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Study The Effect of Water stress Conditions on Some Genotypes of bread Wheat (*Triticum aestivum L.*) Based on Morphological , Physiological Traits and DNA Fingerprinting.

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ABSTRACT

This study was conducted using five genotypes of wheat with different reaction for drought tolerance to know the genetic behavior and directories related to water stress resistance in wheat crop through 2014, 2015 and 2016 seasons. All stages of hybridization between parents were done in 2014 season and all genotypes (parents and their crosses) were grown in 2015 season under normal and drought conditions to study some morphological traits, while genetic analysis were done using half diallel analysis (Griffing technique, mode 1, method 2) to study genetic parameters namely., heterosis over better-parent, combining ability effects with both types besides estimating for drought tolerance indices for five parents of wheat. On the other side of the study, The comparison between the five genotypes of wheat was done using six Random amplified polymorphic DNA (RAPD) primers namely, OPA-2, OPA-4, OPB-14, OPC-5 , OPC-12 and OPA-1, respectively. The results showed that the genotypes ; Sakha 8 and Sakha 94 , Sakha 8 X Sakha 93, Sakha 8 X Sakha 94 and Sakha 8 X Shandweel were the most superior in resisting water stress measuring morphological traits and were different from each other using (RAPD) primers, where six primers detected 68 bands , 29 of them was monomorphic bands and 39 bands were polymorphic and the polymorphism % was 57.35 %. Cluster analysis showed that high diversity observed between the ten genotypes and divided them into two main clusters , where , the first one including (H4 , H5) and the second group including (P1 , P2 , P3 , P4 , P5 , H1 , H2 , H3) , respectively. Cluster analysis showed that the similarity ranged from (58.9 to 96.0 %) , where the lowest similarity was (58.9 %) between P3 and H5 , while the highest value was (96.0 %) among H1 and H2 , respectively.

Keywords: Bread Wheat, Water stress , Half Diallel , DNA Fingerprinting , Heterosis over better-parent,GCA and SCA effects.

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INTRODUCTION

Wheat crop is considered the greatest and most important of all creatures, especially for human, it has known it since the dawn of history as a vital source of food and most need energy not only to human but also for the birds and animals, So it considered the first food for peoples and for him break out of wars and battles. The total cereal production in Egypt is 21.9 million tons in 2013/2014 compared to 24 million tons in 2012/2013, a decline of 9.0%, ,while wheat production totaled 9.3 million tons in 2014/2015 compared to 9.5 million tons in 2013/2014, a decline of 1.9% due low productivity per acre to 2.4%, from the previous year according to estimates by the Egyptian Ministry of Agriculture in season 2015. Thus, we noted a decrease in productive wheat year after year is fast for many reasons, but the problem of drought come in the lead, and it divided into environmental reasons because of the same lack of rainfall or decreasing the water needs and water stress or drought is the part that taken up in some detail and illustration, [1,2].

The development of the root system by increasing the adventitious root growth had a greater impact on the resistance for drought in wheat by increasing the depth and number of roots, [3-5].

Drought effect too much on wheat plants and leads to decline and decreasing in yield at least 40%, So, set off thousands of papers for improving the degree of wheat resistant for drought and give a yield of at least 80%, while maintaining the quality attributes , taxonomic and this is the biggest challenge to confront this danger . Short period of life and early maturation are considered the most important mechanisms for the taxonomic varieties of drought tolerance [6]. Thus, the interest in the science of plant breeding and biotechnology especially RAPD-PCR and ISSR markers were the new , the most greatest methods and trends impact on the development of methods for drought tolerance in the old and new genotypes of wheat under Egyptian conditions, [7].

MATERIALS AND METHODS

The present investigation will be carried out in the farm of agricultural Research Centre (ARC) in Sakha research station ,Department of genetics and cytology , Division of genetic engineering and biotechnology , National research Centre , Dokki ,Giza, Egypt included two experiments with controlled conditions (normal and drought treatments of irrigation) during the period from (2014) to (2015) season.

Where the normal treatment was the normal irrigation of wheat crop at winter season , while drought treatment was divided in to two irrigates only, (the first one at agriculture time of wheat crop and the second irrigate was conducted after one month from the first one and no irrigation was done until harvesting beside the two fields were isolated from each other during conduct the experiment. This work aims to study the genetic behavior of vegetative, yield and some traits related to drought tolerance (physiological traits) in addition to RAPD - PCR analysis using six primers table (1).

Five wheat genotypes with different reactions for drought tolerance were used in half diallel analysis, where the cultivars were ; (P1: Sakha 8) (tolerance for drought),(P2: Sakha 93) (tolerance for drought) ,(P3: Sakha 94) (moderate for drought), (P4: Shandweel 1) and (P5: Sids 1) were tolerance for high temperature and moderate for drought ,respectively. Five wheat genotypes were performed from (Agricultural Research Center, Institute of Field Crops Research, Wheat Research Department) .

The parental genotypes 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 parents in 2014 season. In 2015 season all genotypes (parents and their F1 crosses) were grown in two locations isolated from each of them (normal and water stress conditions).Each location was divided in to three replicates and The package of all other recommendations of wheat planting will be followed in the same season (2015). Heading date, plant height, grain yield per plant, maximum root length , number of roots per plant , relative water content , osmotic pressure and leaf water potential were the traits studied through half diallel analysis using , **[8]**mode 1, method 2.

Drought tolerance indices

Drought tolerance indices were calculated using the following researchers, [9-15] besides some abbreviations as follows:

P1:- Sakha 8 , P2:- Sakha 93 , P3:- Sakha 94 , P4:- Shandweel , P5:- Sids 1 , 0.05: Significant at 5% , 0.01: Significant at 1% , H.D:-Heading date , P.H:-Plant height , G.Y:- Grain yield per plant , M.R.L:- Maximum root length , No.of.R/P:-Number of roots per plant , R.W.C:-Relative water content , O.P:-Osmotic pressure and L.W.P:- Leaf water potential, N:Normal conditions, D:Drought conditions .

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.

Molecular Studies

Molecular studies aimed to determine the phylogenetic tree to figure out the relationships among the different varieties of wheat for drought tolerance.

DNA extraction

DNA was extracted from ten genotypes of wheat varieties (The five parents ; (P1,P2,P3,P4,P5) , the highest three crosses based on physiological traits namely; (H1:P1 X P2,H2: P1 X P3,H3; P1 X P4) , the lowest two crosses from calculating of these parameters namely;(H4:P2 X P4 and H5: P3 X P5), respectively and by Bio basic kits protocol.

PCR- Amplification of RAPD

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 [16]. 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 parents of wheat according to gel analyzer protocol.

RAPD analysis

A set of six random 10-mer primers, (Table 1) was used in the detection of polymorphism among ten lines of wheat which different reaction for drought tolerance. These primers were synthesized at RAPD-PCR and carried out according to the procedure given by [17] with minor modifications.

Table 1: Code and sequences of six- RAPD primers.

Number code	Primer name	Sequence (5`→3`)
1	OPA-02	5'-TGCCGAGCTG-3'
2	OPA-04	5'-AATCGGGCTG-3'
3	OPB-14	5'-TCCGCTCTGG-3'
4	OPC-5	5'-GATGACCGCC-3'
5	OPC-12	5'-TGTCATCCCC-3'
6	OPA-01	5'-CAGGCCCTTC-3'

Data Handling and cluster analysis (Phylogenetic Tree)

Data were 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 [18].

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The similarity coefficients (Dice coefficients) were, then ,used to construct dendograms, 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

After reviewing the results summarized in Table No. (2) Can be summarized and listed the most important results as follows:-

Table 2: Mean Performances For all Traits Studies of The Genotypes of Wheat under Normal and Drought Conditions.

Genotypes	H.D		P.H		G.Y		M.R.L		NO.OF.R/P		R.W.C		O.P		L.W.P	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P1	85.00	74.00	76.00	68.67	74.33	52.67	83.33	69.00	459.33	416.67	100.00	84.00	0.63	0.70	-7.17	-5.24
P2	109.67	117.67	119.00	111.67	22.00	15.67	22.00	14.00	210.67	122.33	22.00	17.00	2.37	2.89	-2.14	-1.45
P3	76.33	66.00	85.33	68.33	63.33	54.00	72.67	63.67	543.00	511.67	97.0	96.14	1.13	1.23	-4.42	-3.45
P4	113.67	120.00	122.67	108.67	21.33	13.67	37.67	22.67	196.33	134.33	33.00	27.67	3.51	4.15	-0.44	-0.34
P5	110.00	115.00	124.00	114.00	26.00	18.67	22.33	15.67	290.33	203.00	37.33	30.33	2.48	2.82	-1.92	-0.58
P1 x P2	78.67	70.00	70.33	60.67	82.54	61.54	101.67	89.67	666.00	562.67	98.0	97.05	0.23	0.25	-5.70	-4.83
P1 x P3	74.00	61.33	65.67	56.33	86.19	64.11	119.00	111.67	987.00	850.67	99.50	97.67	0.15	0.07	-8.94	-7.02
P1 xP 4	74.00	69.67	73.33	60.67	86.73	62.18	124.00	117.67	1126.00	951.00	90.00	86.67	1.35	1.62	-10.27	-11.7
P1 x P5	118.67	126.00	126.00	117.67	29.67	14.33	38.67	14.33	103.00	55.33	18.67	12.00	4.15	4.47	-0.35	-1.56
P2 xP 3	122.33	120.33	128.00	122.00	20.67	13.33	30.67	18.00	116.00	58.67	56.33	25.33	5.12	5.70	-2.81	-1.09
P2 x P4	112.33	120.33	128.67	115.00	15.67	11.00	33.67	24.33	242.33	210.00	43.33	18.00	2.51	3.00	-0.09	-0.45
P2 x P5	120.00	122.67	122.67	114.67	32.67	21.67	37.67	16.00	102.33	59.33	25.00	14.00	3.89	4.68	-0.88	-0.35
P3 x P4	116.00	123.33	121.33	98.00	25.67	15.67	22.33	13.67	201.67	162.33	38.33	14.67	2.59	2.87	-1.65	-1.94
P3 x P5	126.33	121.00	115.00	91.00	17.33	12.33	30.33	18.67	348.00	150.00	39.00	20.33	4.14	4.92	-0.03	-0.17
P4 x P5	119.33	129.33	107.33	97.33	28.67	13.00	33.00	13.67	296.33	168.67	48.33	23.33	5.17	5.54	-0.29	-0.59
LSD 0.05	**9.52**	**8.69**	**8.31**	**9.41**	**7.64**	**7.17**	**13.59**	**10.36**	**48.57**	**67.38**	**17.48**	**11.58**	**1.18**	**1.14**	**1.66**	**1.34**
LSD 0.01	**12.84**	**11.72**	**11.21**	**12.69**	**10.31**	**9.67**	**18.34**	**13.98**	**65.53**	**90.91**	**23.59**	**15.63**	**1.59**	**1.54**	**2.24**	**1.80**

The genotypes ;(P1 and P3 , P1 X P2, P1 X P3 and P1 X P4) were observed the earlier and shorter plants for heading date and plant height traits, As well as they had achieved a better and higher values for grain yield per plant, maximum root length , number of roots per plant and relative water content ,in addition to better and lowest date for osmotic pressure and leaf water potential , respectively. These results are the biggest evidence to confirm that these genotypes were early maturing where it can end their life cycle before increasing drought season . This mean that shorter stature and can carry a large amount number of panicles and spikelet's per plant . For this it can increasing the root system , the length and number of roots per plant. In addition to increasing the amount of water associated with the cells, and this in turn will not be achieved but down osmotic pressure and low leaf water potential , thus accessible to the kernel Egyptian wheat variety more tolerant , resistant for water stress and provide water for the cultivation of other crops more vulnerable to water, So we were successful in contributing to the solution part of the crisis and the problem of shortage of water resources. These results were in agreement with those reported by [19, 20].

Variation and Interactions

Mean squares of the ordinary and genetic analysis for all traits studied under normal and water stress conditions were observed in "Table3". Mean squares of all genotypes studied were exposed to be highly significant for all traits studied under normal and drought conditions. Suggested , significant overall differences among these populations. Both general and specific combining ability variances were found to be highly significant for all traits studied under all conditions. This result would indicate the prominence of both additive and non-additive genetic variances in determining the execution of these traits. GCA/SCA ratio were found to be less than the unity for all traits under normal and drought conditions, which, indicated that non additive gene action played an importance role in controlling these traits. Therefore, it could be concluded that selection procedures based on the accumulation of non- additive effects, would be successful in improving these besides selection by bulk method under normal and drought conditions, Hence it is clear that the basic idea of this analysis is to try, show and prove that these genotypes were difference and a far cry from some of them for the genetic convergence and this is what has already been achieved note that the screening process of natural filtering among themselves to choose the strongest genotype resistance for water stress will be useless in this regard**.** Similar results were obtained by [21].

Table 3: Mean Squares of Different Genotypes of wheat for all Traits Studied under all conditions.

S.O.V	df	H.D		P.H		G.Y		M.R.L		NO.OF.R/P		R.W.C		O.P		L.W.P	
		N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
Replication	**2**	4.42	25.62	1.36	1.36	32.82	20.56	70.47	10.29	2720.16	1187.29	25.49	4.69	0.17	0.09	1.28	0.07
Genotypes	**14**	1175.02**	2085.60**	1726.26**	1738.98**	1516.38**	1138.31**	3934.96**	4366.42**	297859.13**	249595.60**	3425.55**	3707.83**	8.47**	10.79**	21.70**	23.70**
GCA	**4**	741.61**	1536.07**	1231.83**	1204.37**	1200.77**	831.16**	2794.32**	2913.99**	134022.33**	130876.94**	2464.54**	2568.67**	4.58**	6.20**	11.86**	13.94**
SCA	**10**	251.70**	358.85**	312.86**	329.77**	227.34**	198.75**	718.59**	872.07**	85392.00**	64127.17**	612.77**	702.85**	2.12**	2.55**	5.38**	5.48**
Error	**28**	32.40	27.00	24.69	31.64	20.89	18.37	66.09	38.38	843.82	1623.81	109.30	47.98	0.50	0.47	0.99	0.64
Error term		10.80	9.00	8.23	10.55	6.96	6.12	22.03	12.79	281.27	541.27	36.43	15.99	0.17	0.16	0.33	0.21
GCA/SCA		**0.43**	**0.62**	**0.57**	**0.53**	**0.77**	**0.61**	**0.57**	**0.48**	**0.22**	**0.29**	**0.60**	**0.53**	**0.32**	**0.36**	**0.33**	**0.37**

Table 4: Estimates of Heterosis over better-parent for the crosses studied under normal and drought conditions.

Crosses	H.D		P.H		G.Y		M.R.L		NO.OF.R/P		R.W.C		O.P		L.W.P	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P1xP2	**-7.45**	**-5.41**	**-7.46**	**-11.65**	**11.04***	**1.68***	**22.00****	**29.95****	**44.99****	**35.04****	**-2.0**	**15.53***	**-90.14****	**-91.34****	-20.50	-7.82
P1xP3	**-3.06**	**-7.07**	**-13.60***	**-17.56***	**15.95****	**18.72****	**42.80****	**61.84****	**81.77****	**66.25****	**-0.5**	**1.59**	**-86.76**	**-94.57***	24.688*	33.96*
P1xP4	**-12.94***	**-5.86**	**-3.51**	**-11.65**	**16.68****	**18.05***	**48.80****	**70.53****	**145.14****	**128.24****	**-10.0**	**3.17**	**-61.63****	**-60.96****	43.23**	123.28**
P1xP5	**39.61****	**70.27****	**65.79****	**71.36****	**-60.09****	**-72.78****	**-53.60****	**-79.23****	**-77.58****	**-86.72****	**81.33****	**-85.71****	**67.56****	**58.32****	-95.11**	-70.22**
P2xP3	**60.26****	**82.32****	**50.00****	**78.54****	**-67.37****	**-75.31****	**-57.80****	**-71.73****	**-78.64****	**-88.53****	**-4.92****	**-73.65****	**116.48****	**97.58****	-36.42	-69.20**
P2xP4	**2.43**	**2.27**	**8.12***	**5.83**	**-28.79****	**-29.79**	**-10.62**	**7.35**	**15.03**	**56.33***	**31.31**	**-34.94**	**-28.40**	**-27.63***	-97.96*	-68.96
P2xP5	**9.42***	**6.67**	**3.08**	**2.69**	**25.64**	**16.07**	**68.66***	**2.13**	**-64.75****	**-70.77****	**-33.02**	**-53.84****	**56.93***	**62.12****	-58.87	-75.86
P3xP4	**51.97****	**86.87****	**42.19****	**43.41****	**-59.47****	**-70.99****	**-69.27****	**-78.53****	**-62.86****	**-68.27****	**-60.84****	**-84.74****	**-26.31**	**-30.92***	-62.66**	-43.76*
P3xP5	**65.50****	**83.33****	**34.77****	**33.17****	**-72.63****	**-77.16***	**-58.26****	**-70.68****	**-35.91****	**-70.68****	**-59.79****	**-78.85****	**67.03****	**74.38****	-99.32**	-95.07**
P4xP5	**8.48**	**12.46****	**-12.50****	**-10.43***	**10.26**	**-30.36**	**-12.39**	**-39.71**	**2.07**	**-16.91****	**29.46**	**-72.22**	**47.39****	**33.49***	-84.89	-175.38
LSD 0.05	**9.52**	**8.69**	**8.31**	**9.41**	**7.64**	**7.17**	**13.59**	**10.36**	**48.57**	**67.38**	**17.48**	**11.58**	**1.18**	**1.14**	**1.66**	**1.34**
LSD 0.01	**12.84**	**11.72**	**11.21**	**12.69**	**10.31**	**9.67**	**18.34**	**13.98**	**65.53**	**90.91**	**23.59**	**15.63**	**1.59**	**1.54**	**2.24**	**1.80**

Table 5:- Estimates of General combining ability effects for the 5 parental varieties studied for all traits of wheat under normal and drought conditions.

Parents	H.D		P.H		G.Y		M.R.L		NO.OF.R/P		R.W.C		O.P		L.W.P	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P1	-15.31**	-21.10**	-20.97**	-18.46**	21.51**	16.64**	32.34**	31.75**	206.48**	200.90**	27.41**	29.50**	-1.23**	-1.45**	-2.02**	-2.20**
P2	4.30**	6.57**	7.65**	10.54**	-6.20**	-4.65**	-10.85**	-10.44**	-115.33**	-101.62**	-9.35**	-9.50**	0.10	0.21	0.59**	0.58**
P3	-4.46**	-9.24**	-4.78**	-8.27**	3.23**	5.11**	3.44*	5.75**	54.76**	56.90**	9.89**	8.11**	-0.21	-0.28*	-0.36	-0.37*
P4	3.78**	8.57**	5.98**	3.78**	-8.15**	-7.12**	-5.04**	-4.91**	-13.76*	-12.29	-7.59**	-9.22**	0.41**	0.48**	0.34	0.43**
P5	11.69**	15.19**	12.12**	12.40**	-10.39**	-9.98**	-19.90**	-22.15**	-132.14**	-143.90**	-20.35**	-18.89**	0.93**	1.04**	1.45**	1.56**
LSD 0.05	**2.28**	**2.08**	**1.99**	**2.25**	**1.83**	**1.71**	**3.25**	**2.48**	**11.61**	**16.11**	**4.18**	**2.77**	**0.28**	**0.27**	**0.40**	**0.32**
LSD 0.01	**3.07**	**2.80**	**2.68**	**3.03**	**2.47**	**2.31**	**4.38**	**3.34**	**15.67**	**21.73**	**5.64**	**3.74**	**0.38**	**0.37**	**0.54**	**0.43**

Heterosis over better-parent

After reviewing the results described and shown in Table (4), relating in particular to heterosis over better-parent were observed the crosses ; (P1 X P4) for heading date trait under normal conditions and ;(P1 X P3 and P4X P5) for plant height trait under all conditions besides (P1 X P2 and P1 X P4) under normal and drought conditions , (P1 X P3 , P2 X P4 and P3 X P4) under water stress conditions only for osmotic pressure trait in addition to (P1 X P5 , P3 X P4 and P3 X P5) under all situations and the crosses ; (P2 X P3) under water stress conditions and (P2 X P4) under normal conditions only for leaf water potential were detected significantly and highly significantly negatively of heterosis over better-parent, respectively.

On the contrary ; significantly and highly significantly positively of heterosis over better-parent were showed in the three crosses ; (P1 X P2, P2 X P3 and P1 X P4) for the traits of grain yield per plant , maximum root length and number of roots per plant under normal and drought conditions besides the crosses ; (P2 X P5 and P2 X P4) under normal conditions only for maximum root length and under drought conditions only for number of roots per plant traits , respectively. While the two crosses ; (P1 X P2) under drought conditions and (P1 X P5) under normal conditions were recorder significantly and highly significantly positively of heterosis over better-parent for relative water content , respectively.

There is no doubt that heterosis over better-parent had given a strong ability for SCA effects to increase the ability of drought tolerance by increasing maximum root length , number of roots per plant for access to water in the distant depths and increasing also relative water content to keep and use it on hot days characterized by water scarcity , On the other angle decreasing osmotic pressure and leaf water potential as well as early maturity to provide water for agriculture and providing agricultural area for the cultivation of other crops and the short length of the plant to carry a larger number of spikes for high yielding in the three crossing of wheat namely ; (P1 X P2 , P1 X P3 and P1 X P4) depending on the degree of sovereignty and the interaction of it,(Dominance, dominance X dominance and additive X dominance) types of gene action . Similar results were in agreement with those reported by [22].

Combining ability effects

General combining ability effects

Estimates of the GCA effects of the parental varieties under normal and water stress conditions are summarized in "Table 5".

Significant and highly significant negatively of GCA effects were showed in the parents ; (P1 and P3) for heading date and plant height traits under both conditions ,while the same results were observed for osmotic pressure and leaf water potential traits under water stress only, respectively.

In the other antithesis ; the same parents recorded significant and highly significant positively of GCA effects in the traits grain yield per plant , maximum root length , number of roots per plant and relative water content under normal and drought conditions . The other parents ; (P2 , P4 and P5) showed negatively and highly significant values for GCA effects for the same traits under all conditions , respectively , which indicated that the importance of additive and additive X additive types of gene action in the inheritance of these traits and it resulted in a significant and vital role in develop and improve these traits through early maturity , shorter plant height, higher yield , increasing the depth of the root system and the high percentage of water associated with the cells as well as low osmotic pressure and low leaf water potential than his impact in the increase of wheat resistance to water stress. Similar results were obtained by [23].

Specific combining ability effects

The results in table (6), revealed that the crosses ; (P1 X P2, P1 X P3 and P1 X P4) were significantly and highly significantly negatively values of SCA effects in heading date , plant height , osmotic pressure and leaf water potential traits under all conditions except the cross; (P1 X P4) for osmotic pressure under normal and drought conditions and the cross; (P1 X P3) for leaf water potential trait under normal conditions only , while the cross; (P3 X P5) showed the same results for plant height trait under water stress only.

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Table 6: Estimates of Specific combining ability effects for the ten crosses studied of all traits in wheat under normal and water stress conditions.

Crosses	H.D		P.H		G.Y		M.R.L		NO.OF.R/P		R.W.C		O.P		L.W.P	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P1xP2	-14.08**	-19.25**	-22.03**	-25.06**	22.51**	18.59**	26.24**	26.84**	182.30**	155.60**	37.92**	40.92**	-1.27**	-1.50**	-1.47**	-1.69**
P1xP3	-9.98**	-12.11**	-14.27**	-10.59**	1.75	5.16*	29.29**	32.65**	333.20**	285.10**	11.02*	16.30**	-1.03**	-1.20**	-0.77	-0.92*
P1xP4	-18.22**	-21.59**	-17.37**	-18.30**	11.13**	14.06**	42.76**	49.32**	540.70**	454.60**	21.16**	22.63**	-0.46	-0.40	-1.80**	-1.49**
P1xP5	18.54**	28.13**	29.16**	30.08**	-20.30**	-20.41**	-27.71**	-36.78**	-363.90**	-309.40**	-46.41**	-42.37**	1.82**	1.89**	2.72**	2.72**
P2xP3	18.73**	19.22**	19.44**	26.08**	-15.21**	-15.22**	-15.86**	-18.83**	-216.00**	-204.40**	-2.22	-17.03**	2.61**	2.78**	3.38**	3.40**
P2xP4	0.49	1.41	9.35**	7.03*	-8.83**	-5.32*	-4.38	-1.83	-21.10	16.10	2.25	-7.03	-0.63	-0.68	-0.05	-0.04
P2xP5	0.25	-2.87	-2.79	-1.92	10.41**	8.21**	14.48**	7.08*	-42.70**	-2.90	-3.32	-1.37	0.22	0.44	0.63	0.72
P3xP4	12.92**	20.22**	14.44**	8.84**	-8.25**	-10.41**	-30.00**	-28.68**	-231.90**	-190.10**	-21.98**	-27.98**	-0.24	-0.33	0.46	1.41**
P3xP5	15.35**	11.27**	1.97	-6.78*	-14.35**	-10.89**	-7.14	-6.44*	32.80*	-70.80**	-8.56	-12.65**	0.79*	1.17**	2.73**	2.50**
P4xP5	0.11	1.79	-16.46**	-12.49**	8.37**	2.02	4.00	-0.78	49.70**	17.10	18.25**	7.68*	1.20**	1.02**	0.29	0.12
LSD 0.05	**5.87**	**5.36**	**5.13**	**5.81**	**4.72**	**4.42**	**8.39**	**6.39**	**29.98**	**41.59**	**10.79**	**7.15**	**0.73**	**0.71**	**1.02**	**0.82**
LSD 0.01	**7.93**	**7.24**	**6.92**	**7.83**	**6.36**	**5.97**	**11.32**	**8.63**	**40.45**	**56.11**	**14.56**	**9.64**	**0.98**	**0.95**	**1.38**	**1.11**

Table 7: Tolerance indices of the five wheat genotypes through two levels of irrigations.

Genotypes	GYP	GYD	YSI	YI	GMP	DTI	MP	YR	DSI
(G1)	74.33	52.67	0.70	1.41	63.5	0.59	63.5	0.30	2.38
(G2)	22.0	15.67	0.71	1.40	18.83	0.58	18.83	0.29	2.32
(G3)	63.33	54.0	0.85	1.17	58.66	0.54	58.66	0.15	5.69
(G4)	21.33	13.67	0.64	1.56	17.5	0.61	17.5	0.36	1.65
(G5)	26.0	18.67	0.72	1.39	22.33	0.58	44.67	0.28	2.42

For grain yield per plant trait; the crosses; (P1 X P2, P1 X P4 and P2 X P4) under all conditions in addition to the two crosses; (P1 X P3) under drought conditions and (P4 X P5) under normal conditions only showed significant and highly significant positively values of SCA effects ,respectively. While the crosses; (P1 X P2 , P1 X P3 , P1 X P4 and P2 X P4) detected the same results under all conditions for maximum root length trait, respectively, and if we dealt with the rest of the statistical analysis using half diallel of Griffing analysis note that the crosses ;(P1 X P2 , P1 X P3 and P1 X P4) under all conditions besides the hybrids ;(P3 X P5 and P4 X P5) under normal conditions realized significant and highly significant positively values of SCA effects for number of roots per plant, respectively.

Finally the crosses; (P1 X P2 , P1 X P3, P1 X P4) and (P4 X P5) acquired significant and highly significant positively values of SCA effects for relative water content under normal and water stress conditions , These results coincided with [23].

With regard to the individual types of diegetic epistatic gene effects, the three types of gene interaction ; (dominance, dominance X dominance and additive X dominance) were very important in the inheritance of yield components and related characters specially 1000-grain weight, number of filled grains/panicle and grain yield /plant under normal and drought conditions. Finally, it can be concluded that the average percentage of heterosis as a deviation from better-parent were highly significant positive or negative in all studies crosses for grain yield and its major components in the present investigation while, it was differed from character to character and from cross to another, This illustrates the importance of SCA effects to express the degree of progress in the genetic improvement to resistance for water stress in the crosses ; (P1 X P2 , P1 X P3, P1 X P4 and P4 X P5), respectively.

Drought Tolerance indices

From table (7) the genotypes with low DSI values (less than 1) can be considered to be drought susceptible **[24]** because they exhibited higher yield reductions under water stress compared with normal condition than the mean of all genotypes and could be due to lack of yield production under normal conditions rather than an indication of its ability to tolerate water stress. However, the highest values of DSI values may necessarily give a good indication of drought tolerance of genotypes because it was able to reduce the rate of water loss during drought and gave high yields as well as on their ability to access to water in the deeper layers of the soil during the dry season, respectively, [20].

Molecular Studies

RAPD- Markers

RAPD data analysis the fragments were recorded in presence and absence of fragments on gel photographs in Fig,(1) number (a ,b ,c ,d ,e ,f) and Table (8). Using RAPD-PCR technique of six primers showed 68 fragments, where 39 of them were polymorphic bands with (57.35) % and 29 were monomorphic bands with **(42.64%)** , in addition to the average of polymorphism was 53.33% . The band size was range between 100 to 3121bp. The six primers give average of 11 bands **/** primer, as show in Fig (1 ,a, b, c, d, e, f) and Table (8), respectively.

The primer (OPA-2) revealed twelve bands, eight of them were monomorphic bands , four bands were polymorphic with 33.33% polymorphism and the range size of bands was 200 to 2000 bp , Fig. (1,a) and Table (8) in addition to the Primer (OPA-04) showed 13 amplicons bands , two of them were monomorphic bands , twelve of them were polymorphic bands with 84.61% polymorphism and the range size of bands was 458 to 2842 bp, Fig. (1,b) and Table (8).

While the third primer (OPB-14) detected six variable bands , three of them was monomorphic bands and the rest three bands was polymorphic bands with polymorphism 50% , range size of bands was 338 to 1311bp in Fig. (1,c) and Table (8), but, the primer (OPC-5) showed 17 amplicons bands , three of them was monomorphic bands , fourteen of them was polymorphic bands with polymorphism 82.35% and range size of bands was 120 to 3121bp in Fig. (1,d) and Table (8), respectively.

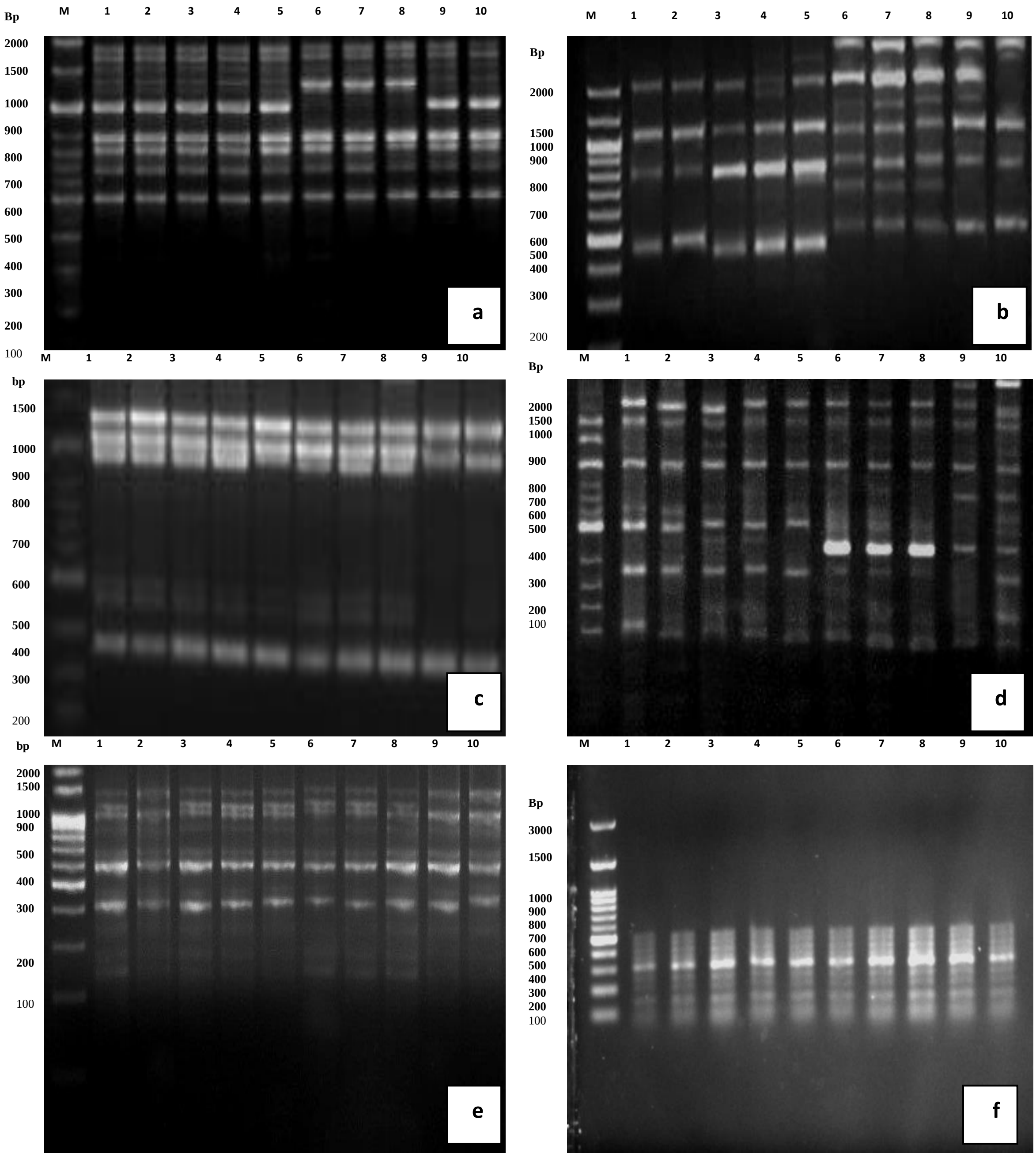

Fig.1 : RAPD- PCR Banding Patterns of Ten wheat Genotypes using (a) Primer, OPA-02. M = 2000bp: 100bp Ladder Marker ,where, 1: P1 , 2: P2 ,3: P3 ,4: P4 ,5: P5 , 6: P1 X P2, 7: P1 X P3 , 8: P1 X P4 , 9 : P2 X P4 , 10 : P3 X P5.
(b) Primer, OPA-04. M = 2000bp: 200bp Ladder Marker. (c) Primer, OPB-14. M = 1500bp: 200bp Ladder Marker.
(d) Primer, OPC-5. M = 2000bp: 100bp Ladder Marker. (e) Primer, OPC-12. M = 2000bp: 100bp Ladder Marker.
(f) Primer, OPA-01. M = 3000bp: 100bp Ladder Marker.

Finally the primers (OPC-12 and OPA-1) showed 11 and 9 amplicons bands, where primer (OPC-12) revealed seven monomorphic bands and four polymorphic bands with polymorphism 36.36% and range size of bands was 252 to1419 bp, while primer (OPA-1) showed six monomorphic bands and three polymorphic bands with polymorphism 33.33%, range size of bands was 100 to 500bp in Figure (1) number (e , f) and table (8), respectively. However knowing that primers (OPA-1 and OPA-2) recorded the lowest polymorphism % (33.33 % and 33.33 %), while primer OPA-4 revealed the highest polymorphism % (84.61%), on the other hand the primer OPB-14 recorded the lowest number of bands (6) with polymorphism % of 50%, while the primer OPC-5 recorded the highest number of bands (17) with polymorphism % of 82.35%, respectively.

Similar results were agreement with those reported by [25-32] studied RAPD-PCR reactions by using 2 random primers, and total 27 scrabble bands were observed in four species of rice varieties ranging from 1600bp to 300bp , While, [33] determined 51 bands ranging from 2344bp to 160bp using seven primers in some genotypes of rice, On the other hand [34] studied nine RAPD-PCR reactions using seven cultivars of Egyptian fig and showed 111 bands divided into 39 monomorphic bands and 72 polymorphic bands with 64.86% polymorphism and [35] revealed 71 fragments in six genotypes of wheat using six primers , where 52 of them were monomorphic bands and 19 bands were polymorphic bands with 26.76% polymorphism , while in this study we observed 68 bands , 29 of them were monomorphic bands and 39 were polymorphic with 57.35 % polymorphism, respectively.

Table 8: Total number, monomorphic, polymorphic bands and percentage of polymorphism as Revealed by Six RAPD primers on Ten Genotypes of Wheat.

Primer code	Total bands	Monomorphic bands	Polymorphic Bands	Unique bands	polymorphism%	Range size of bands (bp)
OPA-2	12	8	4	0	**33.33%**	**200:2000**
OPA-4	13	2	8	3	**84.61%**	**458:2842**
OPB-14	6	3	3	0	**50%**	**338:1311**
OPC-5	17	3	8	6	**82.35%**	**120:3121**
OPC-12	11	7	4	0	**36.36%**	**252:1419**
OPA-1	9	6	3	0	**33.33%**	**100:500**
Total bands	68(100%)	**29 (42.64%)**	**30 (44.11%)**	**9 (13.23%)**	**53.33% (average)**	**100:3121**

If we look at the results shown in Table No. (9), we find that most of all genotypes recorded high numbers of bands ranged from (10 to 12) and (9 to 11) using OPA-02 and OPC-12 primers , respectively, while the primers OPA-04 , OPB-14 , OPC-5 and OPA-01 revealed values ranging from low to medium of the number of bands ,where the values ranged from (4 to 7) , (3 to 6) as low values for OPA-04 and OPB-14 primers and (7 to 12) , (7 to 9) as medium values for the primers OPC-5 and OPA-01 of the ten wheat genotypes , respectively. So (P1 and P2) recorded the lowest number of bands (four bands) using OPA-04 primer in addition to (H4 and H5) revealed the lowest number of bands (three bands) using OPB-14 primer which means that these two crosses were highly susceptible for water stress , while , the parents (P1 , P3 , H1, H2, H3) recorded the highest number of bands using all primers especially (OPA-02 , OPC-12 , OPC-5 , OPA-01) primers and were the most effective and important to discriminate and comparison between all genotypes studied concerning a genetic variation , respectively. Similar results were in agreement with those reported by **[34].**

Table 9: Total bands produced from each primer for the ten genotypes of wheat.

Genotypes	OPA-02	OPA-04	OPB-14	OPC-5	OPC-12	OPA-01
P1	12	4	6	8	10	9
P2	12	4	6	7	10	8
P3	12	5	6	9	11	7
P4	12	5	5	10	11	7
P5	10	6	6	9	10	8
H1	10	7	6	9	9	8
H2	10	7	6	11	10	9
H3	10	7	6	8	11	7
H4	10	7	3	8	10	8
H5	11	5	3	12	9	9

Table 10: Genetic Similarity matrix between ten genotypes of wheat with RAPD markers based on Jaccard coefficients.

Case	P1	P2	P3	P4	P5	H1	H2	H3	H4	H5
P1	**1.0**									
P2	**0.836**	**1.0**								
P3	**0.840**	**0.872**	**1.0**							
P4	**0.837**	**0.812**	**0.893**	**1.0**						
P5	**0.860**	**0.816**	**0.857**	**0.875**	**1.0**					
H1	**0.773**	**0.764**	**0.803**	**0.845**	**0.788**	**1.0**				
H2	**0.811**	**0.804**	**0.807**	**0.823**	**0.792**	**0.960**	**1.0**			
H3	**0.773**	**0.800**	**0.804**	**0.820**	**0.754**	**0.958**	**0.959**	**1.0**		
H4	**0.611**	**0.694**	**0.666**	**0.647**	**0.592**	**0.705**	**0.711**	**0.740**	**1.0**	
H5	**0.625**	**0.611**	**0.589**	**0.629**	**0.607**	**0.625**	**0.660**	**0.619**	**0.750**	**1.0**

Proximity matrix analysis

Detected the genetic relationships among all genotypes were estimated in terms of similarity using Dice coefficient, these results reported in Fig.2 and Table (10).RAPD markers used to figure out the wheat genotypes relationships by UPGMA of the dendrogram , and in the Proximity matrix distinguished relationships among the five parents and the five hybrids.

The similarity ranged from (0.589 to 0.960) , where the lowest similarity was (0.589) between P3 and H5 , while the highest value was (0.960) among H1 and H2, On the other site the middle values of similarity were observed between some genotypes for example (H4 and H5) , (P2 and P4) , (P3 and P4) and (H2 and H3) where the values were (0.750, 0.812 , 0.893 and 0.959) , respectively, These results are considered a strong potential to improve and develop the endurance levels of drought tolerance in wheat crop in the case of using the parents.,(P1,P2,P3) with High possibility , while , we can using the parents (P4 , P5) in a lesser extent in breeding program.

Genetic Similarity

The present investigation aimed to study the RAPD Markers efficiency in determining and accurately the genetic relationships among all the ten wheat genotypes using six primers table (10). Genetic similarity ranged from (0.589 to 0.960) and the mean value of genetic similarity was (0.774) including 45 pairwise comparisons among the ten wheat genotypes , based on 68 bands , 29 of them were monomorphic bands and 39 were polymorphic bands with 57.35% polymorphism.

The phylogenetic tree divided to two main cluster , the first one divided to one sub-cluster including (H4 , H5) , while the second cluster divided including the rest of the ten genotypes of wheat and divided to two sub-cluster ., the first one divided to two groups , the first group included (H3) only and the second group included (H1 , H2) , but sub-cluster number two divided to two groups, the first one including (P2) only and the second group divided in to two sub-groups where the first sub-group including (P1 , P5) and the second sub-group including (P3 , P4) , in fig (2) , respectively. Similar results were in agreement with those reported by [36, 37] who studied and observed that any genotypes from the same geographical area were divided in to different clusters.

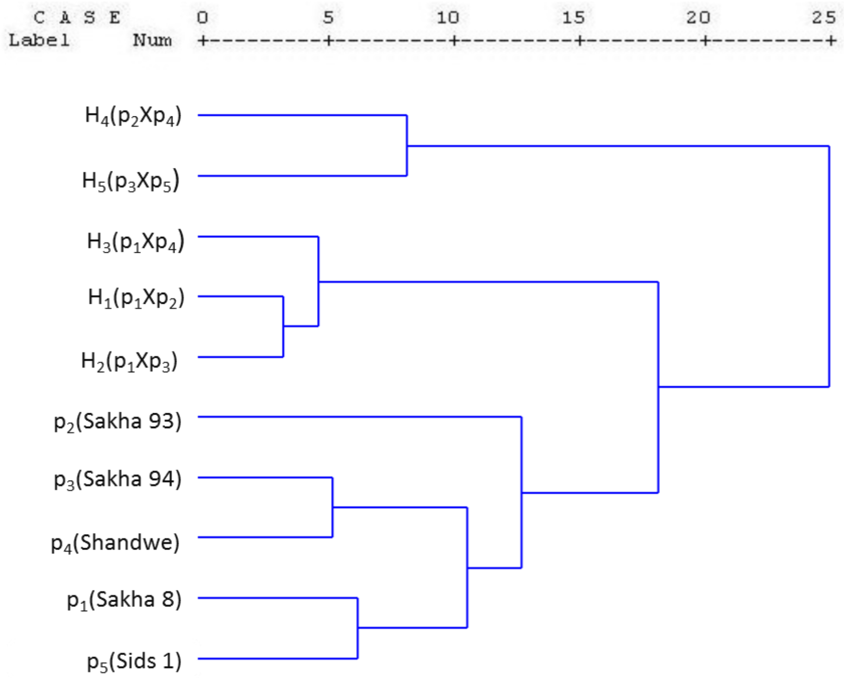

Fig. 2: Dendrogram of Genetic relationships among the ten genotypes of wheat.

CONCLUSION

Five cultivars of wheat with different reaction of drought tolerance and their crosses were used in this study under two levels of irrigation (control and water stress every 15 days) to know the genetic behavior of the ability for drought tolerance using some morphological and physiological traits through half diallel analysis and comparison between the five genotypes of wheat using six primers of RAPD-Markers technique to reach to a distinctive molecular genetic differences for each genotype of wheat and genetic comparison of these differences as indexed a taxonomic markers mind for water stress resistance. Cluster analysis was the most important measurements achieved for the five parents , the highest three crosses for drought tolerance and the lowest crosses of water stress resistance to study the similarity and genetic variability among them , the phylogenetic tree and genetic variability beside the relationships between the ten genotypes were used for improving the ability of drought tolerance between these genotypes and learn more parents closer to each other and the like incorporated in the private wheat breeding program to produce hybrids through hybridization program, and then producing lines of wheat high-yielding and resistant for water stress conditions.

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