Journal of Advances in Biology & Biotechnology
10(1): 1-15, 2016; Article no.JABB.29522
ISSN: 2394-1081

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Breeding Values of 254 Maize (*Zea mays* L.) Doubled Haploid Lines under Drought Conditions 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 data analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/29522
Editor(s):
(1) Fernando José Cebola Lidon, Universidade Nova de Lisboa, Campus da Caparica, Portugal.
Reviewers:
(1) Lawrence Owere, National Agricultural Research Organisation, Uganda.
(2) Violeta Andjelkovic, Maize Research Institute, Zemun Polje, Serbia.
Complete Peer review History: http://www.sciencedomain.org/review-history/16578

Original Research Article

Received 15th September 2016
Accepted 10th October 2016
Published 15th October 2016

ABSTRACT

Breeding value is the main parameter of initial screening through top cross analysis as it represents the general combining ability (GCA) effects of individual test line. Two hundred fifty four top crosses were produced as a result of crossing between 254 doubled haploid lines (DHL) developed by inducer technique and the inbred line tester PHDMF. The main objective was to identify the DHL's of high breeding value under drought at flowering and grain filling to be exploited in a breeding program aiming at developing drought tolerant maize hybrids. A split plot design in lattice (16 x 16) arrangement was used with two replications, where three irrigation treatments (well watering; WW, water stress at flowering; WSF and water stress at grain filling; WSG) were allotted to main plots and genotypes (254 top crosses) to sub-plots. A separate analysis of variance of RCBD was also performed under each irrigation treatment. Results suggested the existence of significant ($p \leq 0.01$) differences among studied DHL's × tester crosses under respective irrigation treatments for all studied traits. For each of the ten studied traits, number of desirable DH lines for GCA effects was identified under WSF and WSG conditions. For grain yield/ha (GYPH), number of

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desirable DH lines for further exploitation in breeding programs; *i.e.* those having positive and significant GCA effects was 66 for drought tolerance at flowering and 82 for drought tolerance at grain filling. The best ten DHL's in GCA effects for GYPH were No. 16, 204, 44, 66, 62, 2, 14, 161, 76 and 160 under WSF and 66, 208, 87, 15, 26, 205, 39, 177, 102 and 153 under WSG conditions. It was observed that for a given trait, the rank of doubled haploid lines for GCA effects was the same rank of their top crosses for mean performance.

Keywords: Top cross analysis; general combining ability; drought tolerance; tester.

1. INTRODUCTION

To reach self-sufficiency of maize production in Egypt, efforts are devoted to extend the acreage of maize; in the desert and to improve the maize productivity from unit area. Growing maize in the sandy soils of low water-holding capacity would expose maize plants to drought stress, which could result in obtaining low grain yields under such conditions. Moreover, the expected future shortage in irrigation water necessitates that maize breeders should pay great attention to develop drought tolerant maize cultivars that could give high grain yield under both water-stress and non- stress conditions.

Maize is particularly susceptible to drought at the flowering stage [1]. Loss in grain yield is particularly severe when drought stress occurs at this stage [2-4]. Grant et al. [3] reported that although yields were most severely reduced (70%) by stress coinciding with silking, yields were reduced by 40-54% from stresses occurring in the period 10 to 31 days after mid-silk, and kernel number was reduced below control for stresses occurring up to 22 days after silking. Several investigators emphasized the role of maize genotypes in drought tolerance. Tolerant genotypes of maize were characterized by having shorter anthesis-silking interval (ASI) [5], more ears/plant [6,7] and greater number of kernels/ear [7,8]. The presence of genotypic differences in drought tolerance would help plant breeders in initiating successful breeding programs to improve such a complicated character.

Maize breeders are always looking for new methods to enrich breeding material of better tolerance to drought stress. Using modern biotechnological techniques in plant breeding could contribute to a great extent in the induction of novel genetic variation, which are not existed in the gene pool, such as somaclonal and gametoclonal variation [9,10]. 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 [11]. 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 between lines for *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 [12]. To our knowledge, all present commercial DH-line breeding programs are based on in vivo induction of maternal haploids [13-15]. Other techniques have proven to be less effective or too genotype specific.

Doubled haploid lines display maximum genetic differentiation for per se and testcross performance from the first generation and allow the breeder to drastically reduce the 'time to market'. As a consequence, most internationally leading seed companies have converted their LD programs to the DH technology during the last years or have initiated this process [12]. The technology has also found its way into research but much slower than in breeding because experienced staff and appropriate experimental facilities are needed to apply it successfully.

Because of the genetic, methodological, and logistic advantages, further progress in maize breeding is expected to increase considerably with the development of DH lines. Yet, the success of employing DH lines depends on a robust and efficient haploid induction technology as well as on breeding strategies that make optimum use of the breeder's genetic, technical, and monetary resources [16-18].

The inbred-tester crosses are in fact half-sib (HS) families. The inbreds involved in the best crosses might carry adequate fixable genetic variance (variance of breeding value) so as to produce better hybrids with a number of other inbreds

[19]. Such inbreds possess high breeding value for the trait of interest. Breeding value is the main parameter of initial screening through top cross analysis as it represents the GCA (general combining ability) effect of individual test inbred [19]. Therefore, operation of additive gene action (warranted by GCA effects (breeding value) is a clean indication in the group of test inbreds. The inbred parents manifested negative breeding value are undesirable for further exploitation.

Top cross analysis is the simplest method of elimination of a considerable number of undesirable lines and identifying those lines of high breeding value, *i.e.* of high GCA effects in the beginning of a breeding program [19]. The topcross method deals with a series of single crosses developed between a single tester and a number of inbred lines to be studied for their genetic constitution. It was first proposed by Jenkins and Brunsen [20] as a method of testing inbred lines of maize in cross-bred combinations. Later, Tysdal and Crandall [21] renamed it "top-cross".

Two hundred fifty four maize doubled haploid (DH) lines developed by DuPontPioneer *via* the *in vivo* (inducer) technique from the crosses between drought tolerant inbreds and good general combiners obtained from Research Department of the Pioneer Hi-Bred Inc. Two hundred fifty four test-cross hybrids were produced as a result of crossing between the 254 DH lines and the inbred line tester PHDMF that shows drought tolerance performance and high general combining ability. These DH line x tester crosses are expected to include test crosses that accumulated favorable genes for both high-yielding and drought tolerance.

The main objective of the present study was to estimate the breeding value (GCA effects) of 254 DH lines under water stress at flowering and grain filling in order to identify the best DH lines of high GCA effects for exploiting them in the breeding program for developing drought tolerant hybrids.

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 at 30°25' 8" N, 31°11' 24" E and Altitude is 74 m above sea level.

2.1 Plant Materials

Seeds of 254 maize (*Zea mays* L.) doubled haploid lines (DHL's) resulted *via* the inducer technique (embryo rescue) used by DuPontPioneer from the crosses between the drought tolerant inbreds (PHM6T – PHJFN – PH1723) and the good general combiners (PH12J4 – PH1CGY – PHM7E) were 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 that shows 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 checks in the evaluation experiment. All the genotypes used were obtained from the germplasm of DuPontPioneer.

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

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
Topcrosses	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

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 of the year 2012, the experimental location was prepared for planting by tractors to get a fairly fine soil to be convenient for the planting by planter. During the tillage process, superphosphate 15.5% with the rate of 30 kg P_2O_5/fed (fed=feddan=4200 m^2) as well 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 meters long with a 1.0 meter long ally between ranges.

2.3 Experimental Design

A split-plot design in simple lattice (16 x 16) arrangement with two replications was used, where main plots were allotted to three irrigation regimes, *i.e.* well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG). Sub-plots were devoted to 256 genotypes (254 top crosses and 2 check cultivars).

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 allotted to the subsurface irrigation tubes (T-tapes), each main line is 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 allotted to the location after 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: 1. Well watering (WW), where the full requirements of water during the whole season was supplied. 2. 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. 3. 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 weed control and after 30 days, hand weed control was made by manual hoeing. Insect control was performed three times during the whole season by spraying the corn borers with Lambada Plus® 21% chlorobirophose active ingredient. Fertilization with nitrogen was done through the irrigation system using liquid fertilizer and with 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/land the soluble anions Cl, CO3 and SO4 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/land 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 (ton)

2.10 Biometrical Analysis

All the data were subjected to analysis of variance (ANOVA) of split plot design in lattice (16 × 16) arrangement using Minitab 17 software and of Comparisons of means were made using least significant difference (LSD) test at P≤.05 and P≤.01 levels of confidence according to Steel et al. [22]. Each treatment was also analyzed separately as randomized complete blocks design.

2.11 Estimating GCA Effects of Individual Inbreds

According to Sharma [19], the main parameter of initial screening through top crosss analysis is the breeding value, which represents the GCA (general combining ability) effects of individual inbreds, which was calculated as follows:

GCA effects = C_i – C/SD (A_i), where C_i = mean of top cross I, C = general mean of top crosses and SD (A_i) = standard deviation = (var. A_i) ^{½}

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance of the split-plot design for 256 maize genotypes, *i.e.* 254 doubled haploid line (DHL) × tester crosses and two check cultivars) evaluated under three irrigation regimes in 2015 season is presented in Table 2. Mean squares due to irrigation regimes for all the traits studied were significant (P≤ 0.05 or 0.01), indicating that preventing 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.

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 [23-28].

It was observed that drought stress effects were more pronounced than genotype effects on five traits, namely leaf rolling (LR), barren stalks (BS), ears/plant (EPP), grain yield/plant (GYPP) and grain yield/ha (GYPH) (Table 2). This was expressed *via* the percentage of sum squares for each component to the total sum of squares, which indicated that irrigation regimes contributed higher percentage to the total variance than genotypes for the above mentioned traits. For the other five studied traits days to anthesis (DTA), days to silking (DTS), anthesis silking interval (ASI), plant height (PH) and ear height (EH), the highest contribution to total variance in this experiment was shown by genotype x irrigation interaction. The effect of genotype was higher than irrigation on the later traits.

Table 2. Analysis of variance of split plot design for all studied traits of 254 DH lines and two check cultivars of maize in 2015 season

SOV	df	% Sum of squares(SS)				
		DTA	DTS	ASI	PH	EH
Rep.	1	0.17	0.33	11.28	0.01	0.02
Irrigation (I)	2	13.61**	6.82**	13.46**	15.54**	5.13**
Error (a)	2	0.09	0.14	2.77	0.00	0.00
Genotypes (G)	255	29.62**	33.59**	26.08**	32.43**	34.17**
G × I	510	53.87**	58.21**	42.32**	52.01**	60.61**
Error (b)	765	2.64	0.91	1.09	0.01	0.06
Total SS	1535	15064	21282	5598	1553034	650926
CV%		1.0	0.7	9.7	0.2	0.6
		LR	BS	EPP	GYPP	GYPH
Rep.	1	0.64	0.17	0.18	0.00	0.01
Irrigation (I)	2	44.95**	29.8**	29.98**	38.70**	43.87**
Error (a)	2	0.60	0.30	0.23	0.02	0.12
Genotypes (G)	255	18.39**	17.5**	17.76**	28.12**	27.12**
G × I	510	32.90**	27.0**	25.93**	20.41**	19.76**
Error (b)	765	2.52	25.2	25.91	12.76	9.11
Total SS	1535	5020	362949	44	1485666	18518
CV%		5.6	24.6	15.5	20.3	16.7

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

For each irrigation treatment, a separate analysis of variance was preformed and presented in Table 3. Mean squares due to genotypes at all studied irrigation treatments were significant (P ≥ 0.01), suggesting the existence of significant differences among studied DHL's × tester crosses under respective irrigation treatments for all studied characters.

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 [25,29-36].

Table 3. Mean squares for studied traits of DHL × tester crosses under well watering (WW), Water stress at flowering (WSF) and water stress at grain filling (WSG) in 2015 season

SOV	df	WW	WSF	WSG	WW	WSF	WSG
		DTA			DTS		
Genotypes	255	14.78**	11.86**	22.68**	16.59**	24.80**	35.23**
Error	256	0.65	0.47	0.59	0.46	0.3	0.39
Total	511	7.7	6.15	11.62	8.51	12.52	17.78
		ASI			PH		
Genotypes	255	4.58**	6.45**	3.99**	1702**	1425**	2015**
Error	256	0.02	2	1.95	0.41	0.26	0.82
Total	511	2.29	4.22	2.97	849.61	711.46	1005.98
		EH			LR		
Genotypes	255	927.8**	618.9**	872.7**	N/A	4.96**	5.14**
Error	256	0.68	1.03	0.55	N/A	0.25	0.49
Total	511	463.36	309.37	435.77	N/A	2.6	2.81
		BS			EPP		
Genotypes	255	43.58	295.6**	294.5**	0.01	0.03**	0.03**
Error	256	39.45	161.62	162.55	0.01	0.02	0.02
Total	511	41.51	228.52	228.42	0.01	0.02	0.02
		GYPP			GYPH		
Genotypes	255	1232**	744**	850**	14.96**	9.01**	10.08**
Error	256	396.21	205.85	139.24	3.84	1.87	0.97
Total	511	813.39	474.81	494.04	9.39	5.43	5.52

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

3.2 Mean Performance

Mean grain yield per plant and per hectare of the best 25 maize DHL x tester crosses and the worst 10 showed wide ranges of performance under well irrigation (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) conditions (Table 4). Grain yield/ha (GYPH) ranged between 11.40 ton for testcross No. 96 and 2.67 ton for testcross No. 130 under WW, from 6.90 ton for testcross No. 16 to 0.90 ton for testcross No. 215 under WSF and from 7.00 ton for testcross No. 66 to 0.97 ton for testcross No. 21under WSG conditions.

Table 4. Mean ($\bar{x}$) grain yield per plant (GYPP) and per hectare (GYPH) of the best 25, the worst 10 test crosses and the two check cultivars under well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) in 2015 season

No.	GYPP (g)						GYPH (ton)					
	WW		WSF		WSG		WW		WSF		WSG	
	Entry	$\bar{x}$	Entry	$\bar{x}$	Entry	$\bar{x}$	Entry	$\bar{x}$	Entry	$\bar{x}$	Entry	$\bar{x}$
The best DHL x tester crosses												
1	87	222	16	146	66	125	96	11.40	16	6.90	66	7.00
2	96	212	90	120	205	121	87	11.23	204	5.80	208	6.43
3	25	174	19	117	153	111	149	8.57	44	5.80	87	5.67
4	16	166	44	110	171	109	153	8.53	66	5.73	15	5.67
5	83	163	60	107	26	106	16	8.43	62	5.67	26	5.63
6	85	161	58	106	81	106	25	8.43	2	5.63	205	5.53
7	149	157	14	104	89	105	14	8.40	14	5.63	39	5.53
8	58	157	117	102	36	103	209	8.40	161	5.57	177	5.53
9	194	155	66	102	95	103	134	8.20	76	5.53	102	5.47
10	153	151	68	101	87	101	53	8.07	160	5.53	153	5.40
11	89	145	76	100	96	101	81	8.00	60	5.47	125	5.40
12	203	144	161	97.9	136	100	194	7.90	58	5.43	95	5.23
13	81	144	140	97.5	15	100	58	7.90	140	5.40	30	5.10
14	34	144	83	96.9	208	100	233	7.90	247	5.23	89	5.07
15	52	144	208	95.5	82	99.5	83	7.87	90	5.20	47	5.07
16	134	143	204	93.8	102	99.1	85	7.87	99	5.20	135	5.00
17	30	141	82	92.4	60	98.4	140	7.83	88	5.20	137	4.97
18	82	141	88	92.1	177	97.8	204	7.83	68	5.13	96	4.97
19	14	141	62	91.4	39	97.2	34	7.73	19	5.10	171	4.97
20	26	140	95	91.3	194	94.9	212	7.67	15	5.07	61	4.87
21	53	139	23	89.7	218	93.6	112	7.63	64	5.00	160	4.87
22	92	139	165	89.3	112	92.2	203	7.53	79	5.00	194	4.77
23	18	138	248	88.7	30	92.2	26	7.50	117	4.97	19	4.73
24	49	137	247	88.7	61	91.7	178	7.50	54	4.90	212	4.70
25	209	137	212	88.4	160	91.3	18	7.43	102	4.90	106	4.67
The worst DHL x tester crosses												
1	130	42.8	215	15.9	238	20.3	130	2.67	215	0.90	21	0.97
2	196	46.5	192	19.1	256	20.5	196	2.70	192	1.13	105	1.03
3	28	51.3	236	25.3	250	20.8	28	2.80	188	1.33	256	1.03
4	231	53.3	223	26	105	21.3	229	2.97	41	1.47	250	1.10
5	8	54.9	41	27.6	21	21.6	8	3.00	223	1.50	133	1.13
6	69	59.3	188	28	133	23	231	3.00	236	1.53	185	1.13
7	229	62.2	201	29.3	71	24.5	129	3.27	228	1.63	71	1.17
8	46	62.2	228	29.5	185	25.8	46	3.33	110	1.67	238	1.23
9	120	62.9	110	30	42	26.2	219	3.50	245	1.73	42	1.50
10	219	65.2	245	30.7	27	27.6	133	3.60	201	1.73	243	1.57
Checks												
1	30N11	100	30N11	78.4	30N11	81.4	30N11	5.90	30N11	3.67	30N11	4.07
2	30R77	123	30R77	84.9	30R77	81.9	30R77	7.00	30R77	5.63	30R77	4.67
LSD 0.05		39.20		28.25		23.24		1.8		1.3		0.9

It is observed from Table 4 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.

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 (Table 4).

The 12 best and the 12 worst genotypes for other studied traits under the three irrigation regimes are presented in Table 5. The earliest DHL x tester crosses for DTA in this study under both WSF and WSG stages were No. 212, 213, 216 and 230 (Table 5). 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 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).

Table 5. 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	
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	
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)
	BP	
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)

Table 5. 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)

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 (Table 5).

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 (Table 6). 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 (Table 5). 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 (1.0) 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 Breeding Value (GCA Effects) of the DH Lines

Breeding value is the main parameter of initial screening through top cross analysis as it represents the GCA (general combining ability) effects of individual test inbred [19]. He reported that high breeding value of some test lines is an indication of operation of additive gene action and suitability for utilization in a breeding program, while the test lines that manifest low breeding value are undesirable for further exploitation. Thus, top cross analysis is the simplest method of elimination of considerable number of undesirable lines in the beginning of a breeding program.

General combining ability effects of the parental DHL's of the studied 254 top crosses were calculated according to Sharma [19] for the studied traits under well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) and values of the best 12 and the worst four DH lines are presented in Table 6.

Table 6. Breeding values (GCA effects) of the best 12 and the worst four doubled haploid lines of maize under well watering (WW), water stress at flowering (WSF) and water stress at grain filling (WSG) conditions

No.	DTA						DTS					
	WW		WSF		WSG		WW		WSF		WSG	
	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA
						The best DHL						
1	12	-2.42**	212	-1.71**	213	-2.45**	12	-2.02**	117	-1.91**	213	-1.98**
2	60	-1.64**	216	-1.71**	250	-2.03**	176	-1.78**	212	-1.71**	117	-1.48**
3	176	-1.64**	16	-1.42**	17	-1.40**	60	-1.53**	149	-1.51**	250	-1.48**
4	64	-1.38**	117	-1.42**	216	-1.40**	96	-1.53**	204	-1.51**	38	-1.14**
5	135	-1.38**	132	-1.42**	38	-1.19**	99	-1.29**	211	-1.51**	51	-1.14**
6	4	-1.12**	170	-1.42**	51	-1.19**	100	-1.29**	16	-1.31**	101	-1.14**
7	57	-1.12**	182	-1.42**	101	-1.19**	124	-1.29**	79	-1.31**	106	-1.14**
8	78	-1.12**	203	-1.42**	106	-1.19**	132	-1.29**	132	-1.31**	107	-1.14**
9	96	-1.12**	211	-1.42**	107	-1.19**	135	-1.29**	170	-1.31**	125	-1.14**
10	98	-1.12**	220	-1.42**	119	-1.19**	182	-1.29**	182	-1.31**	230	-1.14**
11	124	-1.12**	25	-1.13**	125	-1.19**	206	-1.29**	213	-1.31**	26	-0.97**
12	132	-1.12**	79	-1.13**	139	-1.19**	212	-1.29**	220	-1.31**	88	-0.97**
						The worst DHL						
1	130	1.75**	195	1.19**	91	1.54**	245	1.17**	243	1.10**	185	1.38**
2	158	1.75**	8	1.77**	105	1.54**	250	1.17**	8	1.31**	5	1.55**
3	196	1.75**	130	1.77**	155	1.54**	151	1.41**	155	1.31**	56	1.55**
4	248	2.01**	188	3.51**	103	1.75**	248	1.66**	188	2.71**	103	1.55**
SE gi		0.14		0.12		0.14		0.12		0.10		0.11
	(+)	72		94		103		93		108		83
	(-)	93		110		96		104		108		108
				PH						EH		
						The best DHL						
1	83	-2.19**	98	-1.60**	71	-1.98**	90	-2.15**	165	-2.31**	7	-1.50**
2	32	-1.46*	19	-1.33**	91	-1.76**	83	-1.82**	19	-1.91**	64	-1.50**
3	90	-1.46**	75	-1.33**	250	-1.54**	235	-1.50**	98	-1.91**	71	-1.50**
4	12	-1.22**	80	-1.33**	256	-1.54**	30	-1.17**	3	-1.51**	91	-1.50**
5	21	-1.22**	120	-1.33**	81	-1.32**	55	-1.17**	80	-1.51**	153	-1.50**
6	40	-1.22**	165	-1.33**	220	-1.32**	81	-1.17**	83	-1.51**	3	-1.16**
7	103	-1.22**	3	-1.07**	9	-1.09**	85	-1.17**	135	-1.51**	19	-1.16**
8	151	-1.22**	5	-1.07**	16	-1.09**	103	-1.17**	188	-1.51**	55	-1.16**
9	235	-1.22**	10	-1.07**	19	-1.09**	191	-1.17**	195	-1.51**	81	-1.16**
10	238	-1.22**	24	-1.07**	30	-1.09**	227	-1.17**	10	-1.11**	83	-1.16**
11	250	-1.22**	32	-1.07**	32	-1.09**	228	-1.17**	12	-1.11**	130	-1.16**
12	10	-0.97**	33	-1.07**	55	-1.09**	230	-1.17**	14	-1.11**	166	-1.16**
						The worst DHL						
1	140	1.69**	185	1.32**	60	1.58**	219	1.46**	194	1.31**	190	1.55**
2	144	1.69**	190	1.32**	79	1.58**	27	1.79**	205	1.31**	45	1.89**

No.	DTA						DTS					
	WW		WSF		WSG		WW		WSF		WSG	
	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA
3	155	1.69**	230	1.58**	90	1.58**	45	1.79**	27	1.71**	151	1.89**
4	182	1.93**	177	1.85**	190	1.58**	35	2.44**	190	2.11**	159	1.89**
SE gi		0.11		0.09		0.16		0.15		0.18		0.13
	(+)	114		112		81		83		91		76
	(-)	114		109		81		85		58		94

*(+)= Number of DHL's showing significant and positive GCA effects, (-)=Number of DHL's showing significant and negative GCA effects, **indicate significant at 0.01 probability level*

Table 6. Continue

No.	ASI						BS%					
	WW		WSF		WSG		WW		WSF		WSG	
	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA
						The best DHL						
1	5	-1.01**	29	-1.38**	10	-1.60**	30	-1.57	19	-1.48	89	-1.61
2	9	-1.01**	44	-1.38**	48	-1.60**	87	-1.57	165	-1.40	66	-1.43
3	21	-1.01**	149	-1.38**	61	-1.60**	94	-1.57	159	-1.33	245	-1.36
4	70	-1.01**	23	-0.98**	177	-1.60**	96	-1.57	134	-1.33	216	-1.32
5	85	-1.01**	24	-0.98**	183	-1.60**	112	-1.57	1	-1.24	36	-1.27
6	92	-1.01**	32	-0.98**	29	-1.09**	132	-1.57	52	-1.23	150	-1.19
7	93	-1.01**	36	-0.98**	34	-1.09**	149	-1.57	94	-1.23	23	-1.16
8	94	-1.01**	37	-0.98**	46	-1.09**	174	-1.57	45	-1.22	81	-1.15
9	96	-1.01**	56	-0.98**	49	-1.09**	178	-1.57	140	-1.20	86	-1.15
10	99	-1.01**	79	-0.98**	58	-1.09**	216	-1.57	61	-1.17	29	-1.14
11	100	-1.01**	88	-0.98**	88	-1.09**	212	-1.36	53	-1.17	208	-1.12
12	108	-1.01**	89	-0.98**	90	-1.09**	243	-1.35	117	-1.16	213	-1.09
						The worst DHL						
1	227	1.33**	64	1.77**	157	1.41**	102	1.41	128	1.63	238	1.78
2	231	1.33**	152	1.77**	175	1.41**	59	1.52	192	1.78	204	1.91
3	250	1.33**	256	1.77**	197	1.41**	91	1.58	141	2.00	27	2.02
4	196	1.79**	110	2.17**	225	1.41**	158	1.59	215	2.25	105	2.49
SE gi		0.02		0.25		0.25		1.11		2.25		2.25
	(+)	95		83		60		0		0		0
	(-)	161		78		94		0		0		0
	LR						EPP					
						The best DHL						
1	1	NA	7	1.21**	7	1.16**	87	4.61**	19	1.96**	89	1.70**
2	2	NA	8	1.21**	8	1.16**	85	2.21**	165	1.80**	81	1.64**
3	3	NA	13	1.21**	13	1.16**	96	2.18**	159	1.76**	66	1.60**
4	4	NA	15	1.21**	15	1.16**	97	2.07**	94	1.74**	216	1.45**
5	5	NA	25	1.21**	25	1.16**	84	2.02**	134	1.38**	245	1.33**
6	6	NA	26	1.21**	26	1.16**	30	1.93**	140	1.36**	23	1.24**
7	7	NA	29	1.21**	29	1.16**	89	1.64**	1	1.30**	36	1.24**
8	8	NA	55	1.21**	55	1.16**	58	1.60**	52	1.29**	150	1.16**
9	9	NA	56	1.21**	56	1.16**	210	1.58**	45	1.28**	86	1.12**
10	10	NA	62	1.21**	62	1.16**	186	1.57**	61	1.23**	29	1.11**
11	11	NA	67	1.21**	67	1.16**	93	1.57**	53	1.23**	208	1.10**
12	12	NA	69	1.21**	69	1.16**	25	1.49**	117	1.22**	213	1.07**
						The worst DHL						
1	253	NA	233	-1.48**	233	-1.48**	102	-0.97**	128	-1.51**	238	-1.75**
2	254	NA	238	-1.48**	238	-1.48**	59	-1.03**	192	-1.66**	204	-1.87**
3	255	NA	153	-1.93**	153	-1.92**	91	-1.07**	141	-1.87**	27	-1.99**
4	256	NA	135	-2.38**	135	-2.37**	158	-1.08**	215	-2.12**	105	-2.45**
SE gi		NA		0.09		0.12		0.02		0.02		0.02
	(+)	0		138		137		93		141		131
	(-)	0		66		66		155		103		112

*(+)= Number of DHL's showing significant and positive GCA effects, (-)=Number of DHL's showing significant and negative GCA effects, **indicate significant at 0.01 probability level*

Table 6. Continue

No.	GYPP						GYPH					
	WW		WSF		WSG		WW		WSF		WSG	
	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA	DHL	GCA
	The best DHL											
1	87	3.34**	16	3.02**	66	2.11**	96	3.03**	16	2.36**	66	2.37**
2	96	2.87**	90	2.06**	205	1.96**	87	2.96**	204	1.61**	208	2.00**
3	25	1.97**	19	1.94**	153	1.62**	149	1.51**	44	1.59**	87	1.51**
4	16	1.75**	44	1.68**	171	1.57**	153	1.48**	66	1.53**	15	1.49**
5	83	1.66**	60	1.58**	26	1.47**	16	1.45**	62	1.50**	26	1.46**
6	85	1.60**	58	1.54**	81	1.45**	25	1.44**	2	1.46**	205	1.41**
7	149	1.50**	14	1.46**	89	1.42**	14	1.41**	14	1.45**	39	1.40**
8	58	1.48**	117	1.40**	36	1.37**	209	1.40**	161	1.43**	177	1.39**
9	194	1.44**	66	1.39**	95	1.34**	134	1.31**	76	1.41**	102	1.37**
10	153	1.32**	68	1.35**	87	1.31**	53	1.23**	160	1.40**	153	1.31**
11	89	1.14**	76	1.33**	96	1.29**	81	1.20**	60	1.36**	125	1.30**
12	203	1.13**	161	1.24**	136	1.27**	194	1.15**	58	1.33**	95	1.20**
	The worst DHL											
1	231	-1.46**	223	-1.39**	105	-1.44**	229	-1.52**	41	-1.43**	250	-1.51**
2	28	-1.52**	236	-1.42**	250	-1.46**	28	-1.62**	188	-1.53**	256	-1.56**
3	196	-1.66**	192	-1.64**	256	-1.47**	196	-1.68**	192	-1.67**	105	-1.56**
4	130	-1.76**	215	-1.76**	238	-1.48**	130	-1.69**	215	-1.83**	21	-1.60**
SE gi		0.35		0.25		0.21		0.35		0.24		0.17
	(+)	40		64		80		41		66		82
	(-)	42		66		84		43		68		86

*(+)= Number of DHL's showing significant and positive GCA effects, (-)=Number of DHL's showing significant and negative GCA effects, **indicate significant at 0.01 probability level*

From Table 6, it is clearly observed that for a given trait, the rank of parent doubled haploid lines for GCA effects was the same rank of their top crosses for mean performance. Therefore, the best 25 DH lines for GCA effects indicated the best 25 top crosses that include the same 25 DH lines as one of their parents and the worst 10 lines in GCA effects indicated that their top crosses are the worst 10 for a given trait.

Based on top cross analysis, the DH lines having positive and significant GCA effects (breeding values) for GYPP, GYPH, EPP and LR and those having significant and negative GCA effects for DTA, DTS, ASI, PH, EH and BS traits are desirable for future exploitation, because additive gene action operates in these lines. On the contrary, the DH lines having significant or non-significant and negative GCA effects or non-significant and positive GCA effects for GYPP, GYPH, EPP and LR and those having significant or non-significant and positive GCA effects or non-significant and negative GCA effects for DTA, DTS, ASI, PH, EH and BS traits are undesirable and should be eliminated from the beginning of the breeding program.

For grain yield/ha, number of desirable DH lines for further exploitation in breeding programs was 66 for drought tolerance at flowering and 82 for drought tolerance at grain filling; the best 25 DHL's for each group are presented in Table 6. The rest of tested DHL's in the top crosses, *i.e.* 188 and 172 DHL's under WSF and WSG, respectively will be eliminated. The best ten DHL's in GCA effects for GYPH are No. 16, 204, 44, 66, 62, 2, 14, 161, 76 and 160 under WSF and 66, 208, 87, 15, 26, 205, 39, 177, 102 and 153 under WSG conditions. Under wall watering conditions, the best ten DHL's in GCA effects for GYPH are No. 96, 87, 149, 153, 16, 25, 14, 209, 134 and 53.

The best 25 DHL's in GCA effects (breeding value) for GYPP were approximately the same best 25 DHL's in GCA effects for GYPH, but with different ranking. This is also true for the worst 10 DHL's (Table 6).

For DTA, DTS, PH, EH, ASI, LR and EPP traits, number of desirable DH lines for GCA effects were 94, 108, 112, 91, 83, 138 and 141 under WSF conditions and 110, 83, 81, 76, 60,137 and 131 under WSG conditions.

Hybridization between drought tolerant inbreds (PHM6T – PHJFN – PH1723) and good general combiners (PH12J4 – PH1CGY – PHM7E) followed by producing doubled haploid lines *via* inducer (*in vivo*) technique had therefore been

successful in developing transgressive segregants of superior genetic recombinations for grain yield than their parents, which transmitted to their testcrosses making them higher yielders than the best check cultivar in this study under drought stress conditions. This technique besides its advantage in shortening the time (5 to 6 generations of selfing) required for reaching complete homozygosity of the pure (inbred) lines, it proved a great success in developing improved genotypes in many countries such as China, France, Hungary and Canada [37-39].

4. CONCLUSIONS

Using the inducer technique was successful in producing doubled haploid lines (DHL) of maize, which produced DHL's × tester crosses; some of them showed superiority to the best check cultivar in the present study (PH 30R77) in grain yield under drought stress at flowering and grain filling and under well watering. It was concluded from this investigation that for grain yield, the number of desirable DH lines for further exploitation in breeding programs, which showed significant and positive breeding values (general combining ability), was 66 for drought tolerance at flowering and 82 for drought tolerance at grain filling, because additive gene action for grain yield operates in these lines. These desirable DH lines should be evaluated in the second stage to identify the best ones for inclusion in a diallel analysis to identify the best F_1's for specific combining ability. The rest of genotypes could safely be eliminated from the breeding program aiming at developing drought tolerant maize hybrids. Data also concluded that the best DH lines for GCA effects indicated the best top crosses that include the same DH lines as one of their parents and the opposite was true.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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