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Assessment of Phenotypic Divergence and Association Studies in Sunflower (*Helianthus annuus* L.)

S K Dhillon, Phool Chandra and Vikrant Tyagi*

Department of Plant Breeding and Genetics
Punjab Agricultural University, Ludhiana 141 001 (Punjab)

ABSTRACT

Sunflower (*Helianthus annuus* L., 2n=34), one of the important oilseed crops of the world, is a rich source of edible oil and is considered good from cardiac health point of view. In this study, a total of 67 sunflower inbred lines comprising 55 restorer lines and 12 maintainer lines belonging to different geographical origins were evaluated for phenotypic divergence on the basis of eight agro morphological traits and oil content. Among the evaluated traits ,days taken to initiation of disk floret opening, days taken to complete anthesis, days taken to physiological maturity, head diameter, plant height, autogamy per cent, 1000 seed weight, seed yield per plant and oil content revealed significant variation in the material under study. The data pertaining to these traits was subjected to D2 analysis which allowed grouping of the genotypes into nine cluster indicating genetic diversity in the material. The distribution patterns of the genotypes into different clusters indicated that grouping was not according to the source of genotypes. Cluster I has maximum number of genotypes (49). Inter cluster distance were higher than the intra cluster distances supporting the grouping of the genotypes. 1000 seed weight, plant height, initiation of flowering, autogamy per cent and oil content had greater contribution towards the observed genetic divergence. Selection of three CMS lines viz. 207A, 10A, 7-1A and five restorer lines viz.P83R, P81R, PISF-1R, LTRR-341 and R-17 from different clusters based on inter cluster distance and cluster mean values for hybridization is suggested.

Key Words: Sunflower, Genetic divergence, Correlations.

INTRODUCTION

Sunflower (*Helianthus annuus* L. 2n=34) is one of the important oilseed crops of the world and it accounts for nearly 14 per cent of the global production of 9 major vegetable oilseed crops. It is a rich source of edible oil and is considered good from cardiac health point of view due to high concentration of unsaturated fatty acids. Sunflower oil is generally considered premium oil because of its light colour, high level of unsaturated fatty acids and lack of linolenic acid, and high smoke point. Knowledge of genetic parameters is essential for understanding and their manipulations in any crop improvement programme. Seed yield in sunflower being is a quantitative character and dependent on its own component characters. Such interdependence of contributory characters as well as the characters of economic importance often misleads and thus makes correlation coefficient by and large unreliable during selection (Dewey and Lu, 1959), particularly in crop like sunflower, which is highly cross pollinated and heterozygous and envisages enormous variability in succeeding generations. Many researchers (Arshad *et al*, 2004 & 2006: Ghafoor and Ahmed, 2005) have used these techniques along with diversity study for investigating genetic parameters. In a quest to develop hybrids revealing higher magnitude of standard heterosis, greater adaptation with desirable attributes like oil content, tolerance to biotic and abiotic stresses, there is a need to evaluate parental lines for the extent of genetic variability prevailing so that it facilities to develop desired hybrid in a reasonably short time. In this context breeder would

Corresponding Author's Email: vikranttyagi97@gmail.com

choose genetically distinct parents for hybridization since heterotic crosses are expected to arise as a result of hybridization between divergent parental lines (Singh and Sharma, 1989). The D2 analysis has been successfully utilized in sunflower to classify genotypes and determine their inter relationships by many workers (Sankarpandian *et al*, 1996). Therefore, an attempt has been made to study the genetic diversity among parental lines of sunflower comprising mainly CMS A and R lines for further use in hybridization programme.

MATERIALS AND METHODS

The present investigation was carried out at the research fields of the oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. The material for present study consisted of 67 parental lines comprising 55 were restorer lines and 12 maintainer lines. The material was raised in two rows each row of 4.5m length with 60 cm and 30 cm inter and intra row spacing respectively, in the randomized block design. All the agronomic practices recommended for the region were followed to raise a good crop.

The data for morpho physiological traits i.e. days taken to initiation of disk floret opening, complete anthesis and physiological maturity were recorded on the basis of total plants per genotype whereas other characters *viz.*, head diameter, plant height, autogamy per cent, 1000 seed weight, seed yield per plant were recorded for five random plants in the field. From each genotype, five plants were randomly selected and covered with cloth bags on the day the first ray florets opened. These remained covered until harvest to observe seed set, that was used later to calculate per cent autogamy. All other traits were recorded from other five plants left uncovered for open pollination.

The observations on oil content were recorded from the random sample of open pollinated seed using NMR. The mean data of two years with respect to all the traits was subjected to statistical analysis following standard methods to calculate correlations among seed yield and its component traits. The D2 statistic for yield and yield attributes was computed using INDOSTAT, version 7.5 software programme. The D2 values of all the combinations were arranged in descending order. Treating D2 as a generalized statistics, all the genotypes used were clustered into different groups following the method as described by Rao (1952). The intra and inter cluster distances and contribution of individual traits towards divergence were computed following Singh and Chaudhary (1996).

RESULTS AND DISCUSSION

Univariate analysis of variance for each of the nine traits *viz*. Initiation of disk floret opening, complete anthesis, physiological maturity, head diameter, plant height, autogamy , 1000 seed weight, seed yield and oil content revealed highly significant differences among genotypes. The simultaneous testing of significance of difference in mean values between genotypes based on Wilk's criterion revealed highly significant differences (X2=4763.84 with 594 degree of freedom) among genotypes for the aggregate of the nine traits considered. Previous studies conducted by Teklewold *et al* (2000) have also reported significant differences among genotypes in univariate and multivariate analysis of variance.

D2 analysis assigned the test genotypes into nine clusters (Table 1) indicating presence of enough genetic diversity in the material. Cluster I contained maximum number of genotypes (49). Cluster II, IV and VII had seven, four and two genotypes respectively. The cluster III, V, VI, VIII and IX comprised of one genotype each. The cluster 1 which included maximum no. of genotypes (42 R lines and 7 B lines) indicated that the divergence among these lines was rather limited and hence fell in the same cluster. Out of the twelve B lines included in this study, seven B lines developed at different research centres in India fell in group I and, thus may have common ancestors. Maximum number of R lines (43) also fell in group I suggesting

some commonness shared among parents of these forty-three lines.

Intra and inter cluster distance

Intra and inter-cluster distance for 67 lines are given in table 2. Among the 9 clusters formed, inter-cluster D2 values varied from 8.94 (between cluster III and VIII) to 21.07 (between cluster IV and IX). The genotypes falling in these clusters are more diverse from each other. Among the 9 clusters formed cluster I, II, IV and cluster VII had 8.06, 8.41 and 5.34 intra-cluster D2 values, respectively. Intra cluster distances were absent in cluster III, V, VI, VIII and IX because these included only one genotype each. Genotypes grouped in the same cluster presumably diverge little from one another as the aggregate traits were measured. In this context, as the inter-cluster distance was high between cluster IV and IX followed by VII and IX, the genotypes falling in these clusters could be selected for the hybridization programme as these are expected to produce, high heterosis. However, earlier studies by Arunachalam (1981) indicate that too high divergence does not always produce the high heterosis because of internal cancellation of dominance effect at various loci. Environmental variation can also affect the expression of divergence values (Yadav *et al*, 1988). Therefore, assessment of heritable and non-heritable component of variation in total variability is of immense value in choice of the breeding programme.

Cluster mean values and contribution of each trait towards genetic divergence

The Cluster means and contribution of each trait towards genetic divergence is given in table 3. It can be seen from cluster means that each cluster has its own uniqueness that separated it form other clusters. For example cluster I with largest number of genotypes has mean values near to the population mean for all the traits. Cluster II is characterized as having low 1000 seed weight (28.0g). Cluster III has only one genotype, R-17 characterized as having largest head diameter (19.0cm) and high value for autogamy per cent (95.70), this indirectly indicates that large head size produces more number of filled seeds. Cluster IV is characterized as having genotypes with minimum number of days taken for initiation to flowering (53.17) and days to complete anthesis (59.83). Cluster V which has only one genotype 10 B that has maximum seed yield per plant (31.73g).The genotype PISF 13 R falling in cluster VI took maximum number of days to physiological maturity (92.33) and had lowest autogamy per cent of 74.37. The cluster VII comprising of two genotypes is characterized as having highest 1000 seed weight (89.27g). High oil content (41.57%) is the characteristic feature of the genotype 7-1 B falling in cluster VIII. Cluster IX also having one genotype is taking maximum number of days to initiation to flowering (69.97), days to complete anthesis (73.00) but minimum no of days to physiological maturity (82.33), minimum head diameter (8.53cm), lowest 1000 seed weight (23.33g) and minimum seed yield per plant (13.33g).

The per cent contribution of each character to total divergence varied between 1.22 and 35.19 for complete anthesis and 1000 seed weight, respectively. The highest degree of contribution towards genetic divergence was by 1000 seed weight (35.19%) followed by plant height (22.52%), initiation of flowering (15.56%), autogamy (9.45%) and oil content (6.6%). These five traits contributed 89.02% towards divergence. The least contribution to genetic divergence is by days taken to complete anthesis (1.22%). Major contribution of plant height, oil content and day to flowering (Mohan and Seetharam, 2005) oil content and plant height (Manjula *et al* (2001), head diameter and seed yield (Mupidathi *et al* (1995) and Sankarapandian *et al* (1996) towards genetic divergence in sunflower, have earlier been reported.

Genetic diversity is the main consideration for selecting parents to be used in a hybridization programme in crop plants. In this study the highest inter cluster distance was observed between cluster IV and IX followed by cluster VII and IX. Hence the parental lines falling in these clusters could be selected for developing better hybrids in sunflower.

It is common experience of the breeder that variation from diverse origin with reasonable range, when crossed, give maximum heterosis, specific combining ability and transgressive segregants in segregating generations. On the basis of inter cluster distance and cluster mean the crosses viz. 207 A X P83R, 207A X P81R, 207A X PISF1R, 207A X LTRR-341, 10A X R-17, 7-1 A X P83R, 7-1A X P81R, 7-1 A X PISF-1R and 7-1A X LTRR-341 are expected to perform better.

Character association studies

In all breeding programmes, yield is the ultimate objective, which has highly variable expression as it is influenced by several other components. These yield components are related among themselves and with yield either favorably or unfavourably. In general, in most of the crops the association among yield components are reported to be undesirable thereby hindering the rapid progress that could be made. Thus the knowledge of association of various characters with yield and among themselves would provide best criteria for indirect selection through component traits for improvement in yield.

In the present work the character association studies indicated that days to initiation of disk floret opening had strong positive correlation (0.75**) with days to complete anthesis. Head diameter was observed to be highly positively associated with days to physiological maturity (0.38**) which in turn was positively associated with seed weight (0.35**) and seed yield (0.28**). This is an indication that as the no. of days taken for physiological maturity increase the head diameter increases, seed filling becomes better which improves seed weight and ultimately affecting the seed yield in a positive side. This has been strongly supported by the highly positive association of head diameter with seed weight (0.51**) and seed yield (0.49**) in the present study. Autogamy per cent has been observed to be positively correlated with seed weight (0.21*) which in turn has shown significant positive association with seed yield (0.36**). These results were supported by the previous findings by Morinkovi *et al* (1992) and Taklewold *et al* (2000). Efforts were made to correlate seed yield and its component traits with oil content and quality because improving the oil yield and quality is prime

Table 1. Cluster composition with their respective inbred lines/genotypes.

Cluster No.	No. of Genotypes	Name of Genotypes
I	49	PISF-9R, RGA-856, R-272-1-P9, P72R, PISF-12R, P71R, P395R, RHA 271, P87R, 18B, P88R, P35R, P63R, P68R, 243A, 1147-4, P65R, P86R, P70R, PISF-18R, P62R, P78R, RHA-296, P74R, RHA-859, RCR-8297, P66R, P67R, SF-1R, NDR-2, 853B, P73R, P82R, RHA-83R6, 304B, P84R, RHA-297, P75R, RHA-17, 179-2RP2, 31B, P64R, MR-6, P61R, 44-B, RHA-214, PISF-3R, RR-1, RHA-274,
II	7	32-B, 12-B, SF-7R, LTR-1822, RHA-265, P69R, SF-4R
III	1	R-17
IV	4	P83R, P81R, PISF-1R, LTRR-341
V	1	10B
VI	1	PISF-13R
VII	2	R-273, R-801
VIII	1	7-1B
IX	1	207B

Table 2. Inter and intra-cluster distance values.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	8.06	10.59	10.59	13.20	10.10	10.03	11.19	11.13	15.32
II		8.41	11.08	12.30	11.16	12.46	16.9	11.59	13.53
III			0.00	11.52	14.79	14.79	14.12	8.94	16.16
IV				8.41	15.84	15.64	16.08	12.69	21.07
V					0.00	11.20	16.90	15.33	16.12
VI						0.00	10.68	14.24	15.66
VII							5.34	13.90	20.47
VIII								0.00	13.56
IX									0.00

Diagonal and above diagonal values indicated intra-cluster and inter cluster distance respectively.

objective in sunflower breeding. Oil content in the present study did not show any association with the morphological and seed yield components however, it has been reported to correlate negatively with days to flowering, plant height and seed yield per plant, whereas, positive association of oil content with these traits have been reported by Khan *et al* (2003) and Kaya *et al* (2007).

It was observed that palmitic acid showed significant positive correlations with seed weight (0.26*). Oleic acid correlated positively (0.24*) and linoleic acid was associated negatively (-0.24*) with head diameter and this association can be attributed mainly to genotypic effect as the influence of environment on this association was negligible (genotypic correlation almost equal to

Table 3. Cluster means and contribution of traits towards genetic divergence .

Cluster No	Initiation of disk floret opening (days)	Complete anthesis (days)	Physiological maturity (days)	Head diameter (cm)	Plant Height (cm)	Autogamy (%)	1000 seed weight (g)	Seed yield (g/pl)	Oil content (%)
I	61.56	66.69	92.07	14.18	117.22	92.28	52.17	22.32	33.38
II	60.33	66.57	90.67	11.27	110.80	87.27	28.00	17.90	33.96
III	60.33	67.00	90.00	19.00	82.67	95.70	41.07	25.73	28.07
IV	53.17	59.83	89.92	14.26	69.38	91.83	45.12	17.46	35.06
V	61.33	66.67	92.00	15.97	157.27	88.23	37.93	31.73	33.27
VI	62.33	68.33	92.33	12.00	125.13	74.37	62.27	16.47	34.43
VII	60.83	66.50	91.33	14.52	101.20	89.98	89.27	19.30	32.52
VIII	61.67	67.67	83.00	15.70	88.67	95.90	44.87	24.47	41.57
IX	69.67	73.00	82.33	8.53	108.07	83.53	23.33	13.33	31.55
Population Mean	61.1± 0.53	66.9 ± 0.63	90.6 ± 0.85	13.7 ± 0.79	117.8 ± 3.13	91.7 ± 1.44	46.7 ± 2.29	21.4 ± 4.28	33.01 ± 1.20
per cent Contribution of traits towards genetic divergence	15.56	1.22	2.76	5.16	22.52	9.45	35.19	1.54	6.60

Table 4. Genotypic and phenotypic correlations of 13 quantitative characters for 67 inbred lines.

Sr.No.	Characters	IDF	CA	PM	HD	PH	AP	SW	SY	OC	PA	SA	OA	LA
1	IDF	1	0.85	0.18	-0.18	0.37	-0.04	-0.08	-0.02	0.04	-0.04	0.01	0.07	-0.07
2	CA	0.75**	1	0.34	-0.03	0.38	-0.21	-0.07	-0.04	-0.15	0.05	-0.04	-0.03	0.02
3	PM	0.04	0.12	1	0.51	0.13	-0.17	0.48	0.38	-0.14	0.21	-0.01	-0.01	-0.01
4	HD	-0.11	-0.1	0.38**	1	0.35	0.23	0.75	0.67	-0.06	-0.08	-0.03	0.29	-0.28
5	PH	0.3	0.26*	0.06	0.18	1	0.59	0.16	0.37	0.16	-0.14	-0.19	0.13	-0.12
6	AP	-0.05	-0.09	-0.02	0.2	0.15	1	0.38	0.19	-0.15	0.24	-0.33	-0.03	0.04
7	SW	-0.12	-0.14	0.35**	0.51**	0.11	0.21*	1	0.42	-0.15	0.3	-0.15	-0.01	-0.01
8	SY	-0.04	-0.04	0.28**	0.49**	0.23	0.09	0.36**	1	0.32	-0.1	-0.24	0.1	-0.07
9	OC	-0.05	-0.06	-0.02	-0.04	0.07	0.09	-0.1	0.12	1	-0.2	0.26	-0.02	0.02
10	PA	0	0.04	0.15	-0.08	-0.12	0.06	0.26*	-0.12	-0.17	1	-0.2	-0.62	0.56
11	SA	0.01	0	0.02	-0.02	-0.1	-0.18	-0.14	-0.09	0.1	-0.27*	1	0.33	-0.42
12	OA	0.04	-0.04	0.01	0.24*	0.1	-0.01	0.03	0.12	-0.1	-0.57**	0.26*	1	-0.99
13	LA	-0.04	0.03	-0.02	-0.24*	-0.09	0.02	-0.05	-0.1	0.02	0.53**	-0.34**	-0.99**	1

Above diagonal values indicate genotypic correlation and below diagonal values indicate phenotypic correlation

IDF= Initiation of disk floret opening (days); **CA=** Complete anthesis (days); **PM=** Physiological maturity (days); **HD=** Head diameter (cm); **PH=** Plant height (cm); **AP=** Autogamy percent; **SW=** 1000 Seed weight; **SY=** Seed yield (g/pl); **OC=** Oil Content (%); **PA=** Palmitic acid; **SA=** Stearic acid; **OA=** Oleic acid; **LA=** Linoleic acid.

phenotypic correlation).Similarly for other fatty acids i.e. stearic acid showed negative association with palmitic acid (-0.27*). Oleic acid was strongly and negatively associated with palmitic acid (-0.57**) while showed +ve correlation with stearic acid (0.26*). Linoleic acid was observed to have highly significant +ve association with palmitic acid (0.53**) while highly significant –ve correlation with stearic acid (-0.34**) and oleic acid (-0.99**).

CONCLUSION

Diversity analysis indicates enormous quantum of diversity present in the germ plasm which can be exploited and put to use in hybrid breeding programme. The maximum genetic divergence was observed between cluster IV and cluster IX, which represents a good cross combination and may lead to desirable recombinants in the segregating generations. Restorer lines namely, R-17, P83R, P81R, PISF1R and LTRR-341 can be used in hybridization with CMS A lines present in cluster I to synthesize high yielding and good quality hybrids.

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