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ARTICLE

Genetic studies on tissue culture response and some agronomical traits in Egyptian bread wheat

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Abstract The main effort of wheat breeder is the detection of genes and to merge them in a particular genotype using most suitable combination. Five Egyptian cultivars of bread wheat (*Triticum aestivum* L.) were crossed in a half diallel mating design to produce 10 crosses. The genetic potential of embryogenic callus (EC%), plant regeneration (RGP%) response and its association with heading date (HD) and grain yield per plant (GY/P) were investigated. The results showed that GY/P was significantly and positively correlated with EC% and RGP%. The combining ability analysis showed that the magnitudes of general combining ability (GCA) were higher than those of specific combining ability (SCA) for both tissue culture response and agronomic traits. The promising crosses which exhibited desirable SCA effects, showed also high useful heterosis for all studied traits. The magnitudes of additive genetic variance (σ^2A) were larger than those of non-additive ones (σ^2D) for all studied traits except for number of days to heading. The estimates of narrow sense heritability were 84.56%, 82.13%, 43.46% and 70.28% for the percentage of EC%, RGP%, HD and GY/P, respectively. The genetic similarity percents based on RAPD markers ranged from 76% to 93% between the cultivars. The UPGMA cluster analysis revealed that the cultivars could be divided into two main clusters. The range of Euclidean distances based on morphological characters among the cultivars was relatively wide (4.37–27.87), indicating relatively high amount of phenotypic variation. A significant positive correlation between Euclidean distance and RAPD distance (0.72^{**}) was found.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops in the world. It plays a remarkable role in meeting the food requirements and economic stability of the country. One of the main objectives of wheat improvement program is to generate genetically diverse germplasm that has high yield potential. The diallel cross designs are frequently

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used in plant breeding research to obtain information about genetic properties of parental lines or estimates of GCA, SCA and heritability in early generations and particularly suit to autogamous crops like wheat.

Tissue culture techniques are commonly used for the propagation of many plant species. It is known that in many cases, a consistent proportion of the regenerated plants differ from the original parental type when submitted to tissue-culture techniques. Conventional breeding would probably be more efficient if aided by modern tools such as somaclonal variation and molecular markers. Immature embryo of wheat has been widely used as an explant source to study embryogenesis, plant regeneration [1] and somaclonal variation [2]. Embryogenic callus trait was positively and significantly correlated with grain yield per plant in wheat [3,4], also, the earliness in wheat could be correlated with plant regeneration [5]. Quantitatively genetic parameters such as heritability and variance components are useful for designing new breeding programs and allocating resources in field performance trials. The amount of heterosis as well as the GCA and SCA effects are important considerations for hybrid breeding. Furthermore, correlation coefficients between the features are useful because they give information about the effect of the selection on other traits. Heterosis in wheat tissue cultures was reported by [6–9]. Several investigators have reported that both additive and dominance effects contribute to the variation observed among wheat genotypes [9–13]. The GCA and SCA effects were dominant and played a major role in the inheritance of days to heading and grain yield/plant [14]. [15] indicated that a superior performance of the hybrids for some traits depends on the GCA of the parents involved, that progress in improving the desired trait which will be slow if the parental selection is based on per se performance alone. For continued improvement, the selection of parents should be based on per se performance as well as combining ability and heterosis.

One of the most widely used PCR-based marker techniques is Random Amplified Polymorphic DNA (RAPD). RAPD marker is generated by PCR amplification of random genomic DNA fragments with single oligonucleotide primers of arbitrary sequence. RAPD marker analysis could assist for rapidly predicting the genetic diversity among genotypes [16,17]. The level of association between agronomic characterization and DNA marker-based genetic similarity may vary among different crop species. In corn, a close association was found [18], but in others such as, wheat, barley, oat and cotton moderate to low associations have been observed [19–21]. The present study aimed to investigate: (1) Association between tissue culture response and agronomic traits. (2) The genetic parameters of embryogenic callus induction, plant regeneration, heading date and grain yield per plant, and (3) Analysis of RAPD markers to detect the genetic variation among cultivars.

2. Materials and methods

Five Egyptian cultivars of bread wheat (*Triticum aestivum* L.), namely Gemmeiza-3, Sakha-8, Giza-168, Sakha-69 and Giza-164 were used in this study. Seeds of all cultivars were kindly supplied at the Experimental Farm of Faculty of Agriculture, Sohag University, Sohag Governorate, Egypt.

2.1. Field experiment

Half-diallel cross among the five parents was made to produce 10 crosses in the 2010/2011 winter season. In 2011/2012, seeds of the five parents and their 10 F_1 crosses were planted in a complete randomized design with three replicates. Each replication included 15 entries (5 parents and 10 F_1). Field data were recorded on number of days to heading (HD) and grain yield per plant (GY/P g).

2.2. Culture of immature embryos

Immature embryos from all genotypes (5 parents and 10 F_1) were collected 14 days after anthesis. Fifty immature embryos from each genotype in each replicate were dissected aseptically and cultured on callus induction medium with the scutellum side up. Culture induction medium contained the MS inorganic salts [22] supplemented with 150 mg/L L-aspargine, 0.5 mg/L thiamine, 1.0 mg/L 2,4-D, 20 g/L sucrose and 7.0 g/L agar. Immature embryos cultured on the callus induction medium were incubated in the dark at 27 °C for 14 days. Embryogenic calli, which were characterized as compact, yellowish and nodular, were transferred to shoot initiation medium similar to the callus induction medium, except 2,4-D concentration which was reduced to 0.2 mg/L. The cultures were incubated in the growth chamber under 12-h photoperiod at 22 °C for 2 weeks. Regenerable calli, which have green shoot primordia covering the surface, were transferred to hormone-free MS medium and incubated under the same conditions in the growth chamber. The good developed plantlets were transferred to the greenhouse for further growth.

Data were recorded on the percentage of embryogenic calli (number of embryos forming callus per number of immature embryos cultured on the medium × 100) and percentage of regenerated green plants (number of regenerable calli that produced whole plants with a well developed root system per transferred differentiating calli × 100).

2.3. RAPD marker technique

2.3.1. Genomic DNA extraction and PCR procedures

Fresh leaves were frozen in liquid nitrogen, lyophilized, and ground to a fine powder using mortar and pestle. DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method according to [23]. RAPD technique was conducted using 9 arbitrary 10-mer primers (Metabion International AG, Germany).

The RAPD assay was performed in a 25 µl volume containing 12.5 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 3.5 µl of primer 5 pmol, 7 µl of free nuclease water and 2 µl of 150 ng DNA template. The Thermal Cycler (Primus 25, Germany) was programed by an initial denaturation cycle at 94 °C for 5 min. The following 45 cycles were composed of: denaturation step at 94 °C for 1 min, annealing step for 1 min 45 s at 38 °C and elongation step at 72 °C for 2 min. The final cycle of polymerization was performed at 72 °C for 7 min. The amplification products were electrophoresed in a 1% agarose gel stained with 0.4 µl ethidium bromide. The amplified fragments were visualized and photographed using UVP Bio Doc-It imaging system (USA).

2.3.2. DNA banding pattern analysis

The DNA banding patterns generated from RAPD experiments were analyzed by computer program, Gene Profiler (version 4.03). The presence (1) or absence (0) of each band was recorded for each cultivar for the nine primers used. Genetic similarity estimates were determined using Jaccard's coefficient [24]. Dendrogram was generated with the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the computational package MVSP version 3.1.

2.3.3. Combining the Euclidean distance and RAPD distance

Data analysis on the means of all studied traits was initially performed based on the Euclidean distance matrix. The hierarchical cluster analysis [25] was used to investigate patterns of phenotypic diversity existing in these parental cultivars.

The Mantel test is a statistical test of the correlation between two matrices. The similarity matrix of RAPD was converted to dissimilarity matrix. A cophenetic matrix was derived from each matrix to test goodness of fit of the clusters by comparing the two matrices using the Mantel test [26]. Finally, the correlation between each distance pair using computer program NTSYS-pc version 2.1 [27] was calculated.

2.4. Statistical analysis

Variation among parents and F_1 hybrids in a half diallel model for studied traits was analyzed using the [28] method. The Griffing's method for diallel analysis [29] was used to estimate general and specific combining abilities (GCA and SCA). The analyses of correlation were computed using SAS software [30].

3. Results and discussion

3.1. Analysis of variance

Analysis of variance for embryogenic callus induction (EC%), plant regeneration (RGP%), heading date (HD) and grain yield per plant (GY/P g) is presented in Table 1. Highly significant differences existed among genotypes (5 parents and their 10 F_1 crosses) for all studied traits, revealing the presence of genetic diversity among them.

3.2. Performances of genotypes

Mean performances of the 5 parents and their respective 10 crosses for all studied traits are shown in Table 2. The results showed that the most responsive parents for the percentage of embryogenic calli and regenerated green plants were Sakha 8, P_2 (73.1% and 42.6%), Sakha 69, P_4 (69.0% and 49.2%) and Gemmeiza-3, P_1 (60.9% and 46.4%), respectively. For agronomic traits, Gemmeiza-3 (P_1) and Sakha-69 (P_4) were the best parents for earliness (81.1 and 84.6 days) and grain yield per plant (40.5 and 38.1 gm.), respectively. Concerning F_1 hybrids, the cross combinations (P_1xP_2), (P_1xP_4), (P_2xP_4) and (P_4xP_5) were the best hybrids for producing the highest percentage of embryogenic calli and regenerated green plants. However, the cross combinations (P_1xP_3), (P_1xP_4), (P_1xP_5) and (P_4xP_5) were the most promising hybrids for both earliness and high yielding. It could be noticed that the parents and hybrids that have great potential for tissue culture response, were also the best promising genotypes for agronomic traits.

Table 1 Analysis of variance of five bread Egyptian wheat cultivars and their 10 F_1 hybrids for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

SV	DF	EC%	RGP%	HD	GY/P
Genotypes	14	204.11**	117.51**	47.12**	54.33**
Error	45	7.37	5.20	1.54	3.30

**Significant at 1% level of probability.

3.3. Association of tissue culture with agronomic traits

Pearson correlation analyses (Table 3) showed that GY/P was positively correlated to EC% ($r = 0.22$, $P = 0.149$) and was positively and significantly correlated to RGP% ($r = 0.64^{**}$, $P = 0.0001$). In this direction, [4] reported that EC% was positively and significantly correlated with GY/P. Moreover, the results revealed that, EC% was positively correlated with RGP% ($r = 0.80^{**}$, $P = 0.0001$). Therefore, the agronomic traits having high positive direct effects on tissue culture traits are considered suitable predictors of good in vitro plant regeneration. However, insignificant correlation was noticed between HD and both EC% and RGP%. In contrast, [5] obtained a significant positive correlation between earliness and RGP%. The results also showed a significant negative correlation between grain yield per plant and heading date ($r = -0.41^{**}$, $P = 0.005$). Among the reasons for the different correlations are the different factors influencing both quantitatively inherited traits and the different physiological processes behind them [9].

3.4. Estimates of heterosis

Estimates of heterosis over mid and better parent for each cross for all studied traits are presented in Table 4. The results

Table 2 Performances of five wheat parents and their 10 F_1 hybrids for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

Genotypes	EC%	RGP%	HD	GY/P
Gemmeiza-3 (P_1)	60.9	46.4	81.1	40.5
Sakh-8 (P_2)	73.1	42.6	91.4	29.6
Giza-168 (P_3)	48.6	31.5	86.2	34.7
Sakha-69 (P_4)	69.0	49.2	84.6	38.1
Giza-164 (P_5)	48.8	34.8	88.5	33.0
P_1xP_2	70.1	47.1	80.5	38.7
P_1xP_3	57.0	41.9	79.1	41.4
P_1xP_4	71.8	50.7	82.8	43.9
P_1xP_5	66.4	46.1	80.4	40.1
P_2xP_3	67.0	40.6	85.6	34.8
P_2xP_4	74.9	48.8	86.2	36.9
P_2xP_5	68.2	40.5	84.5	35.0
P_3xP_4	61.7	45.3	82.7	40.1
P_3xP_5	59.1	38.6	81.3	36.5
P_4xP_5	72.6	45.4	80.8	40.1
LSD 5%	3.84	3.22	1.78	2.58
LSD 1%	5.11	4.28	2.38	3.46

Table 3 Pearson correlation coefficients of embryogenic calli (EC%) and regenerated green plants (RGP%) with number of days to heading (HD) and grain yield per plant (GY/P g).

Traits	EC%	RGP%	HD	GY/P
EC%	–			
RGP%	0.80**	–		
HD	0.15	−0.09	–	
GY/P	0.22	0.64**	−0.41**	–

**Significant at 1% level of probability.

showed that 9 and 2 out of the 10 crosses exhibited positive heterotic effects against mid and better parents, respectively, for the percentage of embroyogenic calli, the maximum heterosis values above mid and better parent for the same trait were 10.46% and 5.22% for the crosses, (P_1xP_4) and (P_4xP_5), respectively. As for the percentage of regenerated green plants, 10 and 1 out of the 10 crosses showed positive heterotic values over their mid and better parents, respectively. In this respect, the cross (P_3xP_5) was the best hybrid over both mid and better parents for this trait with the highest.

Concerning agronomic traits, the results showed that 9 and 5 out of the 10 crosses exhibited desirable heterotic effects against mid and better parents, respectively, for number of days to heading. The cross (P_3xP_5) displayed the maximum values of heterosis above mid and better parents in earliness which were −6.98% and −5.68%, respectively. The results also showed that the majority of the crosses were significantly better yielding than their mid and best parents. The crosses (P_4xP_5) and (P_1xP_4) were the most promising hybrids for grain yield with the maximum heterotic values of 12.64% and 8.39% over mid and better parents, respectively. Similar results were obtained by [6,8,9,12,31]. Generally, the superiority of some crosses over their mid and better parents reflects the important role of non-additive genetic variance in the inheritance of these traits.

3.5. Combining ability analysis

Mean squares of general and specific combining ability for all studied traits are given in Table 5. The results showed that mean squares of general combining ability (GCA) and specific combining ability (SCA) were highly significant for all studied traits. These results indicate that both GCA and SCA were important in the inheritance of these traits. However, the magnitudes of GCA were higher than those of SCA for all studied traits pointed out the predominance of the additive gene action. Similar finding was obtained by [9,32,33]. However, [11] reported that both additive and non-additive variations are operating to control these traits with predominance of non-additive gene action.

3.6. General combining ability (g_i)

The results of general combining ability effects (g_i) (Table 6) indicated that Sakha-8 (P_2) and Gemmeiza-3 (P_1) were found to be good general combiners for the percentage of embroyogenic calli and percentage of regenerated green plants, respectively. However, Sakha-69 (P_4) proved to be good general combiner for both the percentage of embroyogenic calli and percentage of regenerated green plants. As for the agronomic traits, Gemmeiza-3 (P_1) exhibited negative and highly significant general combining ability effects toward earliness. Whereas, Sakha-8 (P_2) possessed positive and highly significant values of general combining ability effects toward lateness. Concerning grain yield per plant, Gemmeiza-3 (P_1) and Sakha-69 (P_4) were considered to be excellent general combiners.

3.7. Specific combining ability (S_{ij})

Estimates of specific combining ability effects (S_{ij}) of each cross for all studied traits are given in Table 7. The results revealed that the highest desirable SCA effects for the percentage of embroyogenic calli were obtained from the crosses (P1xP4), (P2xP3) and (P4xP5). However, the crosses (P1xP5), (P3xP4) and (P3xP5) gave the highest S_{ij} values for the percentage of regenerated green plants. The cross combinations (P1xP2), (P1xP3), (P3xP5) and (P4xP5) showed desirable negative significant SCA effects for earliness. Concerning grain yield per plant, the crosses (P1xP4) and (P4xP5) were the best yielding crosses.

It could be observed that the promising crosses in all studied traits were obtained from (good × good), (good × poor)

Table 4 Estimates of heterosis over mid (MPH) and better (BPH) parents of each cross for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

Crosses	EC%		RGP%		HD		GY/P	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
P_1xP_2	4.63**	−4.10*	5.84**	1.51	−6.72**	−0.74	10.25**	−4.44**
P_1xP_3	4.01*	−6.40**	7.57**	−9.70**	−5.49**	−2.47**	10.11**	2.22
P_1xP_4	10.46**	4.06*	6.07**	3.05	0.12	2.10**	11.70**	8.39**
P_1xP_5	5.56**	2.47	13.55**	−0.65	−5.19**	−0.86	8.97**	−0.99
P_2xP_3	10.02**	−8.34**	9.58**	−4.69*	−3.60**	−.69	8.07**	0.29
P_2xP_4	5.34**	2.46	6.32**	−0.81	−2.05**	1.89*	8.85**	−3.15*
P_2xP_5	−1.16	−6.70**	4.65**	−4.93**	−6.11**	−4.52**	11.82**	6.06**
P_3xP_4	4.93**	−10.58**	12.27**	−7.93**	−3.16**	−2.24**	9.89**	4.99**
P_3xP_5	4.23*	−8.80**	16.44**	10.92**	−6.98**	−5.68**	7.67**	5.19**
P_4xP_5	8.52**	5.22**	8.09**	−7.72**	−6.70**	−4.49**	12.64**	5.25**
LSD 5%	3.38	3.90	3.20	3.68	1.31	1.52	2.32	2.69
LSD 1%	4.49	5.19	4.25	4.89	1.76	2.02	3.11	3.59

*,**Significant at 5% and 1% levels of probability, respectively.

Table 5 Combining ability analysis of variance for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

SV	DF	EC%	RGP%	HD	GY/P
GCA	4	158.21**	89.18**	21.55**	36.71**
SCA	10	8.16**	5.46**	7.87**	4.33**
Error	45	1.84	1.30	0.39	0.83

**Significant at 1% level of probability.

Table 6 Estimates of general combining ability effects (g_i) of each parent for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

Parents	EC%	RGP%	HD	GY/P
Gemmeiza3 (P_1)	−1.00*	2.68**	−2.47**	2.82**
Sakha-8 (P_2)	4.62**	0.34	2.47**	−2.96**
Giza-168 (P_3)	−7.44**	−4.34**	−0.17	−0.46
Sakha-69 (P_4)	3.56**	4.11**	−0.08	1.68**
Giza-164 (P_5)	0.26	−2.80**	0.25	−1.09**
g_i	0.46	0.39	0.21	0.31

*,**Significant at 5% and 1% levels of probability, respectively.

Table 7 Estimates of specific combining ability effects (S_{ij}) of each cross for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

Crosses	EC%	RGP%	HD	GY/P
P_1xP_2	0.80	0.77	−3.21**	1.28
P_1xP_3	−0.25	0.26	−1.97**	1.48
P_1xP_4	3.55**	0.60	1.64**	1.84*
P_1xP_5	1.45	2.92**	−1.08	0.81
P_2xP_3	4.14**	1.30	−0.41	0.66
P_2xP_4	1.04	1.05	0.10	0.62
P_2xP_5	−2.36*	−0.34	−1.93**	1.49
P_3xP_4	−0.11	2.23**	−0.76	1.22
P_3xP_5	0.59	2.45**	−2.48**	0.49
P_4xP_5	3.09*	0.79	−3.07**	1.95*
S_{ij}	1.18	0.99	0.55	0.79

*,**Significant at 5% and 1% levels of probability, respectively.

and (poor × poor) general combiners. For instance, in the case of grain yield per plant, the best crosses were a result of crossing (good × good) general combiners (P1xP4) and (good × poor) general combiners (P4xP5). Consequently, it is not necessary that parents having high estimates of GCA effects would also give high estimates of SCA effects in their respective crosses. It also noticed that the promising crosses which exhibited desirable SCA effects, showed also high useful heterosis as previously mentioned for all studied traits.

3.8. Estimates of genetic parameters

The results of genetic parameters for all studied traits (Table 8) showed that the magnitudes of additive genetic variance (σ^2A) were higher than those of non-additive ones (σ^2D) for the

Table 8 Estimates of genetic parameters and heritability in broad (H^2_b%) and narrow (H^2_n%) sense for embryogenic calli (EC%), regenerated green plants (RGP%), number of days to heading (HD) and grain yield per plant (GY/P g).

Genetic parameters	EC%	RGP%	HD	GY/P
σ^2A	22.34	12.55	6.05	10.24
σ^2D	6.32	4.16	7.48	3.50
$(\sigma^2D/\sigma^2A)^{1/2}$	0.53	0.57	1.11	0.58
H^2_b%	96.52	95.75	97.20	94.30
H^2_n%	84.56	82.13	43.46	70.28

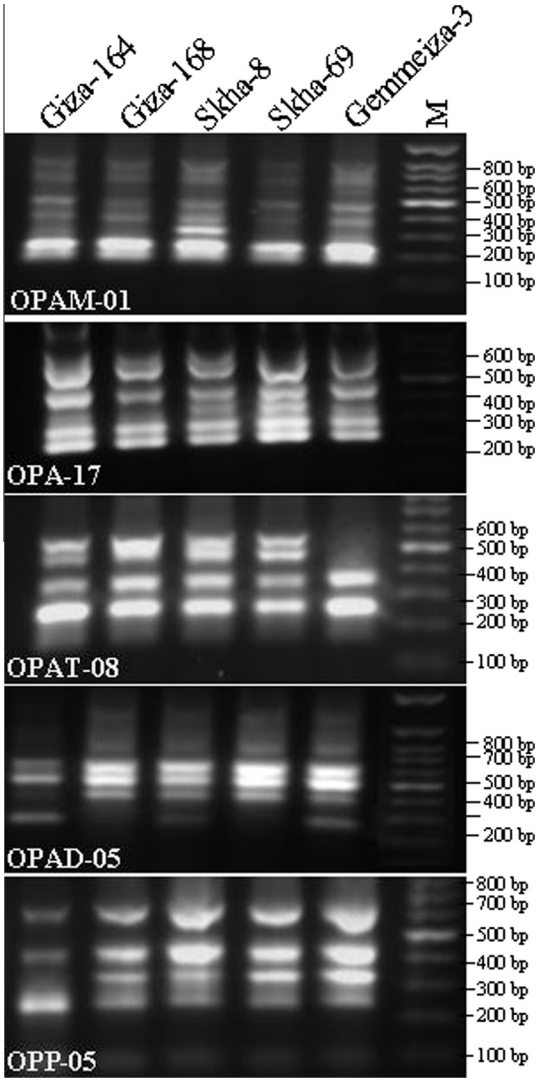

Figure 1 RAPD profiles amplified with five different primers, M = 100 bp ladder size marker.

percentage of embroyogenic calli, percentage of regenerated green plants and grain yield per plant. Whereas, the magnitudes of non-additive genetic variance (σ^2D) were larger than those of additive ones (σ^2A) for the number of days to heading. This finding could be emphasized by the ratio of $(\sigma^2D/\sigma^2A)^{1/2}$ which was less than one, indicating that additive gene action

Table 9 Primers used in RAPD analysis, total number of fragments detected by each primer and polymorphism among 5 Egyptian bread wheat cultivars.

Primer name	Primer sequence (5′–3′)	Amplified bands		Polymorphic bands (%)	Fragments size base pair (bp)	
		Fragments number	Polymorphic bands		Larger	Smaller
OPAM-01	TCACGTACGG	7	4	57.1	850	250
OPA-17	GACCGCTTGT	5	2	40.0	520	200
OPAT-08	TCCTCGTGGG	4	2	50.0	500	250
OPAD-06	AAGTGCACGG	4	2	50.0	700	320
OPP-05	CCCCGGTAAC	5	2	40.0	620	100
OPF-20	GGTCTAGAGG	7	3	42.9	900	200
OPAV-13	CTGACTTCCC	6	4	66.7	680	260
OPW-13	CACAGCGACA	6	2	33.3	880	280
OPAR-05	CATACCTGCC	7	2	28.6	910	250
Total		51	23			
Mean		5.67	2.56			

Table 10 Similarity matrix (%) for five Egyptian bread wheat cultivars according to Jaccard's coefficient obtained from 51 RAPD fragments.

Varieties	Giza-164	Giza-168	Sakha-8	Sakha-69	Gemmeiza-3
Giza-164	100				
Giza-168	93	100			
Sakha-8	82	84	100		
Sakha-69	78	84	92	100	
Gemmeiza-3	76	78	90	90	100

was pronounced in the inheritance of all traits except for earliness. Similar finding was reported by [9,32].

Estimates of broad sense heritability (H^2_b%) were higher than their corresponding narrow sense (H^2_n%) for all studied traits. The values of narrow sense heritability were 84.56%, 82.13%, 43.46% and 70.28% for the percentage of embryogenic calli, regenerated green plants, heading date and grain yield per plant, respectively. These results present additional evidence about the importance of additive genetic variance in expression of these traits. Similar finding was reported by [12,32]. The variance component caused by genotype is relatively low in heading, resulting in a heritability of 0.56, but the heritability in grain yield was the highest with 85% [9].

3.9. RAPD data analysis

Out of 22 primers, only nine 10-mer arbitrary primers produced polymorphic bands (Fig. 1), fifty-one bands were screened among which 23 were polymorphic (45.10%) across the parental cultivars. Similar results obtained by [34], [35] that revealed 46.97% and 46.67% level of polymorphism among wheat genotypes by RAPD and AFLP markers, respectively. This level of polymorphism was lower than the earlier studies conducted by [36,37]. Fragments size ranged from 100 bp to 910 bp and fragments number produced by various primers were from 4 to7 with an average of 5.76 per primer, similar finding was reported by [38,39]. The highest number of DNA fragments (7) was obtained with primers OPAM-01, OPF-20 and OPAR-05 (Table 9).

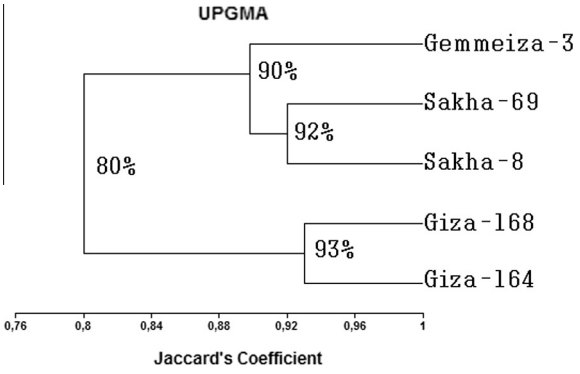

Figure 2 Dendrogram generated by UPGMA cluster analysis using 51 RAPD fragments generated from five Egyptian bread wheat cultivars.

The genetic similarity percent based on RAPD markers (Table 10) ranged from 76% to 93% between Giza-164 and Gemmeiza-3, and between Giza-164 and Giza-168, respectively. The dendrogram constructed based on the similarity matrix (Fig. 2) showed that the five cultivars could be divided into two main clusters. Giza-164 and Giza-168 clustered at 93% level of similarity in the first cluster, showing high genetic similarity among each other. Sakha-8, Sakha-69 and Gemmeiza-3 were grouped in the second cluster that divided into two sub-groups. Sakha-8, Sakha-69 clustered in first sub-group at 92% level of similarity. Gemmeiza-3 variety in the second sub-group clustered at 90% with the first sub-group. The relatively low percent of similarity between the Gemmeiza-3 and (Giza-164 and Giza-168) cultivars in this work may be due to the introduction of varieties of different Egyptian geographical regions, or disclose the use of parents with different genetic content.

3.10. Cluster analysis based on means of studied traits

The dendrogram constructed on the basis of the genetic distances among five Egyptian bread wheat cultivars was

Table 11 Euclidean distances matrix of five Egyptian bread wheat cultivars using means of studied characters.

Varieties	Giza-164	Giza-168	Sakha-8	Sakha-69	Gemmeiza-3
Giza-164	0.00				
Giza-168	4.37	0.00			
Sakha-8	25.91	27.87	0.00		
Sakha-69	25.63	27.27	13.37	0.00	
Gemmeiza-3	19.80	20.81	19.70	9.56	0.00

calculated based on the means of all studied traits (EC%, RGP%, HD and GY/P). The Euclidean distance (Table 11) ranged from 4.37 to 27.87 between Giza-164 and Giza-168, and between Giza-168 and Sakha-8, respectively. The range of Euclidean distance among the varieties was relatively wide. This result indicated that the amount of phenotypic variation among these cultivars was relatively high. These, also reflect the genetic diversity of the loci controlling these traits. These results agree with the findings of [40] who demonstrated that Euclidean distance is from 1.34 to 7.00 among 11 Egyptian bread wheat cultivars.

On the dendrogram (Fig. 3), the five parental cultivars created two distinct clusters. Giza-164 and Giza-168 were grouped together in the first cluster at relatively high Euclidean distance of 24.55. The second cluster formed two sub-groups, which were separated at 16.54 Euclidean distance. The bootstrap values on the dendrogram indicated a high morphological variation pattern among the two main clusters. Morphologically, the varieties in each cluster are different and quite distinct from each other. The first cluster that contained Giza-164 and Giza-168 varieties is less diverse for morphological traits than the second one.

The results of the agronomic characterization and RAPD marker were somewhat similar, indicating that the agronomic characterizing information will continue to be useful to identify diverse germplasm in breeding programs. The correlation between Euclidean distance and RAPD distance was highly significant ($r = 0.72^{**}$) (Fig. 4). [41] reported a moderate correlation (0.47) between RFLP and agronomic relatedness in durum wheat. However, [40] obtained a correlation of −0.20 between RAPD markers and agronomic characterization. In addition, [42] found negative correlation (−0.06) in perennial ryegrass varieties. [43] showed that DNA markers are preferable to morphological ones because they relate variability directly at the genetic level and provide reliable and enormous data that permit a reproducible estimate of genetic diversity in the germplasm.

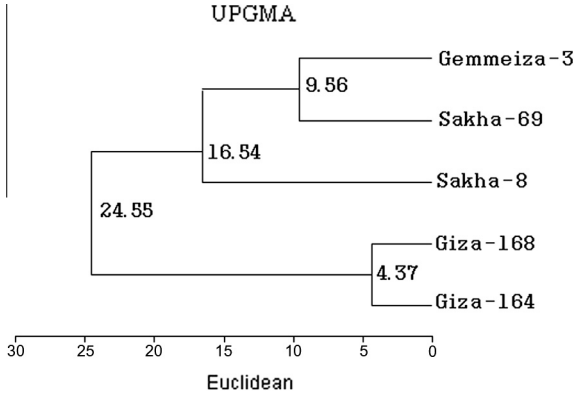

Figure 3 Dendrogram generated by UPGMA cluster analysis based on mean values of embryogenic calli, regenerated green plants, number of days to heading and grain yield per plant in five Egyptian bread wheat cultivars.

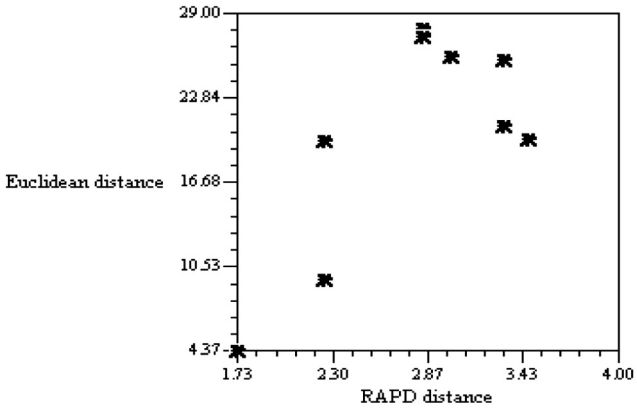

Figure 4 Correlation between Euclidean distance and RAPD distance methods generated by NTSYS-pc Ver 2.1 program.

4. Conclusion

Our results suggest that it is possible to screen genotypes with good tissue culture traits directly at the level of grain yield trait. The magnitudes of additive genetic variance (σ^2A) were higher than those of non-additive ones (σ^2D) for all traits except for heading date. Estimates of narrow sense heritability provide additional evidence about the importance of additive genetic variance in expression of these traits. The genetic markers and the agronomic characterizing information will continue to be useful to identify diverse germplasm in breeding programs.

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