

GENETIC DIVERGENCE IN LINES OF SUNFLOWER DERIVED FROM INTERSPECIFIC HYBRIDIZATION

G.S. MOHAN¹ and A. SEETHARAM^{2*}

SUMMARY

Genetic divergence was assessed in 137 interspecifically derived and known sunflower lines, 1 hybrid, 1 three way cross, and 1 open pollinated variety for 17 characters. The lines, irrespective of their pedigree, segregated into 6 clusters. Cluster I had maximum number (137) of lines. Highest intercluster distance was observed between clusters II and IV, followed by IV and VI, and IV and I. Clusters with highest mean values for various economic characters were identified. Selection of few lines from the clusters based on intercluster distance and cluster mean values for hybridization is suggested. The divergence among the lines was mainly due to five characters (accounting for 67.08%) given in the order of magnitude of contribution *viz.*, head diameter, plant height, seed length, oil content, and days to 50% flowering. The limited divergence observed might be due to high levels of backcrossing to the recurrent parent (cultivated sunflower), which is normally in vogue in wide hybridization programs to obtain desirable agronomic background. However, it was difficult to obtain an optimized blend of genes from wild and cultivated forms leading to limited divergence in the resultant lines. Restricting to BC₁F₂ generation for selecting superior introgressions and intercrossing might help in obtaining lines having higher heterogeneity and *per se* performance.

Key words: Sunflower, interspecific hybridization, genetic divergence

Assessing genetic diversity in breeding material is of paramount importance because crosses effected between lines of diverse origin generally display greater heterosis and generate more useful recombinants than crosses made between closely related lines. This has been observed in fescue, maize, alfalfa, cotton, and other crops (Singh and Narayanan, 2000). The D² technique (Mahalanobis, 1936) helps in the identification of genetically divergent parents. It also measures the degree of diversification and determines the relative proportion of each component character in the total divergence. The forces of differentiation are measured at two levels, *viz.* intracluster and intercluster levels. With this technique, a large number of germplasm can be evaluated together, providing reliable estimates of genetic divergence. In the present study, genetic divergence among interspecific lines of sunflower was assessed.

¹ Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore- 560 065, India

² All India Coordinated Crop Improvement Project (Small Millets), University of Agricultural Sciences, GKVK, Bangalore- 560 065, India

* Correspondence: Dr. A. Seetharam, Emeritus Professor, AICRP (Small Millets), University of Agricultural Sciences, GKVK, Bangalore- 560 065, India. Telefax: 091 80 23332387
Email: annadanarasram@rediffmail.com

MATERIAL AND METHODS

The experiment was conducted at the University of Agricultural Sciences, Bangalore, India and the material was comprised of 144 accessions that included 137 advanced lines, derived from interspecific crosses involving 5 wild species, and 4 other cultivated sunflower lines, 1 hybrid, 1 three way cross, and 1 open pollinated variety (Table 1). The lines were sown with a spacing of 60 cm between rows and 30 cm in the row in a 12 x 12 Simple Lattice design with two replications. Each replication consisted of 12 blocks, with 12 lines in each block. The replications, blocks, and the lines within the block were randomized. Each line, having 12 plants, was grown in a row of 3.6 m. Two seeds were dibbled in each hill to facilitate better emergence and to provide uniform plant stand. A recommended manure dosage was applied. Thinning was attended to 15 days after sowing to retain one plant per hill. The plants were grown under protective irrigation. All the recommended agronomic practices were followed to facilitate good growth and to raise a successful crop. From each line in each replication, five competitive plants were tagged at random. Except for days to 50% flowering, all other observations on quantitative characters were recorded at maturity.

The D^2 statistic for yield and yield attributes was computed using the SPAR I (V.1.1) software program. The D^2 values of all the combinations were arranged in descending order. Treating D^2 as a generalized statistical distance, all the genotypes used were clustered into different groups following Tocher's method (Rao, 1952). The intra- and intercluster distances and contribution of individual characters towards divergence were computed (Singh and Chaudhary, 1996).

Table 1. Pedigree of interspecifically-derived and cultivated sunflower used.

Pedigree	Generation	No. of lines	Line no.
Interspecific derived lines			
<i>H. argophyllus</i> X <i>H. annuus</i> cv. Morden	BC ₄ F ₄	36	1 to 35,127
<i>H. petiolaris</i> X <i>H. annuus</i> cv. Morden	BC ₄ F ₄	34	36 to 65,128 to 130,137
<i>H. annuus</i> (wild) X <i>H. annuus</i> cv. Morden	BC ₃ F ₅	18	66 to 80,131 to 132,136
(<i>H. argophyllus</i> X <i>H. annuus</i> (wild)) X <i>H. annuus</i> cv. Morden	BC ₃ F ₄	30	81 to 107,133 to 135
<i>H. annuus</i> cv. HA 234A X <i>H. debilis</i>	BC ₄ F ₄	19	108 to 126
Cultivated Sunflowers		Description	
Morden			Open pollinated variety
PAC1091			Three way cross hybrid
KBSH-1			Hybrid
6D-1			Parental lines of promising hybrids
R-265			
HA 234			
HA336			

RESULTS

Cluster Composition

The lines were segregated into six clusters. Cluster I had maximum number of lines (137), cluster II had three accessions, and clusters III, IV, V, and VI had one accession each (Table 2). Cluster I included most of the lines (137 out of 144), which indicated that the divergence among the interspecifically-derived lines was rather limited and the introgression of genes from wild species was not forthcoming. It was evident from the cluster composition that majority of the lines with Morden as male parent, irrespective of their female parent, had fallen into the first cluster. In addition, all the lines having *H. petiolaris* and *H. debilis* as their respective female parent and male parent were also present in Cluster I. The scrutiny of pedigree indicates that these lines are derived after 3-4 backcrosses to the recurrent parent Morden or HA 234. Obviously, this has narrowed down the genetic divergence of the lines involved in the study. Out of seven cultivated sunflowers included in the study, Morden, KBSH-1, R-265, and HA-234 were present in this cluster.

Cluster II had lines 80, 6D-1, and HA 336. The presence of two known lines, 6D-1 and HA 336, in this cluster hints about their genetic proximity. Line 80 was separated from Cluster I because of divergence in percent autogamy, seed yield/plant, plant height, days to maturity, oil content, and hull content. Cluster III included only one genotype PAC 1091 and Clusters IV, V, and VI also had one line each.

Table 2. Cluster composition with source, number of lines, and genotypes in 144 sunflower lines.

Cluster	No. of genotypes	Line no.	Source / Parentage
I	137	1-9, 11-35, 127	<i>H. argophyllus</i> X <i>H. annuus</i> cv. Morden
		36-65, 128-130	<i>H. petiolaris</i> X <i>H. annuus</i> cv. Morden
		66-79, 131-137	<i>H. annuus</i> (wild) X <i>H. annuus</i> cv. Morden
		81-105	(<i>H. argophyllus</i> X <i>H. annuus</i> (wild)) X <i>H. annuus</i> cv. Morden
		108-126	<i>H. annuus</i> cv. HA 234 X <i>H. debilis</i>
		138	Morden
		141	KBSH-1
		142	R-265
		143	HA 234
		II	3
140	6D-1		
144	HA 336A		
III	1	139	PAC1091
IV	1	10	<i>H. argophyllus</i> X <i>H. annuus</i> cv. Morden
V	1	106	(<i>H. argophyllus</i> X <i>H. annuus</i> (wild)) X <i>H. annuus</i> cv. Morden
VI	1	107	(<i>H. argophyllus</i> X <i>H. annuus</i> (wild)) X <i>H. annuus</i> cv. Morden

Intra- and Intercluster Distance

Inter- and intracluster distances are given in Table 3. Intracluster distance as expected was lowest in Cluster I (8.98) and marginally higher (10.17) in Cluster II. Intracluster distances were absent in Clusters III, IV, V, and VI as they included only one line each. However, intercluster distances were maximum between Clusters II and IV (22.26), followed by IV and VI (22.24), and I and IV (20.28). Considerable intercluster distances were found between Clusters V and VI, I and V, and I and VI. Based on the intercluster distances, crosses HA 336 (cluster II) with line 10; 10 and 107; and lines of Cluster I with the line of Cluster IV might give high heterosis. Lowest intercluster distances were observed between Clusters III and IV (10.05), followed by III and V (11.15), and IV and V (11.70). The agronomic performance of line 10 in cluster IV was almost identical to that of PAC 1091 in Cluster III except for few characters and, hence, the intercluster distance between these clusters was minimum.

Table 3. Mean intra- and intercluster D-square values of clusters in 144 lines of sunflower.

Cluster	I	II	III	IV	V	VI
I	80.72 (8.98)	193.02 (13.89)	250.65 (15.83)	411.5 (20.28)	310.7 (17.62)	307.56 (17.53)
II		103.49 (10.17)	270.96 (16.46)	495.81 (22.26)	284.94 (16.88)	230.59 (15.18)
III			--	101.13 (10.05)	124.44 (11.15)	285.92 (16.90)
IV				--	137.11 (11.70)	494.75 (22.24)
V					--	321.41 (17.92)
VI						--

Intracluster values on diagonal

Values in parenthesis represent square root of D square values

Contribution of Characters Towards Genetic Divergence

The degree of contribution of each character towards genetic divergence is given in Table 4. The highest degree of contribution towards genetic divergence was by head diameter (16.88%), which ranked first in 1,738 out of 10,296 distance combinations. It was followed by plant height (14.59%), seed length (13.72%), oil content (11.53%), days to 50% flowering (10.56%), and the other characters with less than 5% contribution. The least contribution was from stem girth (0.49%).

It was seen that the divergence among the 144 lines was mainly due to five characters (accounting for 67.08%) given in the order of magnitude of contribution *viz.*, head diameter, plant height, seed length, oil content, and days to 50% flowering. All the remaining characters accounted for only 32.72% of the variation. The large variation in head diameter, plant height, seed length, oil content, and days to 50% flowering in the lines could be due to conspicuous differences for these characters between cultivated sunflowers and the wild species parents.

Cluster Mean Values

The cluster mean values for 17 different characters are presented in Table 5. It can be seen from the cluster means that each cluster has its own uniqueness that separated it

Table 4. Contribution of characters towards genetic divergence in 144 lines of sunflower.

	Trait	Times ranked 1st	% contribution
1	Head diameter(cm)	1738	16.88
2	Plant height(cm)	1502	14.59
3	Seed length (cm)	1413	13.72
4	Oil content (%)	1187	11.53
5	Days to 50 per cent flowering	1087	10.56
6	Seed width(cm)	464	4.51
7	Test weight (g)	448	4.35
8	Days to maturity	440	4.27
9	Seed yield/plant (g)	352	3.42
10	Oil yield /plant(g)	333	3.23
11	No. of leaves in upper 1/3 rd plant	314	3.05
12	Percent autogamy	286	2.78
13	Mean number of leaves/ plant	263	2.55
14	Volume weight(g)	214	2.08
15	Percent Disease Index (<i>Alternaria</i>)	111	1.08
16	Hull content (%)	94	0.91
17	Stem girth (cm)	50	0.49

from other clusters. For example, Cluster I with the largest number of lines was characterized by the lowest mean value for days to 50% flowering (56.54), plant height (94.53 cm), stem girth (1.54 cm), days to maturity (83.02), number of leaves in the upper one third of the plant (5.34), and oil content (24.15%). The lowest average seed yield/plant (6.90g), oil yield/plant (2.51g), head diameter (8.53 cm), average number of leaves/plant (9.03), number of leaves in the upper one third of the plant (5.34), seed length (0.88 cm), hull content (23.39 %), and the highest mean *Alternaria* severity (69.80) among the six clusters characterize Cluster II. Cluster III had only one genotype, PAC 1091, characterized by the highest mean values for seed yield (55.09 g) and seed length (1.07), with high mean values for most of the characters. Cluster IV included line 10 which was identical in performance to PAC 1091 of Cluster III. However, it was distinct for high mean percent autogamy (69.13), oil yield/plant (24.84), oil content (46.20%), and test weight (7.22g), in addition to low *Alternaria* severity (48.19). Cluster V harbored one line (line 106) with high plant height (204.89 cm), stem girth (2.77 cm), head diameter (20.55 cm), number of leaves/plant (25.50), volume weight (42.69g), and seed width (0.48 cm). The lowest percent autogamy (5.60) was also recorded in this cluster. Cluster VI was characterized by a line (line 107) having high values for days to 50% flowering (79.82), days to maturity (106.48), number of leaves in the upper one third of the plant (11.87), hull content (42.3%), and lowest volume weight (22.53g), seed width (0.43 cm), and test weight (2.48g) among the clusters. It seems that the proportion of agronomically undesirable characters was higher in this cluster compared to beneficial characters.

In the present study, Cluster III was superior with respect to seed and oil yield (PAC 1091) and Cluster IV (line 10) was superior for oil yield. The lines in Clusters III and IV had almost similar performance and were superior to the lines in the other clusters

Table 5. Cluster mean values for 17 characters in 144 lines of sunflower.

Cluster	No. of lines	PAU	SYP	OYP	D50F	PHT	SGT	HDM	DMT	PDI	XLP	NUP	VWT	SLN	SWD	OCT	TWT	HCT
I	137	27.48	10.15	2.69	<u>56.54</u>	<u>94.53</u>	<u>1.54</u>	11.43	<u>83.02</u>	69.45	10.58	<u>5.34</u>	27.50	0.91	0.47	<u>24.15</u>	3.47	33.51
II	3	17.69	<u>6.90</u>	<u>2.51</u>	70.89	136.11	1.83	<u>8.53</u>	96.52	<u>69.80</u>	<u>9.03</u>	<u>5.34</u>	33.61	<u>0.88</u>	0.46	36.41	2.66	23.39
III	1	60.22	55.09	22.74	65.23	176.61	2.62	19.52	93.80	53.37	17.00	8.85	36.35	1.07	0.46	41.35	5.37	27.5
IV	1	69.13	53.25	24.84	58.74	170.71	2.22	18.42	91.06	48.19	22.80	11.26	42.32	1.05	0.47	46.20	7.22	31.02
V	1	<u>5.60</u>	42.61	17.67	66.06	204.89	2.77	20.55	100.51	63.11	25.50	10.62	42.69	0.93	0.48	42.09	6.22	37.02
VI	1	15.55	11.09	3.28	79.82	134.49	1.66	11.41	106.48	65.94	13.00	11.87	<u>22.53</u>	0.83	<u>0.43</u>	29.81	<u>2.48</u>	<u>42.3</u>

Numbers in bold indicate highest values among six clusters

Underlined values indicate lowest values among six clusters

PAU = Percent autogamy

SYP = Seed yield/plant

OYP = Oil yield/plant

D50F = Days to 50 per cent flowering

PHT = Plant height (cm)

SGT = Stem girth (cm)

HDM = Head diameter (cm)

DMT = Days to maturity

PDI = Percent Disease Index (*Alternaria*)

XLP = Mean number of leaves/plant

NUP = No. of leaves in upper one third of the plant

VWT = Volume weight (g)

SLN = Seed length (cm)

SWD = Seed width (cm)

OCT = Oil Content (%)

TWT = Test weight (g)

HCT = Hull content (%)

for many other characters such as higher autogamy, days to 50% flowering, plant height, stem girth, head diameter, leaves/plant, leaves in the upper one third of the plant, volume weight, seed length, oil content, test weight, and low *Alternaria* severity. Cluster V was next to cluster III and IV with respect to seed yield and oil yield and had some desirable characters (high plant height, head diameter, and volume weight) as indicated earlier.

DISCUSSION

Intercrossing lines from different clusters may generate large variability and would produce transgressive segregants for yield and yield attributes in population improvement programs. In this study, the highest intercluster distance was observed between Clusters II and IV followed by Clusters IV and VI, and IV and I. Hence, the lines 80 and 144 from Cluster II and the line 107 from cluster VI can be selected for hybridization programs as they are found to be better in respect of characters contributing to the divergence as well as *per se* performance. From Cluster I which had 137 lines, one best-performing line from each of the crosses can be included in the hybridization program. Line 10 from Cluster IV, the best genotype identified among the interspecifically-derived lines in the study, is a common line to be included with all these lines for hybridization that would lead to wide variability. Alternatively, selecting one best line from each of the clusters and crossing them in a diallel fashion may also prove to be beneficial. The resultant divergent lines could be converted either to CMS lines or restorers for future use in a heterosis breeding program in sunflower.

Cluster III recorded high means for seed yield/plant and seed length whereas Cluster IV recorded high mean percent autogamy, oil yield/plant, oil content, and test weight, and the lowest mean percent disease index. Cluster V recorded high mean plant height, stem girth, head diameter, leaves/plant, volume weight, and seed width. Thus, the genotypes with outstanding mean performance from these three clusters may be identified as potential parents and could be utilized in the development of new varieties.

In earlier studies, the contribution of characters towards genetic divergence was varied (Anand and Chandra, 1980; Muppudathi *et al.*, 1995; Alvarez *et al.*, 1996; Sankarapandian *et al.*, 1996; Manjula, 1997; Mirza *et al.*, 1997; Sujatha, 2000). In this study, head diameter, plant height, seed length, oil content, and days to 50% flowering contributed together to the divergence to the extent of 67.08%. These characters should be given more emphasis in further selection of lines in hybridization programs.

The limited divergence observed in the sunflower lines for agronomically important characters was due to the high level of backcrossing to the cultivated species during the prebreeding process. While introgressing genes from wild gene pool, it is a common practice to resort to backcrossing with the recurrent cultivated parent in order to obtain a desirable agronomic background. However, it is difficult to obtain an optimized blend of genes from wild and cultivated groups leading to greater tilt towards the cultivated forms. Obviously, many lines harboring genes coming mostly from cultivated species may fail to provide additional advantages and, hence, may have limited utility. On the contrary, restricting to the BC₁F₂ generation for selecting superior introgressions and their intercrossing may be more rewarding in getting lines having high heterogeneity and *per se* performance.

ACKNOWLEDGEMENT

This work was supported through a fellowship provided by the Council of Scientific and Industrial Research, New Delhi, India, for the Ph.D. research of the first author.

REFERENCES

- Alvarez, M.P., N. Mancuso, and E. Frutos. 1996. Genetic divergence among open pollinated populations of sunflower (*Helianthus annuus* L.). In: *Proc. of the 14th Int. Sunflower Conf., June 12-20 1996, Beijing/Shenyang, China*. 1:230-235.
- Anand, I.J., and S. Chandra. 1980. Genetic diversity and interrelationships of oil yielding traits in sunflower. *Sunflower Newsl.* 4(1):5-8.
- Mahalanobis, P.C. 1936. On the generalized distance in statistics. *Proc. Am. Soc. Animal Prod.* 33:293-301.
- Manjula, K. 1997. *Genetic variability, diversity and path coefficient analysis in non-oil seed sunflower (Helianthus annuus L.) genotypes*. M. Sc. (Agri.) Thesis. Univ. Agric. Sci. Dharwad, India.
- Mirza, S.H., M.S. Hassan, and D. Nessa. 1997. Genetic diversity amongst fifty sunflower (*Helianthus annuus* L.) genotypes. *Bangladesh J. Bot.* 26(1):51-54.
- Muppidathi, N., R. Sankarapandian, and S. Rajarathinam. 1995. Genetic divergence, correlation, and path analysis in sunflower. *Crop Improv.* 22(2):221-224.
- Phundan, S., and S.S. Narayanan, 2000. *Biometrical Techniques in Plant Breeding*. Kalyani Publishers, New Delhi, India. p. 249.
- Rao, C.R.. 1952. *Advanced Statistical Methods in Biometrical Research*. John Wiley and Sons, New York, USA. pp. 357-363.
- Sankarapandian, R., N Muppidathi, S. Rajarathinum, and S. Chidambaram. 1996. Genetic divergence in sunflower. *Madras Agric. J.* 83:637-639.
- Singh, R.K., and B.D. Chaudhary. 1996. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi, India. p. 318.
- Sujatha, H.L. 2000. *Characterization and evaluation of new sunflower (Helianthus annuus L.) inbreds*. M.Sc. (Agri) Thesis, Univ. Agric. Sci., Bangalore, India.