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Genetic diversity among wheat genotypes using RAPD markers and its implication on genetic variability of diallel crosses

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Hybrids involving diverse parental genotypes generally produce high specific combining ability effects and hybrid vigor. Therefore, the choice of the suitable breeding program depends on the inheritance of the quantitative traits. This research aims at detecting the extent of the genetic diversity (GD) among 8 wheat parental genotypes, and its implication on F_1 crosses for biological and grain yield plant^{-1}. Thus the 8 parents were screened using ten RAPD primers. These primers produced 89 fragments, 58 of them were polymorphic. A GD value as high as 0.474 was found just one cross (P1xP4). Half diallel mating, design involving 8 parents, was made to initiate a set of 28 F_1 crosses. Parents and their crosses were tested under normal and drought stress conditions for biological and grain yield per plant. Genotypes, general (GCA) and specific (SCA) combining ability were significant for the two traits. The non-additive gene action is substantial in controlling these traits. Five crosses showed all showed relatively high mean performance and SCA effects for both traits in both and across normal and drought environment. For biological and per plant grain yield, Pearson's correlation coefficients with GD were 0.34 and 0.69. Hence, RAPD marker can be used as a tool for determining the extent of genetic diversity among wheat genotypes and to precisely predict the yield performance value for F_1 hybrids.

Keywords: combining ability, drought stress, *GCA, RAPD marker, SCA,* Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L) yield is exposed to a wide range of damaging drought stress in many production areas around the globe due to climate change conditions. One way to guard against these harmful changes is to develop wheat crosses that exhibit relatively more water stress resistance.

Molecular techniques are now the most reliable, easy, cheap and valuable tool for assessing the genetic diversity in genome research (Tonk et al., 2014 and EL Saadoown et al., 2018). Random amplified polymorphic DNAs tool (RAPDs) has been proved to be independent of the confounding effects of environmental factors.

Drought, among abiotic stress factors, is considered the most wide spreading and limiting to crop productivity. (EL Saadoown et al., 2017) illustrated that many factors like, potential evapotranspiration, evapotranspiration, precipitation, temperature, humidity individually or in combination leads to drought stress. Water stresses can happen at any stage of plant growth and development; therefore, studying the genetic nature of resistance and productivity is needed.

Diallel crosses schemes are extremely used in plant breeding research to get information

about genetic properties of parental lines and/or to estimate both general combining ability (GCA) and specific combining ability (SCA) (Shrief et al., 2017). In addition, this technique provide prior information on the genetic behavior of these attributes in F_1 (Chowdhry et al., 1992 and Topal et al., 2004). Heterosis is a complex phenomenon, which depends on the balance of different combinations of gene effects, as well as on the distribution of plus and minus alleles in the parents of a mating system. In self-pollinated crops, like wheat, the scope for utilizing heterosis depends mainly upon the direction and magnitude of heterosis. Heterosis over better parent may be useful in identifying the best crosses might show immense practical value if they involve the best cultivars of the area (El Hosary and Nour El Deen 2015).

Therefore, this investigation aims at applying the RAPD-PCR marker technique to detect DNA polymorphism, to identify parents and to estimate genetic diversity among wheat genotypes. In addition to using a half diallel crosses among eight genotypes to assess genetic variability under drought and normal irrigation treatments and to correlate F_1 performance to genetic divergence of the parental genotypes.

MATERIALS AND METHODS

Plant material

Eight wheat genotypes, P_1-P_8 (Table 1) were crossed in 8x8 diallel cross excluding reciprocals giving a total of 28 crosses in the first season of 2015/2016.

Laboratory Experiment

DNA extraction

Ten seeds of parental genotypes were planted in pots. Then, leaf tissue was collected from 5-7 days old germinated seedlings of each parent. The samples were bulked, lyophilized and ground with a mortar and pestle. Genomic DNA was isolated and extracted according to the manufacturer's protocol carefully using DNeasy® Plant mini Kit for DNA isolation. The purity, quantification and qualification of the extracted DNA were tested.

RAPD-PCR

Random amplified polymorphic DNA (RAPD reactions) were conducted using a set of ten 10-mer oligonucleotide (Primer from Operon Technologies, lnc.) i.e. OP A2, A9, B5, B7, C6, C20, D2, D10, D16 and E7 were screened for the ability to provide a suitable band pattern with various parents. These primers give polymorphic results for parents under study. All PCR reactions were performed as reported by Williams et al., (1990). Products of amplification were separated by electrophoresis on 1.2% agarose gels, stained with ethidium bromide, then photographed under UV-trans illuminator by digital camera with UV filter adaptor.

Field layout and management, and experimental design

Two field trials were conducted during 2016/2017 seasons at the Moshtohor Experiment Research Station (30° 21´ 07´´ N and 31° 13´ 34´´ E), Al-Khalubiah, Egypt. In each trail, seeds of 8 parents and its resultant 28 F_1 crosses (thereafter named P_ixP_j), were planted on 4 December 2016 in three completely randomized blocks. The first trial was irrigated only once after planting irrigation and the second one was normally irrigated 5 times. Both parent and F1 seeds were put in 0.20-m hills in 3.0 m x 0.30 m single-row plots. All field and crop management practices were followed as needed.

Meteorological data in season 2016/ 2017 were obtained from the Agro-meteorological Station at Moshtohor, Benha Univ. from December to May, the Maximum temperatures were 19.7, 17.7, 20.4, 25.8, 29.1 and 34.5°C, and the minimum temperatures were 9.2, 6.1, 7.8, 11.4, 14.4, and 19.0 °C, relative humidity were 51.3, 55.9, 47.2, 37.3, 38.9 and 32.1% and the mean precipitation were 0.5, 1.6, 0.8, 0.4, 0.3 and 0.00 mm respectively.

Sampling and studied traits

A 10-plant random sample was selected from each individual plot to calculate mean biological yield and per plant grain yield.

Data analysis

Separated bands for each RAPD marker were filed as binary data matrices, where 0 for absence and 1 presence of band. Similarity coefficients between a pair of parental genotypes were calculated according to Jaccard (1908). A dendrogram tree was constructed by the UPGMA clustering algoritm from the SAHN option of NTSYS-PC version 2.1 (Rohlf, 2000).

Table (1): The name, pedigree and source of the studied parental genotypes

.NO	Entry name	Pedigree	Source
1	Yakora Rojo	Ciano 67/Sonora 6411 Klien Rendidor/3/1L815626Y-2M-1Y-0M-302M	CIMMYT
2	Sakha 93	S 92/TR 810328 S8871-1S-2S-1S-0S	Egypt
3	Masr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	Egypt
4	Drought 4	Landraces developed by Prof. Dr. M. El Bedawy	Egypt
5	Shandawel 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/B HC	Egypt
6	Gemiza 9	ALD"S"/HUAC"S"//CMH74A.630/5X	Egypt
7	Giza 171	SAKHA 93 / GEMMEIZA 9 S.6-1GZ-4GZ-1GZ-2GZ-0S	Egypt
8	Sides 13	KAUZ"S"//TSI/SNB"S"	Egypt

NOVA was conducted as outlined by Steel and Torrie (1980) for all characters. Both GCA and SCA mean squares were calculated according to Griffing's (1956) Method II, Model I. A combined analysis of the two experiments was carried out whenever homogeneity of mean squares was detected (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

RAPD Polymorphism

The polymorphic amplification products set of ten RAPDs primers were presented in table (2). Per primer fragment mean was 8.9 that was generated in a range of 5-13 fragments, among a total of 89 generated fragments.

Among of them 58 were polymorphic within the eight genotypes (Table 2). Other studies indicated different results (Cao et al., 2002, Guo-yue and Hui, 2007, Tahir, 2008, Siddiqui et al., 2010, and Kumar et al., 2017).

Genetic similarity

The genetic similarity matrix was produced from the RAPD data using Nei and Li's formula (1979) (Table 3). Genetic similarity ranged from 0.526 for P1 and P4 to reach 0.812 for P1 and P2, with and overall mean of 0.669 among all genotypes.

Cluster analysis:

Based on RAPD data, the dendrogram constructed from cluster analysis (Fig. 1) shows two main clusters. Each of P4, P5, and P7 are in Cluster 1, and the rest of genotypes is in Cluster 2. The latter one shows two sub clusters: one contains P8, and in the other one, P1 and P2 were closely related.

Field experiment

Table 4 shows the ANOVA for both per plant biological and grain yields for each of and across irrigations. For the two yield characters, differences between the two irrigations (environments) were significant ($p<0.01$). Genotypes and its components (parents, crosses and parent *vs* crosses) mean squares were significant for studied yield traits indicating wide diversity among all genotypes used in this work. Moreover, significant mean squares between each of genotypes, parents and crosses by environment interaction were detected for biological and grain weight/ plant. For both yield characters, the interaction effects of irrigation regime with each of genotypes, parents, and crosses greatly varied ($p<0.01$) (Table 4). In addition, the same trend existed for all main effects of genotype, and for parent, cross, and parent vs. cross.

Mean trait performance

Across environment (irrigation regime), per plant biological yield widely ranged from about 111 to 212 g and from about 29 to 52 g for per plant grain weight among all parents and the 28 crosses (Table 5). This 2-fold range for both yield traits implied how genotypes unevenly responded to greatly varied irrigational environments. Percentage performance improvement, for the parent and F_1 hybrids, showed significant differences ($p < 0.05$) relative to average genotypes (Table 5). Concerning Across environment (irrigation regime), per plant biological yield widely ranged from about 111 to 212 g and from about 29 to 52 g for per plant grain weight among all parents and the 28 crosses (Table 5).

Table (2): Primer used in RAPD analysis of eight wheat genotypes and total number of fragments detected by each primer and number of polymorphic fragments.

Primers	Sequence	TSB	TF	NPF	PPF
OPA-02	TGCCGAGCTG	75	11	7	63.64
OPA-09	GGGTAACGCC	76	13	7	53.85
OPB-05	TGCGCCCTTC	49	10	8	80.00
OPB-07	GTCCACACGG	53	11	9	81.82
OPC-06	GAACGGACTC	49	9	6	66.67
OPC-20	ACTTCGCCAC	46	9	6	66.67
OPD-02	GGACCCAACC	29	5	4	80.00
OPD-10	GGTCTACACC	33	6	3	50.00
OPD-16	AGGGCGTAAG	43	6	3	50.00
OPE-07	TCAGGGAGGT	55	9	5	55.56
Total		508	89	58	-
Mean		50.8	8.9	5.8	65.17

Where: TSB = Total number of scorble bands, TF= Total number of fragments, NPF = Number of polymorphic fragments, PPF = Percentage of polymorphic fragments.

Table (3): Similarity matrix based on Nei and Li's coefficient for eight genotypes in wheat revealed by RAPD.

Rows/ cols	p1	p2	p3	p4	p5	p6	p7	p8
p1	1							
p2	0.812	1						
p3	0.792	0.806	1					
p4	0.526	0.600	0.569	1				
p5	0.615	0.567	0.617	0.779	1			
p6	0.788	0.753	0.760	0.544	0.573	1		
p7	0.608	0.620	0.609	0.794	0.792	0.605	1	
p8	0.685	0.724	0.618	0.637	0.600	0.657	0.704	1

Where, P1 = Yakora Rojo, P2 = Sakha 93, P3 = Masr 1, P4 = Drought 4, P5 = Shandawel 1, P6 = Gemiza 9, P7 = Giza 171, P8 = Sides 13

Table (4): Mean squares for yield traits under drought stress condition and normal irrigation as well as the combined over them.

S.O.V	df		Biological yield/ plant			Grain weight / plant		
	S	**C**	**Drought**	**Normal**	**Combined**	**Drought**	**Normal**	**Combined**
Irrigation (I)		**1**			136604.74**			1628611**
Rep/ I	**2**	**4**	64.33*	30.7	47.519**	17.76**	2.12	9.94
Genotypes (G)	**35**	**35**	3213.69**	1751.33**	3555.66**	176.72**	149.35**	233.03**
Parent (P)	**7**	**7**	1992.57**	1454.07**	1914.43**	102.05**	146.03**	185.69**
Cross (C)	**27**	**27**	3639.48**	1843.45**	4060.93**	195.69**	149.42**	227.73**
P vs C.	**1**	**1**	265.01**	672.45**	1401.94**	187.20**	170.52**	357.53**
G x I		**35**			1409.36**			103.04**
p x I		**7**			1532.21**			62.40**

C x I		**27**			1422.01**			117.39**
P.vs.C x I		**1**			207.95**			0.19
Error	**70**	**140**	13.29	12.54	12.91	5.43	8.17	6.8
GCA	**7**	**7**	1007.41**	701.48**	701.08**	12.98**	38.84**	28.68**
SCA	**28**	**28**	1087.18**	554.35**	1306.25**	70.39**	52.52**	85.76**
GCA x L		**7**			1007.80**			23.14**
SCA x L		**28**			335.28**			37.15**
Error	**70**	**140**	4.43	4.18	4.3	1.81	2.72	2.27
GCA/SCA			0.93	1.27	0.54	0.18	0.74	0.33
GCA x L/GCA					1.44			0.81
SCA x L/SCA					0.26			0.43

* and ** significant at 0.05 and 0.01 levels of probability, respectively.

Table (5): Mean performance for the studied traits under normal irrigation and drought stress condition as well as the combined over them.

	Biological yield/ plant (g)			**Grain yield/ plant (g)**		
	Drought	**Normal**	**Combined**	**Drought**	**Normal**	**Combined**
P1	159.67	174.00	166.83	24.73	46.30	35.52
P2	121.67	165.67	143.67	25.87	33.20	29.53
P3	94.33	191.33	142.83	21.20	44.43	32.82
P4	151.67	216.33	184.00	34.43	44.27	39.35
P5	154.33	226.00	190.17	35.03	51.47	43.25
P6	123.00	180.33	151.67	26.53	52.37	39.45
P7	163.67	168.67	166.17	35.30	51.00	43.15
P8	166.00	185.00	175.50	35.57	55.47	45.52
1x2	124.00	212.67	168.33	28.27	36.97	32.62
1x3	193.33	224.33	208.83	41.20	47.83	44.52
1x4	134.00	162.00	148.00	31.57	60.37	45.97
1x5	148.67	188.00	168.33	33.73	56.17	44.95
1x6	160.67	201.67	181.17	35.20	45.33	40.27
1x7	178.67	187.67	183.17	37.93	54.73	46.33
1x8	192.67	198.00	195.33	39.13	48.97	44.05
2x3	123.33	211.67	167.50	27.63	42.47	35.05
2x4	113.67	207.67	160.67	41.50	57.40	49.45
2x5	212.00	213.00	212.50	45.33	58.03	51.68
2x6	121.00	177.67	149.33	23.33	56.87	40.10
2x7	188.33	236.00	212.17	39.03	48.63	43.83
2x8	148.67	170.67	159.67	33.53	53.47	43.50
3x4	170.00	216.67	193.33	46.67	57.43	52.05
3x5	124.33	216.67	170.50	28.80	39.20	34.00
3x6	119.33	171.00	145.17	22.80	43.97	33.38
3x7	147.33	223.33	185.33	33.47	50.13	41.80
3x8	132.00	222.00	177.00	36.33	63.07	49.70
4x5	119.67	221.00	170.33	23.30	46.40	34.85
4x6	126.67	198.33	162.50	30.50	55.30	42.90
4x7	81.33	141.67	111.50	21.10	44.70	32.90
4x8	117.33	179.33	148.33	22.53	50.80	36.67
5x6	200.00	218.33	209.17	45.47	55.07	50.27
5x7	81.33	149.00	115.17	19.00	45.23	32.12
5x8	171.00	216.00	193.50	37.40	55.87	46.63
6x7	144.67	164.00	154.33	38.03	40.90	39.47
6x8	117.00	197.00	157.00	22.23	54.57	38.40
7x8	184.67	188.00	186.33	38.97	39.50	39.23
Average of genotypes	144.72	195.02	169.87	32.30	49.66	40.98
L.S.D 5%	5.92	5.75	5.75	5.92	5.75	5.75

Table 6. Estimates of combining ability effects for yield traits in both and across environments.

Parent	Biological yield/ plant			Grain weight/ plant		
	Drought	Normal	Combined	Drought	Normal	Combined
P1	14.88**	-3.28**	5.80**	0.58	-0.40	0.09
P2	-2.82**	0.55	-1.13**	-0.03	-2.67**	-1.35**
P3	-10.42**	11.32**	0.45	-1.14**	-1.40**	-1.27**
P4	-13.65**	0.42	-6.62**	-0.46	1.40**	0.47**
P5	6.32**	11.88**	9.10**	1.24**	1.19*	1.22**
P6	-6.72**	-6.65**	-6.68**	-2.00**	0.98*	-0.51**
P7	3.12**	-12.82**	-4.85**	0.75	-2.11**	-0.68**
P8	9.28**	-1.42**	3.93**	1.06**	3.02**	2.04**
L.S.D(0.05) gi	1.24	1.2	0.48	0.79	0.97	0.35
L.S.D(0.05) gi-gj	1.87	1.82	0.91	1.20	1.47	0.66
P1xP2	-32.79**	20.38**	-6.20**	-4.58**	-9.62**	-7.10**
P1xP3	44.14**	21.28**	32.71**	9.46**	-0.03	4.71**
P1xP4	-11.96**	-30.15**	-21.05**	-0.85	9.71**	4.43**
P1xP5	-17.26**	-15.62**	-16.44**	-0.39	5.71**	2.66**
P1xP6	7.78**	16.58**	12.18**	4.32**	-4.91**	-0.29
P1xP7	15.94**	8.75**	12.35**	4.31**	7.58**	5.95**
P1xP8	23.78**	7.68**	15.73**	5.19**	-3.32**	0.94
P2xP3	-8.16**	4.78**	-1.69	-3.50**	-3.12**	-3.31**
P2xP4	-14.59**	11.68**	-1.45	9.70**	9.01**	9.36**
P2xP5	63.78**	5.55**	34.66**	11.82**	9.85**	10.84**
P2xP6	-14.19**	-11.25**	-12.72**	-6.93**	8.90**	0.99
P2xP7	43.31**	53.25**	48.28**	6.02**	3.76**	4.89**
P2xP8	-2.52	-23.49**	-13.00**	0.21	3.46**	1.83
P3xP4	49.34**	9.91**	29.63**	15.97**	7.77**	11.87**
P3xP5	-16.29**	-1.55	-8.92**	-3.60**	-10.26**	-6.93**
P3xP6	-8.26**	-28.69**	-18.47**	-6.36**	-5.27**	-5.81**
P3xP7	9.91**	29.81**	19.86**	1.56	3.98**	2.77**
P3xP8	-11.59**	17.08**	2.75**	4.11**	11.78**	7.95**
P4xP5	-17.72**	13.68**	-2.02	-9.78**	-5.85**	-7.81**
P4xP6	2.31	9.55**	5.93**	0.67	3.26**	1.97**
P4xP7	-52.86**	-40.95**	-46.90**	-11.48**	-4.25**	-7.86**
P4xP8	-23.02**	-14.69**	-18.85**	-10.36**	-3.28*	-6.82*
P5xP6	55.68**	18.08**	36.88**	13.93**	3.23*	8.58**
P5xP7	-72.82**	-45.09**	-58.95**	-15.29**	-3.51*	-9.40**
P5xP8	10.68**	10.51**	10.60**	2.80*	1.99	2.40**
P6xP7	3.54	-11.55**	-4.00**	6.99**	-7.63**	-0.32
P6xP8	-30.29**	10.05**	-10.12**	-9.12**	0.91	-4.11**
P7xP8	27.54**	7.21**	17.38**	4.86**	-11.07**	-3.10**
LSD5%(sij)	3.8	3.69	2.61	2.43	2.98	1.89
LSD5%(sij-sik)	5.62	5.46	3.86	3.59	4.41	2.80
LSD5%(sij-skL)	5.3	5.15	1.29	3.39	4.15	0.93

** p< 0.05

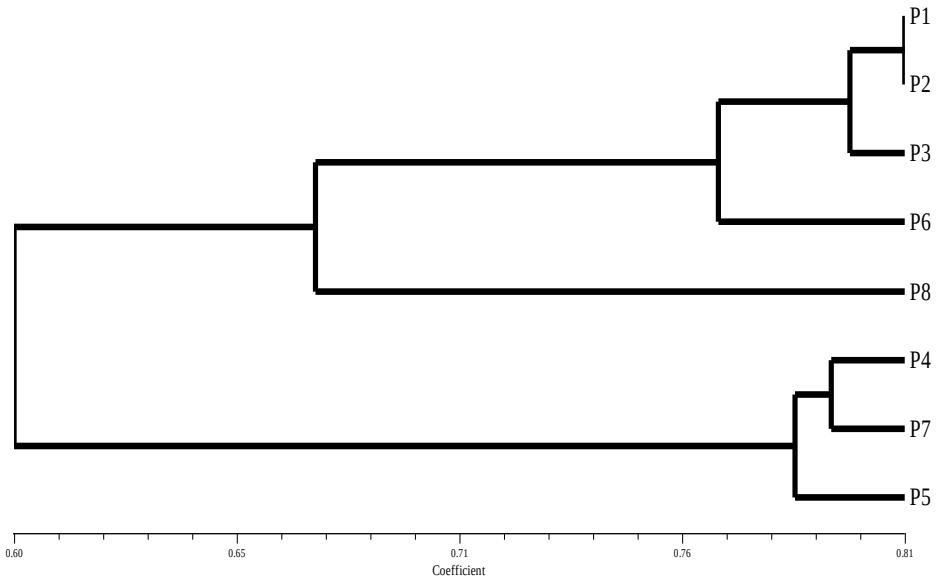

Figure 1. Phonogram generated by UPGMA cluster analysis based on Nei and Li, coefficients showing clustering of eight genotypes.

Where, P1 = Yakora Rojo, P2 = Sakha 93, P3 = Masr 1, P4 = Drought 4, P5 = Shandawel 1, P6 = Gemiza 9, P7 = Giza 171, P8 = Sides 13

This 2-fold range for both yield traits implied how genotypes unevenly responded to greatly varied irrigational environments.

The parents 4 and 5 and crosses:P1xP3, P1xP6, P2xP5, P2xP7, P3xP4, P5xP6 and P5xP8, out yielded the average genotypes in in both and across irrigation treatments. As for grain yield/ plant the parent No. 8 and the crosses P2xP4, P3xP4 and P5xP6 differed significantly relative to mean genotype.

Combining ability

General (GCA) and specific (SCA) combining ability were highly significant for all studied traits in both and across environments (Table 4). Moreover, the ratios between GCA and SCA were less than unity for the two studied traits at both and across environments except, for biological yield plant^{-1} at normal environment. Hence, non-additive types of gene action are more relatively distinct than additive and additive x additive gene action in controlling these traits. For the exceptional case, the additive and additive x additive types of gene action are more likely pronounced than non-additive gene action in controlling this trait when subjected to such conditions. The genetic variance was previously reported (Abd El-Aty and Katta, 2002, El Hosary et al., 2012, and Gomaa et al., 2014) to be mostly due to additive effects for grain yield/ plant.

The GCA x irrigation and SCA x irrigation mean squares were significant differed ($p<0.05$) for the studied traits. Such result indicated that the additive and non-additive types of gene action differed significantly from one environment to another for these traits. Similar results were reported by Abd El-Aty and Katta, (2002), El Hosary et al., (2012), and Gomaa et al., (2014). The additive type of gene action was much more influenced by environments than non-additive genetic one because the ratio GCA x environment/ GCA was much higher that of SCA x irrigation/ SCA treatments for the two studied traits. Such results are in harmony with those obtained by EL Saadoown et al., 2018.

General combing ability (GCA) effects

A parent's (P_i) relative average performance (Table 6) is assessed to be advanced to further breeding programs for drought resistance selection. For per plant biological yield, P_1 ranked first for a positive ĝi effect when grown under stress environment, and it responded similarly when averaged over both environments. Also, for the same trait, P_7 had a significant positive ĝi effect under stress conditions. However, P_3 had a desirable ĝi effect yet under favorable conditions. For per plant grain yield, P_4 had significant positive ĝi effect when tested under normal

irrigation conditions and over both environments. It also took a relative top rank over both environments. For both yield traits, P_5 considered a good combiner when grown in any of the two current environments and over both of them. This response of P_5 extended to P_8 except for per plant biological yield at normal environment.

Specific combining ability $\hat{s}_{ij}$ effects

For per plant biological yield, crosses varied in their significant positive ŝij effects under each of the two irrigation regimes and averaged over both (Table 6). Among the 28 crosses, 36% (10 crosses) were under drought, 64% (18 crosses) under normal, and 46% (13 crosses) when averaged over both environments. In case of per plant grain yield, 46% (13 crosses) of all crosses had significant positive ŝij effects for each environment and over both. Only 25%, seven crosses, had in common significant positive ŝij effects at each environment and when averaged over the two growing conditions (Table 6). Based on the above, these crosses might be of interest in breeding programs in favor of developing pure line varieties for relatively higher biological, and grain yields/ plant under drought conditions. If a cross shows a high specific combining ability and involves only one good combiner, such combinations would result in desirable transgressive segregates. Providing that an additive genetic system is present in a good combiner and a complementary and epistatic effects are present in a cross. All act in the same direction to reduce undesirable plant characteristics and maximize the character in view.

Correlation between genetic distance and each of mean performance and SCA

For the 28 hybrids, Pearson's coefficients of correlation, r, between GD and mean performance for per plant biological yield, were 0.353, 0.205, and 0.334 at drought, normal, and over both environments. Its values were 0.417 ($p<0.05$), 0.201, and 0.366 between GD and SCA, which did not differ much from GD's r values with per plant biological yield. On the other hand, the r values between GD and per plant grain yield were quite higher ($p<0.05$): 0.520, 0.615, and 0.695. These results thrower light on how GD is related to heterosis for both yield characters. The reason for high morbidity may be due to the number of genes that control the inheritance of grain yield compared to biological yield. This study showed that GD can be used to precisely predict the yield performance and heterosis value for F_1 hybrids. This suggests that the more primers/markers be applied, the better detection of differences between parents.

CONCLUSION

To sum up, specific combining ability effects, high mean performance and hybrid vigor produce from hybridization between diverse parental. Genetic diversity (GD) can be estimated from RAPD-PCR marker. Thus, its easy to predict the hybrid performance from screening parents by RAPD primers.

CONFLICT OF INTEREST

All authors contributed in collecting and analyzing data. All authors participated in writing every part of this study. All authors read and approved the final version.

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AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author A.A. El-Hosary designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors A.A. El-Hosary, A.A.A. El-Hosary and M. El. M. El-Badawy supervised the study and managed the literature searches. Author M. El. M. El-Badawy, A.A.A. El-Hosary, Tamer El Akaad and A. El-Fahdawy managed the experimental process and performed data analyses. All authors read and approved the final manuscript. The Authors have read and are fully aware of the journal policy.

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