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Tolerance of 254 Maize Doubled Haploid Lines × Tester Crosses to Drought at Flowering and Grain Filling

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMMAN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AMAA and AMAG managed the literature searches. Author EHMH managed the experimental process and performed the statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

The doubled haploid lines (DHL) of maize in their top crosses are expected to include genotypes that accumulated favorable genes for both high-yielding and drought tolerance. The objectives of this study were: (i) to screen 254 DHL's x tester crosses for tolerance to water stress at flowering (WSF) and grain filling (WSG), (ii) to estimate the superiority of tolerant (T) over sensitive (S) crosses under WSF and WSG and (iii) to identify the selection criteria for drought tolerance in maize. Two hundred fifty four top crosses developed from crossing between 254 DHL's and an inbred tester along with two checks were evaluated under three watering treatments; *i.e.* well watering (WW), WSF and WSG using split plot design with two replicates. Based on drought tolerance index (DTI), maize genotypes (254 top crosses and 2 check cultivars) were grouped into three categories, namely tolerant (T), moderately tolerant (MT) and sensitive (S). The number of T, MT and S genotypes were 120, 108 and 28 under WSF and 123, 105 and 28 under WSG,

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respectively. The crosses classified amongst the drought tolerant and high yielding were No. 16, 14, 204, 96, 58, 140, 161, 2, 66 and 44 under WSF and No. 87, 96, 66, 208, 26, 153, 205, 177, 15 and 39 under WSG. Grain yield/ha (GYPH) of T was greater than that of S test crosses by 78.78 and 82.52% under WSF and WSG, respectively. DTI showed a significant (p≤0.01) and positive correlation coefficient (r_g) with grain yield/plant (0.88 and 0.86), GYPH (0.82 and 0.80) and ears/plant (0.55 and 0.44) under WSF and WSG conditions, respectively. Moreover, DTI had small, but significant and negative correlation coefficients with days to anthesis, days to silking, anthesis silking interval, barren stalks and leaf rolling.

Keywords: Zea mays; drought tolerance index; rank correlation; top crosses.

1. INTRODUCTION

Drought tolerance is defined as the mechanism(s) causing minimum loss of yield in a drought environment relative to the maximum yield in constraint-free *i.e.* optimal environment for the crop [1]. Some authors prefer the term ‘dehydration’ to ‘drought’ and consequently refer to ‘dehydration tolerance’ [2,3]. Drought tolerance can also be described with reference to resource economics. Drought and drought tolerance were used in terms of yield in relation to a limited supply of water. Plants with better growth under limited water supply were considered to be drought-tolerant [2,4].

Maize (*Zea mays* L.) is considered more susceptible than most other cereals to drought stresses at flowering, when yield losses can be severe through barrenness or reductions in kernels per ear [5]. Susceptibility of maize yield to stresses at flowering has been documented in early Corn Belt Germplasm [6,7]. Their studies showed that the sensitive period extended from around one week before to two weeks after 50% silking. Studies of more recent hybrids suggest that this period of susceptibility may have moved towards early grain filling [8,9].

Breeding for tolerance to drought is difficult because the genetic mechanism that controls the expression of such tolerance in crop plants is poorly understood and because of the polygenic nature of such a complicated character [10]. Selection for increased drought tolerance is associated with a significant reduction in anthesis-silking interval (ASI) and barrenness, and an increase in ears plant-1, stay green and harvest index [8,9,11-16].

Maize breeders are always looking for new methods to enrich breeding material of better tolerance to drought stress. Use of modern biotechnological techniques in plant breeding could contribute to a great extent in the induction of novel genetic variation, such as somaclonal and gametoclonal variation which do not exist in the gene pool [17,18]. The in vivo (inducer) technique helps in developing doubled haploids, in a short time from maize crosses that show new genetic variation amenable for efficient selection for drought tolerant genotypes [19]. Recently, doubled haploid (DH) lines are routinely applied in many commercial hybrid maize breeding programs. Major advantages of DH lines compared to selfed lines include: (i) maximum genetic variance among lines per se and testcross performance from the first generation; (ii) reduced breeding cycle length; (iii) perfect fulfillment of DUS (distinctness, uniformity, stability) criteria for variety protection; (iv) reduced expenses for selfing and maintenance breeding; (v) simplified logistics, and (vi) increased efficiency in marker-assisted selection, gene introgression, and stacking genes in lines [20]. To our knowledge, all present commercial DH-line breeding programs are based on in vivo induction of maternal haploids [21-23].

Two hundred and fifty four (254) maize doubled haploid lines (DHL) developed by DuPontPioneer via the in vivo (inducer) technique from crosses between drought tolerant inbreds and good general combiners were obtained from Research Department of the Pioneer Hi-Bred Inc. Two hundred and fifty four test-cross hybrids were developed as a result of crossing between the 254 DHL's and the inbred line tester PHDMF. These DHL's x tester crosses are expected to include genotype(s) that accumulated favorable genes for both high-yielding and drought tolerance. The objectives of this study were: (i) to screen a number of 254 DHL's x tester crosses for tolerance to drought at flowering and grain filling in order to identify the most drought tolerant crosses, (ii) to estimate the superiority of tolerant (T) over sensitive (S) genotypes under drought at flowering and grain filling and (iii) to identify the selection criteria for drought tolerance in maize.

2. MATERIALS AND METHODS

This study was carried out in the summer seasons of the years 2011 and 2012 in DuPontPioneer Research Station at Sandanhur, Benha, Qaliubiya, Egypt. The station is located at30°25' 8" N, 31°11' 24" E and Altitude is 74 m above sea level.

2.1 Plant Materials

Seeds of 254 maize doubled haploid lines (DHL's) resulted via the inducer technique and embryo rescue used by DuPontPioneer from the crosses between the drought tolerant inbreds (PHM6T – PHJFN – PH1723) and the good general combiners (PH12J4 – PH1CGY – PHM7E) obtained from Research Department of the Pioneer Hi-Bred Inc. Seeds of 254 test cross hybrids were produced as a result of crossing between the 254 double haploid lines and the inbred line tester PHDMF and these exhibit drought tolerance performance and high general combining ability. Two hybrids; one single cross hybrid (PHN11) and one three-way cross hybrid (PHR77) with high yield potential and drought tolerance performance (Table 1) were used as controls in the evaluation experiment.

2.2 Methods

2.2.1 First season (Crossing blocks)

On the 1st of April 2011, the 254 DH lines and the tester parent PHDMF were planted at DuPontPioneer Research Station, Sandanhur, Benha, Qaliubiya, in a crossing block to produce the top crosses (single cross hybrids). The DH lines (females) were planted in 4 meters long rows and 4 ranges each range about 63 to 64 rows, while the tester inbred line PHDMF (male) was planted in one range of 65 rows which is equivalent to (1 : 4) (Tester : DH lines).

During the flowering stage, the female shoots were covered before the emergence of the silks in 10 plants for each DH inbred line to control the hybridization process and eliminate contamination with pollen grains. In the same stage, the male tassels of the tested inbred PHDMF were covered one day before artificial pollination to make sure that the pollen captured in the bags is the required pollen. The result of this year was seeds of 254 single cross hybrids (top crosses) that were used in the second year of this study.

2.2.2 Second season (Evaluation experiment)

On the 1st of May, the experimental field was disc ploughed with tractors to get a fairly fine soil. The maize seeds were planted with a planter. During the tillage process, superphosphate 15.5% at the rate of 30 kg P_2O_5/fed (fed=feddan=4200 m^2) as well as 25 kg K_2SO_4/fed of potassium sulfate 48% were added to the soil. After the tillage was done, laser leveling was performed to the location. During the seedbed preparation, the seeds of the 254 hybrids and the two check cultivars were packed in small easy tear bags each of 45 kernels; also the planting arrangements were prepared to get ready for the planting process. On the 15th of May the seeds were planted by 4 rows Vacuum Plot planter SRES®; this type of planter is equipped with a device to bury the irrigation tubes (T-Tapes) under the soil. The large number of top crosses (254) that has been obtained in the first season plus two check cultivars with a total of (256) genotypes were sown in the field in two replicates; each experimental plot included two rows of 0.7 meter width and 4.0 meter long with a 1.0 meter long ally between ranges.

Table 1. Pedigree and drought tolerance for all the genotypes used in the current study

Genotype	Pedigree	Drought tolerance
Doubled haploid lines (DHL) from DHL1 to DHL254	Doubled haploid lines resulting from crossing between the drought tolerant inbreds (PHM6T – PHJFN – PH1723) and the good general combiners (PH12J4 – PH1CGY – PHM7E)	Unknown
PHDMF	Inbred line tester	Tolerant
Top crosses	254 top crosses resulted from crossing between the tester PHDMF and the DH lines (DHL1 to DHL254)	Unknown
Check cultivars:		
PH-30N11	Yellow single cross hybrid	Tolerant
PH-30R77	Yellow three-way cross hybrid	Tolerant

Source: All genotypes are owned by DuPont Pioneer, PH= Pioneer Hybrid

2.3 Experimental Design

A split-plot design in simple lattice (16 x 16) was used with two replicates. The main plots were three irrigation regimes; well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG). The 256 genotypes (254 top crosses and 2 check cultivars) were used as sub-plots.

2.4 Irrigation System

The irrigation method used in this study is one of the most advanced methods of irrigation systems in the world; it is one of the subsurface irrigation methods called T-Tape Drip Tape® by John Deer irrigation (16 mm/30 cm/1.3 LPH). It is a type of drip irrigation system which gives the chance to supply a specific amount of water for each plant separately, the main irrigation lines (Lay Flats) were connected to the subsurface irrigation tubes (T-tapes), each main line being operated by a pressure reducing valve to control the water pressure in the irrigation system and to control the water regime application during the season.

Water availability during the water regime is very important to understand if the treatment is actually under stress or not. For that reason, a very sophisticated advanced tool (Diviner)® was used to record soil water content 15 days from planting. Each treatment has 2 tubes fixed under the two replicates of the check cultivar PH-30N11 to take readings for the water content in the soil for 1.0 meter depth and each 10 cm a separate reading.

2.5 Water Regimes

Three different water regimes were used: (i) Well watering (WW), where the full requirements of water during the whole season was supplied. (ii) Water stress at flowering stage (WSF), where irrigation water was withheld 10 days prior to anthesis and lasted for a complete 30-day period making a stress period of 25 days. (iii) Water stress at the grain filling stage (WSG), where irrigation water was withheld 10 days post 80% anthesis and lasted till harvest without any irrigation.

2.6 Agricultural Practices

During the season, chemical weed control was done by applying Gesbrim® and Harness® as pre-emergence herbicides and after 30 days manual hoeing was used to remove the weeds. Insect control was performed three times during the whole season by spraying the corn borers with Lambada Plus® 21% chlorobirophose active ingredient. Nitrogen fertilizer was applied through the irrigation system using liquid fertilizer at the rate of 150 kg N per feddan (357 kg N per hectare).

2.7 Soil and Water Analysis

The soil of the experimental site contained clay (49.35%), silt (18.92%), fine sand (15.08%) and coarse sand (16.65%). Soil type was clay; SP was 74%; pH was 7.14 and EC was 0.70 dSm^{-1}. The soluble cations of soil Ca, Mg, Na and K were 2.61, 1.30, 2.40 and 0.69 mEqu/l and the soluble anions Cl, CO_3 and SO_4 were 4.10, 2.20 and 0.70 mEqu/l, respectively. Irrigation water pH was 7.15 and EC was 0.47 dSm^{-1}. The soluble cations of water Ca, Mg, Na and K were 3.70, 0.60, 9.18 and 0.64 mEqu/l and the soluble anions Cl, CO_3 and SO_4 were 1.40, 2.20 and 10.50 mEqu/l, respectively.

2.8 Meteorological Data

A weather station was installed at the location to collect the required weather data for the site. On May, June, July, August and September, minimum temperature was 20, 23, 25, 25 and 25; maximum temperature was 32, 35, 36, 36 and 36, mean temperature was 26, 29, 30, 30 and 30, and average relative humidity was 39, 48, 55, 49 and 49%, respectively.

2.9 Data Recorded

1. Days to 50% anthesis (DTA)
2. Days to 50% Silking (DTS)
3. Anthesis-silking interval (ASI)
4. Plant height (PH)
5. Ear height (EH)
6. Leaf rolling (LR)
7. Barren stalks (BS%)
8. Ears per plant (EPP)
9. Grain yield per plant (GYPP)
10. Grain yield per hectare (GYPH)

2.10 Biometrical Analysis

All the data were subjected to analysis of variance (ANOVA) of split plot experiment using Minitab 17 software. Comparisons of means were made using least significant difference

(LSD) test at p≤ 0.05 and p≤ 0.01 levels of confidence according to Steel et al. [24].

Drought tolerance index (DTI) is the factor used to differentiate between the genotypes from tolerance point of view and it is calculated using the equation proposed by Fageria [25] as follows:

$$DTI = (Y1/AY1) \times (Y2/AY2)$$

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 0.5-<1, it indicates that genotype is moderately tolerant (MT) to drought. If DTI is <0.5, it indicates that genotype is sensitive (S) to drought.

2.11 Spearman's Rank Correlation Coefficients

Rank correlation coefficients were calculated between drought tolerance index (DTI) and all studied traits under each environment, using Genstat software and the significance of the rank correlation coefficient was tested according to Steel et al. [24]. The correlation coefficient (r_s) was estimated for each pair of any two parameters as follows:

$$r_s = 1- (6 \Sigma d_i^2)/(n^3-n)$$

Where, d_i is the difference between the ranks of the i^{th} genotype for any two parameters, n is the number of pairs of data. The hypothesis Ho: $r_s= 0$ was tested by the r-test with (n-2) degrees of freedom.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Mean squares due to irrigation regimes for all studied characters (Table 2) were significant (p≤ 0.05 or p≤ 0.01), indicating that skipping irrigation at flowering or grain filling stages has an obvious effect on all studied traits. Mean squares due to maize genotypes were significant (p≤ 0.01) for all studied traits, suggesting existence of genetic differences among studied test cross hybrids and check cultivars for all studied characters. This also indicates that DH lines differ in their top cross combinations, *i.e.* in their hybrid ability. Such genotypic differences in studied traits under well watering as well as water stress at flowering and grain filling were also recorded by previous investigators in maize [26-33].

Mean squares due to the interaction between genotypes and irrigation regimes were significant (p≤ 0.01) for all studied traits, indicating that genotypes behaved differently under different irrigation regimes for studied traits and the possibility of selection for improved performance under a specific water regime as confirmed by previous investigators [29,30,34-36].

3.2 Mean Performance

Mean grain yield per plant and per hectare of the 25 best and the 10 worst maize DHL's x tester crosses showed wide ranges of performance under well irrigation (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) conditions (Table 3). Grain yield/ha (GYPH) ranged between 11.44 ton for testcross No. 96 and 3.81 ton for testcross No. 130 under WW, from 6.67 ton for testcross No. 16 to

Table 2. Analysis of variance of split plot design for all studied traits of 254 DH lines and two check cultivars of maize in 2012 season

SOV	df	Mean squares				
		DTA	**DTS**	**ASI**	**PH**	**EH**
Irrigation (I)	2	**	**	**	**	**
Genotypes (G)	255	**	**	**	**	**
G × I	510	**	**	**	**	**
CV%		1.0	0.7	9.7	0.2	0.6
		LR	**BS**	**EPP**	**GYPP**	**GYPH**
Irrigation (I)	2	**	**	**	**	**
Genotypes (G)	255	**	**	**	**	**
G × I	510	**	**	**	**	**
CV%		5.6	24.6	15.5	20.3	16.7

*** indicate significant at 0.01 probability level, respectively*

1.91 ton for testcross No. 215 under WSF and from 7.15 ton for testcross No. 66 to 1.41 ton for testcross No. 21 under WSG conditions.

It is observed from Table 3 that most of the best testcrosses in GYPH under all irrigation treatments are the best testcrosses in grain yield/plant (GYPP). The testcrosses No. 16, 204, 58 and 14 were among the best 25 crosses in GYPH under both WW and WSF environments. The testcrosses No. 26, 153, 96 and 212 were among the best 25 crosses in GYPH under both WW and WSG environments. Moreover, the testcrosses No. 66, 15 and 160 were among the best 25 crosses in GYPH under both WSF and WSG stressed environments.

Table 3. List of test crosses showing the 12 highest and 12 lowest top crosses and ranges for studied traits under well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) conditions

Water stress	Best top crosses	Range
	DTA	
WW	12,60,176,64,135,4,57,78,96,98,124,113	(61 - 66)
WSF	212,216,16,117,132,170,182,203,211,220,25,79	(62 - 64)
WSG	213,250,17,216,38,51,101,106,107,119,125,139	(59 - 65)
	DTS	
WW	12,176,60,96,135,124,132,99,100,206,212,254	(64 - 67)
WSF	117,212,211,149,204,16,132,170,182,220,79,213	(62 - 65)
WSG	213,250,117,51,101,106,107,125,230,216,139	(62 - 68)
	ASI	
WW	130,21,85,154,5,129,92,94,216,70,93,108	(0 - 1)
WSF	29,44,149,219,89,168,184,2335,32,56,128,137	(0 - 1)
WSG	61,10,48,177,183,93,94,200,236,95,162,224	(0 - 1)
	PH (cm)	
WW	83,90,32,250,151,238,12,40,103,235,21,126	(230 - 180)
WSF	98,19,75,80,120,165,3,5,10,24,32,33	(180 - 200)
WSG	71,91,250,256,81,220,9,16,19,30,32,55	(160 - 200)
	EH (cm)	
WW	90,83,235,241,228,81,55,248,85,227,230,191	(70 - 100)
WSF	165,19,98,83,135,188,3,195,80,39,23,14	(70 - 100)
WSG	7,64,153,91,71,166,83,3,19,130,169,239	(80 - 90)
	LR	
WW	n/a	
WSF	26,89,136,8,15,99,7,62,29,78,55,69	(9 - 8)
WSG	78,69,67,99,29,26,15,70,62,8,56,89	(9 - 8)
	BS %	
WW	27,150,94,194,174,214,95,45,18,136,212,64	(0 - 1)
WSF	49,159,1,134,140,61,45,52,117,35,170,145	(0 - 3)
WSG	66,81,216,245,36,86,23,91,150,78,29,112	(0 - 3)
	EPP	
WW	87,85,96,97,84,30,89,58,210,186,93,25	(1 - 1.5)
WSF	19,165,159,94,134,140,1,52,45,61,53,117	(1 - 0.9)
WSG	89,81,66,216,245,23,36,150,86,29,208,213	(1 - 0.9)
	GYPP (g)	
WW	96,87,149,153,16,25,14,209,134,53,81,194	(221.9–144.3)
WSF	16,204,44,66,62,2,14,161,76,160,60,58	(146.4–97.5)
WSG	66,208,15,87,26,177,205,102,39,125,153,95	(124.8- 100.2)
	GYPH (ton)	
WW	96,87,149,153,16,25,14,209,134,53,81,194	(11.44–8.10)
WSF	16,204,44,66,62,2,14,161,76,160,60,58	(6.67–5.24)
WSG	66,208,15,87,26,177,205,102,39,125,153,95	(7.15–5.24)

Table 3. Continue

Water stress	Worst top crosses	Range
	DTA	
WW	248,130,71,75,90,158,196,127,229,191,151,40	(75 - 78)
WSF	188,130,8,33,85,22,41,87,143,195,12,40	(72 - 80)
WSG	103,71,91,105,155,5,56,202,27,229,12,96	(79 - 77)
	DTS	
WW	248,151,130,127,229,40,49,103,8,109,113,120	(77 - 79)
WSF	188,8,155,12,40,47,73,75,201,243,176,110	(77 - 85)
WSG	103,5,56,71,91,105,155,118,185,12,96,111	(83 - 81)
	ASI	
WW	196,18,83,126,250,80,72,227,231,15,11,73	(4 - 6)
WSF	110,64,152,256,176,67,78,154,112,142,120,155	(9 - 6)
WSG	78,20,77,84,145,157,175,197,114,225,141,17	(6 - 5)
	PH (cm)	
WW	182,155,144,140,51,35,22,45,202,117,221,50	(350 - 320)
WSF	177,230,27,46,50,79,170,184,185,190,8,26	(310 - 280)
WSG	45,60,79,90,190,4,151,155,78,104,127,154	(320 - 300)
	EH (cm)	
WW	35,45,27,140,51,117,201,219,101,184,182,155	(210 - 170)
WSF	190,27,177,46,184,194,26,4,134,160,106,96	(180 - 160)
WSG	45,151,159,190,4,104,172,171,78,158,253,178	(180 - 160)
	LR (score)	
WW	n/a	
WSF	135,153,233,9,144,158,201,238,139,1,134,170	(4 - 1)
WSG	135,153,158,139,201,238,144,233,9,45,162,198	(4 - 1)
	BS %	
WW	154,231,4,203,111,91,213,158,102,59,116	(5 - 6)
WSF	215,141,128,192,238,158,243,133,162,236,110,227	(23 - 17)
WSG	238,105,27,130,157,243,141,244,204,46,13,120	(16 - 20)
	EPP	
WW	158,91,59,102,29,222,203,220,237,231,213,239	(0.7 - 0.8)
WSF	215,141,192,128,243,238,133,110,188,236,227,162	(0.3 - 0.5)
WSG	105,27,204,238,13,243,46,256,248,103,71,225	(0.2 - 0.4)
	GYPP (g)	
WW	130,196,28,229,8,231,129,46,219,133,120,69	(65.2 - 42.9)
WSF	215,192,188,41,223,236,228,110,245,201,231,69	(30.7–15.9)
WSG	21,105,156,250,133,185,71,238,42,27,243,120	(27.7–20.3)
	GYPH (ton)	
WW	130,196,28,229,8,231,129,46,219,133,120,69	(3.81 – 2.86)
WSF	215,192,188,41,223,236,228,110,245,201,231,69	(1.91 –0.95)
WSG	21,105,156,250,133,185,71,238,42,27,243,120	(1.43 –0.95)

The best DHL x tester cross for GYPP was No. 87 (222 g) under WW, No. 16 (146 g) under WSF and No. 66 (125 g) under WSG conditions as compared with the best check (30R77), which showed mean GYPP of 123, 84.9 and 81.9 g under WW, WSF and WSG conditions, respectively.

The earliest DHL x tester crosses for DTA in this study under both WSF and WSG stages were No. 212, 213, 216 and 230. They were earlier by 3 and 9 days than the earliest check (PH 30R77) under WSF and WSG, respectively. Earliness of these testcrosses, which is favorable for drought tolerance, could be due to their parental doubled haploid lines No. 212, 213, 216 and 230, which were developed from their parental crosses between the drought tolerant inbreds (PHM6T – PHJFN – PH1723) and the good general combiners (PH12J4 – PH1CGY – PHM7E).

The DHL x tester crosses No. 117, 213, 119 and 97 were the earliest for DTS in this study under both WSF and WSG environments. Anthesis-silking interval (ASI) ranged from 0 to 9 days in this experiment. The DHL x tester crosses No.

29, 88 and 149 were of very short ASI (0-1 day) under both WSF and WSG conditions.

Seventeen DHL x tester crosses in this study did not show any symptoms of leaf rolling under both drought stress (WSF and WSG) conditions. These crosses had the DH lines No. 67, 78, 70, 7, 8, 13, 55, 69, 26, 15, 25, 62, 56, 89, 99, 136 and 29 as one of their parents. On the contrary, the worst cross (No. 135) for LR showed tightly rolled leaves under WSF and WSG.

The DHL x tester crosses No. 3, 19, 33 and 165 had the shortest plants in the experiment (favorable for drought tolerance) under both WSF and WSG environments (≤ 200 cm), but the tallest plants under these stresses were No. 177 (310 cm) and No. 45 (320 cm).

Moreover, the DHL x tester crosses No. 19, 32 and 130 showed the lowest ear position (≤ 100 cm) under both WSF and WSG conditions, but the worst cross for EH was No. 190 (180 cm) under WSF and No. 45 (180 cm) under WSG.

The best DHL x tester crosses for barren stalks were No. 19, 165 and 94 under WSF and No. 89, 66 and 81 under WSG, which did not show any barren stalks. The worst DHL x tester cross for BS was No. 215 (23%) under WSF and No. 105 (20%) under WSG conditions.

For ears/plant, the best DHL x tester crosses were No. 87 (1.5) under WW, No. 19, 165, 159 and 94 (1.01-1.04) under WSF stress and No. 89, 81 and 66 (1) under WSG stress conditions. The worst cross for EPP was No. 215 (0.33) under WSF and No. 105 (0.30) under WSG conditions.

3.3 Drought Tolerance Index

Drought tolerance index (DTI) values of studied genotypes estimated using the equation suggested by Fageria [25] under the stressed environments WSF and WSG are presented in Table 4. According to our scale, when DTI is ≥1.0, it indicates that genotype is tolerant (T), if DTI is 0.5 - <1, it indicates that genotype is moderately tolerant (MT) and if DTI is <0.5, it indicates that genotype is sensitive (S).

Based on DTI values, the 256 studied maize genotypes were grouped into three categories under water stress at flowering, namely tolerant (120 genotypes), moderately tolerant (108 genotypes) and sensitive (28 genotypes) (Table 4). Number of tolerant (T), moderately tolerant (MT) and sensitive (S) genotypes were 123, 105 and 28 under water stress conditions at grain filling, respectively.

The highest DTI under the two stressed environments (WSF and WSG) was exhibited by the DHL x tester cross No. 96 (rank 3 and 2, respectively). The ten top crosses No. 16, 14, 96, 204, 209, 38, 140, 160, 161 and 134; these DHL x tester crosses are the most tolerant genotypes to drought at flowering in descending order. They are more tolerant than the best check cultivar 30R77 (Table 4). It was observed that out of the 12 most drought tolerant top crosses, five crosses were also among the best performing in GYPH, namely No. 16, 66, 14, 161 and 160; these top crosses were among the most drought tolerant at flowering and among the highest yielding top crosses under the same conditions. On the contrary, the most drought sensitive DHL x tester crosses under WSF conditions were the top crosses No. 215,130, 231, 28, 196, 229, 228, 110, 236 and 69 (Table 4).

The 12 most drought tolerant top crosses under WSG conditions were No. 87, 96, 66, 153, 208, 26, 177, 194, 30, 81, 205 and 212; these crosses and No. 160, 203, 15, 53, 19, 112, 233, 58 and 47 were bore tolerant than the most tolerant check cultivar 30R77. Out of the 12 most drought tolerant top crosses under WSG conditions, eight crosses were also among the best performing in GYPH under the same conditions, namely No. 66, 208, 87, 26, 205, 177, 153 and 30; these top crosses were among the most drought tolerant at grain filling and among the highest yielding top crosses under the same conditions. On the other hand, the most sensitive 10 crosses under WSG conditions were No. 133, 256, 21, 185, 250, 105, 120, 238, 229 and 91 (Table 4).

Data indicated that the most drought tolerant top crosses (DTI ≥ 1.5) were No. 96 followed by No. 16, 66, 153, 26, 58, 160, 19, 208, 209, 53, 81, 177, 194, 212, 15, 79 and 52 under both WSF and WSG, No. 205 followed by No. 68, 54, 62 and 132 under WSF only and No. 205, 203, 112, 125, 150, 137, 181 and 171 under WSG only. The corresponding DH lines of these top crosses should be recommended to maize breeding programs aiming at improving drought tolerance under corresponding drought stressed environments.

Table 4. Drought tolerance categories of 254 top crosses and 2 check cultivars based on drought tolerance under water treatments

Drought tolerance categories	(DTI)	No. of genotypes	Top crosses	Mean	DTI range
			WSF		
Tolerant	**DTI > 1**	**120**	16,14,96,204,209,58,140,160,161,134,**30R77**, 66,19,83,149,44,53,76,79,117,183,68,212,88, 81,153,52,34,26,6,,54,87,62,132,235,249,208, 177,194,15,18,90,86,45,159,169,25,30,178,233,12,102,73,99 ,11,6,29,247,23,64,226,9,37,92, 166,199,82,255,240,213,100,239,24,106,84,128,39,173,165, 7,184,47,211,43,89,148,77,7,51, 198,107,10,190,70,200,114,243,4,50,93,22, **30N11**,95,170,145,142,214,123,80,181,125,32,179,187,5, 251,112,205,48,21	1.4	(2.8 - 1.001)
Moderately tolerant	**DTI (> 0.5 - 1)**	**108**	168,63,151,207,137,191,150,206,241,174,27, 253,36,109,127,202,49,94,144,136,220,3,203, 135,55,57,17,227,237,186,189,139,42,59,85,35,197,146,193, 157,121,108,147,180,230,152, 217,131,20,116,171,40,12,6,31,218,164,210,97,33,101,222,2 46,167,252,225,242,113,67,182, 103,105,195,254,13,115,248,71,163,162,119, 61,154,78,65,216,56,104,143,158,98,185,111, 38,155,232,244,172,124,141,250,238,234,122,17,221,75,118 ,156	0.78	(0.99 - 0.501)
Sensitive	**DTI (< 0.5)**	**28**	224,133,175,120,256,46,201,129,223,91,41, 245,74,219,138,192,188,8,69,236,110,228,229,196,28,231, 130,215	0.37	(0.48 - 0.20)
			WSG		
Tolerant	**DTI > 1**	**123**	87,96,66,153,208,26,177,194,30,81,205,212, 160,203,15,53,19,112,233,58,47,**30R77**,16,89,39,125,102,25 ,150,79,181,107,209,145,137,	1.39	(3.24 - 1.001)

Drought tolerance categories	(DTI)	No. of genotypes	Top crosses	Mean	DTI range
Moderately tolerant	**DTI (> 0.5 - 1)**	**105**	171,166,52,84,70,161,178,11,4,83,82,117,140,14,7,60,95,23, 164,36,149,199,106,29,136,90, 49,173,159,64,7,6,198,253,251,135,18,230,151,34,213,76,**30 N11**,24,80,22,176,235,152,210, 41,180,144,174,94,101,134,202,183,179,10,40,218,86,241,4 4,85,109,226,35,249,246,88,115, 43,99,100,93,67,55,48,163,237,92,207,220,223, 214 61,123,255,57,65,113,141,104,17,118,245,139,54,247,114,1 67,20,193,225,169,189,146,3,97, 77,72,187,132,170,190,252,78,63,5,62,124,111,98,222,142,2 16,242,121,244,195,69,128,9,12,32,221,68,148,200,168,51,2 27,182,119,240,126,175,143,154,73,234,165,59,56,186,217, 45, 211,236,188,157,204,131,156,232,192,228,31,191,184,75,25 4,239,122,206,147,219,38,129,	0.76	(0.99 - 0.501)
Sensitive	**DTI (< 0.5)**	**28**	243,103,158,127,201,116,27,197,33,50,162 172,224,108,155,110,138,13,215,74,8,71,42,248,28,130,196, 46,231,91,229,238,120,105,250, 185,21,256,133	0.36	(0.49 - 0.20)

3.4 Superiority of Drought Tolerant (T) to Sensitive (S) Genotypes

Based on grain yield/ha and drought tolerance index (DTI) the best five top crosses were No. 16, 204, 44, 66 and 62 under WSF and No. 66, 208, 87, 15 and 26 under WSG, while the drought sensitive and lowest yielding top crosses were 215, 192, 188, 41 and 223 under WSF and No. 21, 105, 256, 250 and 133 under WSG conditions. Data averaged for each of the two groups (T and S) under WSF and under WSG indicated that GYPH of drought tolerant (T) was greater than that of the sensitive (S) test crosses by 78.78 and 82.52% under drought at flowering (WSF) and grain filling (WSG), respectively (Table 5).

Superiority of drought tolerant (T) over sensitive (S) top crosses in GYPH under drought at flowering (WSF) and grain filling (WSG) was associated with superiority in higher GYPP (78.55 and 79.86%), higher EPP (45.49 and 35.24%), lower BS (-242.3 and –305.5%), better LR (25.71 and 29.27%) shorter ASI (-166.67 and –42.86%), less DTA (-10.09 and -3.53%) and DTS (-14.97 and 5.08%), respectively. However, tolerant top crosses had taller plants (7.26 and 20.16%) and higher ear placement than sensitive top crosses (9.38 and 18.46%) under drought at flowering (WSF) and grain filling (WSG), respectively.

CIMMYT breeders found that maize grain yield under drought was closely related to some secondary traits such as more ears per plant, *i.e.* less barrenness, short ASI and late leaf senescence, *i.e*. stay grain [37-40].

Reduction in barren stalks and shortening in ASI of tolerant as compared to sensitive top crosses in the present study are desirable and may be considered as important contributors to drought tolerance. Similar conclusions were reported by [16,27,40-45].

3.5 Grouping Genotypes

3.5.1 Based on water efficiency and responsiveness

According to efficiency under water stress (either WSF or WSG) and responsiveness to well watering, studied top crosses were classified into four groups, *i.e.,* water stress efficient and responsive to well watering (E-R), water stress efficient and non-responsive (E-NR), water stress non-efficient and responsive (NE-R) and water stress non-efficient and non-responsive (NE-NR) based on GYPH.

For the relationship of GYPH between WW and WSF, the top crosses No. 96, 16, 14, 149, 204, 66, 2, 44, 19 and 134 were classified among water efficient under WSF and responsive crosses, while top crosses No. 215, 291, 130, 196, 28, 236, 188, 69 and 219 were classified among water non-efficient under WSF and non-responsive crosses (Fig. 1). The first group of top crosses (E-R) was amongst the highest GYPH under WW and WSF, *i.e.*; they could be considered as the most water stress efficient under WSF and the most responsive genotypes in this study (Fig. 1). On the contrary, the second group of crosses (NE-R) had the lowest GYPH under both WW and WSF and therefore could be considered inefficient and non-responsive (Fig. 1). The crosses No. 62, 211, 56 and 127 were

Table 5. Superiority (%) of the five most tolerant (T) over the five most sensitive (S) top crosses for studied traits under water stress at flowering (WSF) and water stress at grain filling (WSG) conditions

Trait	WSF			WSG		
	T	S	% Superiority	T	S	% Superiority
DTA (day)	65.4	72.0	-10.09**	68.0	70.4	-3.53**
DTS (day)	66.8	76.8	-14.97**	70.8	74.4	-5.08**
ASI (day)	1.8	4.8	-166.67**	2.8	4.0	-42.86**
PH (cm)	248	230	7.26**	258	206	20.16**
EH (cm)	128	116	9.38**	130	106	18.46**
BS (%)	13.0	52.6	-305.6**	12.5	42.9	-242.3**
LR	7.0	5.2	25.71**	8.2	5.8	29.27**
EPP	0.87	0.47	45.49**	0.88	0.57	35.24**
GYPP (g)	108.7	23.3	78.55**	106.5	21.5	79.86**
GYPH (ton)	5.98	1.27	78.78**	6.08	1.06	82.52**

*% Superiority = 100 × [(T – S)/S].*and ** indicate significant at 0.05 and 0.01 probability levels, respectively*

amongst the group of water efficient and non-responsive (high GYPH under WSF but low GYPH under WW). The crosses No. 87, 85, 112, 192, 139, 143 and 203 had low GYPH under WW and under WSF, *i.e.* water stress inefficient and responsive.

For the relationship of GYPH between WW and WSG (Fig. 2), the top crosses No. 96, 16, 66, 87, 153, 26, 208, 177, 30 and 205 were classified among water efficient under WSG and responsive crosses, while top crosses No. 8, 46, 133, 256, 185, 21, 105, 231, 229, 120 and 248 were classified among water non-efficient under WSF and non-responsive crosses.

The first group of top crosses (E-R) was amongst the highest GYPH under WW and WSG, *i.e.*; they could be considered as the most water stress efficient under WSG and the most responsive genotypes in this study (Fig. 2). The second group of crosses (NE-R) had the lowest GYPH under both WW and WSF and therefore could be considered inefficient and non-responsive (Fig. 2). The crosses No. 95, 135, 62, 163, 69 and 175 were amongst the group of water efficient and non-responsive (high GYPH under WSF but low GYPH under WW). The crosses No. 149, 134, 204, 95, 92, 24 and 14 had low GYPH under WW and under WSF, *i.e.* water stress inefficient and responsive (Fig. 2).

According to Fageria and Baligar [46-48] genotypes (progenies) belonging to the 1st group "efficient and responsive" (above all) and 2nd group "efficient and non-responsive" (to a lesser extent) appear to be the most desirable materials for breeding programs that deal with adaptation to water stress.

It was observed that the top crosses No. 96, 66 and 16 occupied the first group (E-R) under both WSF and WSG conditions; they 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.

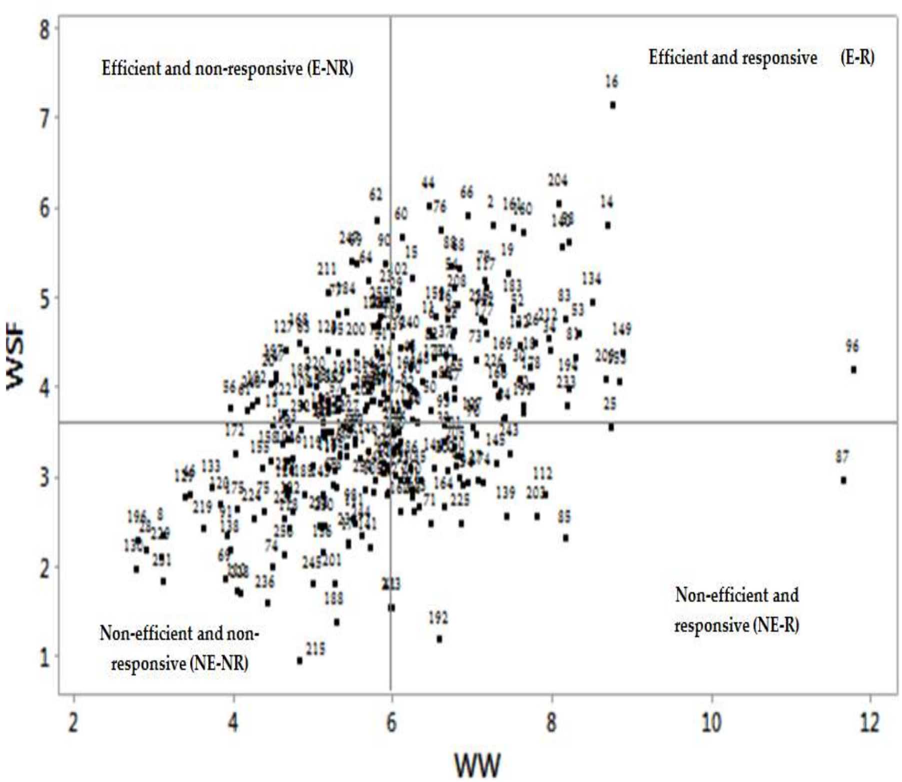

Fig. 1. Relationships between GYPH of top crosses under well watering (WW) and water stress at flowering (WSF)

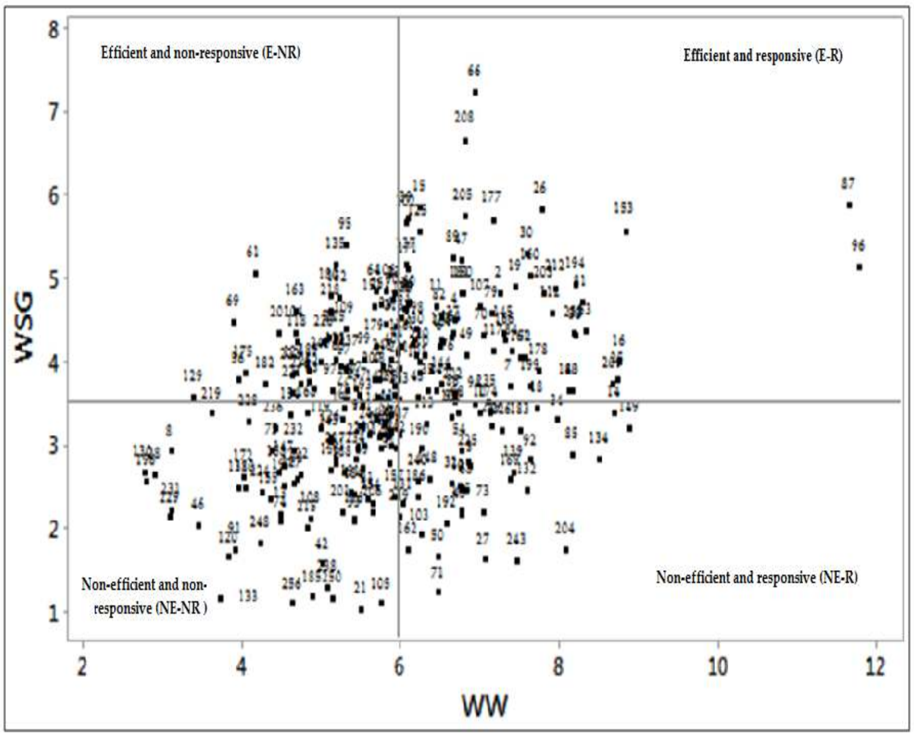

Fig. 2. Relationships between GYPH of top crosses under well watering (WW) and water stress at grain filling (WSG)

3.5.2 Based on drought tolerance and grain yield

Mean grain per hectare of studied top crosses under water stress at flowering (WSF) and grain filling (WSG), was plotted against same trait of the same genotypes under WW (Fig. 3 and 4, respectively), which made it possible to distinguish between four groups, namely tolerant high- yielding, tolerant low-yielding, sensitive high-yielding and sensitive low-yielding under WW [49-51].

Under water stress at flowering (WSF), the top crosses No16 followed by No. 14, 204, 96, 58, 140, 161, 2, 66 and 44 were classified amongst the drought tolerant and high yielding crosses *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. 3). The crosses 108, 197, 189, 57, 135, 22o, 127, 206, 191 and 168 were among the group of sensitive and high yielding crosses under WSF, *i.e.* water stress sensitive and responsive to water stress. The top crosses No. 87, 25, 107, 70, 243, 93, 145 and 181 occupied the group of tolerant and low yielding under WSF. The top crosses No 215, 192, 188, 236, 228, 223, 231, 69, 245 and 130 were classified amongst the water stress sensitive and low yielding and therefore could be considered sensitive and low yielding (Fig. 3).

Under water stress at grain filling (WSG), the top crosses No. 87, 96, 66, 208, 26, 153, 205, 177, 15 and 39 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. 4). On the contrary, top crosses No. 256, 21, 133, 185, 105, 250, 238, 42, 71, 27 were classified as water stress sensitive and low yielding (Fig. 4). The top crosses No. 174, 14, 249, 149, 187, 134, 18, 94, 202 and 200 occupied the group of water stress tolerance and low GYPH under WSF. The crosses 180, 163, 20, 69, 104, 118, 237, 189, 175 and 252 were sensitive and high yielding under WSF (Fig. 4).

Summarizing the above-mentioned classifications, it is apparent that the top crosses No. 96, 16, 14, 204, 66, 2, 44, 19 and 134 were the best crosses 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 top crosses No. 96, 66, 87, 153, 26, 208, 177 and 205 were the best crosses 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.

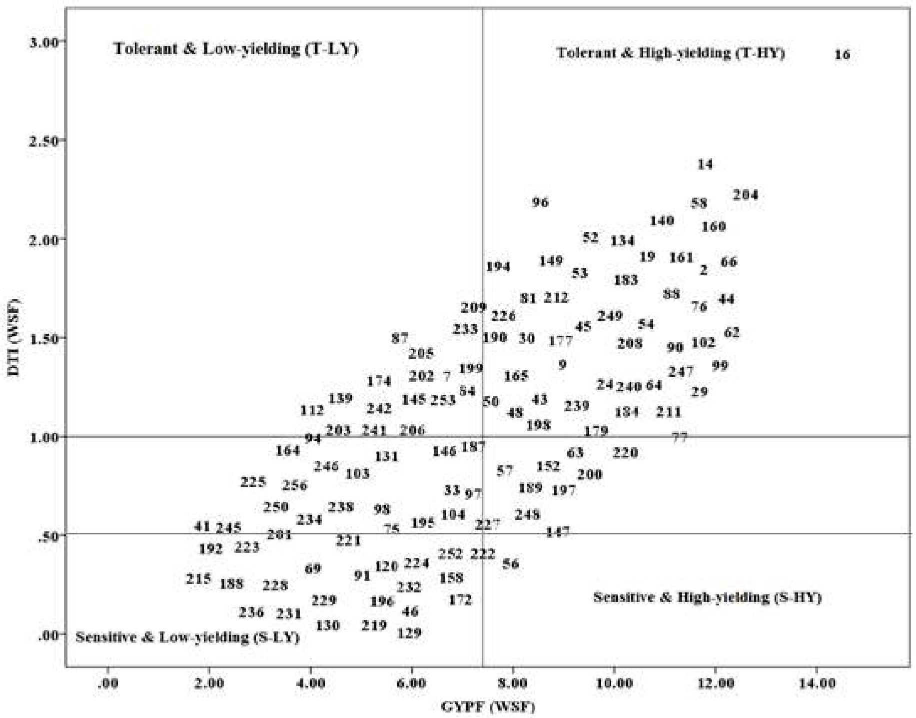

Fig. 3. Relationships between drought tolerance index and means of GYPH of top crosses water stress at flowering (WSF)

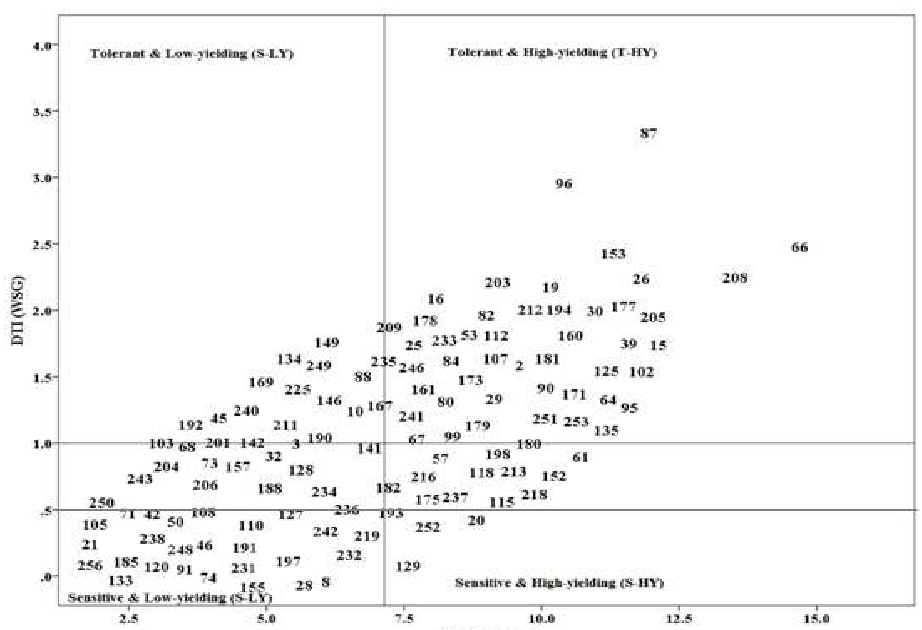

Fig. 4. Relationships between drought tolerance index and means of GYPH of top crosses water stress at grain filling (WSG)

It was observed that the two top crosses No. 96 and 66 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 as well as WW.

3.6 Correlation between Drought Tolerance and Studied Traits

Estimates of rank correlation coefficients between DTI and all studied traits under each of the two stressed environments (WSF and WSG) were calculated across all top crosses and presented in Table 6.

Drought tolerance index had a significant ($p \leq 0.01$) and positive correlation with grain yield/plant (r_g= 0.88 and 0.86) and grain yield/ha (r_g= 0.82 and 0.80) under WSF and WSG conditions, respectively. This indicates that grain yield was the best indicator of drought tolerance in this experiment.

Table 6. Rank correlation coefficients between DTI and all studied traits across 245 top crosses under WSF and WSG environments

Trait	WSF	WSG
DTA	-0.20*	-0.17*
DTS	-0.27*	-0.20*
ASI	-0.28*	-0.19*
BS	-0.55**	-0.44**
PH	0.11	0.07
EH	-0.03	0.04
LR	-0.18*	-0.19*
EPP	0.55**	0.44**
GYPP	0.88**	0.86**
GYPH	0.82**	0.80**

WW = well watering, WSF= water stress at flowering, WSG= water stress at grain filling, DTI= drought tolerance index, and** indicate significant at 0.05 and 0.01 probability level, respectively*

Drought tolerance had a medium of magnitude, significant and positive correlation coefficient, with number of ears/plant (r_g = 0.55** and 0.44**) and a significant and negative correlation coefficient with percent of barren stalks (r_g= 0.55** and 0.44**). Moreover, drought tolerance index had a small in magnitude, but significant and negative correlation coefficient with DTA, DTS, ASI, BS and LR traits. This indicates that drought tolerant top crosses under both WSF and WSG conditions are characterized by early DTA and DTS, short ASI, less BS and less LR. This conclusion is in accordance with other investigators [9,13,16,37,52,53]. These traits could be considered as selection criteria for drought tolerance in maize.

4. CONCLUSIONS

The study identified drought tolerant and highest yielding DHL's x tester crosses and their parental doubled haploid lines. The identified maize genotypes could be used in breeding programs aiming at developing drought tolerant and high yielding maize hybrids. These DH lines were No. 16, 14, 204, 96, 58, 140, 161, 2, 66 and 44 under water stress at flowering (WSF) and No. 87, 96, 66, 208, 26, 153, 205, 177, 15 and 39 under water stress at grain filling (WSG) conditions. Developing drought tolerant (T) hybrids of maize is very important target in plant breeding programs, because of their superiority under WSF and/or WSG to the sensitive (S) hybrids in grain yield. Superiority of T over S hybrids in grain yield was associated with superiority in anthesis silking interval (ASI), days to anthesis (DTA), days to silking (DTS), barren stalks (BS), leaf rolling (LR) and ears per plant (EPP). Results concluded that grain yield was the best indicator of drought tolerance. The drought tolerant genotypes were characterized by early DTA and DTS, short ASI, less BS and LR. These traits could be considered good selection criteria for improving drought tolerance in maize under both WSF and WSG conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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