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Identification of a Molecular Markers Linked to Drought Tolerance in Some Genotypes of Barley

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Abstract: Drought is by far the most important environmental stress in agriculture and many efforts have been made to improve croup productivity under water-limiting conditions. While natural selection has favoured mechanisms for adaptation and survival, breeding activity has directed selection towards increasing the economic yield of cultivated species. More that 80 years of breeding activities have led to some yield increase in drought environments for many crop plants. Meanwhile, fundamental research has provided significant gains in the understanding of the physiological and molecular responses of plants to water deficits, but there is still a large gap between yields in optimal and stress conditions. minimizing the 'yield gap' and increasing yield stability under different stress conditions are of strategic importance in guaranteeing food for the future. This studies aimed to study the genetic behavoiur of drought tolerance for some genotypes of barley using half diallel analysis under normal and drought conditions, in addition to molecular markers to identify the bands of drought tolerance using HAL-137 primer as index for root xylem vessel number trait, HAL-139 primer as index for root volume trait and OS-R16 primer as index for waxy layers of leafs trait and water-soluble protein in the cultivars Giza 123, Giza 129, Giza 130, Giza 132 and Giza 2000, respectively. The results showed that the best genotypes using traditional breeding and molecular markers techniques " Specific – PCR " were Giza 123, Giza 132, Giza 2000, Giza 123 × Giza 2000, Giza 129 × Giza 132 and Giza 132 × Giza 2000 for drought tolerance in barley.

Key words: Barley, breeding, drought tolerance, yield components, molecular markers.

INTRODUCTION

Drought is the most significant environmental stress in agriculture worldwide and improving yield of barley under drought is a major goal of plant breeding. A review of breeding progress pointed out that selection for high yield in stress-free conditions has, to a certain extent, indirectly improved yield in many water-limiting conditions. Further progress requires the introduction of traits that reduce the gap between yield potential and actual yield in drought-prone environments. To achieve this three main approaches can now be exploited: (i) plant physiology has provided new insights and developed new tools to understand the complex network of drought-related traits, (ii) molecular genetics has discovered many QTLS affecting yield under drought or the expression of drought tolerance-related traits, (iii) molecular biology has provided genes useful either as candidate sequences to dissect QTLS or for a transgenic approach. The extent of information that breeders have now offers them new tools for breeding, such as markers for QTLS and single genes for plant transformation. Breeders are thus asked to blend together all knowledge on the traits sustaining yield under drought and to accumulate the most effective QTLS and/or transgenes into elite genotypes without detrimental effects on yield potential. This strategy will lead to new cultivars with high yield potential and high yield stability, that in turn will result in superior performance in dry environments for some genotypes of barley.

MATERIALS AND METHODS

The present investigation was carried out in the farm of mansourah and department of genetic and cytology, national research center from 2009 to 2011 seasons. This work aimed to study the genetic behavior of some genotypes of barley under normal and drought conditions. Five barley cultivars with different reactions for drought were used in ahalf diallel analysis using Griffing (1965) model 1, methods 2.

Five barley parents namely; Giza 123, Giza 129, Giza 130, Giza 132 and Giza 2000 were grown in three planting dates with ten days interval in order to overcome the differences in flowering time between plants to make a hybridization from December 2009 to April 2010.

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In 2011 season, the genotypes parents and their F1 crosses were grown in a randomized complete block design, exactly from December 2010 to April 2011 and replicated three times under normal and drought conditions.

Normal conditions was three irrigations, the first one was the sowing irrigation " the end of December ", the second irrigation was after one month from the first one " At maximum tillering stage " and the third irrigation was after one month from the second irrigation " At maturity stage ", while drought conditions was the sowing irrigation only.

All other recommendation of barley planting was followed in the same season from December 2010 to April 2011 in the farm of mansourah.

Table 1: The main characteristics for some genotypes of barley using half diallel design.

Genotypes	Parentages	Drought tolerance	Duration " Day "
Giza 123	Giza 117 / FAO 86	Tolerance	140
Giza 129	Deir Alla 106 / cel // As 46 / A ths*2"	Susceptible	135
Giza 130	CC229 // Bco Mr / DZ02391 / 3 / Deir Alla 106	Moderate	139
Giza 132	Rihane-O5 // AS46 / Aths*2" Aths / Lignee 686	Tolerance	144
Giza 2000	Giza 117 / Bahtim 52 // Giza 118 / FAO 86	Tolerance	140

Traits Studied:

Thirty plants were taken from the parents and F1 crosses at random from each replicate to determine all traits studied:

1- **Heading date (days):** Number of days from sowing to 50% heading.

2- **Plant height (cm):** Was measured in centimeter from soil surfaces to the top of the main stem at harvesting.

3- **Grain yield (ardab/fed):** Estimated by weighting the total grain yield/plot after threshing and then converted to (ardab/fed).

4- **Maximum root length (cm):** Measured in centimeters from the tillering plateau to the longest root tip.

5- **Root volume:** Volume of all root system was determined in cubic centimeters using standard column.

6- **Root xylem vessel number:** The average xylem vessel number of roots of the same plant was counted under light microscope.

Statistical Analysis:

The analysis of variance and expected mean squares of all genotypes " parents and their F1 crosses " was computed according to IRRI STA T V.6. and Griffing 1956 model 1, method 2.

Estimation of Heterosis:

The heterosis of an individual cross was determined for each trait as the increase of the F1 hybrid men over its better parent, (i.e. heterobeltiosis) as follows:

Heterosis over the better parent % = $\frac{\overline{F_1} - \bar{B}.\bar{P}.}{\bar{B}.\bar{P}.} \times 100$

Where :

$\overline{f_1}$ = Mean value of the first generation

$\bar{B}.\bar{P}.$ = Mean value of the better parent.

LSD. values were calculated to test the significance of the heterosis effect, according to the following formula suggested by Wyanne *et al*. (1970).

LSD for better parent heterosis = $t\sqrt{\frac{2MSe}{r}}$

t = Tabulated value at the specified level of probability for the experimental error.
MSe = The experimental error mean squares.
r = Number of replications.

Molecular Genetic Studies:

SDS-Protein Electrophoresis:

SDS – Polyacrylamide gel electrophoresis (SDS - PAGE) analysis was performed for water soluble proteins according to Laemmli (1970) and Modifies by Studier (1973).

Total Seed Proteins:

Total proteins were extracted from seeds of the parental lines of indifidual plant. Seeds were hulled manually and their grouned to afire powder using amortar and pestle total proteins were extracted from each genotype with 1 ml of sodium dodecyl sulfate (SDS). After the gels had been stained with coomassie Blue and destained with a 50% methanol and 10% glacial acetic acid mixture.

Specific – PCR Protocol:

DNA was extracted from the leaves of the selected plants of five barley parents which different reaction of drought tolerant, moderate and sensitive according to the method of Graham et al. (1997).

Gel electrophoretic buffers:

TBE buffer	10 x
Tris	10.80 g
Boric acid	5.50 g
EDTA	0.74 g
H2O (dd)	up to 100 ml

Loading buffer:

Tris	10.8 g
Boric acid	5.5 g
EDTA	0.74 g
H_2O (dd)	up to 100 ml

Agarose Gel Electrophoresis:

PCR amplification products were analyzed using 1.5% agarose gel electrophoresis in 1 × TBE buffer and stained with ethidium bromide. The run was performed at 100 V in Bio Rad submarine. The bands of amplified DNA ladder of 100 to 2000 base pairs, and photographed with gel documentation system.

Gel Analysis:

Gels were photographed under Ultra violet light with Polaroid film 667 and scanned with Bio-Rad video densitometer Model 620 at a wave length of 557 software data analysis for Bio-Rad model 620 USE densitometer and computer were used.

Table 2: The sequences of the three primers using Root xylem vessel number, root volume and waxy layers of leafs traits as index for drought tolerance in barley.

Primer names		Sequences
HAL-137	Reverse	5\ CATGAATCCGGCTTACTGCA 3\
	Forward	5\ ACGGTACCTAACGATTCTGC 3\
HAL-139	Reverse	5\ TCTAAGGCCATCCTAGGATG 3\
	Forward	5\ AATCCTGTTGCCGGAATCTG 3\
OS-R16	Reverse	5\ AACCTAGGTTCCCGGGAAAC 3\
	Forward	5\ CCAAAATTTTCCGGGATCCA 3\

* The first primer for root xylem vessel number trait.
** The second primer for root volume trait.
*** The third primer for waxy layers of leafs trait.

RESULTS AND DISCUSSION

A. variations and Interaction:

The analysis of variance for half dialed analysis of all traits studied of barley under all conditions are showed in table (3).

Mean squares of all genotypes were found to be highly significant for all traits studied under normal and drought conditions indicating over all differences among these populations of barley. Both general and specific combining ability effects were showed to be highly significant also for all traits studied and observed the importance of both additive and non-additive genetic variances in determining the importance of these traits. similar results were reported by Abo-Elenein et al. (1981), Ahmed et al. (1998) and El-Mouhamady (2010).

B. Mean Performance:

The results in table (4), showed that the earlier plants were Giza 123, Giza 2000, Giza 123 × Giza 132, Giza 129 × Giza 132 and Giza 130 × Giza 132 under normal and drought conditions and the mean values were ranged from 81.69 to 94.76 "day" and from 77.45 to 90.32 "day" under normal and drought conditions respectively for heading date trait while, the genotypes; Giza 129, Giza 130, Giza 123 × Giza 132, Giza 123 × Giza 2000, Giza 129 × Giza 132, Giza 130 × Giza 132 and Giza 132 × Giza 2000 recorded the lowest values for plant height under all conditions.

Table 3: Mean Squares estimates of ordinary analysis for some traits of barley under normal and drought conditions.

S.O.V	D.F.	M.S											
		Heading date (day)		Plant height (cm)		Grain yield		Maximum root length (cm)		Root xylem vessel number		Root volume	
		N	D	N	D	N	D	N	D	N	D	N	D
Genotypes	14	** 426.30	** 152.0	** 116.43	** 112.13	** 102.70	** 143.18	** 108.32	** 86.72	** 100.0	** 104.30	** 170.18	** 90.0
Parents	4	** 173.32	** 148.60	** 83.57	** 60.14	** 50.12	** 37.32	** 28.73	** 19.70	** 170.13	** 113.20	** 40.70	** 32.10
Crosses	9	** 95.42	** 136.18	** 60.13	** 70.18	** 73.15	** 80.19	** 50.18	** 40.30	** 60.12	** 80.0	** 14.70	** 22.13
P. VS. C.	1	** 39.16	** 45.18	** 106.32	** 117.40	* 16.08	** 37.13	** 70.41	** 106.33	** 80.33	** 70.63	** 41.60	** 30.50
Error	28	0.32	0.18	0.56	0.72	0.93	0.52	0.28	0.36	0.19	0.27	0.33	0.45
GCA	4	** 96.83	** 127.63	** 56.0	** 43.0	** 76.73	** 207.31	** 166.30	** 144.32	** 172.0	** 96.70	** 64.0	** 22.30
SCA	10	** 142.70	** 138.80	** 87.72	** 109.06	** 56.70	** 117.27	** 76.32	** 90.18	** 40.0	** 23.70	** 100.73	** 86.0
GCA/SCA		0.33	0.74	0.54	0.18	1.07	1.03	1.04	1.00	1.12	1.05	0.82	0.73
Error	28	0.46	0.37	0.27	0.68	0.78	0.30	0.35	0.43	0.13	0.19	0.52	0.70

* : Significant at 0.05 N:Normal conditions D:Drought Conditions
** : Significant at 0.01

For grain yield, the best genotypes were obtained from Giza 123, Giza 130, Giza 132, Giza 2000, Giza 123 × Giza 132, Giza 129 × Giza 132 and Giza 130 × Giza 132 under normal and drought conditions, which indicated that these genotypes were highly tolerant for drought in barley under Egyptian conditions, while, the genotypes; Giza 123, Giza 130, Giza 132, Giza 2000, Giza 123 × Giza 130, Giza 123 × Giza 132, Giza 129 × Giza 132, Giza 130 × Giza 132 and Giza 132 × Giza 2000 were the highest mean values for maximum root length under all conditions.

With respect to root xylem vessel number, the most desirable genotypes were showed from Giza 123, Giza 130, Giza 132, Giza 2000, Giza 123 × Giza 132, Giza 129 × Giza 130, Giza 129 × Giza 132, Giza 130 × Giza 132 and Giza 132 × Giza 2000 under normal and drought conditions, while, Giza 130, Giza 132 and Giza 2000, Giza 123 × Giza 129, Giza 123 × Giza 130, Giza 123 × Giza 132, Giza 130 × Giza 132 and Giza 132 × Giza 2000 were recorded the highest mean values under all conditions for root volume, which, indicated that maximum root length, root xylem vessel number and root volume traits were index for drought tolerance in barley. similar results were detected by Abo-Elenein et al. (1981), Gorham and Chapman (1992), Vish Wakarma and Sharma (1998), El-Said (2007) and Weerakoon et al. (2008).

Table 4: The genotypes mean performance for all traits studied of barley under normal and drought conditions.

Genotypes	Heading date (day)		Plant height (cm)		Grain yield		Maximum root length (cm)		Root xylem vessel number		Root volume	
	N	D	N	D	N	D	N	D	N	D	N	D
Giza 123	84.31	81.29	108.54	105.40	20.74	17.92	36.40	42.12	11.73	10.13	37.27	45.81
Giza 129	88.50	83.39	100.34	94.0	17.84	12.36	19.18	20.43	7.18	5.32	19.43	12.70
Giza 130	91.73	89.50	106.54	103.42	23.32	20.23	48.71	56.30	12.66	15.49	87.13	92.73
Giza 132	92.04	90.03	112.43	109.40	27.63	26.00	65.19	71.23	14.67	19.32	121.05	130.14
Giza 2000	86.43	81.07	113.87	108.42	21.74	18.03	53.13	58.71	20.58	23.14	62.57	40.12
Giza 123 × Giza 129	85.73	82.03	112.18	106.04	15.40	11.09	29.31	26.74	13.71	10.45	51.30	37.92
Giza 123 × Giza 130	88.07	86.79	110.64	106.32	24.89	22.67	52.37	57.04	15.87	17.03	101.52	123.14
Giza 123 × Giza 132	81.69	77.45	106.14	104.05	32.19	30.78	49.39	54.12	17.93	22.43	200.17	231.02
Giza 123 × Giza 2000	86.12	82.09	111.77	106.59	22.14	19.66	41.43	44.70	14.93	16.72	16.72	19.56
Giza 129 × Giza 130	90.73	86.49	114.84	110.72	25.46	23.02	46.21	51.43	15.81	17.13	69.71	77.62
Giza 129 × Giza 132	85.34	81.04	109.50	106.72	28.43	26.72	81.83	84.02	20.15	24.63	50.08	57.12
Giza 129 × Giza 2000	94.76	90.32	115.83	111.49	23.17	20.45	24.31	22.17	13.43	11.08	34.72	39.53
Giza 130 × Giza 132	83.14	80.66	104.91	100.18	32.42	29.73	84.17	91.73	30.43	37.84	132.91	142.07
Giza 130 × Giza 2000	84.74	79.18	110.37	106.13	26.77	23.92	61.53	65.93	18.94	26.86	90.13	100.84
Giza 132 × Giza 2000	87.16	82.08	109.16	107.30	29.34	26.11	70.41	73.54	41.76	44.18	96.78	104.82
LSD at 0.05	0.44	0.35	0.26	0.65	0.73	0.29	0.33	0.41	0.12	0.18	0.50	0.67
LSD at 0.01	0.59	0.48	0.35	0.88	0.99	0.39	0.45	0.55	0.16	0.22	0.67	0.91

N:Normal Conditions
D:Drought Conditions

C. Heterosis Over Better Parent:

The results in table (5). Showed that, four out of ten crosses detected highly significant and negative values of heterosis over better – parent under all conditions for heading date, which indicated that crosses namely; Giza 123 × Giza 132, Giza 129 × Giza 132, Giza 130 × Giza 132 and Giza 130 × Giza 2000 were greater importance for additive gene action to control this trait.

For plant height; The crosses; Giza 123 × Giza 132, Giza 130 × Giza 132 and Giza 132 × Giza 2000 showed highly significant and negative values of heterosis over better – parent under normal and drought conditions, which observed the importance of additive and additive x additive types of gene action. similar results were obtained by Vish Wakarma and Sharma (1998), El-Said (2007) and El-Mouhamady et al. (2010).

With respect to grain yield, All crosses were detected highly significant and positive values of heterosis over better – parent under all conditions except Giza 123 × Giza 129, which observed that these crosses were good combiners of this trait to improve drought tolerance of barley. These results were in agreement with those reported by El-Mouhamady et al. (2010).

The crosses; Giza 123 × Giza 130, Giza 129 × Giza 132, Giza 130 × Giza 132, Giza 130 × Giza 2000 and Giza 132 × Giza 2000 showed highly significant and positive values of maximum root length under all conditions, while, the crosses; Giza 123 × Giza 129, Giza 123 × Giza 130, Giza 123 × Giza 132, Giza 129 × Giza 130, Giza 129 × Giza 132, Giza 130 × Giza 132, Giza 132 × Giza 2000 under normal and drought condition and Giza 130 × Giza 2000 under drought condition only were expected highly significant and positive values of heterosis over better – parent for root xylem vessel number. These results means the great role of additive and additive x additive gene action to inheritance these traits similar results were obtained by El-Said (2007).

For root volume; highly significant and positive values of heterosis over better – parent were observed from the crosses Giza 123 × Giza 129 under normal condition only, Giza 123 × Giza 130, Giza 123 × Giza 132, Giza 130 × Giza 132 and Giza 130 × Giza 2000 under all conditions which indicated that, these crosses were great importance for specific combining ability effects and improve drought tolerance in barley. These results were in agreement with those reported by Abo-Elemein et al. (1981). Ahmed et al. (1998a), El-Said (2007) and Aermae (2011).

It could be concluded that the most desirable genotypes for all traits studied under all conditions were Giza 123, Giza 132, Giza 2000, Giza 123 × Giza 132, Giza 129 × Giza 132, Giza 129 × Giza 2000 and Giza 132 × Giza 2000.

Table 5: Estimates of heterosis% over-better-parent for some genotypes of barley under normal and drought condition.

Crosses	Heading date		Plant height (cm)		Grain yield (Ardap / Fed)		Maximum root length (cm)		Root xylem vessel number		Root volume	
	N	D	N	D	N	D	N	D	N	D	N	D
Giza 123 × Giza 129	** 1.68	** 0.91	** 11.79	** 12.80	** -25.74	** -38.11	** -19.47	** -36.51	** 16.87	** 3.15	** 37.64	** -17.22
Giza 123 × Giza 130	** 4.45	** 6.76	** 3.84	** 2.80	** 6.73	** 12.06	** 7.51	** 1.31	** 25.35	** 9.94	** 16.51	** 32.79
Giza 123 × Giza 132	** -3.11	** -4.72	** -2.21	** -1.28	** 16.50	** 18.38	** -24.23	** -24.02	** 22.22	** 16.09	** 65.36	** 77.51
Giza 123 × Giza 2000	** 2.14	** 1.25	** 2.97	** 1.13	** 1.84	** 9.04	** -22.02	** -23.86	** -27.45	** -27.74	** -73.27	** -57.30
Giza 129 × Giza 130	** 2.52	** 3.71	** 14.45	** 17.78	** 9.17	** 13.79	** -5.13	** -27.79	** 24.88	** 10.58	** -19.99	** -16.29
Giza 129 × Giza 132	** -3.57	** -2.82	** 9.12	** 13.53	** 2.89	** 2.76	** 25.52	** 17.95	** 37.35	** 27.48	** -58.62	** -56.11
Giza 129 × Giza 2000	** 9.63	** 11.41	** 15.43	** 18.60	** 6.57	** 13.42	** -54.24	** -62.23	** -34.74	** -52.11	** -44.51	** -1.47
Giza 130 × Giza 132	** -9.36	** -9.87	** -1.53	** -3.13	** 17.33	** 14.34	** 29.11	** 28.78	** 107.43	** 95.85	** 52.54	** 9.16
Giza 130 × Giza 2000	** -1.95	** -2.33	** 3.59	** 2.62	** 14.79	** 18.24	** 15.81	** 12.29	** -7.96	** 16.07	** 3.44	** 8.74
Giza 132 × Giza 2000	** 0.84	** 1.24	** -2.90	** -1.03	** 6.18	** 0.42	** 8.00	** 3.24	** 102.91	** 90.92	** -20.05	** -19.45
LSD at 0.05	0.44	0.35	0.26	0.65	0.73	0.29	0.33	0.41	0.12	0.18	0.50	0.67
LSD at 0.01	0.59	0.48	0.35	0.88	0.99	0.39	0.45	0.55	0.16	0.22	0.67	0.91

* : Significant at 0.05
**: Significant at 0.01

D. Combining Ability:

D.1. General Combining Ability Effects:

Estimates of the GCA effects of individual parental lines for all traits studies under all conditions are showed in table (6).

The parents; Giza 123, Giza 132 and Giza 2000 showed highly significant and negative values of GCA effects for heading date and plant height under normal and drought conditions, while, the parents; Giza 123, Giza 132 and Giza 2000 were showed highly significant and positive values for the other traits under all conditions.

It is clear that these varieties indicating that additive x additive types of gene action were of greater importance for inheritance of these traits. similar results were obtained by El-Said (2007) and El-Mouhamady (2010).

D.2. Specific Combining Ability Effects:

SCA effects for the crosses studied are showed in table (7).

The crosses; Giza 123 × Giza 132, Giza 123 × Giza 2000 and Giza 132 × Giza 2000 were showed highly significant and negative values of SCA effect for heading date and plant height under normal and drought conditions, while, the same crosses in addition to the cross; Giza 130 × Giza 2000 detected highly significant and positive values of SCA effects for the other traits under all conditions, which indicated the importance of both additive and non additive types of gene action in the inheritance of these traits to improve drought tolerance in barley under Egyptian conditions. These results were in agreement with those reported by Vish Wakarma and Sharma (1998), El-Said (2007) and Aermae (2011).

It was obvious in table (7). That the most of crosses with high SCA effects involved diverse parents. The superior F' shaving SCA effects are expected to produce desirable trangressive segregates, pro vides that the desirable complementary genes and epistatic effects are coupled in the same direction to maximize these traits in view.

Table 6: Estimates of general combining ability effects for some traits in barley under normal and drought conditions.

Crosses	Heading date		Plant height (cm)		Grain yield (Ardap / Fed)		Maximum root length (cm)		Root xylem vessel number		Root volume	
	N	D	N	D	N	D	N	D	N	D	N	D
Giza 123	** -8.15	** -4.16	* -1.60	* -1.12	** 107.13	** 50.70	** 12.41	** 81.07	** 106.70	** 111.32	** 5.07	** 3.16
Giza 129	0.32	0.18	** 1.97	** 13.60	-0.26	* -1.12	** -16.02	** -7.22	** -68.14	** -6.04	** -10.0	** -4.0
Giza 130	0.58	1.15	0.86	0.34	** 1.19	** -2.05	** 6.13	** 12.70	** -1.13	** -2.07	** -9.13	** -70.0
Giza 132	** -11.30	** -18.64	** -12.70	** -14.68	** 19.39	** 42.16	** 18.06	** 37.54	** 9.15	** 75.13	** 80.03	** 2.09
Giza 2000	** -9.14	** -8.19	** -28.52	** -16.40	** 11.72	** 15.68	** 11.80	** 7.16	** 8.06	** 14.47	** 4.60	** 8.07
LSD at 0.05 (gi)	0.76	1.20	1.02	0.92	0.34	0.28	0.64	0.13	0.56	0.92	0.54	0.47
LSD at 0.01(gi)	0.88	1.76	1.66	1.18	0.88	1.14	1.05	0.48	0.87	1.23	0.76	1.18

* : Significant at 0.05
**: Significant at 0.01
N: Normal
D: Drought

Table 7: Estimates of specific combining ability effects for some traits in barley under normal and drought conditions.

Crosses	Heading date		Plant height (cm)		Grain yield		Maximum root length (cm)		Root xylem vessel number		Root volume	
	N	D	N	D	N	D	N	D	N	D	N	D
Giza 123 × Giza 129	0.056	0.086	** 28.037	** 20.14	** -4.123	** -10.18	** -41.02	** -9.07	** -5.04	** -2.01	** -75.40	** -60.13
Giza 123 × Giza 130	** 13.84	** 12.720	** 4.056	** 3.021	** -17.12	** -8.12	** -14.62	** -8.15	** 1.072	** -8.032	** -11.89	** -19.08
Giza 123 × Giza 132	** -104.32	** -52.180	** -32.12	** -18.43	** 19.72	** 84.113	** 14.60	** 80.13	** 108.60	** 72.13	** 104.70	** 92.70
Giza 123 × Giza 2000	** -172.11	** -79.06	** -17.41	** -6.19	* 18.60	** 29.40	** 50.11	** 43.06	** 21.09	** 16.30	** 30.17	** 17.62
Giza 129 × Giza 130	** 6.182	** 9.134	** 19.872	** 51.032	** 46.03	** -7.011	** -11.12	** -15.34	** -30.52	** -107.80	* -26.04	** -39.07
Giza 129 × Giza 132	** 136.140	** 102.94	** 26.324	** 17.102	** -12.54	** -9.17	** -54.60	** -10.103	** -7.182	** -12.60	** -18.76	** -9.072
Giza 129 × Giza 2000	** 34.106	** 30.108	** 13.20	** 10.104	** -0.580	** -0.713	** -62.73	** -80.14	** 0.563	** -7.37	** -14.68	** -27.13
Giza 130 × Giza 132	** 7.506	** 14.206	** 41.320	** 37.112	** -26.39	** -40.03	** -13.728	** -5.132	** -1.18	** -2.03	** -80.19	** -6.321
Giza 130 × Giza 2000	** 11.712	** 13.082	** 1.112	** 2.063	** 22.09	** 70.12	** 4.06	** 13.09	** 4.03	** 10.17	** 71.89	** 37.11
Giza 132 × Giza 2000	** -74.98	** -11.43	** -12.93	** -171.13	** 13.92	** 19.07	** 7.16	** 8.13	** 28.13	** 12.60	** 63.71	** 160.740
LSD at 0.05	0.128	0.113	0.426	0.027	0.113	0.092	0.071	0.821	0.172	0.140	0.567	0.426
LSD at 0.01	0.196	0.154	0.864	0.103	0.227	0.105	1.03	1.09	0.205	1.032	0.729	0.609

* : Significant at 0.05
**: Significant at 0.01
N: Normal
D: Drought

E. Total Soluble Proteins (TSP):

Total soluble proteins using five parents of barley namely, Giza 123, Giza 129, Giza 130, Giza 132 and Giza 2000 were observed in fig (1) and table (8).

All bands were observed in the five cultivars of barley, thus these bands were common bands in these cultivars, but the bands number 1 and 6 with molecular weight of 200 and 80 KDa, respectively, weren't appeared in the parents Giza 129 and Giza 130 only this appearance for the other bands maybe referred to the synthesis of specific protein bands linked with classification of these genotypes of barley.

F. Specific – PCR – Markers:

The results in Fig (2); revealed that the band number 4 with molecular weight of 1600 bp was appeared in all cultivars of barley, which indicated that this band was specific marker for drought tolerance in barley using HAL-137 primer by high number of root xylem vessel number.

The band number 4 with molecular weight of 1350 bp was appeared in all parents of barley, except Giza 2000, while the band number 3 with molecular weight of 1400 bp was showed in Giza 2000 only in fig (3), using HAI-139 primer, which indicated that these two bands were index for drought tolerance by increasing root volume trait in barley.

Using OS-R16 primer as index for drought tolerance in barley by increasing number of waxy layers of leafs in fig (4). The band number 3 with molecular weight of 1800 bp was observed in the five cultivars of barley, which means that this band was specific band for drought tolerance using waxy layers of leafs to decrease the level of water loss during the peried of maturity.

From the previous results, it could be included that the traits root xylem vessel number, root volume and waxy layers of leafs were greater importance for drought tolerance in barley by increasing the activation of root system to get the water from the deepest layer of soil during drought conditions.

Similar results were reported by Albayrak and Gozukirmizi (1999), Ribaut and Betran (2000), Singh et al. (2004), Quinirin et al. (2005), El-Said (2007) and Weerakoon (2008).

The strategy of pyramiding of partial resistance loci of drought to derive tolerance cultivars (horizontal resistance) is actually suggested, with possible contribution to durable resistance. High levels of tolerance, reaching near immunity , have already been obtained by intercrossing with many such un-known resistance genes (Singh et al. 2004). Therefore, it is important to assess critically the utility of such diversity and precisely identify the loci responsible for the high tolerance, to ensure its systematic utilization in breeding for durable drought tolerance in barley.

Table 8: The Densitometric analysis of water soluble protein for some cultivars of barley.

No. of Band	Molecular weight (KDa)	Giza 123	Giza 129	Giza 130	Giza 132	Giza 2000
1	200	1	0	1	1	1
2	165	1	1	1	1	1
3	130	1	1	1	1	1
4	125	1	1	1	1	1
5	85	1	1	1	1	1
6	80	1	1	0	1	1
7	70	1	1	1	1	1
8	65	1	1	1	1	1
9	55	1	1	1	1	1
10	45	1	1	1	1	1
11	40	1	1	1	1	1
Total of band		11	11	10	11	11

Conclusion:

* The most desirable mean value, positive and highly significant for heterosis, general and specific combining ability effects for all traits studied under all conditions were detected from the genotypes; Giza 123, Giza 130, Giza 2000, Giza 123 × Giza 2000, Giza 129 × Giza 132 and Giza 132 × Giza 2000 for drought tolerance in barley.

** Three primers were index for drought tolerance in barley using root xylem vessel number, root volume and waxy layers of leafs namely; HAL-137, HAL-139 and OS-R16 primer respectively, included Giza 123, Giza 129, Giza 130, Giza 132 and Giza 2000 and water soluble protein to identify these cultivars for the reaction of drought tolerance .

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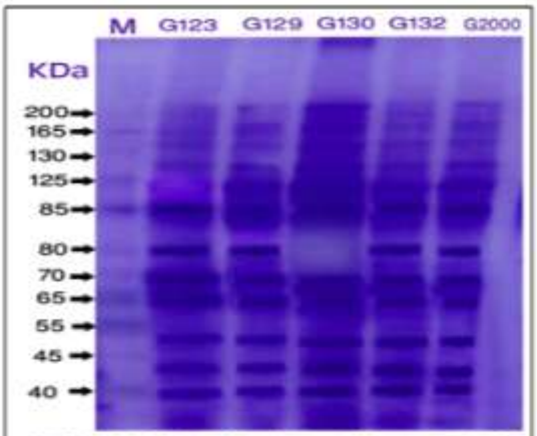
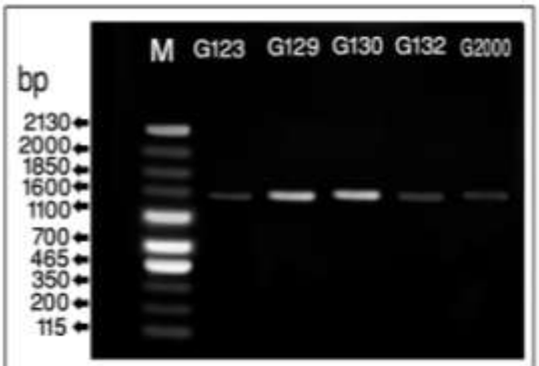
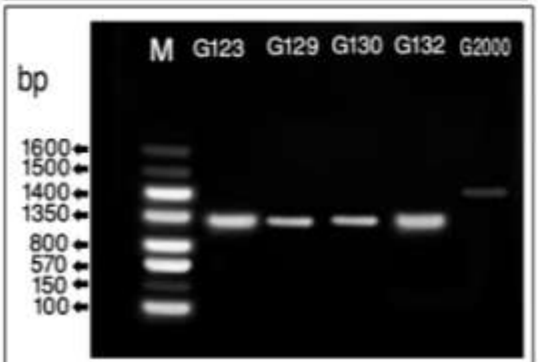


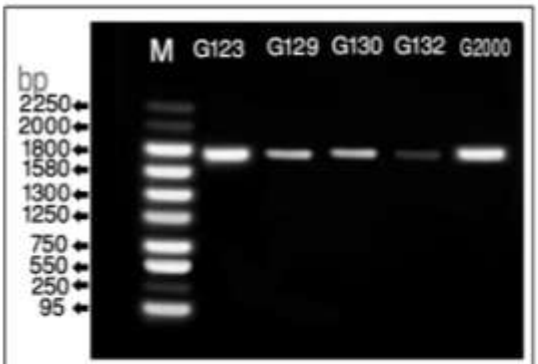
Fig(1) :SDS-PAGE of leafs protein(water-soluble protein)using some cultivars of barley



Fig(2):The densitometric analysis of specific PCR using roor xylem vessel number trait as index for drought tolerance of barley by HAL-137 primer



Fig(3):The densitometric analysis of specific PCR using roor volume trait as index for drought tolerance of barley by HAL-139 primer



Fig(4):The densitometric analysis of specific PCR using waxy layers of leafs trait as index for drought tolerance of barley by OS-R16 primer

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