. [10] (2011) for maize root traits and Al-Naggar et al. (2016a) [14, 15] for grain yield. Mean squares due to the the 1st and 2nd order interaction were significant for some root and yield traits, indicating 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 as proposed by Al-Naggar et al. (2009, 2011, 2016 b, 2017 a,b) [16-20]. Combined analysis of variance of RCBD under each of the four environments indicated the significance of differences among studied genotypes for the majority of studied root traits and grain yield under each irrigation regime.

#### 3.3. The effect of genotype

Average, minimum and maximum values of all studied traits of 22 genotypes across all irrigation treatments combined across two seasons are presented in Table (3).

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No. 8) to 11.2 g-(genotype No. 20).

Parameter			Tra	aits		
	BW (No.)	BN (No.)	BA (score)	BB (score)	CN (score)	CA (score)
Average	2.5	37.1	6.7	4.9	3.2	6.7
Min	1.9 (8)	25.6 (21)	5.5 (1)	3.4 (18)	1.9 (21)	5.6 (7)
Max	3.0 (10,11,17)	49.0(10)	7.7(19)	6.2(9)	4.5(6)	8.1(10)
LSD <sub>.05</sub>	0.36	6.8	0.74	1.09	0.86	0.76
	CB (score)	CRL (cm)	RC (cm)	RDW (g)	GYPP (g)	GYPH (ton)
Average	4.2	22.8	32.7	22.3	107.3	7.18
Min	3.0 (21)	20.4 (18)	25.9 (21)	11.2 (20)	62.5(22)	2.69(22)
Max	6.5 (8)	26.1 (5)	38.1 (8)	36.8(8)	158.5(6)	13.03(8)
LSD <sub>.05</sub>	0.91	2.57	2.85	6.05	9.72	0.39

Means of minimum and maximum are followed by genotype No. (Between brackets). BW= Number of aboveground 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, GYPH= grain yield/ha.

Genotypes varied for grain yield/fed from 13.03 ton (genotype No. 8) to 2.69 ton (genotype No. 22), grain yield/plant from 158.5 g (genotype No. 6) to 62.5 g (genotype No. 22), number of above-ground whorls occupied with brace roots from 3.0 from (genotype No. 17) to 1.9—(genotype No. 8), number of brace roots from 49.0—(genotype No. 10) to 25.6—(genotype No. 21), angle of 1<sup>st</sup> arm of the brace roots originating from whorl 1 from 7.7 (genotype No. 19) to 5.5—(genotype No. 1), branching density of brace roots from 6.2—(genotype No. 9) to 3.4 (genotype No. 18), number of crown roots from 4.5—(genotype No. 6) to 1.9—(genotype No. 21), crown roots angle from 8.1—(genotype No. 10) to 5.6—(genotype No. 7),—branching density of crown roots from 6.5—(genotype No. 8) to 3.0—(genotype No. 21), crown root length from 26.1 cm—(genotype No. 5) to 20.4 cm—(genotype No. 18), root circumference from 38.1 cm (genotype No. 7) to 25.9 cm—(genotype No. 21) and roots dry weight from 36.8 g—(genotype

The genotype No. 8 (Pioneer-3444) exhibited the highest mean values for four traits [GYPH, root circumference (RC), crown root branching (CB) and roots dry weight (RDW)] and second highest for GYPP, brace root branching (BB), number of crown roots (CN), crown root length (CRL), *i.e.* most important yield and root traits. The genotype No. 6 (SC-128) developed by ARC-Egypt was the highest in GYPP and number of crown roots and second highest in crown root branching. The genotype No. 4 (Egaseed 77) developed by Fine Seed Co. showed the third highest in grain yield and the highest in brace root angle (BA). The genotype No. 5 (SC-10) developed by ARC-Egypt showed the highest means for one trait (crown root length; CRL); it gave the fourth highest grain yield per plant and per hectare.

On the contrary, the genotype No. 22 (Pop. Sweepstakes 5303) exhibited the lowest means for two traits, namely GYPP, GYPH. The genotype No. 21 (Pop. Golden Republic) exhibited the lowest means for two traits, namely BN and CN. The genotype No. 18 (Pop. Nubaria) showed the lowest means for two traits (BB and CRL).

Means of the 22 maize genotypes showed wide ranges of performance (difference between minimum and maximum values) for all studied root and yield traits across all irrigation treatments. Three commercial varieties showing the highest grain yield showed also the highest means for a number of root traits. The superiority of these three commercial varieties in six root traits (RC, CB, RDW, BB, CN and CRL) for Pioneer-3444, two traits (CN and CB) for SC-128, one trait (BA) for Egaseed 77 and one trait (CRL) for SC-10 might be the reason of their superiority in grain yield, because good roots may help the plants to uptake more water and nutrients from the soil for their biological activities, especially under drought conditions [4, 21, 22] (Wright and Nageswara, 1994; Lynch, 1995; Henry et al., 2011).

In general, the commercial varieties P-3444, SC-128, Egaseed-77 and SC-10 were the best genotypes in our experiment; they showed the highest grain yield and the best root architectural traits across all studied irrigation treatments; they could be recommended for farmers use under a range of different environments as well as for maize breeding programs. On the contrary, it is observed that most of root and yield traits with undesirable mean values were exhibited by populations and the *vice versa* for traits with desirable means, which were mostly shown by the single crosses.

#### **Genotype** × water stress interaction

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Min

Aver. Min Max LSD.05 Aver.

Max

Aver.

Min

Max

**Parameter** 

LSD.05

LSD.05 Aver.

Min

WWF

2.52

2(2)

3.1(17)

0.7

6.7

5(1)

8.3 (19)

1.62

3.82

1.7 (18)

6 (12)

2.2

4.6

3(2)

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3.7 2.2 (19)

For root traits (Table 4), data were measured under WWF, WWG, WSF and WSG.

Under WWF, WWG, WSF and WSG, for BW the lowest mean was exhibited by genotypes No.

2, 13, 17 and 21 and the highest mean was shown by genotypes No. 17, 19, 4 and 10, for BN the

lowest mean by genotypes No. 21, 12, 4 and 21 and the highest mean by genotypes No. 11, 11,

10-and 10, for BA the lowest by genotypes No. 1, 9, 14 and 1 and the highest mean was shown

by genotypes No. 19, 21, 21 and 19, for BB the lowest by genotypes No. 18, 18, 13 and 20 and

the highest mean was shown by genotypes No. 5, 15, 6 and 9, for CN the lowest by genotypes

No. 18, 19, 13 and 13 and the highest mean was shown by genotypes No. 12, 8, 6 and 3, for CA the lowest by genotypes No. 2, 5, 7 and 1 and the highest mean was shown by genotypes No. 10,

10, 21 and 10, for CB the lowest by genotypes No. 21, 17, 19 and 19 and the highest by

genotypes No. 8, 8, 6 and 8, for CRL the lowest by genotypes No. 14, 18, 22 and 22 and the

highest mean by genotypes No. 8, 5, 9 and 4, for RC the lowest by genotypes No. 18, 19, 19 and 21 and the highest by genotypes No. 7, 8, 7 and 8 and for RDW the lowest by genotypes No. 20,

Table 4. Average, minimum (Min) and maximum (Max) values under each irrigation treatment

WSG

2.64

1.5 (21)

3.3(10)

0.81

6.5

4.7(1)

7.5 (19)

1.25

3.05

1.8 (13)

5(3)

1.47

WWF

39

27.3 (21)

47(11)

16.58

5.3

3.3 (18)

7 (5)

2.38

6.8

5.7(2)

8 (10)

1.6

22.4

WWG

37.1

22.7 (12)

54.7(11)

14.5

4.7

2 (18)

7 (15)

2.66

6.5

5.3 (5)

8 (10)

1.92

Brace Root No.

**Brace Root Branching (Score)** 

Crown Root Angle (Score)

WSF

31.5

23 (4)

43.3(10)

7.3

4.9

3 (13)

6.8 (6)

1.66

6.9

5 (7)

8 (21)

1.2

WSG

40.8

25.2 (21)

59(10)

14.76

4.7

2.3 (20)

6.2 (9)

2.02

6.5

5.2(1)

18, 19 and 21 and the highest by genotypes No. 8, 8, 5 and 8, respectively.

WSF

2.29

1.8 (17)

2.9 (4)

0.57

6.9

5.8 (14)

7.5 (21)

1.02

3.38

1.8 (13)

5.3 (6)

1.3

4.6

3.2 (19)

for all studied root traits and grain yield across two seasons.

**Brace Root Whorls No.** 

**Brace Root Angle (Score)** 

Crown Root Number (Score)

Crown Root Branching (Score)

WWG

2.48

1.66 (13)

3.33(19)

0.81

6.7

5 (9)

7.3 (21)

1.88

2.66

1(19)

4(8)

1.8

4.1

2(17)

18.6 (14) 18.8 (18)

21.2 (22)

23.2 23.9

Crown Root Length (cm)

21.76

16.9 (22)

8.5 (10) 1.25

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Max	6 (8)	7.3 (8)	6.3 (6)	6.5 (8)	25.9 (8)	28.1(5)	26.2 (9)	26 (4)
LSD <sub>-05</sub>	1.95	2.35	1.49	1.54	6.67	5.1	4.1	4.4
		Root Circur	nference (cm)			Root Dry	Weight (g)	
Aver.	34.7	30.7	34.4	30.9	26.2	21	18.8	23.3
Min	28.1(18)	23.3 (19)	26.5(19)	23.3(21)	8.2 (20)	8.2 (18)	9.8 (19)	9.9 (21)
Max	40.4(7)	41(8)	42.5(7)	36.6(8)	40.7 (8)	44.9 (8)	33.6 (5)	40.1(8)
LSD <sub>-05</sub>	6.48	6.5	4.97	4.95	14.36	12.96	9.53	11.53
	Gra	in Yield/Pla	nt (g)		,	Gra	in Yield/ha(t	on)
	ww	WSF	WSG			ww	WSF	WSG
Aver.	128.2	91.4	102.2			9.03	5.8	6.72
Min.	82.9 (19)	31.8 (22)	58.9 (15)			3.91 (22)	1.39 (22)	2.77 (22)
Max.	168.1(1,5)	156.4(6,4)	179.7(8,6,4)			15.25(8,5,6)	10.55(4,8.6)	13.45(8,6)
LSD.05	23	13.3	12.7			0.75	0.63	0.71

Maks of minimum and maximum are followed by genotype No. (Between brackets).

294 295

296 297

298

299

For grain yield (Tables 5 and 6), data were measured under WW, WSF and WSG.—The lowest mean GYPP was shown by genotypes No. 19, 22 and 15 and the highest by genotypes No. 1, 6 and 8 under WW, WSF and WSG, respectively. For GYPH, the lowest mean was exhibited by Genotypes No. 22, 22 and 22 and the highest mean was shown by Genotypes No. 8, 4 and 8 under WW, WSF and WSG, respectively.

300 301

302

303

**Table 5.** Means of grain yield/plant and grain yield/ha for each genotype under each irrigation regime-(well watering; WW, water stress at flowering; WSF and water stress at grain filling; WSG) across 2016 and 2017 seasons.

Genotype	ww	WSF	Ch%	WSG	Ch%	ww	WSF	Ch%	WSG	Ch%
		Grai	in yield/pl	ant			G	rain yield	/ha	
1	168.1	78.0	53.6	102.7	38.9	9.95	4.40	55.8	6.30	36.7
2	131.7	73.3	44.3	92.0	30.1	8.51	3.79	55.5	5.51	35.2
3	124.0	75.6	39.1	109.0	12.2	7.98	4.29	46.3	6.29	21.2
4	151.6	147.9	2.5	132.5	12.6	9.56	8.35	12.7	6.36	33.5
5	166.3	123.2	25.9	126.0	24.2	10.22	5.96	41.7	6.65	34.9
6	150.4	156.4	-4.0	168.7	-12.2	10.05	8.14	19.1	8.38	16.6
7	128.5	131.2	-2.1	106.8	16.9	7.34	6.41	12.6	4.76	35.2
8	150.4	137.6	8.5	179.7	-19.5	12.11	8.21	32.2	10.67	11.9
9	134.4	105.6	21.4	121.0	9.9	8.12	5.64	30.6	6.69	17.7
10	134.3	98.9	26.4	117.7	12.3	8.32	5.31	36.2	6.43	22.8
11	125.5	78.5	37.4	84.7	32.5	7.61	4.02	47.2	4.50	40.9
12	119.4	91.0	23.8	111.5	6.6	7.79	5.12	34.2	6.09	21.8
13	149.4	111.1	25.6	120.7	19.2	9.28	5.96	35.8	7.16	22.8

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14	133.6	89.7	32.9	81.9	38.7	5.65	4.15	26.5	3.86	31.7	Formatted: Tab stops: Not at 3.25" + 6.5"
15	125.4	84.7	32.5	58.9	53.1	4.96	3.79	23.6	3.05	38.5	Formatted: Tab stops: Not at 3.25" + 6.5"
16	118.6	56.2	52.6	81.9	30.9	4.30	2.84	33.9	4.12	4.1	Formatted: Tab stops: Not at 3.25" + 6.5"
17	110.9	65.0	41.4	70.8	36.2	4.86	2.80	42.4	3.62	25.6	Formatted: Tab stops: Not at 3.25" + 6.5"
18	110.5	74.2	32.9	85.8	22.4	5.37	3.22	40.1	4.54	15.4 🕶 -	Formatted: Tab stops: Not at 3.25" + 6.5"
19	82.9	59.4	28.4	75.8	8.5	3.83	2.33	39.1	3.38	11.9 🕶 -	Formatted: Tab stops: Not at 3.25" + 6.5"
20	106.6	79.7	25.2	91.4	14.3	4.64	3.00	35.4	3.63	21.9 🕶 –	Formatted: Tab stops: Not at 3.25" + 6.5"
21	100.8	61.8	38.7	70.4	30.2	3.79	2.60	31.5	3.04	19.8 🕶 –	Formatted: Tab stops: Not at 3.25" + 6.5"
22	96.9	31.8	67.2	58.9	39.3	3.10	1.11	64.2	2.19	29.4	Formatted: Tab stops: Not at 3.25" + 6.5"
Average	128.2	91.4	28.7	102.2	20.3	7.15	4.61	35.5	5.33	25.5	Formatted: Tab stops: Not at 3.25" + 6.5"
Min.	82.9	31.8		58.9		3.10	1.11		2.19	4	Formatted: Tab stops: Not at 3.25" + 6.5"
Max.	168.1	156.4		179.7		12.11	8.35		10.6		
LSD <sub>-05</sub>	23	13.3		12.7		0.6	0.5		0.6		
LSD <sub>-01</sub>	30.5	17.6		16.8		0.8	0.7		0.8		

Ch% = 100(WW-WSF or WSG)/WW

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On the contrary, the worst genotypes were No. 22 (Sweepstakes) in 3 traits (GYPP, GYPH, CRL) under WSG, 3 traits (GYPP, GYPH, CRL) under WSF and one trait (GYPH) under WW, the genotype No. 21 (Golden Republic) in 4 traits (BW, BN, RC, RDW) under WSG, two traits (BN,CB) under WWF, the genotype No. 19 (Nebraska) in one trait (CB) under WSG, and 3 traits (CB, RC, RDW) under WWG and the genotype No. 18 (Nubaria) in two traits (CN, RC) under WWG and one trait (GYPP) under WW.

The four highest and the four lowest performing genotypes under water stress at flowering (WSF) and grain filling (WSG) across seasons are presented in Table (6). Under WSF conditions, the highest mean grain yield/ha was achieved by the single cross Egaseed-77 (developed by Egaseed Co.), followed by P-3444 (developed by Pioneer Co.), SC 128 (developed by ARC, Egypt) and HT-2066 (developed by Hi Tec Co.) in a descending order. The single cross Egaseed-77 was amongst the four highest genotypes under WSF for GYPH, GYPP, BA and CRL. The single cross P-3444 was amongst the four highest genotypes under WSF for GYPH, GYPP, CN, CB and CRL. The single cross SC-128 was amongst the four highest genotypes under WSF for GYPH, GYPP, BB, CN, CB, RC, and RDW. The single cross HT-2066 was amongst the four highest genotypes under WSF for GYPH, GYPP, CN and RC.

**Table 6.** The four highest and the four lowest genotypes for studied traits under water stress at flowering (WSF) and grain filling (WSG) across seasons.

WSF WSG WSF WSG WSF WSG

Brace root whorls No.

Brace root No.

Brace root angle (score)

Highest

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SC-128 P-3444 Midland Eg-77 HT-2066 Giza							
SC-128	Pop-45	32D99	32D99	32D99	Nebraska	Nebraska	
TWC-360	HT-1100	HT-1100	TWC-352	TWC-352	Golden	SC-10	
Fine 1005   Eg-77   Fine 1005   P-3444   SC-128   TWC-352	32D99	TWC-360	Pop-45	HT-1100	Fine 1005	Golden	
Fine 1005   Eg-77   Fine 1005   P-3444   SC-128   TWC-352   SC-128   P-3444   Midland   Eg-77   HT-2066   Giza   Giza   F-3444   Golden   Eg-77   Golden   Golden   Golden   Golden   Golden   TWC-360   HT-2031   TWC-352   SC-128   P-3444   HT-2066   SC-128   Fine 1005   Golden   SC-166   SC-128   Fine 1005   Golden   SC-166   SC-128   P-3444   HT-2031   32D99   Nebraska   SC-166   P-3444   HT-2066   SC-128   Midland   Midland   Midland   S2D99   SC-10   TWC-352   HT-1100   TWC-324   Golden   TWC-352   HT-100   TWC-324   Golden   TWC-352   HT-100   TWC-324   Golden   TWC-324   Golden   TWC-324   TWC-324   HT-2031   HT-2031   HT-2031   TWC-324   HT-2031   HT-2036   HT-	TWC-360	Pop-45	HT-1100	TWC-360	Eg-77	Sweep	
SC-128		•	Lov	vest	-	•	
Fig-77   30K09   Golden   30K09   SC-166   TWC-324	Fine 1005	Eg-77	Fine 1005	P-3444	SC-128	TWC-352	
P-3444         Golden         Eg-77         Golden Urbanching (score)         TWC-360         HT-2031           Brace root branching (score)         Crown root number (score)         Crown root angle (score)           Highest           SC-128         SC-128         Fine 1005         Golden         32D99           TWC-352         SC-128         P-3444         HT-2031         32D99         Nebraska Golden           SC-166         P-3444         HT-2066         SC-128         Midland         Midland           James Born         SC-10         TWC-352         HT-1100         TWC-324         Golden           Golden         Nubaria         Eg-77         SC-166         TWC-360         P-3444           Giza         Wat-11         Sweep         Midland         P-3444         HT-1100           Nebraska         Golden         TWC-324         TWC-324         HT-2031         HT-2066           Crown root branching (score)         Crown root length (cm)         Root circumference (cm)         Root circumference (cm)           Highest         FB-3444         FB-3444         FB-3444         FB-2066         FB-3444         FB-2066         FB-3444         FB-2066         FB-3444         FB-2066	SC-128	P-3444	Midland	Eg-77	HT-2066	Giza	
SC-128   SC-106   SC-128   P-3444   HT-2031   HT-2031   HT-2031   HT-2031   HT-2034   Highest   SC-128   P-3444   HT-2031   HT-2066   P-3444   HT-1100   SC-166   P-3444   TWC-352   30K09   TWC-352   HT-2066   SC-10   HT-1100   TWC-352   TWC-352   SC-166   SC-128   Eg-77   SC-10   HT-1100   TWC-352   TWC-352   SC-128   HT-2031   HT-2066   Golden   Midland   Midland   Midland   Giza   Sc-128   P-3444   SC-128   P-3444   Eg-77   SC-128   TWC-352   TWC-	Eg-77	30K09	Golden	30K09	SC-166	TWC-324	
Highest   SC-128   SC-128   Fine 1005   Golden   32D99   TWC-352   SC-128   P-3444   HT-2031   32D99   Nebraska   SC-166   P-3444   HT-2066   SC-128   Midland   Midland   Midland   32D99   SC-10   TWC-352   HT-1100   TWC-324   Golden   Lowest   SC-166   TWC-360   P-3444   HT-2031   Golden   TWC-324   TWC-324   HT-2031   HT-2031   HT-2031   TWC-324   Midland   Golden   Golden   Golden   Golden   Golden   Golden   HT-2066   HT-2031   HT-2066   HT-2066   HT-2066   HT-2066   HT-2066   HT-2031   HT-2066   HT-2066   HT-2031   HT-2031   HT-2066   HT-2066   HT-2031   HT-2066   HT-2066   HT-2031   HT-2066   HT-2066	P-3444	Golden	Eg-77	Golden	TWC-360	HT-2031	
SC-128   SC-166   SC-128   Fine 1005   Golden   32D99     TWC-352   SC-128   P-3444   HT-2031   32D99   Nebraska     SC-166   P-3444   HT-2066   SC-128   Midland   Midland     32D99   SC-10   TWC-352   HT-1100   TWC-324   Golden	Brace root be	ranching (score)	Crown root n	umber (score)	Crown root	angle (score)	
TWC-352         SC-128         P-3444         HT-2066         SC-128         Midland         Midland           32D99         SC-10         TWC-352         HT-1100         TWC-324         Golden           Lowest           Lowest           Golden         Nubaria         Eg-77         SC-166         TWC-360         P-3444           Giza         Wat- 11         Sweep         Midland         P-3444         HT-1100           Nebraska         Golden         TWC-324         TWC-324         HT-2031         HT-2031           TWC-324         Midland         Golden         Golden         HT-2066         HT-2066           Crown root length (cm)         Root circumference (cm)           HT-2066         F-3444         F-3444         F-3444         TWC-352         TWC-352         30K09           TWC-352         HT-2066         SC-10         HT-1100         TWC-352         TWC-352 <td></td> <td></td> <td>Hig</td> <td>hest</td> <td></td> <td></td> <td></td>			Hig	hest			
SC-166							
SC-10							
Colden		P-3444					
Golden	32D99	SC-10	TWC-352	HT-1100	TWC-324	Golden	
Giza         Wat-11         Sweep         Midland         P-3444         HT-1100           Nebraska         Golden         TWC-324         TWC-324         HT-2031         HT-2031           TWC-324         Midland         Golden         Golden         HT-2066         HT-2066           Crown root branching (score)         Crown root length (cm)         Root circumference (cm)           Highest           SC-128         P-3444         Fg-77         HT-2066         P-3444           P-3444         HT-1100         SC-166         P-3444         TWC-352         30K09           TWC-352         HT-2066         SC-10         HT-1100         TWC-352         TWC-352           SC-166         SC-128         Eg-77         SC-10         SC-128         HT-2031           Lowest           Fine 1005         Golden         Pop-45         Nubaria         Nubaria         Nubaria         Nebraska           Eg-77         32D99         HT-2066         Golden         Midland         Midland         Midland         Midland         Midland         Midland         Midland         Mebraska         Golden         Golden         Nebraska         TWC-324         Midland         Rep-77         <							
Nebraska   Golden   TWC-324   TWC-324   HT-2031   HT-2031			C				
TWC-324         Midland         Golden         Golden         HT-2066         HT-2066           Crown root branching (score)         Crown root length (cm)         Root circumference (cm)           SC-128         P-3444         P-3444         Eg-77         HT-2066         P-3444           P-3444         HT-1100         SC-166         P-3444         TWC-352         30K09           TWC-352         HT-2066         SC-10         HT-1100         TWC-352         TWC-352           SC-166         SC-128         Eg-77         SC-10         SC-128         HT-2031           Lowest           Fine 1005         Golden         Pop-45         Nubaria         Nubaria         Nebraska           Eg-77         32D99         HT-2066         Golden         Midland         Midland         Midland         Midland         Midland         Midland         Midland         Midland         Midland         Mebraska         Golden         Nebraska         Golden         Grain yield/ha         Fig-77         P-3444         Eg-77         P-3444         Fig-77         P-3444         Fig-77         P-3444         Fig-77         P-3444         Fig-77         P-3444         Fig-77         P-3444         Fig-77         P-3444							
Crown root branching (score)							
Highest   SC-128   P-3444   P-3444   Eg-77   HT-2066   P-3444   P-3444   HT-1100   SC-166   P-3444   TWC-352   30K09   TWC-352   HT-2066   SC-10   HT-1100   TWC-352   TWC-352   SC-166   SC-128   Eg-77   SC-10   SC-128   HT-2031   Lowest   SC-128   HT-2031   Lowest   SC-128   SC-1							
SC-128	Crown root b	ranching (score)			Root circum	ference (cm)	
P-3444							
TWC-352         HT-2066         SC-10         HT-1100         TWC-352         TWC-352           SC-166         SC-128         Eg-77         SC-10         SC-128         HT-2031           Lowest           Fine 1005         Golden         Pop-45         Nubaria         Nubaria         Nebraska           Eg-77         32D99         HT-2066         Golden         Midland         Midland         Midland         Midland         Midland         Midland         Midland         Midland         Mebraska         Golden         Nubaria         Nebraska         Golden         Nubaria         Nubaria         Golden         TWC-352         Nebraska         Golden							
SC-166         SC-128         Eg-77         SC-10         SC-128         HT-2031           Lowest         Lowest         Lowest         Fine 1005         Golden         Pop-45         Nubaria         Nubaria         Nebraska           Eg-77         32D99         HT-2066         Golden         Midland         Midland         Midland         Midland         Midland         Midland         Nebraska         Golden         Nubaria           Nebraska         Nebraska         Sweep         Sweep         Nebraska         Golden         Golden         Nebraska         Golden         Midland         F-3444         Eg-77         P-3444         Fg-77         P-3444         SC-128         P-3444         SC-128         P-3444         SC-128         P-3444         SC-128         P-3444         SC-128         TWC-324         TWC-352         TWC-324         TWC-352         TWC-324         TWC-352         TWC-352         Nebraska         Golden         Fop-45         Nebraska         Golden         TWC-352         Nebraska         Golden         TWC-352         Nebraska         Golden         TWC-352         Nebraska         Golden							
Lowest							
Fine 1005         Golden         Pop-45         Nubaria         Nubaria         Nebraska           Eg-77         32D99         HT-2066         Golden         Midland         Midland           TWC-324         TWC-324         Midland         Giza         Golden         Nubaria           Nebraska         Sweep         Sweep         Nebraska         Golden           Root dry weight (g)         Grain yield/plant (g)         Grain yield/plant           Highest           SC-10         P-3444         SC-128         P-3444         Eg-77         P-3444           Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden	SC-166	SC-128	•		SC-128	HT-2031	
Eg-77         32D99         HT-2066         Golden         Midland         Midland           TWC-324         TWC-324         Midland         Giza         Golden         Nubaria           Nebraska         Nebraska         Sweep         Sweep         Nebraska         Golden           Root dry weight (g)         Grain yield/plant (g)         Grain yield/ha           Highest           SC-10         P-3444         SC-128         P-3444         Eg-77         P-3444           Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden							
TWC-324         TWC-324         Midland         Giza         Golden         Nubaria           Nebraska         Nebraska         Sweep         Sweep         Nebraska         Golden           Root dry weight (g)         Grain yield/plant (g)         Grain yield/ha         Grain yield/ha           Highest           SC-10         P-3444         SC-128         P-3444         Eg-77         P-3444           Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest         Lowest         Lowest         Webraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden							
Nebraska         Nebraska         Sweep         Sweep         Nebraska         Golden           Root dry weight (g)         Grain yield/plant (g)         Grain yield/ha           Highest           SC-10         P-3444         SC-128         P-3444         Eg-77         P-3444           Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden							
Root dry weight (g)         Grain yield/plant (g)         Grain yield/ha           SC-10         P-3444         SC-128         P-3444         Eg-77         P-3444           Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden				400			
SC-10							
SC-10         P-3444         SC-128         P-3444         Eg-77         P-3444           Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden	Root dry	y weight (g)		1 60	Grain y	/ield/ha	
Fine 1005         HT-1100         Eg-77         SC-128         P-3444         SC-128           SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden	00.10	D 2444			F 77	D 2444	
SC-128         SC-128         P-3444         Eg-77         SC-128         TWC-324           TWC-352         HT-2031         HT-2066         SC-10         HT-2066         SC-166           Lowest           Midland         Nebraska         Golden         Pop-45         Pop-45         Nebraska           TWC-324         Midland         Nebraska         Golden         Golden         TWC-352           Golden         Nubaria         Giza         TWC-352         Nebraska         Golden							
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Under WSG conditions, the highest mean grain yield/ha was achieved by the single-cross P-3444 (developed by Pioneer) followed by SC-128 (developed by ARC), TWC-324 (developed by ARC) and SC-166 (developed by ARC) in a descending order. The single cross P-3444 was amongst the four highest genotypes in GYPH, GYPP, BB, CB, CRL, RC and RDW, i.e. most important grain yield and root architecture traits. The single cross SC-128 was amongst the four highest genotypes in GYPH, GYPP, BB, CN, CB and RDW (the most important grain yield and root architecture traits). The single cross SC-166 was amongst the four highest genotypes in GYPH and BB.

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Results from Tables (4 and 5) concluded that the best genotypes were No. 8 (P-3444) in 5 traits (GYPP, GYPH, CB, RC, RDW) under WSG, 4 traits (CN, CB, RC, RDW) under WWG, 3 traits (CA, CRL, RDW) under WWF and one trait (GYPH) under WW, the genotype No. 6 (SC 128) in 4 traits (GYPP, BB, CA, CB) under WSF, the genotype No.5 (SC 10) in two traits (BB and CRL) under WWF and WWG, respectively, the genotype No. 7 (Hi-Tec 2066) in one trait (RC) under WSF and RC under WWF, the genotype No. 4 (Egaseed 77) in one trait (GYPH) under WSF, and the genotype No. 2 (30K09) in one trait (GYPH) under WSF.

The best genotypes in grain yield under drought at either flowering or grain filling were-characterized by one or more desirable root architecture traits. Accumulating genes of more desirable root characteristics in one genotype might help plants to search water and nutrients in the soil and consequently help plant to accomplish its biological activities and achieve almost its potential grain yield under drought stress at flowering or grain filling stages [4, 10, 21-24] (Wright and Nageswara, 1994; Lynch,1995; Hund et al.,2009 b;Hund,2010; Henry et al., 2011;Trachsel et al. (2011). The studied single-cross hybrids P-3444, Egaseed-77 and SC-128 were considered drought tolerant genotypes under drought stress at flowering and/or grain filling stages and would be offered to future breeding programs to utilize their genes of desirable root architecture and grain yield traits in improving maize drought tolerance under Egyptian conditions. It should be mentioned that the hybrid P-3444 was characterized in this experiment by its ability to stay green even under water stress, which might help it to tolerate water stress at grain filling stage in a way much better than other tested hybrids and populations.

#### 3323Drought tolerance index

Drought tolerance index (DTI) values of studied genotypes under the stressed-environments WSF and WSG are presented in Table (7). According to our scale, when DTI is  $\geq 1.0$ , 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 7). Under water stress conditions at grain filling, number of tolerant (T), and sensitive (S) genotypes were 11, and 11, respectively.

Table 7. Drought tolerance index (DTI) of each genotype under WSF and WSG environments.

Genotype No. Designation WSF WSG Genotype No. Designation WSF WSG WSG

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1	Hi-Tec-2031	1.3	1.6	12	Watania -11	1.2	1.2	4
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	•

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The highest DTI under both the two stressed environments (WSF and WSG) was exhibited by the genotype No. 8 (P-3444). The 2<sup>nd</sup> and 3<sup>rd</sup> 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 1<sup>st</sup>, but P-3444 and SC-128 ranked 3<sup>rd</sup>. Under WSG, P-3444, SC-128 and SC-10 ranked 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>, 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 were the lowest.

### 3.3. 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 Sweepstakes, 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 189.0and 131.3 % under drought at flowering (WSF) and grain filling (WSG), respectively (Table 8).

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**Table 8.** Superiority (Sup.%) of the three most tolerant (T) to the three most sensitive (S) genotypes for selected traits under the stressed environments WSF and WSG, combined across 2016 and 2017 seasons.

Trait WSF WSG

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	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.

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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.

#### 3.4. Correlations between drought tolerance and root traits

Drought tolerance index had a strong significant ( $p \le 0.01$ ) and positive correlation withgrain yield/plant (r = 0.912\*\* and 0.941\*\*) under WSF and WSG conditions, respectively (Table 9). 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.

**Table 9.** 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	Trait	WSF	wsg 🎺
Grain yield/plant	.912**	.941**	Crown root angle	319	203
Brace root whorls number	598**	288	Crown root branching	.381	.489°
Brace root Number	250	231	Crown root length	.693**	.561**
Brace root angle	183	193	Root circumference	.440*	.499° 🔩
Brace root Branching	.169	.506*	Root dry weight	.410*	.592**
Crown root number	.469*	.320			<b>*</b> ,

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

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#### 3.5. Correlations between grain yield and root traits

Estimates of rank correlation coefficients among grain yield/plant and all studied roottraits 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 (10). Under well Formatted: Space After: 0 pt

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watering, grain yield/plant had a significant ( $p \le 0.01$ ) 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 (10) showed that under WSF, grain yield/plant was significantly ( $P \le 0.01$ ) and positively correlated with each of RC (r=0.33) and CN (r=0.27). Under water stress at grain filling (WSG), grain yield/plant had a significant and positive correlation (p $\le$ 0.01 or p $\le$ 0.05) with CRL (r=0.33), CB (r=0.25), RDW (r=0.23), BB (r=0.18) and RC (r=0.17).

**Table 10.** Correlation coefficients between grain yield/plant and each of studied root traits of maize underwell watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) across two years.

)						700	700 700		
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.

#### **Grouping genotypes**

#### Based on drought tolerance and grain yield

Mean grain yield/fed of studied genotypes under water stress at flowering (WSF) and grain filling (WSG), was plotted against drought tolerance index of the same genotypes under WSF and WSG; respectively (Fig. 2), which made it possible to distinguish between four groups, namely tolerant and high- yielding, tolerant and low-yielding, sensitive and high-yielding and sensitive and low-yielding according to Sattelmacher *et al.*, 1994 [25], Worku *et al.* (2007) [26] and Al-Naggar *et al.* (2015) [27].

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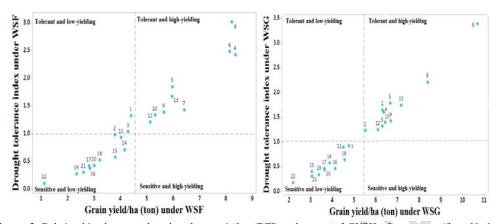
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No.22) under water stress at flowering (WSF) and grain filling (WSG) combined across seasons. Broken lines represent mean grain yield/fed and DTI.

Under water stress at flowering (WSF), the genotypes No 8 followed by No. 4, 6, 5, 7, 13, 9, 10 and 12 were classified as the drought tolerant and high yielding genotypes, *i.e.* they could be considered as the most water stress tolerant and the most responsive genotypes to water stress at flowering in this study (Fig. 2). There was no genotype belonging to the group of sensitive and high yielding genotypes under WSF. The genotypes No. 1 and 3 occupied the group of tolerant and low yielding under WSF. The genotypes No 22, 19, 21, 16, 17, 20, 18, 15, 14, 11 and 2 were classified as water stress sensitive and low yielding and therefore could be considered sensitive and low yielding.

Under water stress at grain filling (WSG), the genotypes No. 8 followed by 6, 13, 5, 1, 4, 9, 10, 3, 12 and 2 were classified as drought tolerant and high yielding, they could be considered as the most water stress tolerant and the most responsive genotypes to water stress at grain filling in this study (Fig. 3). On the contrary, genotypes No. 22, 21, 15, 19, 20, 17, 16, 14, 18, 11 and 7 were classified as water stress sensitive and low yielding (Fig. 2).

According to Fageria and Baligar (1994 and 1997a and b) [28-30] genotypes belonging to the 1<sup>st</sup> group "tolerant and high yielding" (above all) and 2<sup>nd</sup> group "tolerant and low yielding" (to a lesser extent) (we did not have) appear to be the most desirable materials for breeding programs that deal with adaptation to water stress. It was observed that the genotypes No. 8, 6, 4, 13, 5, 9, 10 and 12 occupied the first group (E-R) under both WSF and WSG conditions; they

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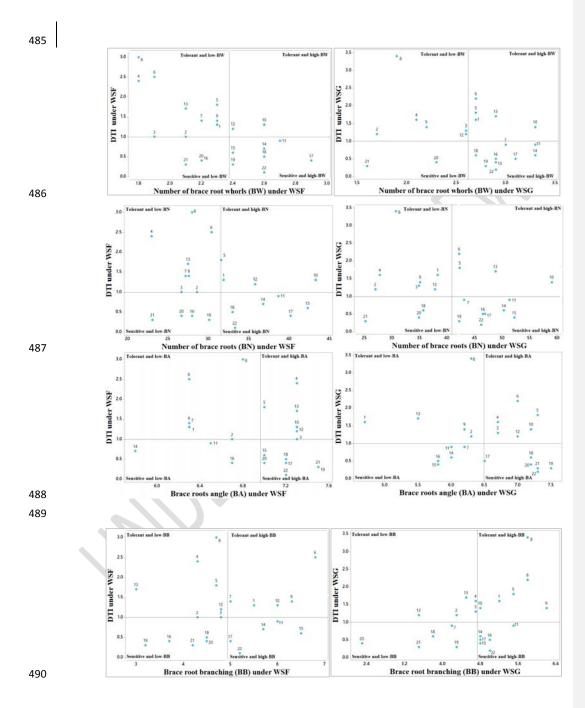
had genes of high water efficiency; i.e. drought tolerance to both WSF and WSG stages and genes for high yield under well watering conditions.

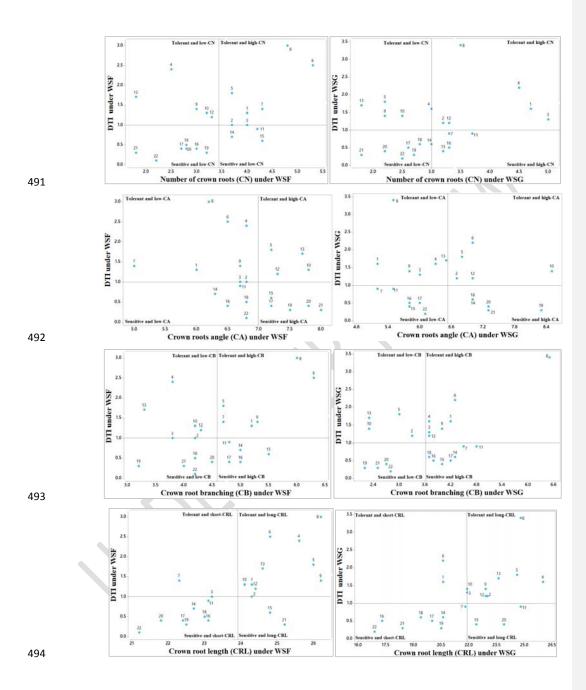
Summarizing the above-mentioned classifications, it is apparent that the genotypes No. 8 (P-3444) followed by 6 (SC-128), 4 (Egaseed-77), 5 (SC-10),13 (TWC-324), 7 (Hi Tec-2066), 9 (SC-166), 10 (P-32D99) and 12 (Watania 11) were the best genotypes that occupied the first group (best one) in both classifications; they are the most efficient, most drought tolerant, the highest yielder under WSF—as well as WW. The genotypes No. 8 (P-3444) followed by 6 (SC-128), 13 (TWC-324), 5 (SC-10),1 (Hi Tec-2031),4 (Egaseed-77), 9 (SC-166),10 (P-32D99), 3 (Fine 1005), 12 (Watania 11) and 2 (P-30K09) were the best genotypes that occupied the first group (best one) in both classifications; they are the most efficient, most drought tolerant, the highest yielder under WSG—as well as WW.

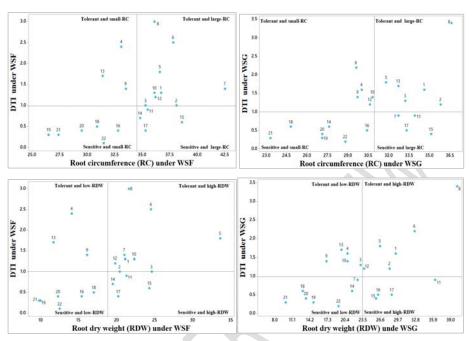
It was observed that the genotypes No 8 (P-3444) followed by 6 (SC-128), 4 (Egaseed-77), 5 (SC-10), 13 (TWC-324), 7 (Hi Tec-2066), 9 (SC-166), 10 (P-32D99) and 12 (Watania 11) were the best in the first group for both stresses WSF and WSG; they are the most efficient, most drought tolerant and the highest yielders under WSF and WSG as well as WW. In accordance to these results, a previous study by Al-Naggar *et al.* (2011) [17], proved that the single cross hybrid SC-128 (genotype No. 6 in the present study) was the most water efficient (drought tolerant) under WSF and the most responsive to WW based on grain yield, ears/plant, kernels/plant, ASI and leaf senescence.

#### Based on drought tolerance and root traits

 Means of root traits of studied genotypes under water stress at flowering (WSF) and grain filling (WSG), were plotted against drought tolerance index (DTI) of the same genotypes under WSF and WSG; respectively (Fig. 3), which made it possible to distinguish between four groups, namely tolerant and high value of root trait, tolerant and low value of root trait, sensitive and high value of root trait and sensitive and low value of root trait.—According to Fageria and Baligar [29] (1997a), genotypes belonging to the 1<sup>st</sup> group "tolerant and high value of root trait" (above all) appear to be the most desirable materials for breeding programs.







**Fig-ure 3.** Relationships between drought tolerance index (DTI) and means of number of whorls carrying brace roots, brace root branching, crown root number, crown root branching, root circumference, crown root length, and root dry weight, of genotypes (from No. 1 to No.22) under water stress at flowering (WSF) and grain filling (WSG) combined across seasons. Broken lines represent mean DTI and root trait.

Figure (3) indicates that the 1<sup>st</sup> group "tolerant and high value of root trait" included the genotypes No. 10 and 12 under WSF, No. 10, 13, 6, 5 and 1 under WSG for number of whorls carrying brace roots, No. 10, 12, 1 and 5 under WSF, No. 10, 13, 1, 5 and 6 under WSG for number of brace roots, No. 4, 13, 10, 12, 3 and 5 under WSF, No. 5, 6, 10, 12, 4 and 3 under WSG for brace root angle, No. 6, 9, 10, 1 and 7 under WSF, No. 9, 6, 5, 1 and 10 under WSG for brace root branching, No. 6, 8, 7, 1, 5, 3 and 2—under WSF, No. 3, 1, 6, 8, 12 and 2 under WSG for number of crown roots, No. 10, 13, 12 and 5—under WSF, No. 10, 6, 12, 5 and 2 under WSG for crown root angle, No. 6, 8, 9, 1, 7 and 5 under WSF, No. 8, 6, 1, 9, 4, 3 and 12 under WSG for crown root branching, No. 8, 4, 5, 6, 9, 12, 1, 10 and 2—under WSF, No. 8, 4, 5, 13, 9, 2, 3, 10 and 12 under WSG for crown root length, No. 7, 6, 8, 5, 1, 10, 12, 2 and 3—under WSF, No. 8, 2, 1, 3, 13 and 5 under WSG for root circumference and No. 5, 6, 8, 10, 7, 1, 12, 3 and 2 under WSF, No. 8, 6, 1, 5, 2 and 12 under WSG for root dry weight.

Mechanisms of drought tolerance of the most tolerant and high-yielding genotypes:

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515	The above-mentioned results (Figs. 2 and 3) helped us to identify the root traits that
516	characterize the most drought tolerant and high-yielding genotypes, in descending order, as
517	follows:
518 <b>1.</b>	Genotype No. 8 (SC-P-3444): Five traits (high CN, CB, large RC, long CRL and heavy RDW) Formatted: Indent: Left: -0.25"
519	under both WSF and WSG.
520 <b>2.</b>	Genotype No. 6 (SC-128): Four traits (high CN, CB, BB, large RC and heavy RDW)under both
521	WSF and WSG.
522 <b>3.</b>	Genotype No. 4 (SC-Egaseed-77): Two traits (steep brace root; i.e. large BA and long CRL)
523	under both WSF and WSG.
524 <b>4.</b>	Genotype No. 5 (SC-10): Six traits (high CN, CB, BA,RC, long CRL and heavy RDW) under
525	WSF and five traits (high BA, CA, large RC, long CRL and heavy RDW) under WSG.
526 <b>5.</b>	Genotype No. 13 (TWC-324): Two traits (steep brace root; i.e. large BAand long crown root
527	(CRL) under WSF and two traits (large RC and long CRL) under WSG.
528 <b>6.</b>	Genotype No. 9 (SC-166): Two traits (high CB and long crown root CRL) under both WSF and
529	WSG.
530 <b>7.</b>	Genotype No. 10 (SC-P-32D99): Four traits (steep crown root; CA steep brace root; BA, long
531	crown root; CRL and heavy root dry weight; RDW) under both WSF and WSG and one trait
532	(heavy RDW) under WSF.
533 <b>8.</b>	Genotype No. 12 (Watania TWC-11): Seven traits (BW, BN, BA, CA, CRL, RC and RDW)
534	under WSF and six traits (BA, CN, CA, CB, CRL and RDW) under WSG.
535	The present study suggested that further investigation should be conducted to determine the
536	underlying root mechanisms contributing to the selection of water-efficient hybrids of maize.
537	In a recent study [31] (Shao et al., 2019), maize genotypes with less variation in root
538	size, medium root size, medium broad root system and more inter-row root distribution help to
539	reduce root-to-root competition and tend to have higher yield at high planting density.
540	CONCLUSIONS
541	Correlation analysis of the present study concluded that drought tolerance in maize had a* Formatted: Space Before: 0 pt, After: 0 pt
542	strong and positive association with crown root length, root circumference and root dry weight
543	under both WSF and WSG, a negative correlation with brace root whorls, and a positive
544	correlation with crown root number under WSF and brace root branching and crown root

branching-under WSG. These root traits could be considered as putative mechanisms of drought

- tolerance. The present study suggested that further investigation should be conducted to 546 determine the underlying plant mechanisms contributing to the selection of water-efficient 547 hybrids of maize. The cultivars Pioneer-3444, SC-128, Egaseed-77, SC-10 and TWC-324 548 showed the most drought tolerance and the highest yielding in a descending order; each had a 549
- number of such drought tolerance mechanisms. These cultivars should be retested for drought 550
- tolerance and grain productivity under drought stress and could be offered to plant breeding 551
- programs for improving tolerance to drought and high grain yield. 552

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