DIALLEL AND FINGERPRINTING ANALYSES OF FIVE SUNFLOWER PARENTS

H.M.E. TAHER[1], Y.M. ABDEL-TAWAB[2] and R.E.A. EL-SHARIHI[1]

1. Oil Crops Research Dept., Field Crops Research Institute, Agricultural Research Center.
2. Seed Technology Research Dept., Field Crops Research Institute, Agricultural Research Center.

ABSTRACT

All possible cross combinations were made, without reciprocals, among five inbred lines. The F_1 of the ten crosses and the five parents were evaluated. Data revealed that the mean squares of genotypes, parents and crosses were highly significant for all studied characters. Positive and negative heterosis over better parent were detected for all studied characters indicating that parental genotypes were genetically diverse. The parent line 92 was the best combiner, which showed substantial and highly significant positive general combining ability (gca) effects for all characters except 100-seed weight and showed highly significant negative (gca) for 50% flowering. Also, the parent line 62 was good combiner which showed a highly significant positive (gca) effects for all characters except head diameter. The cross line 102 X line 46 showed highly significant positive specific combining ability (sca) for all characters except 100-seed weight and showed negative (sca) for 50% flowering. Meanwhile, the cross line 102 X line 62 showed highly significant positive (sca) for all characters except seed yield/plant. The crosses line 102 X line 92, line 46 X line 62, line 46 X line 92 and line 92 X line 82 had significant (sca) effects for seed yield/plant and 100-seed weight. These crosses could be used as superior and promising hybrids in sunflower breeding programs.

To evaluate the genetic diversity of the five sunflower genotypes and to determine the correlation between genetic distance and single-cross hybrid performance, random amplified polymorphic DNA (RAPD), a Polymerase Chain Reaction (PCR) technique was used. Fifteen different primers were employed giving a total of 101 reproducible amplification products, 59 of them (58.4%) being polymorphic. Cluster analysis divided the samples into two distinct groups. The genetic distances were correlated with important agronomic traits for single-cross hybrids and heterosis. The results indicated that RAPD can be used as a tool for determing the extent of genetic diversity among sunflower genotypes, for allocating genotypes into different groups, and also to aid in the choice of the superior crosses to be made among sunflower genotypes, so reducing the number of crosses required under field evaluation.

Key words: *Sunflower, Helianthus annuus, Heterotic effect, Combining ability, Diallel analysis, RAPD.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the one of the main crops used for edible oil production in many countries of the world, including Egypt. The major problem facing oil production in Egypt is the wide gap between production and consumption. Egypt's production covers less than 10% of the national consumption. Although the main oil crop cultivated in Egypt is

sunflower, both the area and the average yield per unit area were decreased gradually. Consequently, great effort should be given to improve sunflower cultivars in Egypt.

Developing hybrids and synthetic varieties are most effective in utilizing heterosis in sunflower (Gundaev 1966 and Putt 1966). In the earlier studies, high heterosis for seed yield and some yield components of sunflower were reported by Chaudhary and Anand (1984). Heterotic performance of hybrid combinations depends upon combining ability of parents. Combining ability studies are frequently used by plant breeders to evaluate parents and crosses for a number of objectives, including the development of superior synthetics or hybrids. Many breeders have reported that general combining ability (gca) and specific combining ability (sca) variances were significant for all characters in sunflower.

Since sunflower is a cross-pollinated crop, the wide genetic variation is being exploited to produce commercial varieties. Knowledge of the relative genetic distance among individuals or populations is useful in a breeding program because it permits organization of germplasm and provides more efficient sampling of genotypes (Nienhuis *et al* 1993). At the inception of a breeding program, knowledge of the genetic relationships among genotypes could be used to complement phenotypic information in the development of breeding populations. Ultimately, knowledge of the genetic similarity between genotypes may facilitate the choice of individuals to cross in hybrid combinations to optimize expression of heterosis (Melchinger *et al* 1990).

Classifying germplasm and breeding materials exclusively based on a few discrete morphological traits may not provide an accurate indication of genetic similarity since it is highly influenced by the environment (Menkir *et al* 1997) and also time intense. DNA based markers are now widely used owing to their virtues (Paris *et al* 2003 and Lu *et al* 2003). Williams *et al* (1990) and Welsh and McClelland (1990) demonstrated the utility of single short oligonucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly throughout the genome, and further the amplified bands were utilized for the determination of their relatedness (Sun *et al* 2001). Previously, the reality of RAPD has been well exploited to determine the genetic similarity of sunflower genotypes (Arias and Reisberg 1995 and Sivalop and Soledenko 1998).

For the purpose of the present study, five sunflower genotypes were crossed in a diallel design without reciprocals. The main objectives of the present study were: (1) to estimate the magnitude of the general and specific combining ability effects among the parents, (2) to find out the extent of heterosis in the material used, (3) to distinguish among the sunflower

genotypes using RAPD markers, (4) to evaluate the genetic divergence of sunflower genotypes; and, (5) to correlate single-cross performance and heterosis to the genetic divergence of the parental genotypes.

MATERIALS AND METHODS

Field experiments

Five parental inbred lines were used in this study as the genetic material. Those parents were chosen from the breeding nursery of Oil Crops Research Section, Institute of Field Crops, Agricultural Research Center. Hereafter, numbers 1 through 5 will identify parents. These were IN102 (P_1) Egypt, IN46 (P_2) Romania, IN62 (P_3) USA, IN92 (P_4) Romania and IN82 (P_5) Yugoslavia. The parental inbred lines were sown at the Research station farm, Agric. Res. Center, Giza, in 2005 summer season. The plants used as female were hand emasculated. Possible cross combinations without reciprocals were made between the parents giving ten crosses. The seeds from the ten crosses and the five parents were sown in summer season in 2006, in a randomized complete block design with three replicates. Each plot in the experiment consisted of two ridges, each ridge was three m. long and 60 cm. wide. The seeds were sown in hills spaced 20 cm. and the plants were thinned three weeks after sowing to secure one plant/hill. The inner rows were harvested at the physiological stage and seeds were air-dried. Data were recorded on an individual plant basis for days to 50% flowering, plant height, stem diameter, head diameter, seed yield/plant, 100-seed weight and oil percentage.

Oil content (seed oil percentage)

Oil percentage was determined in the powdered seeds according to the procedure prescribed by the AOAC (1990) using Soxhlet apparatus for 16 hours with petroleum ether (boiling point 40-60°C).

DNA extraction

DNA was extracted from 100 mg of young leaves for each line using mi-Plant Genomic DNA Isolation Kit (metabion). The concentration and purity were determined by spectrophotometer.

RAPD-PCR analysis

RAPD analysis was carried out according to Williams *et al* (1990). Fifteen 10-mer oligonucleotide primers were selected as potentially useful, their universal code and sequences are shown in Table (6). PCR reactions were optimized and mixtures (25 µl total volume) were composed of dNTPs (200 µM), Mg Cl$_2$ (1.5 mM), 1x buffer, primer (0.2 µM), DNA (50 ng), Taq DNA polymerase (2 units). Amplification was carried out in a Thermo

Cycler (PTC 200) programmed for 94 °C for 3 min (one cycle); followed by 94 °C for 30 sec, 36 °C for 1 min and 72 °C for 2 min (36 cycle); 72 °C for 10 min (one cycle), then 4 °C (infinitive). Amplification products (15 µl) were mixed with 3 µl loading buffer and separated on 1.3% agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 100 bp DNA Ladder marker.

Data analysis

Data were statistically analyzed using computer statistical program MSTAT-C. General and specific combining ability effects were estimated according to Griffing (1956) diallel cross analysis designated as Method 2 Model I. The results of RAPD analysis were entered in a computer file as binary matrices where 0 stands for the absence of a band and 1 stands for band presence in each individual sample. Similarity coefficients were calculated according to Dice matrix (Nei and Li 1979). Construction of the dendrogram tree was performed using the unweighted pair group method based on arithmetic mean (UPGMA) as implemented in the SPSS program version 10.

RESULTS AND DISCUSSION

It is clear from Table (1) that the analysis of variance revealed significant differences among the studied genotypes. These findings indicated that the genotypes had a wide genetic diversity. The results emphasized the importance of combining ability studies and indicated good prospects for selection of suitable parents and crosses for the development of appropriate varieties and hybrids. Moreover, estimates of highly significant gca, sca variances and also the mean sum of squares due to parents vs. crosses for all characters except oil percentage indicated the importance of both additive and non-additive genes in the expression of all studied characters except oil percentage. Hence, any approach that facilitates simultaneous exploration of additive and non-additive gene effects would be most desirable for the improvement of these traits. Significant mean squares related to parents were detected for all traits. Significant mean squares related to crosses were detected for all traits, revealing over all differences among these genotypes. For all traits, the analysis of variance from the diallel crosses showed significant mean square values for gca and sca (Table 1), indicating the presence of both additive and non-additive gene effects among the parents.

Table 1. Analysis of variance and combining ability for all the studied traits.

S.V.	df	50% flowering	Plant height	Stem diameter	Head diameter	Seed yield/plant	100 seed weight	Oil percentage
Genotypes	14	49.50**	802.42**	0.43**	23.94**	749.66**	9.90**	24.49**
Parents	4	45.60**	282.57**	0.38**	5.96**	197.64**	11.70**	53.93**
Crosses	9	41.24**	812.74**	0.41**	17.98**	470.07**	8.62**	13.80**
P Vs F$_1$	1	139.38**	2788.90**	0.80**	149.51**	5474.04**	14.24**	2.88
Error	28	1.04	25.94	0.02	0.82	6.77	0.12	0.85
GCA	4	25.02**	346.66**	0.21**	7.14**	175.04**	4.59**	11.26**
SCA	10	13.09**	235.80**	0.12**	8.31**	279.82**	2.78**	6.92**
Error	28	0.35	8.65	0.01	0.27	2.26	0.04	0.28

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

Occurrence of both additive and non-additive gene effects with preponderance of non-additive gene action for yield and important yield components in sunflower were reported by Marinkovic (1993), Ortegon and Mendoza (1993), Taha (1993), Kashef (1996), Gangappa *et al* (1997), Limbore *et al* (1997), Ali and Korkut (1998), Ahmed (1999), Salih (2000), El-Azzony (2001), Goksoy *et al* (2002), Taher (2002) and Ortis *et al* (2005).

Regarding the parental and hybrids mean values, Table (2) emphasized that line62 followed by line102 were the highest yielding sunflower genotypes under the study. On the contrary, line46 was the lowest yielder. Moreover, line62 proved to be superior in terms of 100-seed weight and oil percentage comparing with other studied genotypes. On the other hand, the highest F$_1$ mean values of seed yield/plant, 100-seed weight and oil percentage were recorded for line42 x line92, line102 x line92 and line62 x line92, respectively. Moreover, the hybrids line102 x line92, line102 x line62, line62 x line92 and line46 x line92 were the earliest hybrids, the tallest with thick stems and big head, respectively comparing with other sunflower hybrids. Data indicated that if the yield and its components were the most important selection criteria, these mentioned hybrids will be the best in a breeding program. For most of the studied characteristics, hybrids, in general, had higher values than parental lines. This indicated the importance of heterosis.

Data in Table (3) illustrate percentage of heterosis relative to better parent. For 50% of flowering, negative value of heterosis for this trait is desirable. These crosses manifested heterosis values ranging from -26.54 (P$_3$ x P$_4$) to -4.00 (P$_4$ x P$_5$). With respect to plant height, four crosses showed positive significant heterosis, these crosses exhibited heterosis values ranging from 13.82 (P$_2$ x P$_3$) to 42.76 (P$_1$ x P$_3$). Regarding stem diameter

Table 2. Mean performance of the genotypes for all the studied traits.

Genotype	50% flowering (day)	Plant height (cm)	Stem diameter (cm)	Head diameter (cm)	Seed yield/plant (g)	100-seed weight (g)	Oil percentage
P_1	44.00	89.67	1.37	13.67	43.07	10.23	32.77
P_2	46.00	93.00	1.67	14.67	31.33	7.23	37.65
P_3	54.00	101.33	1.77	13.33	51.83	10.83	39.76
P_4	50.00	114.00	2.23	16.93	35.53	6.20	39.94
P_5	50.00	94.00	2.13	14.47	35.47	8.00	34.11
P_1 x P_2	41.00	115.00	2.10	19.53	63.47	9.10	34.84
P_1 x P_3	44.67	144.67	2.33	19.33	61.07	12.03	36.95
P_1 x P_4	39.67	122.00	2.20	17.67	78.33	11.50	38.40
P_1 x P_5	46.67	90.00	1.43	15.00	44.00	6.90	40.60
P_2 x P_3	48.00	115.33	2.17	20.50	66.40	10.50	40.35
P_2 x P_4	46.00	107.33	2.53	21.77	82.43	11.27	39.95
P_2 x P_5	47.00	92.33	1.57	14.33	46.57	8.13	34.63
P_3 x P_4	39.67	131.00	2.60	20.67	72.00	10.33	42.31
P_3 x P_5	50.00	111.67	2.17	17.00	57.30	8.07	37.47
P_4 x P_5	48.00	121.67	2.07	19.00	56.87	9.10	41.32
L.S.D 5%	1.70	8.52	0.22	1.51	4.35	0.57	1.54

Table 3. Heterosis relative to better parent for all the studied traits.

Hybrid	50% flowering	Plant height	Stem diameter	Head diameter	Seed yield/plant	100-seed weight	Oil percentage
P_1 x P_2	-10.87**	23.66**	26.00**	33.18**	47.37**	-11.06**	-8.58**
P_1 x P_3	-17.28**	42.76**	32.08**	41.46**	17.81**	11.08**	-10.54**
P_1 x P_4	-20.67**	7.02	-1.49	4.33	81.89**	12.38**	-4.48*
P_1 x P_5	-6.67**	-21.05**	-35.82**	-11.42*	2.17	-32.57**	1.91
P_2 x P_3	-11.11**	13.82**	22.64**	39.77**	28.10**	-3.08	-1.03
P_2 x P_4	-8.00**	-5.85	13.43**	28.54**	131.99**	55.76**	-0.10
P_2 x P_5	-6.00**	-19.01**	-29.85**	-15.35**	31.05**	12.44**	-15.25**
P_3 x P_4	-26.54**	14.91**	16.42**	22.05**	38.91**	-4.62	4.57*
P_3 x P_5	-7.41**	-2.05	-2.99	0.39	10.55*	-25.54**	-9.14**
P_4 x P_5	-4.00*	6.73	-7.46	12.21**	60.04**	46.77**	3.91

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

five out of the ten crosses exhibited positive significant heterosis these crosses showed heterosis values ranging from 13.43 (P_2 x P_4) to 32.08 (P_1 x P_3). For head diameter, six out of the ten crosses showed positive significant heterosis value ranging from 12.21 (P_4 x P_5) to 41.46 (P_1 x P_3). With respect

to seed yield/plant, all crosses showed positive significant heterosis except one, these crosses ranging from 10.55 (P_3 x P_5) to 131.99 (P_2 x P_4). For 100-seed weight, five out of the ten crosses showed positive significant heterosis, these crosses had heterosis value ranging from 11.08 (P_1 x P_3) to 55.76 (P_2 x P_4). Regarding oil percentage, only one cross exhibited positive significant heterosis, this cross is P_3 x P_4. These results suggested the possibility of developing yield components traits from these crosses combinations. These results agree with those of Sing *et al* (1984), Giriraj *et al* (1986) and Goksoy *et al* (2002) who reported high heterosis for seed yield and some traits in sunflower.

Highly significant gca variances were obtained for all the characters observed. The estimates of sca variance were also significant for all the characters observed (Table 1). Since gca and sca provided an estimate for additive and non-additive gene actions, respectively (Sprague and Tatum 1942), our results were in good agreements with those of Sindagi *et al* (1979), Rao (1980) and Goksoy *et al* (2002) who found that most of the total genetic variability for a number of agronomic characters in sunflower crosses was due to additive gene effects. Only two parents (line62 and line92) had significant positive gca effects for seed yield/plant (Table 4). On the other hand, only two parents (line102 and line62) showed significant positive gca effects for 100-seed weight. Parents line62 and line92 had significant positive gca effect for oil percentage, out of which parent line92 showed significant positive effect for head diameter. Parents line62 and line92 showed significant positive effect for stem diameter and plant height. However, parents line102, line46 and line92 exhibited significant negative effect for 50% flowering. This means that those parents are earlier than the others (Table 4).

Table 4. General combining ability effects for all the studied traits.

Genotype	50% flowering	Plant height	Stem diameter	Head diameter	Seed yield/plant	100 seed weight	Oil percentage
P_1	-2.55**	-0.89	-0.19**	-0.61**	0.39	0.60**	-1.78**
P_2	-0.55**	-5.89**	-0.06*	0.33	-1.25*	-0.33**	-0.64**
P_3	1.78**	6.88**	0.10**	0.15	4.31**	0.98**	0.61**
P_4	-0.65**	7.54**	0.25**	1.40**	4.35**	-0.17*	1.56**
P_5	1.97**	-7.65**	-0.09**	-1.27**	-7.80**	-1.08**	0.24
LSD 5% g_i	0.41	2.04	0.05	0.36	1.04	0.14	0.37
LSD 5% g_i-g_j	0.64	3.22	0.08	0.57	1.64	0.21	0.58

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

Data on specific combining abilities of some promising crosses (Table 5) suggested that the hybrids line102 x line46, line102 x line92, line46 x line62, line46 x line92, line62 x line92, line62 x line82 and line92 x line82 had the highest significant SCA effects for seed yield/plant. The hybrids line102 x Line62, line102 x Line 92, Line46 x Line62, Line46 x Line92 and Line 92 x Line 82 had highest significant SCA effects for 100-seed weight. High significant sca effects were shown by the hybrids line102 x Line46, line102 x Line62 and Line46 x Line82 for oil percentage. The earliest hybrids were line102 x Line46, line102 x Line92 and Line62 x Line92, which showed high significant sca.

Table 5. Specific combining ability effects for all the studied traits.

Hybrid	50% flowering	Plant height	Stem diameter	Head diameter	Seed yield/plant	100 seed weight	Oil percentage
P_1 x P_2	-2.21**	12.24**	0.33**	2.62**	9.28**	-0.47*	2.10**
P_1 x P_3	-0.87	29.14**	0.41**	2.61**	1.32	1.16**	2.97**
P_1 x P_4	-3.44**	5.81*	0.12	-0.32	18.55**	1.77**	0.52
P_1 x P_5	0.94	-11.00**	-0.31**	-0.31	-3.63**	-1.92**	-2.31**
P_2 x P_3	0.46	4.81	0.11	2.83**	8.29**	0.56**	-0.97*
P_2 x P_4	0.89	-3.86	0.33**	2.84**	24.29**	2.47**	-0.48
P_2 x P_5	-0.73	-3.67	-0.30**	-1.92**	0.57	0.25	3.04**
P_3 x P_4	-7.78**	7.05*	0.23**	1.93**	8.30**	0.23	-0.18
P_3 x P_5	-0.06	2.90	0.14*	0.93	5.75**	-1.12**	-4.18**
P_4 x P_5	0.37	12.24**	-0.11	1.67**	5.27**	1.05**	-2.30**
LSD 5% Sij	1.05	5.26	0.14	0.93	2.69	0.35	0.95
LSD 5% Sij-Sik	1.58	7.89	0.20	1.40	4.03	0.53	1.43
LSD 5% Sij-Ski	1.44	7.20	0.19	1.28	3.68	0.48	1.30

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

In general, it is desired to identify parents and hybrids having positive and significant gca and sca effects for yield and yield components in hybrid breeding (but negative for 50% flowering). Parent Line92 was observed to be a good general combiner for all traits except 100-seed weight. Also, parent Line62 was the second good combiner in terms of high positive significant sca for all traits except head diameter and 50% flowering.

These Lines appeared to transmit additive genes for high grain yield to their progenies and hence could be used as parents in a crossing program for improving yield performance. The best hybrid line102 x Line46 showed highly significant negative gca for 50% flowering and highly significant positive gca for all traits except 100-seed weight. The hybrid Line62 x Line92 showed the highest negative effects and was found to be the best

specific combiner for earliness. With regards to plant height, a highly significant positive value for plant height in the F_1 generation was found in the combinations (Line46 x Line62) and (Line92 x Line82), which had been obtained by crossing a parent with a poor gca for plant height with another one that had a highly positive gca for this trait (Table 4). These results support those obtained by Skoric *et al* (2000), who determined that crosses with a good plant height sca usually involve one parent with high and one with low gca values.

RAPD analysis

Many random primers were pre-tested for the ability to detect polymorphism in the five sunflower genotypes. Fifteen random primers succeed to anneal with the five genotypes and produce a high degree of polymorphism. These primers gave a total of 101 reproducible bands, 59 (58.4%) from them were polymorphic, while 42 bands were monomorphic (Table 6 and Fig. 1).

Table 6. Summary of data obtained by RAPD analysis for the five sunflower genotypes.

Primer	Sequence 5→3	TAB	PB	Genotypes										TSM
				1		**2**		**3**		**4**		**5**		
				AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	
A04	AATCGGGCTG	6	4	2	0	2	0	3	0	5	0	6	1	1
A11	CAATCGCCGT	7	4	5	1	3	0	6	2	3	0	4	0	3
A12	TCGGCGATAG	9	7	3	2	5	1	6	0	5	0	6	1	4
B03	CATCCCCCTG	6	4	6	0	6	0	5	0	2	0	2	0	0
B04	GGACTGGAGT	11	10	6	4	1	0	5	0	5	0	7	1	5
B05	TGCGCCCTTC	5	1	4	0	5	0	4	0	5	0	4	0	0
B06	TGCTCTGCCC	5	2	4	0	5	1	4	0	3	0	3	0	1
B07	GGTGACGCAG	6	1	5	0	5	0	5	0	5	0	6	1	1
B09	TGGGGGACTC	9	2	8	2	8	0	8	0	8	0	8	0	2
B14	TCCGCTCTGG	6	3	4	0	4	0	4	0	4	0	5	1	1
B16	TTTGCCCGGA	4	3	4	0	4	0	1	3	4	0	4	0	3
B18	CCACAGCAGT	4	2	2	0	2	0	3	1	3	1	2	0	2
IS01	TTGGCCGAGC	6	3	3	0	3	0	6	3	3	0	3	0	3
IS08	CCCCCCTTAG	6	4	5	1	4	1	5	0	4	1	4	0	3
IS10	TTAACCGGGG	11	9	5	1	4	0	6	0	3	0	7	3	4
Total		101	59											33

TAB= Total amplified bands, PB= Polymorphic bands, TSM= Total specific markers,
AB= amplified band and SM= specific marker.

An average of 6.7 bands per primer was obtained, ranging in size from 160 to 1460 bp. The least number of polymorphic bands was detected for primers B05 and B07 (one band), while the largest number of polymorphic bands was detected for primer B04 (10 bands). Thirty three out of 101 RAPD-PCR bands were found to be useful as genotype-specific
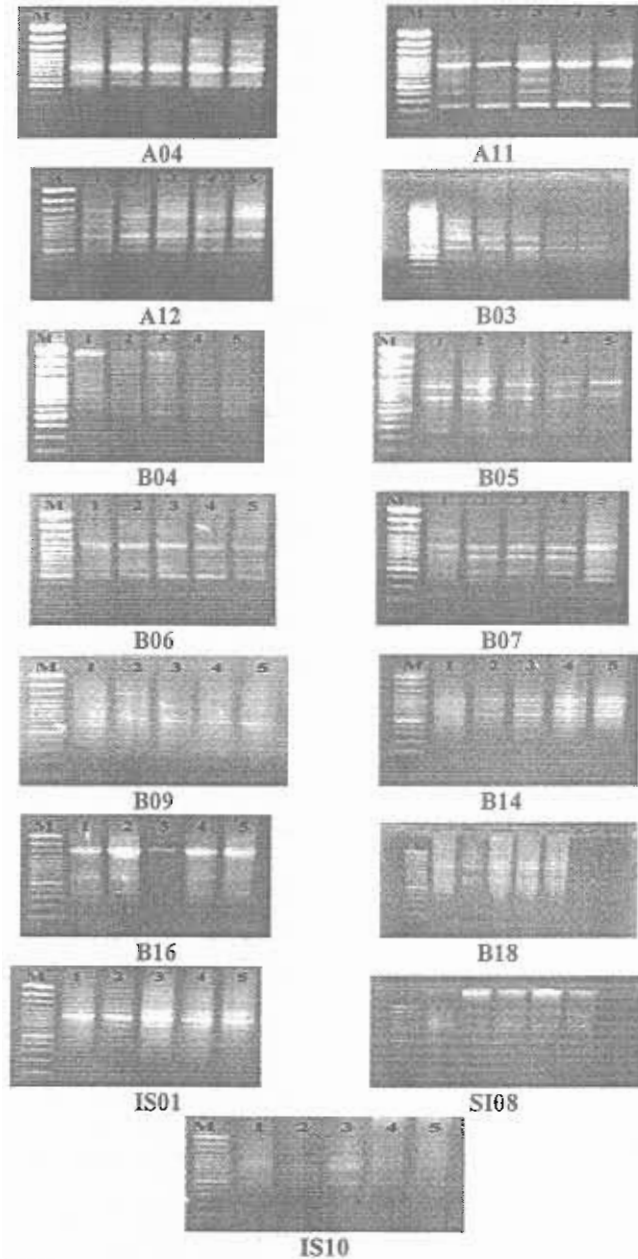
Figure 1. RAPD-PCR polymorphism of DNA extracted from five sunflower genotypes (Lanes 1-5, IN102, IN46, IN62, IN92 and IN82, respectively) using random primers. M refers to 100 bp DNA Ladder plus.

markers. Twenty-six out of 33 were positive markers, while seven were negative. The largest number of specific markers was scored for genotype 1 (11 markers), while the lowest number of specific markers was scored for genotype 4 (2 bands). Meantime, primer B04 generated the largest number of specific markers (5 markers), while primers A04, B06, B07 and B14 gave the lowest number of specific markers (one marker for each primer). On the other hand, primers B03 and B05 did not produce any specific markers.

In conclusion, all primers used allowed for distinction among the genotypes. Genotype-specific markers can be used in subsequent experiments to detect molecular markers for polymorphic genes with economic importance among these and other genotypes.

Genetic distance among genotypes

The similarity indices and the dendrogram tree among genotypes utilizing RAPD markers are shown in Table (7) and Fig. (2), respectively. The lowest genetic similarity (56%) was obtained between genotypes 1 and 4, while the highest genetic similarity (75%) was scored between genotypes 4 and 5. Cluster analysis classified the five genotypes into two groups. The first group included three genotypes (3, 4, and 5), while the second group included two genotypes (1 and 2). The first group subdivided into two subgroups. One of them included the genotype 3 only, while the other combined the two genotypes 4 and 5 with a genetic similarity (75%).

Table 7. Similarity matrix among the five sunflower genotypes based on RAPD analysis.

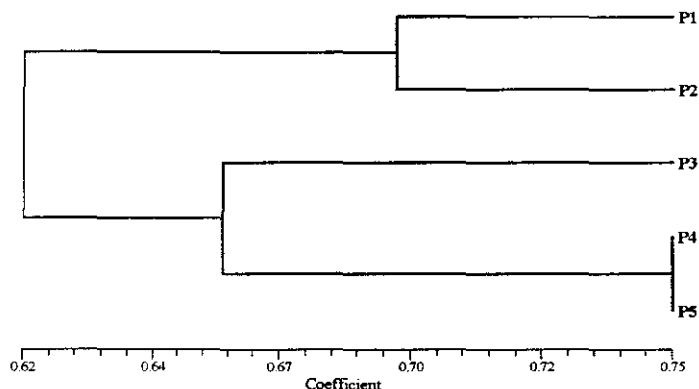| Genotype	1	2	3	4
1. IN102				
2. IN46	0.69			
3. IN62	0.59	0.69		
4. IN92	0.56	0.68	0.68	
5. IN82	0.57	0.59	0.63	0.75

Figure 2. Dendrogram cluster analysis of the genetic distances among five sunflower genotypes based on RAPD analysis.

Genetic distances and single cross performance

The results demonstrated good correlation between RAPD based genetic distance and sunflower single cross seed yield, 100-seed weight and oil percentage. The results assess the potentiality of the RAPD technology for characterizing at the molecular level and for generating unique fingerprint for each genotype. This could have great impact in plant improving programs of sunflower.

REFERENCES

Ahmed, H.K. (1999). Heterosis and combining ability in sunflower (*Helianthus annuus* L.) M.Sc. Thesis, Fac. of Agric., Assiut University.

Ali, A. and K.Z. Korkut (1998). Characterization of some broomrape resistant lines for their combining ability effects to utilize in sunflower hybrid breeding. Helia 21: 23-28.

AOAC. (1990). Official Methods of Analysis of The Association of Official Analytical Chemists 15th ed. Washington DC, USA.

Arias, D.M. and L.H. Reisberg (1995). Genetic relationships among domesticated and wild sunflower (*Helianthus annuus* L.). Economic Bot. 49: 239-248.

Chaudhary, S.K. and I.D. Anand (1984). Heterosis and inbreeding depression in sunflower. Genet. Crop Improvement 11: 15-19.

El-Azzony, E.F.H. (2001). Genetical studies on sunflower (*Helianthus annuus* L.). M.Sc. Thesis, Fac. Of Agric., Mansoura University.

Gangappa, E., K.M. Channakrishnaiah, M.S. Harini and S. Ramesh (1997). Studies on combining ability in sunflower (*Helianthus annuus* L.). Helia 20: 73-84.

Giriraj, K., N. Shivaraju and S.R. Hiremath (1986). Studies on heterosis and inbreeding depression in selected crosses combination of sunflower. Oilseed Res. 3: 67-72.

Goksoy, A.T., A. Turkec and Z.M. Turan (2002). Quantitative inheritance in sunflower (*Helianthus annuus* L.). Helia 25: 131-140.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Austr. J. Biol. Sci. 9: 463-493.

Gundaev, A.I. (1966). Prospects for breeding sunflowers for heterosis. Sborn. Rab. Masl. Kul't (Rec. Wk. Oil Pl.), 3: 15-21.

Kashef, E.A.A. (1996). Breeding studies on sunflower(*Helianthus annuus* L.). Ph.D thesis, Fac. Of Agric. Moshtohor, Zagazig Univeristy, Benha Branch.

Limbore, A.R., D.G. Weginwar, B.D. Gite and R.B. Ghorade (1997). Combining ability in sunflower(*Helianthus annuus* L.). Journal of Soils and Crops 7: 39-42.

Lu, B.R., K.L. Zheng, H.R. Oian and J.Y. Zhuang (2003). Genetic differentiation of wild relatives of rice as assessed by RFLP analysis. Theor. Appl. Genet. 106: 101-106.

Marinkovic, R. (1993). Combining ability of some inbred sunflower (Helianthus annuus L.) lines. J. Genet. and Pl. Breed. 53: 299-304.

Melchinger, A.E., M. Lee, K.R. Lankey, A.R. Hallauer and W.L. Woodman (1990). Genetic diversity restriction fragment length polymorphisms and heterosis for two maize inbreds. Theor. Appl. Genet. 80: 488-496.

Menkir, A., P. Goldsbrough and G. Ejeta (1997). RAPD based assessment of genetic diversity in cultivated races of sorghum. Crop Sci. 37: 564-569.

Nei, M. and W.H. Li (1979). Mathematical model of studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

Nienhuis, J., M.K. Slocum, D.A. De Vos and R. Muren (1993). Genetic similarity among *Brassica oleracea* genotypes as measured by restriction fragment length polymorphism. J. Amer. Soc. Hort. Sci. 26: 1678-1778.

Ortegon, M.A.S. and A.E. Mendoza (1993). Combining ability in sunflower hybrids (*Helianthus annuus* L.) for achene yield and oil. Helia 16: 45-54.

Ortis, L., G. Nestares, E. Frutos and N. Machado (2005). Combining ability analysis for agronomic traits in sunflower (*Helianthus annuus* L.). Helia, 28: 125-134.

Paris, H.S., N. Yonash, V. Portnoy, N. Mozes-Daube, G. Tzuri and N. Katzir (2003). Assessment of genetic relationship in *Cucurbita pepo* (cucurbitaceae) using DNA markers. Theor. Applied Genet. 106: 971-978.

Putt, E.D. (1966). Heterosis and combining ability and predicted synthetics from a diallel crosses in sunflower. Can. J. Pl. Sci. 46: 59-67.

Rao, P.K.A. (1980). Diallel analysis of ten quantitative characters in sunflower (*Helianthus annuus* L.). Univ. Agric. Sci., Bangalore, India. Thesis Abstracts, 6: 111-112.

Salih, M.A. (2000). Breeding studies of some economic characters on sunflower (*Helianthus annuus* L.). Ph. D. Thesis, Fac. Of Agric. Moshtohor, Zagazig Univeristy (Benha Branch).

Sindagi, S.S., R.S. Kulkarni and A. Seetharam (1979). Line x tester analysis of combining ability in sunflower (*Helianthus annuus* L.). Sunflower Newsletter, 3: 11-12.

Sing, S.B., K.S. Labana and D.S. Virk (1984). Heterosis in variety x inbred crosses of sunflower. Crop Improvement, 11: 35-38.

Sivalop Yu, M. and A.E. Soledenko (1998). Inter-and Intra-species differentiation in the genus *Helianthus* by RAPD analysis. Helia 21 (29): 9-17.

Skoric, D., S. Jocic and I. Molnar (2000). General and specific combining ability in sunflower. Proceeding of 15th International Sunflower Conference Toulouse, France, 12-15 June. pp. E23-E29.

Sprague, G.F. and L.A. Tatum (1942). General vs. specific combining ability in single crosses of corn. J. Am. Soc. Agron. 45: 923-932.

Sun, G.L., B. Salomon and R. von Bothmer (2001). Analysis of tetraploid *Elymus* species using wheat microsatellite markers and RAPD markers. Genome 40: 806-814.

Taha, R.S. (1993). Heterosis and combining ability in sunflower (*Helianthus annuus* L.). Egypt. J. Appl. Sci. 8 (12).

Taher, H.M.E. (2002). Genetical studies on sunflower (*Helianthus annuus* L.). Ph. D. Thesis, Fac. Of Agric., El-Azhar University.

Welsh, J. and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acid Res. 19: 303-306.

Williams, J.G.K., A.R. Kubelk, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res. 18: 6231-6235.

Zhang, X.Y., R.R.C. Wang and Y.S. Dong (1996). RAPD polymorphisms in *Aegilops geniculata*. Roth. Genet. Res. And Crop Evol. 43: 429-433.

الهجن الدائرية والبصمة الوراثية لخمسة تراكيب من دوار الشمس

هشام محمد البغدادي طاهر¹ – ياسر محمد عبد التواب² – رضا السيد أحمد السيد الشرايحى¹

١- معهد بحوث المحاصيل الحقلية – قسم بحوث المحاصيل الزيتية – مركز البحوث الزراعية.
٢- معهد بحوث المحاصيل الحقلية – قسم بحوث تكنولوجيا البذور – مركز البحوث الزراعية.

لتحسين محصول دوار الشمس تلزم الاستفادة من قوة الهجين عن طريق التهجين بين السلالات النقية بعد اختبار قدرتها علي التوافق من خلال الهجن التبادلية. لذلك تم عمل كل الهجن الممكنة عدا العكسية بين خمسة سلالات، وفى الموسم الصيفي لعام 2005 زرعت الآباء وهجنها العشرة (الجيل الأول) بمزرعــة محطــة بحــوث الجيزة. أظهرت النتائج وجود قوة هجين عن الأب الأعلى في الهجن العشرة بالنسبة ل 50% تزهير، وأربعة هجن بالنسبة لطول النبات، وخمسة هجن بالنسبة لقطر الساق، وستة هجن بالنسبة لقطر القرص، وتسعة هجن بالنسبة لمحصول النبات من البذور، وخمسة هجن بالنسبة لوزن 100 بذرة، وهجن واحد بالنسبة لنسبة الزيت. أظهـرت

200

السلالة 92 أفضل قدرة عامة على الائتلاف لكل الصفات المدروسة ماعدا صفة وزن 100 بـذرة، كمـا أظهـرت السلالة 62 أفضل قدرة عامة على الائتلاف لكل الصفات المدروسة ماعدا صفتي 50% تزهير وقطر القرص ممـا يساعد على استخدامهما كمختبرات جيدة في برنامج التربية لهذه الصفات. كما أظهرت بعض الهجن تـأثيرا عاليـا ومرغوبا للقدرة الخاصة على الائتلاف ومنها الهجين سلالة 102 x سلالة 46 لجميع الصفات المدروسـة ماعـدا صفة وزن 100 بذرة، والهجين سلالة 102 x سلالة 62 أيضا لكل الصفات المدروسة ماعـدا صـفتي محـصول النبات من البذور و50% تزهير. وكانت الهجن سلالة 102 x سلالة 92، سلالة 46 x سـلالة 62، سـلالة 46 x سلالة 92، سلالة 92 x سلالة 82 قد أظهرت تأثيرا عاليا ومرغوبا لصفتي محصول النبـات مـن البـذور و وزن 100 بذرة. هذه الهجن يمكن الاستفادة منها في برنامج التربية كهجن مبشرة.

كما تم تقدير التباعد الوراثي بين التراكيب الوراثية الخمسة معمليا باستخدام طريقة RAPD باستخدام خمسة عشر بادئ عشوائي. وقد أظهرت النتائج 101 حزمة منها 59 حزمة متباينة (58.4%). كما قـسم تحليـل الشجرة التطورية التراكيب الوراثية إلى مجموعتين. كما دلت النتائج على إمكانية الاستفادة من طريقـة RAPD لتحديد درجة التباعد الوراثي بين التراكيب الوراثية لتقسيمها إلى مجموعات مختلفة وبالتالي إمكانية اختيار أفـضل الآباء التي يمكن تهجينها للحصول على أعلى محصول وبالتالي تقليل عدد الهجن التي يتم تقيمها في الحقل.