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Molecular Genetic Studies on Some Barley Entries for Drought Tolerance

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Abstract : Five barley lines with different reaction for drought tolerance were hybridization using half diallel analysis without reciprocals to produce F1 hybrids. All genotypes (5 Parents and their 10 F1 crosses) were estimated on the farm of (Nubaria , Beheira governorate under normal and water deficit conditions) for knowing the genetic behavior responsible for drought stress tolerance based on studding morphological and physiological traits beside appreciation DNA-fingerprinting using (RAPD-PCR) through five primers for the five parents and the best five crosses revealed from the highest calculated data resulting from the genetic parameters of the aforementioned traits under drought stress conditions compared with the control treatment. Plant height, days to maturity, grain yield/plant, maximum root length, number of roots/plant, relative water content, osmotic pressure and canopy temperature traits were the most measurements calculated under both treatments in all genotypes of barely based on genetic parameters such as mean performances, analysis of variance, heterosis over better–parents, combining ability effects with both types in addition to tolerance indices. From the previous results can be seen that the entries ; (P1, P2, P1 X P2, P1 X P3, P1 X P4, P2 X P4, P2 X P5) considered as the most desirable genotypes for drought stress tolerance where they achieved the highest values and detected significant and highly significant positively values of heterosis over better-parent, GCA and SCA effects in all traits under both treatments of irrigation beside exhibited highly data of tolerance indices especially(MP , GMP , YSI , DTI ,YI) and lowest data of (DSI , YR) respectively. Five different primers were recorded total of 43 reproducible amplification products, where 30 of them were polymorphic bands with (69.76 %) polymorphism and 13 fragments were monomorphic. The genetic similarity ranged from 54.50 to 95.20%, with an average of 74.85%. Cluster analysis divided the ten barley entries into two main clusters. The first cluster contained the hybrid H3 (P1 X P4) only, while the second cluster divided into two sub-clusters; the first sub-cluster contained the genotype H5 (P2 X P5) only, while the second sub-cluster contained the other entries, respectively.

Keywords: Barley, Drought stress, GCA, SCA effects, Half Diallel analysis, DNA – RAPD markers.

Introduction

Barley crop is considering an important grain globally and locally and occupies the fourth place in terms of importance after wheat, maize and rice. Barley is using as food for humans and animals for more than ten

Centuries BC. This crop is containing some traits recipes unique from the rest of the most important grain crops such as highly recipes wide regionalization environmentally more than any other cereal crop and using to feed humans, animals and malt extract of it used in the manufacture of beer, which was superposing from the abstracts of other crops.

Beside that barley flour makes up the large gap in the municipal baking industry. Thus, the importance of this crop is not less than the importance of other strategy crop. There are a number of environmental barriers that reduce the production of barley and limit its spread, such as lack of an appropriate temperatures needed for growth and flowering, high toxicity of heavy metals, high or low the (PH) about natural limit appropriate for growth, as well as the problem of soil salinity and water deficit conditions. Drought stress problem is a main abiotic stress that diversely effects on barley production spread on all the world. Thus, research into crop administration exercises that boost drought stress resistance and plant growth when moisture nutrition is decreased has be accretion fundamental. Barley germplasm is a fortune trove of profitable genes and supply wealthy exporters of genetic divergence of crop development. [1] Revealed that the average yield decreasing in 11 barley lines caused by drought stress conditions was 28.05%.The capability of a genotype to manufacture highest and favorable yield above a beamy ambit of stress and non-stress environments is very paramount. [2]Ratifies in order to settlement over ambiences and yield prospect are more or less autonomous of each other. [3]Proposed that one technique of breeding for boosting performance under water deficit conditions might be to breed for superior yield under optimum conditions on the supposition that the preferable entries might like wise execute quite under sub optimum conditions. [4] Marked out that a high yield base line that authorizes a genotype to do well over the limit of environments does not glimpse drought stress tolerance. They acquainted water deficit tolerance like the ability to diminish yield damage in the non-attendance of soil water availability. The exemplary status would be to have a highly stabilized entry with high yield potential [5,6] .The incorporation of high yield steadiness and high proportional yield under drought stress has been proposition like advantageous selection standard for charactering genotypic performance under converting degree of water deficit conditions[7] . [8]Established collection of drought stress sensibility indicator (calculate of yield stabilization) *vs.* genealogical yield beneficial indistinguishing entries with yield prospect and comparatively steady yield performance under several stresses of drought.[9]Studied drought stress tolerance in some barley entries and revealed that tolerance indices specially (MP, GMP, DTI, YI, YSI) were recorded the highest values in the tolerance entries of barley compared with the control treatment of irrigation .Many researchers worked hard to know the effect of drought and salinity stress on the structure of DNA and also the role of mutations for induction the resistance for water stress through exposure to (EMS)[10,11].The objectives of this investigation, thence, were to screen barley lines with high yield potential and steadiness under water deficit conditions. The tended research recent to address the problem of drought stress through importing the resistant lines which high yielding and tolerance for stresses like water deficit conditions from abroad and hybridized it with the Egyptian varieties which sensitive and moderate sensitivity to drought stress tolerance, Then continuing in planting these crosses several generations and make selection for the strongest plants of drought tolerance after the end of each segregation generation to reach to stable genetically lines, high yield and high resistance for water deficit under the Egyptian conditions in addition to the new methods of biotechnology such as RAPD-PCR , ISSR, SSR markers to determine the genetic and alleles responsible for water deficit resistance in barley, compared between all entries under study and this is the most important goals of this investigation.

Materials and Methods

The present investigation was conducted during the period from September 2015 to march 2016 on the farm of (Nubaria, Beheira governorate, Genetics and Cytology Department, Genetic Engineering and Biotechnology Division , National Research Centre , Dokki , Giza , Egypt) using five barley lines with different reaction for drought tolerance and their 10 F1 crosses through two treatments irrigation (normal and drought stress conditions).The five lines of barley were grown in a randomized complete block design through three planting dates with ten days interval in order to overcome the differences in flowering time between these lines for crossing under greenhouse conditions in agriculture research center in September 2015 to produce F1 hybrids using half diallel analysis without reciprocals. All entries (Parents and F1 crosses) were grown under two treatments of irrigation (normal and drought stress conditions) with three replicates for each treatment in the farm of national research center in (Nubaria , Beheira governorate in the period from the half of November 2015 to 25 march 2016 , respectively. The first irrigation treatment (normal condition) was irrigated two times after planting irrigation and the period of time between each two irrigates was one month, while the second

treatment (drought stress conditions) was given planting irrigation only and the preceding crop was cotton, respectively. The traits studied were plant height , days to maturity , grain yield/plant , maximum root length , number of roots/plant , relative water content , osmotic pressure and canopy temperature , respectively. Classification of greenhouse: Clay soil, optimum temperature for planting was (18-20^{o}C), while the optimum temperature for flowering was (20-22^{o}C) in addition to the flowering and maturity was needed to 13 light clock/day.

Note:-The two treatments of irrigation were isolated from each other and isolation distance was ten meters between the two treatments of irrigation to prevent nominated water from normal irrigation to drought treatment, the five lines of barley were performed from agriculture research Centre, Field crops research, Barley department, Egypt.

Estimation of tolerance indices:

All tolerance indices were estimated according to [12,13,14,15,16,17,18].

Table (1):- The pedigree and reaction for Drought Tolerance in the five barley lines.

Entries Name	Pedigree	Reaction for Drought Tolerance
Line (1) Beacher	Introduced to Egypt from USA and named Giza-118	Tolerance
Line (2)	Ssn/Bda//Arar/3/Arabayan-01//CI07117-9/DeirAlla 106	Tolerance
Line (3)	Lignee 527/Chn-01/Gustoe/5/Alanda-01/4/WI2291/3/Api/CM67//L2966- 69	Moderate
Line (4)	Giza 119/3/ESCOBA/BRB2//ALELI	Moderate
Line (5)	ACSAD 1182/Harmal-02/Salmas/3/Saico	Susceptible

Abbreviations as follows:

GYP: mean yield under normal conditions, GYD: mean yield under drought conditions , YSI: Yield stability index, YI: Yield index , GMP : geometrical mean productivity, YI: yield index, DTI : drought tolerance index , MP: mean productivity , Yr : yield reduction ratio , DSI: drought susceptibility index, P.H: plant height, D. of. M: Duration of maturity, G.Y/P: Grain yield/plant, M.R.L: Maximum root length, NO.OF.R/P: Number of roots/plant, R.W.C: relative water content , O.P: osmotic pressure, C.T: Canopy temperature, LSD 0.05 or *: Significant at 5% , LSD 0.01 or ** : Significant at 1% or highly significant.

Statistical analysis:

Analysis of calculated date for all traits studied under both treatments of irrigation was performed by the formula of [19](method 2, model 1) for estimating heterosis over better-parent and general and specific combining ability effects, respectively.

Molecular Markers:

DNA Isolation:

The genomic DNA was extracted from fresh leaf of ten barley lines (The five parents which different reaction for drought tolerance and the best five crosses resulting from these parents using half diallel analysis and revealed highly tolerance of drought stress according to the results of all genetic parameters under drought stress treatment compared with the control treatment as follows:-

P1: (Line 1) Beacher , P2: Line 2 , P3: Line 3 , P4 :Line 4 , P5: Line 5and the crosses ; H1: (P1 X P2) , H2: (P1 X P3) ,H3: (P1 X P4) , H4 : (P2 X P4) , H5: (P2 X P5) according to the protocol of Biospin plant genomic DNA extraction Kit (Bio basic) , respectively.

Polymerase chain reaction (PCR) procedure:

A set of five random 10-mer primers, (Table 2) was used in the detection of polymorphism among ten genotypes of barley. These primers were synthesized at RAPD-PCR and carried out according to the procedure given by [20]with minor modifications.

Table (2): Sequences of the 10-merRAPD primers (5'-3')

No.	Code name	5'-3' Sequences
1	OPC10	TGTCTGGGTG
2	OPF-4	GAATGCGGAG
3	OPA-17	GACCGCTTGT
4	OPG-05	CTGACGTCAC
5	OPAM-01	TCACGTACGG

Amplification reaction was carried out in 25µl reaction mixture contained 2µl of genomic DNA, 3µl of the primer, 2.5µl of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase and 200 mm of each dNTPs. The following PCR program was used in a DNA Thermocycler (PTC-100 PCR version 9.0-USA). Initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 42°C for 90 sec. for annealing temperature, 72°C for 90 Sec. and final extension at 72°C for 2 min. Products by RAPD- PCR were separated on 1.5% agarose gels in 1X TAE buffer and detected by staining with ethidium bromide according to [21].

DNA ladder 100bp was used and PCR products were visualized by UV-trans illuminator and photographed by gel documentation system , Biometra-Bio Documentations, the amplified bands were scored as (1) for presence and (0) for the absence of all studied rice according to gel analyzer protocol.

Data Handling and cluster analysis (Phylogenetic Tree):-

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Pairwise components of the ten genotypes based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to[22]**.** The similarity coefficients [23]were, then ,used to construct dendrograms, using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential , Agglomerative , Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System) , version 1.80 (Applied Biostatistics Program).

Results and Discussion

Mean performance:

The mean values of all traits studied namely ; plant height, days to maturity, grain yield/plant, maximum root length, number of roots/plant , relative water content, osmotic pressure and canopy temperature under normal and water deficit conditions were revealed in table (3), respectively.

The most desirable values in all traits studied under both treatment of irrigation were observed in the entries ; (P1, P2, P1 X P2 , P1 X P3 , P1 X P4 , P2 X P4 , P2 X P5), which indicated that these genotypes of barley would be considered highly resistance for drought stress conditions because its exhibited the lowest mean values for days to maturity, in addition revealed shorter plants for plant height , lowest values for osmotic pressure and canopy temperature , while the same entries revealed the highest mean values for the rest traits especially grain yield/plant, maximum root length, number of roots/plant and relative water content, respectively,[9,24,25,26,27,28,29].

There is no doubt that drought stress is one of the biggest environmental problems facing the strategic crops such as barley, because the strong plant of barley which was the most tolerant for drought stress has to be a short or medium-length to carry the largest number of spikes, high yield, early maturity to be able to end its life cycle when beginning water deficit season, It contains maximum root length to reach to water in the distant depths of soil during water shortage case , Including the higher number of roots / plant especially the occasional or adventitious roots, as well as increasing the amount of relative water content in cells which necessary for all biological processes such as photosynthesis during water shortages time, Reducing both canopy temperature and osmotic pressure during water deficit conditions to reduce water loss by transpiration and maintain an appropriate level of enough water for all the physiological operations. So the previous genotypes considered are the most tolerant and resistant for drought stress.

Variation and Interaction:-

The results obtained in table (4), detected that mean squares of all traits studied were highly significant variances for all barley entries under the control of irrigation and water deficit treatment and the same results were revealed for GCA and SCA effects for all traits of both treatments where were exhibited significant and highly significant variances. The GCA/SCA ratio was less than the unity for all traits under all conditions except plant height trait for the stress treatment, which was higher than the unity, respectively. Interpretation of previous results confirms that non-additive type of gene action was more influential and very importance in the inheritance and control of these traits under all treatments of irrigation. Thus, the selection will be effective through using bulk method not pedigree method, [9, 25,26,30,31].

Before starting in plant breeding program for drought tolerance in barley plants, firstly we have to choosesuperior genotypes in this regard and must contain the number of genes in order to cover all the qualities and quantity traits desired presence in the perfect plant for drought stress resistant. The second step is to test these entries through hybridization among them and sure about found the biggest differences from each other. Hybrids obtained will be superior and better than all parents in production and this has already happened in this study. We note through (ANOVA) test that GCA effects was very high among tolerance parents for drought stress and so we will have succeeded in raising the values of additive gene action and leap it across genetic and isolated generations of crosses to obtained superior stability lines for drought stress tolerance.

Table (3):- Mean performances of the 5 parents and their F1 crosses for all traits studied in barley under control and drought stress conditions.

Genotypes	P.H		Days to maturity		G.Y/P		M.R.L		NO.OF.R/P		R.W.C		O.P		C.T	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1	88.00	78.33	116.00	112.00	38.00	33.33	53.00	47.67	487.00	434.67	57.67	44.67	1.47	1.52	22.40	23.23
P2	87.67	75.00	117.00	109.00	40.50	37.00	59.33	54.00	373.00	316.67	63.67	54.33	1.07	1.17	20.25	20.73
P3	105.33	102.67	125.00	122.00	24.00	20.67	32.33	23.33	123.67	105.67	37.67	27.67	2.57	3.27	24.40	25.20
P4	108.50	104.67	122.43	119.83	26.33	24.00	28.33	24.33	187.67	143.00	30.17	27.00	1.92	2.47	23.63	24.13
P5	114.00	111.50	127.50	124.33	27.50	25.50	36.67	28.33	162.67	124.67	41.00	31.33	3.76	4.80	26.00	27.50
P1 X P2	84.00	73.33	114.00	106.00	47.33	45.33	65.00	60.33	746.00	641.67	78.33	67.67	0.75	0.82	19.73	20.23
P1 X P3	81.83	76.00	114.00	108.00	52.00	45.33	66.83	64.00	571.67	499.67	72.00	64.00	1.20	1.23	21.93	22.67
P1 X P4	83.67	75.33	117.00	114.67	47.50	45.33	57.33	51.67	648.67	515.33	85.67	73.33	0.92	0.64	21.67	22.03
P1 X P5	115.83	112.33	121.00	119.00	22.83	19.67	34.33	27.00	288.67	207.67	38.67	26.67	5.33	4.86	24.83	26.00
P2 X P3	85.00	79.33	122.00	120.00	30.00	24.00	27.67	20.67	188.67	141.00	38.00	30.67	3.47	3.83	24.83	25.90
P2 X P4	80.00	72.67	114.33	108.00	46.33	42.67	65.33	59.00	448.00	421.00	84.33	69.67	0.94	1.09	19.87	20.10
P2 X P5	80.83	69.33	115.33	107.67	53.00	48.67	65.33	60.33	715.00	556.67	87.67	77.67	0.66	0.93	19.87	20.27
P3 X P4	114.00	95.33	126.00	120.67	33.83	21.67	39.00	33.67	260.00	215.00	35.00	27.67	3.67	4.52	25.90	26.70
P3 X P5	112.33	106.00	125.67	121.33	30.83	26.67	28.33	21.67	147.67	127.33	30.33	23.33	4.37	5.17	25.40	26.30
P4 X P5	116.33	112.00	123.00	121.67	24.33	22.00	30.33	26.00	217.00	160.33	35.67	29.33	5.00	6.00	26.50	27.10
LSD 0.05	**4.00**	**5.02**	**3.25**	**4.11**	**3.73**	**3.96**	**5.89**	**7.20**	**59.76**	**83.18**	**9.19**	**9.11**	**0.87**	**0.90**	**1.04**	**1.35**
LSD 0.01	**5.39**	**6.78**	**4.38**	**5.54**	**5.03**	**5.34**	**7.95**	**9.72**	**80.63**	**112.21**	**12.40**	**12.29**	**1.17**	**1.22**	**1.40**	**1.82**

Table (4):- Mean Squares of the half diallel analysis for all studied traits under control and water deficit conditions.

S.O.V	df	P.H		Days to maturity		G.Y/P		M.R.L		NO.OF.R/P		R.W.C		O.P		C.T	
		Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Replication	2	0.172	16.24	1.66	12.01	1.69	0.14	31.04	11.47	6645.36*	7814.49*	148.21*	29.07	1.032*	0.45	0.79	0.53
Genotypes	14	623.20**	761.96**	70.54**	126.31**	347.58**	350.89**	763.40**	841.90**	141394.83**	103842.78**	1463.40**	1244.67**	8.17**	10.76**	18.28**	22.18**
GCA	4	506.38**	693.22**	58.45**	99.39**	145.88**	162.68**	442.16**	488.95**	85886.04**	66816.93**	753.75**	616.56**	4.814**	7.67**	14.17**	16.97**
SCA	10	88.27**	78.29**	9.54**	19.19**	103.85**	98.68**	179.39**	197.31**	31629.84**	21733.19**	381.42**	334.22**	1.887**	1.96**	2.86**	3.57**
Error	28	5.714	9.03	3.77	6.04	4.98	5.60	12.41	18.56	1277.38	2474.16	30.23	29.66	0.268	0.29	0.38	0.65
Error term		1.90	3.01	1.26	2.01	1.66	1.87	4.14	6.19	425.79	824.72	10.08	9.89	0.09	0.10	0.13	0.22
GCA/SCA		0.65	1.02	0.77	0.63	0.16	0.18	0.28	0.28	0.30	0.35	0.22	0.21	0.29	0.45	0.57	0.56

Heterosis over better-parent:

Table (5) presented the percentages of heterosis over better parents for all traits studied under both treatments of irrigation (normal and drought stress conditions).

With respect to plant height trait, the hybrids (P1 X P3, P1 X P4, P2 X P4) under normal irrigation, (P3 X P4) under water deficit conditions and the cross (P2 X P5) under all conditions revealed significant and highly significant negatively values of heterosis over better-parent , while , days to maturity , osmotic pressure and canopy temperature traits no detected any significant and highly significant negatively values of heterosis over better-parent for both treatments of irrigation , respectively.

On the other side , the hybrids (P1 X P2 , P1 X P3 , P1 X P4 , P2 X P4 , P2 X P5) under both treatments and the cross (P3 X P4) under normal irrigation for grain yield/plant trait , (P1 X P3 , P3 X P4) under all treatments and (P2 X P4 , P2 X P5) under normal conditions only for maximum root length trait , the genotypes ; (P1 X P2 , P2 X P4 , P2 X P5) under normal irrigation and drought stress conditions and (P1 X P3 , P1 X P4 , P3 X P4) under normal irrigation only for number of roots/plant trait , in addition to the hybrids (P1 X P2 , P1 X P3 , P1 X P4 , P2 X P4 , P2 X P5) under both treatments of irrigation for relative water content trait were exhibited significant and highly significant positively values of heterosis over better-parent , respectively.

From the previous results it could be concluded that the heterosis percentages as deviation from better parents was significant and highly significant negatively in (four and two) crosses out of ten hybrids studied under all conditions for plant height trait , while (six and five)hybrids for grain yield/plant , (four and two)hybrids for maximum root length , (six and three)hybrids for number of roots/plant and (five and five)hybrids for relative water content traits out of ten hybrids studied were revealed significant and highly significant positively percentages of heterosis over better-parent under all conditions , respectively. Thus, Heterosis over better-parent is considering the most genetic effective way to determine the degree of divergence of parents from each other , differential from these hybrids and determine also the percentage difference between the obtained hybridizations from the parents involved in the hybridization program of barley Accordingly, the aforementioned hybrids has proven transgressive segregation because they contain genes superior and special qualities of higher yielding , resistance to drought stress conditions and revealed the importance of these crosses for SCA effects and (Dominance and Dominance X Dominance) gene action for controlling these traits for drought tolerance,[9,24,25,26,32,33].

Combining ability effects:

General Combining ability effects:

The parents ; (P1 and P2) detected highly significantly and negatively values of GCA effects under both treatments of irrigation (Control and drought stress) for plant height , days to maturity , osmotic pressure and canopy temperature traits in addition to the parent (P5) under the control treatment only for canopy temperature trait was revealed the same results, while the parents (P1 and P2) were exhibited highly significantly and positively values of GCA effects under normal and water shortage conditions for the rest of traits in table (6), respectively. These results may be pointed to the importance of (additive and additive X additive) types of gene action in the inheritance and control these traits for drought stress resistance in barley entries, [9, 24, 25,26,32,33].

Generally ; the parents number (1 , 2) exhibited highly significant positive of GCA effects for grain yield/plant , maximum root length , relative water content and number of roots/plant under the two conditions. This might be due to the higher values of its components traits for grain yield and the rest positive traits and considering good combiner for these traits under normal and water deficit conditions. On the other side, The parents number (3, 4, 5) revealed significant and highly significant negatively values of GCA effects for the same traits under both conditions of irrigation ,this means that these entries (which recorded negative and highly significant values of GCA effects) seem to be poor combiners in these traits for improving drought stress resistance under Egyptian conditions. It was suggested that population involving the parents number (1, 2) could be considered in making multiple crossing because they might possess eligible genes for earliness and/or short stature as well as high grain yielding ability under both treatments of irrigation , accordingly, these parents would be the best choice as base populations.

Table (5):-Estimates of heterosis over better-parent for the 10 crosses of all traits studied in barley under normal and drought stress conditions.

Crosses	P.H		Days to maturity		G.Y/P		M.R.L		NO.OF.R/P		R.W.C		O.P		C.T	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1 X P2	-4.18	-2.22	-1.72	-2.75	16.84**	22.51**	9.55	11.72	53.18**	47.62**	23.02**	24.55**	-29.90	-29.91	-2.56	-2.41
P1 X P3	-7.01 **	-2.97	-1.72	-3.57	36.84**	36.00**	26.09**	34.25**	17.38**	14.95	24.84**	43.27**	-18.36	-19.07	-2.09	-2.41
P1 X P4	-4.92 *	-3.82	0.86	2.38	25.00**	36.00**	8.16	8.39	33.10**	18.55	48.55**	64.15**	-37.41	-57.89	-3.25	-5.16
P1 X P5	31.62**	43.40**	4.31**	6.25**	-39.92**	-40.98**	-35.22**	-43.36**	-40.72**	-52.22**	-32.94**	-40.29**	262.58**	219.73**	10.84**	11.92**
P2 X P3	-3.04	5.77	4.27**	10.09**	-25.92**	-35.13**	-53.36**	-61.72**	-49.41**	-55.47**	-40.31**	-43.54**	224.29**	227.35**	22.61**	28.02**
P2 X P4	-8.74**	-3.10	-2.28	-0.91	14.39**	15.32**	10.11*	9.25	20.10*	32.94*	32.44**	28.23**	-12.14	-6.83	-1.87	-0.64
P2 X P5	-7.80**	-7.56*	-1.42	-1.22	30.86**	31.54**	10.11*	11.72	91.68**	75.78**	37.69**	42.95**	-38.31	-20.51	-1.87	0.19
P3 X P4	8.23**	-7.14**	2.91*	0.70	28.48**	-9.70	20.63*	38.38*	38.54*	50.34	-7.08	0.00	91.14**	82.99**	9.60**	10.65**
P3 X P5	6.64**	3.24	0.53	-0.54	12.10	4.58	-22.74**	-23.50	-9.63	2.13	-26.02*	-25.53	70.03**	58.10**	4.09	4.36
P4 X P5	7.21**	7.00**	0.46	1.25	-11.52	-13.72	-17.28*	-8.22	15.62	12.11	-13.00	-6.38	160.41**	142.91**	12.14**	12.30**
LSD 0.05	**4.00**	**5.02**	**3.25**	**4.11**	**3.73**	**3.96**	**5.89**	**7.20**	**59.76**	**83.18**	**9.19**	**9.11**	**0.87**	**0.90**	**1.04**	**1.35**
LSD 0.01	**5.39**	**6.78**	**4.38**	**5.54**	**5.03**	**5.34**	**7.95**	**9.72**	**80.63**	**112.21**	**12.40**	**12.29**	**1.17**	**1.22**	**1.40**	**1.82**

Table (6): Estimates of GCA Effects for the 5 Parental Entries studied of all Traits of Barley under normal and Drought stress conditions.

Parents	P.H		Days to maturity		G.Y/P		M.R.L		NO.OF.R/P		R.W.C		O.P		C.T	
	Normal	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**
P1	-7.14**	-7.58**	-3.16**	-3.14**	3.99**	4.23**	7.69**	8.22**	143.27**	127.08**	9.10**	7.29**	-0.53**	-0.90**	-0.85**	-0.83**
P2	-9.31**	-11.30**	-2.92**	-4.86**	5.70**	5.99**	9.48**	9.65**	88.22**	78.50**	12.76**	12.05**	-0.98**	-1.13**	-2.01**	-2.18**
P3	1.79**	2.18**	2.51**	2.90**	-3.30**	-4.82**	-7.02**	-7.73**	-115.83**	-92.83**	-10.81**	-9.86**	0.43**	0.62**	1.14**	1.25**
P4	2.81**	2.56**	0.73	1.57**	-1.87**	-1.87**	-3.86**	-3.11**	-39.59**	-35.21**	-3.62**	-2.29*	-0.07	0.04	0.33**	0.14
P5	11.86**	14.13**	2.84**	3.52**	-4.53**	-3.53**	-6.29**	-7.02**	-76.07**	-77.54**	-7.43**	-7.19**	1.15**	1.38**	1.39**	1.63**
LSD 0.05	**0.96**	**1.20**	**0.78**	**0.98**	**0.89**	**0.95**	**1.41**	**1.72**	**14.29**	**19.88**	**2.20**	**2.18**	**0.21**	**0.22**	**0.25**	**0.32**
LSD 0.01	**1.29**	**1.62**	**1.05**	**1.33**	**1.20**	**1.28**	**1.90**	**2.32**	**19.27**	**26.82**	**2.97**	**2.94**	**0.28**	**0.29**	**0.33**	**0.44**

Specific combining ability effects:

Significant and highly significant negatively values of SCA effects were observed in the hybrids ; (P1 X P3, P1 X P4 , P2 X P3, P2 X P4) for plant height trait, (P1 X P3 , P2 X P4, P2 X P5) for days to maturity trait, (P1 X P2, P1 X P3, P2 X P4, P2 X P5) for osmotic pressure and canopy temperature traits under both treatments of irrigation in table (7) , respectively. On the other side the hybrids ; (P1 X P2 , P1 X P3 , P1 X P4 , P2 X P4, P2 X P5) under all conditions for grain yield/plant and maximum root length traits, the cross(P3 X P5) only under all treatment for grain yield/plant, (P1 X P2) under water deficit conditions only for grain yield/plant beside the cross (P3 X P4) under normal irrigation only for grain yield/plant and under both treatments for maximum root length were revealed significant and highly significant positively values of SCA effects in table (7), respectively.

In addition to the crosses; (P1 X P3 , P1 X P4 , P2 X P5) under both treatments of irrigation for number of roots/plant and relative water content traits and the cross (P2 X P4) only under all conditions for relative water content trait were revealed Significant and highly significant positively values of SCA effects in table (7) , respectively , which detected the importance of (Dominance and Dominance X Dominance) types of gene action in the inheritance and improving the ability of drought resistance in the entries of barley under Egyptian conditions, [9,24,25,26,32,33].

Evaluation of SCA effects of the10barleyhybrids under both treatments of irrigation were calculated for all studied traits and revealed in Table (7). Distinct hybrids detected eligible SCA effects for the studied traits. The superior hybrids showing eligible SCA effects for earliness and short stature were obtained from (P1 X P3, P2 X P4). These hybrids could be used in breeding programmers as early and short stature donors either under normal and drought irrigation, depending on their non-additive gene effects. Also, eligible SCA effects for grain yield and the rest traits studied were observed from distinct hybrids such as ;(P1 X P3 , P1 X P4 , P2 X P4 , P2 X P5) under normal and drought stress treatments .It was obvious that most of the hybrids revealed high significant positive SCA effects, included assorted parents. The eminent F_1's having SCA effects are expected to produce eligible transgressive segregates, provided that the eligible integral genes and epistatic effects are coupled in the same orientation to maximize the trait in judgment. In ruling of the existing findings, it can be stated that the hybrids (P1 X P2, P1 X P3, P1 X P4,P2 X P4and P2 X P5) might be bespoke to be exercised in hybrids barley breeding and could be used as a base population. The population would possessed eligible genetic for grain yield and the rest traits. Also, this different origin of these parents would widen the genetic base for selection.

Table (7):- Estimates of SCA Effects for The 10 Crosses from Half Diallel analysis of all Traits Studied of Barley under normal and Drought stress conditions.

Crosses	P.H		Days to maturity		G.Y/P		M.R.L		NO.OF.R/P		R.W.C		O.P		C.T	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1 X P2	1.90	1.09	0.06	-1.61	1.35	2.99**	1.89	2.33	143.50**	128.70**	2.09	3.33	-0.22	0.03	-0.56*	-0.62
P1 X P3	-11.37**	-9.72**	-5.37**	-7.37**	15.02**	13.80**	20.22**	23.38**	173.20**	158.10**	19.33**	21.57**	-1.17**	-1.31**	-1.51**	-1.62**
P1 X P4	-10.56**	-10.77**	-0.59	0.63	9.09**	10.85**	7.56**	6.43**	174.00**	116.10**	25.80**	23.33**	-0.96**	-1.32**	-0.97**	-1.14**
P1 X P5	12.56**	14.66**	1.30	3.01**	-12.91**	-13.15**	-13.02**	-14.33**	-149.6**	-149.2**	-17.39**	-18.43**	2.24**	1.57**	1.14**	1.33**
P2 X P3	-6.03**	-2.67*	2.39**	6.34**	-8.70**	-9.29**	-20.73**	-21.38**	-154.7**	-152.0**	-18.34**	-16.52**	1.55**	1.52**	2.56**	2.96**
P2 X P4	-12.06**	-9.72**	-3.49**	-4.33**	6.21**	6.42**	13.77**	12.33**	28.30	70.30**	20.80**	14.90**	-0.48*	-0.64**	-1.60**	-1.73**
P2 X P5	0.73	-1.63	-4.61**	-6.61**	15.54**	14.09**	16.20**	17.57**	331.80**	248.30**	27.94**	27.81**	-1.98**	-2.14**	-2.66**	-3.06**
P3 X P4	10.85**	-0.53	2.75**	0.58	2.71**	-3.77**	3.94**	4.38*	44.40**	35.70	-4.96*	-5.19*	0.83**	1.04**	1.28**	1.44**
P3 X P5	0.13	-1.44	0.30	-0.71	2.37*	2.90**	-4.30**	-3.71*	-31.50*	-9.70	-5.82*	-4.62*	0.32	0.35	-0.28	-0.45
P4 X P5	3.11**	4.18**	-0.59	0.96	-5.56**	-4.72**	-5.47**	-4.00*	-38.40*	-34.30	-7.67**	-6.19**	1.45**	1.77**	1.63**	1.46**
LSD 0.05	**1.95**	**2.45**	**1.58**	**2.01**	**1.82**	**1.93**	**2.87**	**3.52**	**29.16**	**40.59**	**4.49**	**4.44**	**0.42**	**0.44**	**0.51**	**0.66**
LSD 0.01	**2.63**	**3.31**	**2.14**	**2.71**	**2.46**	**2.61**	**3.88**	**4.74**	**39.34**	**54.76**	**6.05**	**6.00**	**0.57**	**0.59**	**0.68**	**0.89**

Drought Susceptibility index:

With respect to (DSI) , the genotypes (P3 , P4 , P5 , P1 X P5 , P2 X P3 , P3 X P5 , P4 X P5) for plant height and days to maturity , (P2 , P4 , P5 , P1 X P2 , P1 X P4 , P2 X P4 , P2 X P5 , P4 X P5) for grain yield/plant , (P1 , P2 , P1 X P2 , P1 X P3 , P1 X P4 , P2 X P4 , P2 X P5) for maximum root length , (P1 , P2 , P3 , P1 X P2 , P1 X P3 , P2 X P4 , P3 X P5) for number of roots/plant , (P2 , P4 , P1 X P2 , P1 X P3 , P1 X P4 , P2 X P5) for relative water content , (P1 , P2 , P1 X P2 , P1 X P3) for osmotic pressure and (P2 , P4 , P1 X P2 , P1 X P4 , P2 X P4 , P2 X P5 , P3 X P4 , P4 X P5) for canopy temperature traits were achieved and recorded values less than the unity which indicated that these entries can be considered and gazed to be water deficit resistant and highly tolerance for drought because they revealed smaller yield reductions under water deficit conditions compared with the control treatment of all the means for all genotypes. However, the low DSI values may not necessarily give a good indication of drought tolerance of genotype. Low DSI values of a variety could be due to lack of yield production under normal treatments rather than an indication of its ability to tolerate water deficit conditions and the same results were revealed in the rest of traits (table, 8), respectively, where the traits recorded values of (DSI) less than the unity were highly resistance and tolerance for drought stress conditions for the above-mentioned reasons. These results were agreement with those reported by [9,32,34].

Table (8):- Estimates of Drought Susceptibility Index (DSI) for all Traits Studied in Barley.

Genotypes	P.H	Days to maturity	G.Y/P	M.R.L	NO.OF.R/P	R.W.C	O.P	C.T
P1	1.46	0.94	1.07	0.80	0.63	1.31	0.24	1.18
P2	1.92	1.86	0.75	0.71	0.88	0.85	0.66	0.76
P3	0.34	0.65	1.21	2.20	0.85	1.54	1.94	1.05
P4	0.47	0.58	0.77	1.12	1.39	0.61	2.04	0.68
P5	0.29	0.68	0.63	1.80	1.36	1.37	1.97	1.84
P1 X P2	1.68	1.91	0.37	0.57	0.81	0.79	0.66	0.81
P1 X P3	0.95	1.43	1.12	0.34	0.73	0.64	0.18	1.08
P1 X P4	1.32	0.54	0.40	0.78	1.20	0.83	-2.16	0.53
P1 X P5	0.40	0.45	1.21	1.69	1.64	1.80	-0.63	1.50
P2 X P3	0.88	0.45	1.74	2.00	1.47	1.12	0.74	1.38
P2 X P4	1.22	1.51	0.69	0.77	0.35	1.01	1.13	0.37
P2 X P5	1.24	1.81	0.71	0.61	1.29	0.66	2.91	0.64
P3 X P4	2.17	1.15	3.13	1.08	1.01	1.21	1.65	0.99
P3 X P5	0.75	0.94	1.18	1.86	0.80	1.34	1.30	1.13
P4 X P5	0.49	0.30	0.84	1.13	1.52	1.03	1.42	0.72

Tolerance Indices:

The results detected in table (9), revealed that the genotypes (P2 , P4 , P5 , P1 X P2 , P1 X P4 , P2 X P3 , P2 X P4) were recorded the lowest values for (YR) which indicated that these entries were highly resistance for drought stress conditions in barley because the reduction % of yield was low in these entries, while, the genotypes ; (P1 X P2, P1 X P3 , P1 X P4, P2 X P4 , P2 X P5) were exhibited the highest values for (MP, GMP, DTI, YSI, YI) and this is the definitive guide on highly resistance for water deficit conditions in these entries, respectively, [9,34,35].

After careful consideration and depth to these special results with tolerance indices we can say that these lines in addition to the previous hybrids have achieved a remarkable advantage to drought stress resistance.

Table (9):- Estimates of Tolerance indices for 15 Genotypes of Barley Through two levels of irrigation.

Genotypes	GYP	GYD	MP	GMP	YSI	YI	DTI	YR
P1	38.0	33.33	35.66	35.58	0.87	1.03	0.96	0.13
P2	40.50	37.0	38.75	38.71	0.91	1.15	1.13	0.09
P3	24.0	20.67	22.33	22.27	0.66	0.64	0.37	0.34
P4	26.33	24.0	25.15	25.13	0.91	0.74	0.48	0.09
P5	27.50	25.5	26.5	26.48	0.92	0.79	0.53	0.08
P1 X P2	47.33	45.33	46.33	46.31	0.95	1.41	1.63	0.05
P1 X P3	52.00	45.33	48.66	48.55	0.87	1.41	1.79	0.13
P1 X P4	47.50	45.33	46.41	46.40	0.95	1.41	1.63	0.05
P1 X P5	22.83	19.67	21.25	21.19	0.86	0.61	0.34	0.14
P2 X P3	30.00	24.0	27	26.83	0.80	0.74	0.54	0.20
P2 X P4	46.33	42.67	44.5	44.46	0.92	1.33	1.50	0.08
P2 X P5	53.0	48.67	50.83	50.78	0.91	1.51	1.96	0.09
P3 X P4	33.83	21.67	27.75	27.07	0.64	0.67	0.55	0.36
P3 X P5	30.83	26.67	28.75	28.67	0.86	0.83	0.62	0.14
P4 X P5	24.33	22.0	23.16	23.13	0.90	0.68	0.40	0.10

After reviewing all results of statistical analysis for this investigation of the genetic programs (half diallel analysis without reciprocal), we find that these genotypes were achieved the first condition of this analysis is the presence of significant and highly significant differences, Thus it is considered the most successful and feasible to engage in a crossing model of plant breeding program to get different hybrids from each other , So estimation of all genetic parameters which gives the first evidence on the importance of parents , the difference between them and then expected the positive form from them crosses to improve the genetics quantitative values for the development of the efficiency of barley plants for confrontation non-favorable conditions beside maintaining highly yielding suitable for these conditions.

With rapidly review of the mean values for all entries studied and used in this investigation for each calculated traits under all circumstances, we found that parents and their hybrids had given clear contrast when exposed for lack of water conditions compared to the control and appeared so evident, especially in the trait of grain yield/plant and then because the exposure for drought stress may lovers best tolerated crosses through treated with it and biggest proof of that , these genotypes revealed the highest ranges of hybrid vigor in the positive track specially for grain yield/plant , root system and physiological traits in addition ,the entries (P1 , H5) were detected the highest index for drought stress tolerance, respectively.

So, GCA effects played an important role to clarify the actual role for additive and additive X additive types of gene action in the inheritance the fruitful traits responsible for water deficit resistance from the combiner parents (specially P1) across several generations of hybridization to produce new lines of barley highly stability and tolerance for stresses because manufacturing new genetic alleles to meet this environmental challenge.

If we dealt with the tolerance indices calculated we will find that the first parent and hybrid number five also have achieved a certain, strong index for drought tolerance and continue to survive, grow and give a good yield in spite under all of these circumstances, as well as the sensitivity for water stress of these genotypes guide was less than one ,drought tolerance index was high and the rate of decline in the yield under drought stress was low compared to the controlled experiment.

Molecular Description Using RAPD Marker:

Five RAPD primers were used to identify and characterize the ten genotypes of barley (Fig. 1) shows the banding patterns produced from each primer for the ten barley entries, where the highest number of PCR-amplified fragments by using all primers was presented in the parent P5 (34 fragments), while the genotype H3 (P1 X P4) gave the lowest number (22 fragments), as shown in Table (10). The other genotypes displayed different numbers of amplified fragments. The primer OPAM-01 gave the highest number of amplified fragments (105 fragments) for all studied genotypes, while the primer OPC10 showed the lowest number of amplified fragments (26 fragments).

Two RAPD specific markers of MW 687 and 369 bp were detected in the electrophoretic patterns of the H3 (P1 X P4) using the primer OPAM-01. In addition, four specific RAPD markers of 127, 217, 292 and 395 bp were detected in patterns of the most tolerance parent P1 using the primer OPF-4.

The polymorphism revealed by the five RAPD primers used for identification of ten barley entries is shown in Table (11). The primer OPAM-01 gave the highest number of polymorphic loci in all varieties (14 fragments) with 87.5 % polymorphism, while primer OPC10 gave the lowest number of polymorphic loci (1 fragment) with 33.3 % polymorphism.

Four specific markers out of 10 amplified fragments were detected for primer OPF4 and two specific markers out of 16 amplified fragments were generated for primer OPAM-01. On the other hand, three common fragments were revealed for primers OPA-17, two common fragments were observed in primers OPC10, OPG-O5 and OPAM-01, while four common fragments were detected for primers OPF4.

The results of amplification by PCR for the ten barley entries studied with five RAPD primers indicated relationship of these barley entries. A total of 43 amplified DNA fragments ranging in size from (127 – 770 bp) base pairs were presented, whereas 30 fragments were polymorphic, 13 fragments were monomorphic. Therefore, out of 43 loci, 30.23 % were monomorphic, and 69.76 % were polymorphic with average of 11.87 polymorphisms per primer. The number of DNA fragments for each primer varied from 3 (OPC10) to 16 (OPAM-01).

Table (10): Total bands produced from each primer for the barley genotypes and all amplified fragments in each genotype.

Genotypes	Primers					
	OPC10	**OPF-4**	**OPA-17**	**OPG-05**	**OPAM-01**	*****
P1	3	6	4	9	11	**33**
P2	3	6	3	9	6	**27**
P3	3	4	4	9	12	**32**
P4	3	5	5	9	10	**32**
P5	3	5	5	9	12	**34**
H1	2	5	4	8	11	**30**
H2	2	5	4	8	12	**31**
H3	2	5	3	4	8	**22**
H4	3	6	5	8	10	**32**
H5	2	6	5	2	13	**28**
Total bands	26	53	42	75	105	**301**

*** Refer to presence of all amplified fragments in each variety.**

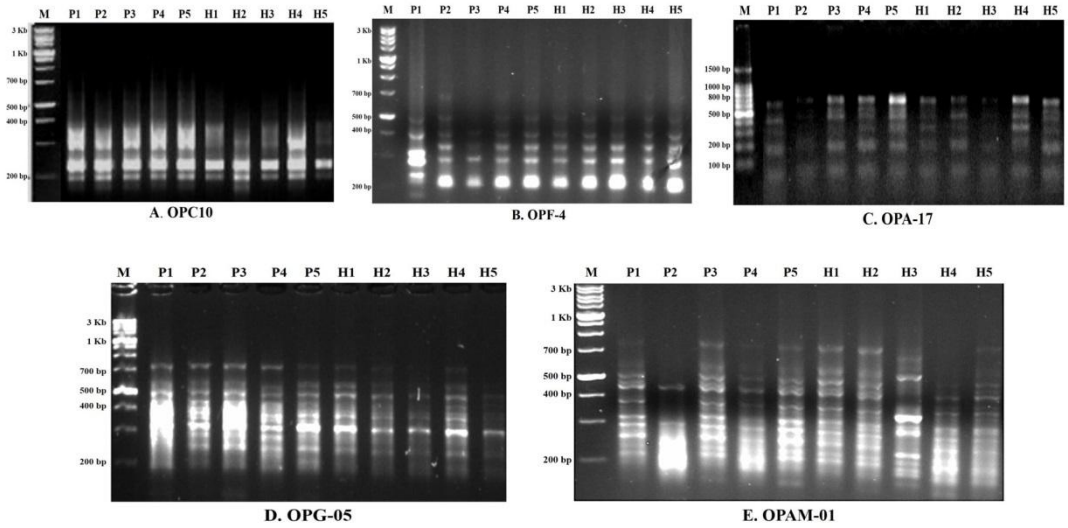

Fig.(1): PCR fragments with using five RAPD primers (A, B, C, D and E) of ten barley genotypes (1:10), M; DNA ladder (100bp) as markers.

Table (11):- The polymorphic loci amplified by the five RAPD primers.

Primer Code	Loci	Polymorphic bands	Monomorphic bands	Polymorphism%	Specific bands
OPC10	3	1	2	33.3	-
OPF-4	10	6	4	60	4
OPA-17	5	2	3	40	-
OPG-05	9	7	2	77.7	-
OPAM-01	16	14	2	87.5	2
Total Loci	43	30	13	69.7	6

Table (12): Genetic similarity percentages of the ten genotypes of barley (Five parents and five selected crosses) based RAPD banding patterns.

	P1	P2	P3	P4	P5	H1	H2	H3	H4	H5
P1	1									
P2	0.733	1								
P3	0.862	0.814	1							
P4	0.831	0.881	0.906	1						
P5	0.836	0.82	0.939	0.939	1					
H1	0.825	0.807	0.903	0.903	0.906	1				
H2	0.844	0.793	0.952	0.889	0.923	0.951	1			
H3	0.545	0.694	0.63	0.667	0.643	0.692	0.679	1		
H4	0.769	0.881	0.844	0.938	0.909	0.839	0.825	0.704	1	
H5	0.656	0.691	0.767	0.8	0.839	0.793	0.814	0.68	0.833	1

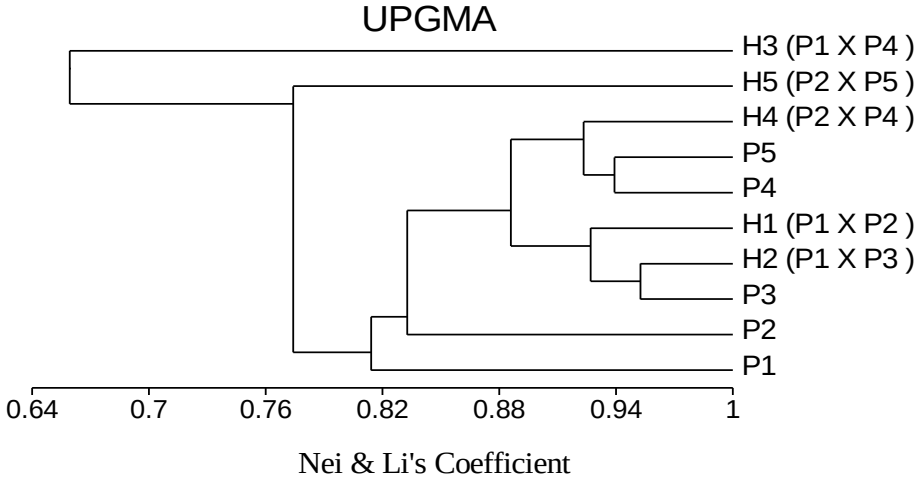

Fig. (2): Dendrogram representing the genetic relationship among the ten barley entries using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from RAPD markers.

Genetic Similarity:

The genetic similarity index and dendrogram tree of the ten barley entries under study were performed using [36] similarity index. On the basis of RAPD amplified fragments as presented in (Table 12 and Fig. 2). Identified relationships among the five parents and the five selected hybrids.

The genetic similarity ranged from 54.50 to 95.20%, with an average of 74.85%. Some genotypes showed high genetic similarity with others, such as parent P3 and hybrid H2 (P1 X P3) (95.2%), the hybrids H1 (P1 X P2) and H2 (P1 X P3) (95.10%), parents P3 and P5 (93.9%), P4 and P5 (93.9%), P4 and H4 (P2 X P3) (93.80%). On the contrary, some genotypes displayed low genetic similarity, such as P1 and H3 (54.50%), H3 and P3 (63%), respectively.

The present study aimed to know the RAPD Markers efficiency in defining and minutely the genetic diversity and relationships between all the ten barley entries using five primers. The dendrogram resulting from UPGMA cluster analysis showed that the ten barley entries could be divided into two main clusters. The first cluster contained the hybrid H3 (P1 X P4) only, while the second cluster divided into two sub-clusters; the first sub-cluster contained the most tolerance hybrid for drought H5 (P2 X P5) only. The second sub-cluster contained two main groups; the first group included the most tolerance parent P1 only. The second group divided into two sub- group; the first one contained the tolerance parent P2, while the second sub- group included the rest of genotypes parents (P3, P4, and P5) and hybrids (H1, H2 and H4).

These results indicate a genetic relationship to drought tolerance among used barley genotypes and provide basic information about the genetic relationships for breeding purposes. Consequently, it is possible to identify superior cultivars, and evaluate the genotypic performance under drought conditions. In addition, results obtained were confirmed by clustering made by RAPD tool on the base of DNA analysis, which is compatible with the arrangement obtained with statistical method.

These results were agreement with those reported by [37]whoinvestigated RAPD analysis in 27 inbred barley lines with varying amounts of common ancestry and in 20 doubled-haploid (DH) lines from a biparental cross. They found that RAPD markers can be used to gain information about genetic similarities or differences that are not evident from pedigree information. Similar investigations were reported by many researchers.[38]Used ten RAPD and ten ISSR primers to identification of 16 barley cultivars from different countries. analyses of RAPD and ISSR PCR markers provides a quick, reliable and highly informative system for DNA fingerprinting and also permit to establish genetic relationships which agree with , by other means, known origin of the cultivars**.**[39]Analyzed Drought tolerance of 32 barley (*Hordeum vulgare* L.) by two RAPD-PCR primers P6 and P7. They found that primers (P6 and P7 marker) revealed characteristic loci at 920 bp region in 75% of genotype electrophoretic profiles and 750 bp region in 78% of the analyzed barley genotypes

respectively. Comparative analysis of the RAPD spectra showed that characteristic for both markers fragments had been synthesized in 59% of the genotypes. This confirms an existence of a special locus associated with drought tolerance in the barley genotypes.

[40]Investigated the variation between wild barley lines originated from Turkey which were comparatively at the molecular level with RAPD technique. They found that the results can be used in the selection of commercially important traits (such as resistance against disease, drought etc.) present in wild barley lines. Thus, development of high quality barley cultivars will be possible.[41]Tested thirty eight RAPD and twenty-five ISSR primers for polymorphism among parental genotypes (cross between drought sensitive and tolerant genotype) and F2 population to identify molecular markers linked to flag leaf senescence gene in wheat under water-stressed conditions as indicator for drought tolerance. They indicated that four RAPD and two ISSR markers were linked to the QTL for the flag leaf senescence gene as indicator of drought tolerance. These markers can be used in wheat breeding programs, as a selection tool in early generations. While, [42]evaluated the genetic diversity by using RAPD and ISSR analyses among natural populations and Korean wheat cultivars (*Triticum aestivum*). Ninety three populations were evaluated with fifty RAPD and three ISSR primers. They found that this study provides basic information about the genetic relationships for breeding purposes. On the same track,[43]used RAPD and ISSR markers to establish the genetic characterization of twenty five registered durum wheat cultivars. They detected that polymorphism and genetic variation values indicate narrow genetic base of the tested cultivars. This study could be useful for selection of suitable parents in breeding programs involving germplasm. while[44]revealed 71 amplicons in six wheat entries using six primers , where 52 of them were monomorphic fragments and 19 amplicons were polymorphic with 26.76% polymorphism beside [45]revealed 51 fragments ranging from 2344bp to 160bp using seven primers in some lines of rice where 13 of them were monomorphic and 38 fragments were polymorphic with 74.51% polymorphism.

[33]studied drought stress tolerance in some entries of rice with different reaction for drought resistance using 7 ISSR primers and detected that 51 fragments were generated through these primers, where 37 of them were polymorphic bands with 71.15% polymorphism and 14 amplicons were monomorphic, while in this study we revealed 43 fragments, 13 of them were monomorphic bands and 30 fragments were polymorphic with 69.76 % polymorphism, respectively.

Molecular genetics science is considering one of the most important branches of genetics to reach and stand on the molecular genetic differences in a clear and quick to distinguish between the entries of the same plant or the distinction between plant groups and various assets. This modern trend in science as well as of heredity quantity genetic helps us to know and understand the major role in the genetic change resulting from exposure to conditions of water shortages and its impact on all stages of growth and productivity**.**

Use of RAPD-PCR assays adopted primarily on the selection of the best barley entries used under normal and drought stress conditions measurements with all traits, then the trade-offs between them to identify those genetic alleles and mechanisms responsible for drought stress tolerance and this also proves true the results of statistical genetic analysis and then using the results as molecular genetics in determining the degree of genetic divergence and convergence networking by cluster analysis.

Conclusion

Five lines of barley with different reaction for drought stress resistance were used in this study under two treatments of irrigation (the control and water deficit treatment), respectively. These entries were tested in agricultural research center for diseases resistance and the stability of yield in more than one location, while in this study the resistance for drought stress conditions was the only factor under test for these lines and their F1 hybrids using half diallel analysis (model 1 and method 2) without reciprocal on the farm of (Nubaria , Beheira governorate) and (National Research Centre , Dokki , Giza , Egypt) in season 2016 . For this, the desired objective of this study is trying to understand the genetic behavior responsible for drought tolerance in these entries and then track the impact of this additive gene action in the resulting hybrids among them to get genetic stability lines of barley resistant for water deficit conditions. Some morphological and physiological traits such as; plant height, days to maturity, grain yield/plant, maximum root length, number of roots/plant, relative water content, osmotic pressure and canopy temperature were the most desirable measurements calculated under normal and drought stress conditions beside RAPD –PCR fragments to compare between the five parents and the five more hybrids resistant for drought stress conditions. Based on a summary of the most

important results can be summarized that the genotypes ; (P1 , P2 , P1 X P2 , P1 X P3 , P1 X P4 , P2 X P4 , P2 X P5) were the most desirable entries for drought tolerance through calculating mean performance , (ANOVA) of half diallel analysis , heterosis over better-parent , combining ability effects with both types and tolerance indices parameters under both treatments of irrigation. Five different primers were scored total of 43 fragments, where 30 of them were polymorphic with (69.76 %) polymorphism and 13 bands were monomorphic. Cluster analysis divided the ten barley entries into two main clusters, where the first cluster contained the hybrid H3 (P1 X P4) only, while the second cluster divided into two sub-clusters. The first sub-cluster contained the genotype H5 (P2 X P5) only, butthe second sub-cluster contained the rest of entries.

References

1. Vaezi, B.; Bavei, V.; Shiran, B. Screening of barley genotypes for drought tolerance by agro-physiological traits in field condition . Afric J of Agri Res., 2010 ; 5(9):881-892.
2. Finlay,K.W .The significance of adaptation in wheat breeding .Australian Academy of Sciences,1968 ; 742–54.
3. Blum, A .Genetic improvement of drought resistance in crop plants .Stress Phys in Crop Plants,1979; 495–545.
4. Sojka, R.E.; Stolzy, L.H.; Fischer, R.A. Seasonal drought response of selected wheat cultivars. Agron J,1981 ; 73: 838–45.
5. Finlay, K.W.; Wilkinson, G.N . The analysis of adaptation in plant breeding programmers, Aust J Agric Res,1963 ; 14: 742–54.
6. Smith, E.L . Heat and drought tolerant wheats of the future, Proc. of the Natio Wheat Res,1982 ; 141-147.
7. Pinter, J.r.; Zipoli ,P.J.; Reginato, G.; Jackson, R.J.; Idso, S.B. Canopy temperature as an indicator of differential water use and yield performance among wheat cultivars. Agric Water Manag ,1990;18: 35–48.
8. Ahmad, R.; Stark, J.C.; Tanveer, A.; Mustafa, T. Yield potential and stability indices as methods to evaluate spring wheat genotypes under drought. Agric Sci 1999 ; 4: 53–9.
9. Mansour, M.; El-Shawy, E.E.; Abaas, S.I. Genetic improvement of water stress tolerance in some barley genotypes,J. Plant Breed, 2016 ;20(1).119-134.
10. Heiba, S. A. A.; Sara, E. I. Eldessouky.; A. A. El-Mouhamady.; I.S. El-Demardash. ; A.A. Abdel–Raheem.Use of RAPD and ISSR Assays for the Detection of Mutation Changes in Wheat (Triticum aestivum L.) DNA Induced by Ethyl-Methane Sulphonate (EMS) ,International Journal of ChemTech Research ,2016;9(3): 42-49.
11. Mostafa , E. A. H. ; H. El-Atroush.; Z. M. El-Ashry.; F. I. Mohamed.; S. E. El-Khodary and S. A. Osman . Genetic Variation and Agro-morphological Criteria of Ten Egyptian Barley Under Salt Stress , International Journal of ChemTech Research , 2016 ,9(7): 119-130.
12. Hossain, A.B.; Sears, A.G.; Cox, T.S.; Paulsen ,G.M. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. , Crop Sci,1990 ; 30: 622-627.
13. Fernandez, G.C.J. Effective selection criteria for assessing stress tolerance. Procee of the Inter Symp on "Adap of Veg and Other Food Crop in Temp and Dro stre ,1992 ; 257-270.
14. Gavuzzi, P.; Rizza, F.; Palumbo, M.; Campaline, R.G.; Ricciardi, G.L.; Borghi, B. Can. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. J. Plant Sci ,1997 ; 77: 523-531.
15. Lin, C.S.; Binns, M.R.;Lefkovitch, L.P. Stability analysis: where do we stand.Crop Sci ,1986 ; 26: 894-900.
16. Bouslama ,M.; Schapaugh, W.T. Stress tolerance in soybean, Crop Sci, 1984 ; 24: 933-937.
17. Golestani, S.A.; Assad , M.T.Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat, Euphytica, 1998 ; 103: 293-299.
18. Fischer, R.A.; Maurer, R. Drought resistance in spring wheat cultivars. I. Grain yield response, Aust. J. Agric. Res, 1978 ; 29: 897–907.
19. Griffing, J.B. Concept of general and specific combining ability in relation to diallel crossing systems. Australian J.of Biol. Sci,1956 ; 9: 463-493.
20. Williams, J.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A.; Tingey, S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res,1990 ; 18: 6531-6535.

21. Sambrook, J.; Fritsch, K.F.; Maniatis, T. Molecular cloning, secondedition (cold spring Harbor, NewYork ,1989.
22. Jaccard,P . Bull, Soc.Nouvelles Researchers Sur La Istribution Lorale ,Vaud Sci Nat, 1908 ; 44:223-270.
23. Dice, L.R. Ecology, 1945; 26: 297-302.
24. El-Seidy, E.H.; El-Moselhey, M.A.; El-Gammaal1, A.A.; El-Naggar, A.A. Combining Ability And Genetic Component Analysis For Barley Genotypes (*Horduem vulgare* L.) Under Stress And Non-Stress Environments. J. Plant Prod., Mansoura Univ,2010 ; 1 (11): 1451 – 1466.
25. Amer, K.; Eid, A.; Elakhdar, A.; El-Shawy, E. Yield potential and stability indices as methods to evaluate spring wheat genotypes under drought. Egyptian J Agric Res ,2012 a ; 90: 105-116.
26. Amer, K.;Eid, A.; El-Sayed, M.; Elakhdar, A. Estimation of some genetic parameters for yield and its components in some barley genotypes. Egyptian J Agric Res , 2012b , 90: 117-130.
27. Abdi, H.; Azizov, E.; Bihamta, M.R.; Chogan, R.; Aghdam, K.N. Assessment and determination of the most suitable drought resistance index for figures and advanced lines of bread wheat. International Journal of Agri. Science,2012 ; 1: 78 -87.
28. Nabil, S.A.M. ; Mohamed, A . Int J of Agri Res, 2013; 8(1):17-25.
29. Mossa, M.M.; Mamati, E. , Reda, T. Evaluation of Physiological and agronomic Responses as Screening Techniques for Yield and Water Stress Tolerance in Wheat Cultivars in Tigray ,Acad. J. A gric. Res,2016 ; 4(1): 006-017.
30. Sathya, R.; Jebaraj, S. Evaluation of aerobic hybrid analysis of combining ability in three line hybrids in Rice (*oryza sativa* L.) under aerobic Conditions, Afri J of agri Res, 2015 ; 10(18): 1971-1981.
31. Ammar, M.H.; Anwar, E.H.; El-Harty, H.M.; Migdadi, S.M.; Abdel-Khalik, S.A ; Al-Faifi , A.; Alghamdi, S.S. J. Agro. Crop Sci ,2015 ; 201:280-287.
32. El-Mouhamady, A.A.; El-Ashary, Z.M.; Mohamed, F.I ; Elewa, T.A.; Aboud, K.A. Study The Effect of Water stress Conditions on Some Genotypes of bread Wheat (*Triticum aestivum L*.) Based on Morphological , Physiological Traits and DNA Fingerprinting. Research Journal of Pharmaceutical, Biological and Chemical Sciences,2016 ; 7(4):2065-2077.
33. Eldessouky, E.I.S.; Heiba, S.A.A.; El-Mouhamady, A.A.; Abdel-Tawab,Y.M. DNA Fingerprinting and Half Diallel Analysis of Some Rice Genotypes under Water Deficit Conditions. RJPBCS,2016 ; 7(4) : 985-997.
34. Kumar, R.; Kaul, J.; Dubey, R.B.; Singode, A.; Chikkappa, G.K.; Manivannan, A.; Debnath, M.K. Assessment Of Drought Tolerance In Maize (Zea *mays* L.) Based On Different Indices, Sabrao. Journal of Breeding and Genetics,2015 ; 47 (3): 291-298.
35. Esmail, R.M. ; Sara E.I. Eldessouky , Sherin A. Mahfouze and I.S. EL-Demardash. Evaluation of new bread wheat lines (Triticum aestivum L) under normal and water stress conditions , International Journal of ChemTech Research ,2016,9(5): 89-99.
36. Nei, M.; Li, W.H .Proceedings of the National Academy of Sciences of the USA,1979 ; 76: 5269 – 5273.
37. Tinker, N.A.; Fortin, M.G.;Mather, D.E . Random amplified polymorphic DNA and pedigree relationships in spring barley,Theor Appl Genet,1993 ; 85 (8):976-84.
38. Fernández, M.; Figueiras, A.; Benito, C. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known originTheoretical and Applied Genetics, 2002 ; 104: (5) 845–851.
39. Huseynova, M.S.M.; Rustamova, M.Y.; Nasrullayeva , Aliyev, J.A. Screening of Barley Genotypes for DroughtTolerance Using Molecular Markers. International Journal of Plant Science and Ecology, 2015 ; 1 (3) :88-92.
40. Gülruh, A.; Nermin, G. Tr. RAPD Analysis of Genetic Variation in Barley, J. of Agriculture and Forestry, 1999 ; 23: 627-630.
41. Milad, S.I.; Wahba, L.E.; Barakat, M.N. Identification of RAPD and ISSR markers associated with flag leaf senescence under water-stressed conditions in wheat (*Triticum aestivum* L.), Australian J. of Crop Sci, 2011 ; 5 (3): 334-340.
42. Cheol, Y.W.; Seok, K.M.; Up, K.Y.; Seong, J.C.; Weon, S.Y.; Don, L.S. , Hee , K.K.; Cheol, M.J.; Kyu, L.S.; Moo, L.B. Evaluation of genetic diversity among Korean wheat using RAPD and ISSR analysis Korean, J. Breeding Sci, 2007 ; 39 (3) :309-315.
43. Karaca, M.; Izbirak, A. Comparative analysis of genetic diversity in Turkish durum wheat cultivars using RAPD and ISSR markers, J. Food Agric. Environ,2008 ; 6 219-225.

44. Tharwat, El Ameen . African J of Biotech 2013; 12(21): 3148-3152.
45. Heiba, S.A.A.; El-Mouhamady, A.A.; Eldessouky, S.E.I. ; Hoda, B.M.A. ; Elewa , T.A . Study the Genetic Variations Related to the Resistance of Heavy metals Toxicity in Some Rice Genotypes Using RAPD Markers Int J Curr Microbiol App Sci 2016 ; 5(1): 174-189.

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