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GENETIC DIVERGENCE STUDY IN SUNFLOWER (*Helianthus annuus L.*)

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Abstract

This study has been undertaken to investigate the seventy five hybrids developed by making crosses between fifteen female parents (lines) and five male parents (testers) in line x testers mating design along with one standard checks (HSFH 848). Hybrids and parents were evaluated under four different environments *i.e.* Summer 2014, last week of August (E_1) and First week of Sept. (E_2) and during spring 2015, *i.e.* first week of February (E_3) and last week of February (E_4). Randomly five plants are selected for each genotype and replication to recorded the data of different quantitative characters viz. plant height (cm), head diameter (cm), stem diameter (cm) days to 50% flowering, days to maturity, hundred seed weight (g), seed yield per plant (g), oil content (%), hull content (%), percent seed filling, germination (%), electrical conductivity ($\mu Scm^{-1}g^{-1}$), viability (%), vigour index and fatty acids (%) in all the test environments. On the basis of Euclidean minimum distance, all the genotypes were grouped into six clusters which showed the presence of enough amount of genetic diversity in the present material. The cluster IV was having highest numbers of genotypes followed by clusters V,I,VI, II, III .The intra-cluster distances were less than that of inter cluster distances which showed that there was narrow genetic variation within the clusters while there was maximum genetic variation in between the clusters. The use of genotypes in hybridization from these results is likely to produce more heterotic combination in future.

Key words: D^2 statistics, genetic divergence, clusters, sunflower

Introduction

Sunflower is an important oilseed crop widely adopted and accepted for its high quality edible oil. Sunflower (*Helianthus annuus* L.) belongs to the genus *Helianthus* of the family Asteraceae, which includes 20 genera with 400 species. Its basic chromosome number is n = 17. In plant breeding, genetic diversity is important as hybrids between lines of diverse origin generally display a greater heterosis than those between closely related parents. D^2analysis is a statistical method for genetic divergence that provides better choice of parents in any breeding program. The D^2 statistics equip to discriminate between different cultivars according to the diversity present (Mahalanobis, 1936). It furnishes a pleasant idea about the diverse nature of population. As per the ward method (Ward, 1963) of the Euclidean method, the clusters were used to determine the distance between and within clusters.

Material and Methods

The present investigation was carried out at experimental area and laboratories of Oilseed Section, Department of Plant Breeding and Genetics, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The experimental material consisted of 15 CMS (cytoplasmic male sterile) lines used as seed parents and five restorers (R line) used as a pollen parents which were grown in paired rows and crossed in Line X Tester design to obtain 75 F_1

hybrids, and there is a commercial check hybrid HSFH 848. Each CMS and restorer lines were grown in 2 rows of 4 meter length with a spacing of 45cm x 30 cm. The evaluation of 75 hybrids and a check HSFH 848 was conducted over 4 environments during 2014 -2015.The genetic diversity existing between the genotypes with respect to the set of characters was estimated using Mahalanobis' D^2 statistic (Mahalanobis, 1936). Treating D^2 as a generalized statistical distance, the criterion used by Ward (1963) was applied for determining the group constellation. Average intra and inter-cluster distances were determined following the method of Singh and Chaudhary (1977).

Results and Discussion

The analysis of variance revealed significant variability among the genotypes for all the traits, but the extent of genetic diversity could not be explained, therefore, cluster analysis was performed to quantify the genetic divergence among all the genotypes using Mahalanobis' D^2 statistics (1936) as described by Ward (1963). The genotypes were grouped into different clusters on the basis of minimum genetic distance using Euclidean method (Ward, 1963).

Cluster analysis classified all the 96 genotypes into 6 clusters based on the relative magnitude of their D^2 values, in such a way that genotypes within each cluster had smaller D^2 value than between the clusters. Cluster pattern (Table 1) revealed that cluster IV and V had the maximum with 35 and 32 genotypes, respectively followed by cluster I (15 genotypes), cluster VI (8 genotypes), cluster II (5 genotypes) and clusters III (1 genotype). Genotypes from different sources were grouped in the same cluster thereby indicating that genetic divergence had little relationship with the geographic distance. Reddy *et al.* (2012) reported the similar result while analyzing the genetic diversity in germplasm accessions of *Helianthus annuus* L which also suggested that geographical divergence does not necessarily represent genetic diversity.

The genotypes included in the same cluster are considered genetically similar with respect to the aggregate effect of the characters examined; the hybridization attempted between these could not expect to yield desirable recombinants. Therefore, putative parents for crossing programme should belong to different clusters characterized by large inter-cluster distance. Earlier, Mohan and Seetharam (2005) also observed similar clustering pattern of genotypes among clusters, as some clusters were unique having only single genotype.

Intra and Inter cluster distance

The intra and inter cluster distances among various clusters involving ninety six genotypes are presented in table 2 and figure 2. The inter cluster distances were greater than intra cluster distance which indicates the presence of narrow genetic variation within a cluster and divergence among the different clusters. The maximum intra cluster D^2 value was observed for cluster I (5.451) followed by cluster II (5.099), V (3.800), and VI (3.711) which reveals the existence of maximum differences among the genotypes that fall in these clusters.

When diversity was studied among the clusters based on the inter-cluster D^2 values, intercluster distance ranged from 4.126 to 17. 040 (Table 2 and Figure 1). Clusters IV and V showed minimum inter-cluster distance of 4.126 indicating close relationship among the genotypes included in these clusters. Clusters III and V showed maximum inter-cluster distance of 17.040, followed by clusters III and IV (16.785), clusters I and III (16.190), clusters III and VI (15.898) and clusters II and III (14.929) which indicates that genotypes in these clusters are genetically diverse and can be used as promising parents for hybridization in a breeding programme.

Clusters mean value of different clusters

The cluster means for seed yield per plant and its component characters are presented in Table 3. The data revealed considerable differences among all the clusters for most of the characters studied.

From the table 3 (a), it was evident that genotypes present in cluster VI had higher plant height (cm) with a highest cluster mean value (136.97), while the lowest mean value (89.96) was observed for genotypes present in cluster III. Cluster VI also had highest value for head diameter (14.95 cm) and stem diameter (7.37 cm), while cluster III had lowest mean value (10.19) for head diameter and cluster I had lowest value (5.03) for stem diameter. Days to flowering was recorded lowest in cluster III (43.33), whereas higher mean value was observed in cluster I (62.05). Cluster III consist of lower mean value for days to maturity (69.42), while the highest value of days to maturity was recorded in cluster V (96.49).

Cluster VI showed the higher value (7.72) for 100 seed weight (g), while the minimum value was showed in cluster III (4.79). Cluster VI comprised of genotypes which have high cluster mean value for seed yield per plant (35.15), while lowest value was evident in cluster I (23.5). Cluster II showed higher value for oil content (48.33 %) and lowest value in cluster III (29.67). The minimum value for hull content (%) was recorded in cluster III (33.87)

and maximum in cluster V (51.69). Seed percent filling had highest value in cluster V (85.83), while lowest value in cluster III (42.72).

Maximum germination per cent was recorded in cluster I (91.20 %) and minimum mean value in cluster III (51.02). Cluster IV had lowest mean value (0.51) for electrical conductivity ($\mu Scm^{-1}g^{-1}$), while the maximum mean value was observed in cluster II (1.16).

From the table 3 (b), the maximum mean value for viability was observed in cluster I (86.05) and minimum mean value (43.64) in cluster III. Vigour index I showed highest mean value in cluster IV (2685.20) and lowest mean in cluster III (1300.48).

Cluster IV had the highest mean value (35.93) for vigour index II, while the cluster II had the lowest mean value (18.84). Palmitic acid displayed highest mean in cluster V (7.77) and lowest mean (4.87) for cluster III. Stearic acid had lowest mean value in cluster III (2.67), while the highest mean value in cluster VI (5.72). The maximum value for oleic and linoleic acid was recorded in cluster V (42.37) and cluster VI (47.12) respectively, where the minimum mean value for oleic (25.49) and linolenic (25.24) was recorded in cluster III.

The overall comparison indicated that cluster IV, V and VI, had better cluster means for characters *viz.*, 100 seed weight (g), head diameter (cm), stem diameter (cm), palmitic acid (%), stearic acid (%), vigour index II, oleic and linoleic acid (%). Therefore, cluster IV, V and VI could be considered while selecting genotypes for sunflower improvement for above traits. Neelima *et al.* (2016) reported that in their studies cluster XI had high mean value for seed yield per plant (g), Cluster X had higher mean value for plant height (cm) and head diameter (cm) and cluster XII had higher test weight (g). The maximum inter cluster distance was recorded between cluster XI and XII followed by cluster X and XII and clusters V and XII. It was suggested that if the diverse accessions from these diverse groups are used in the breeding programme, it is expected to produce a wide range of genetic variability in the population.

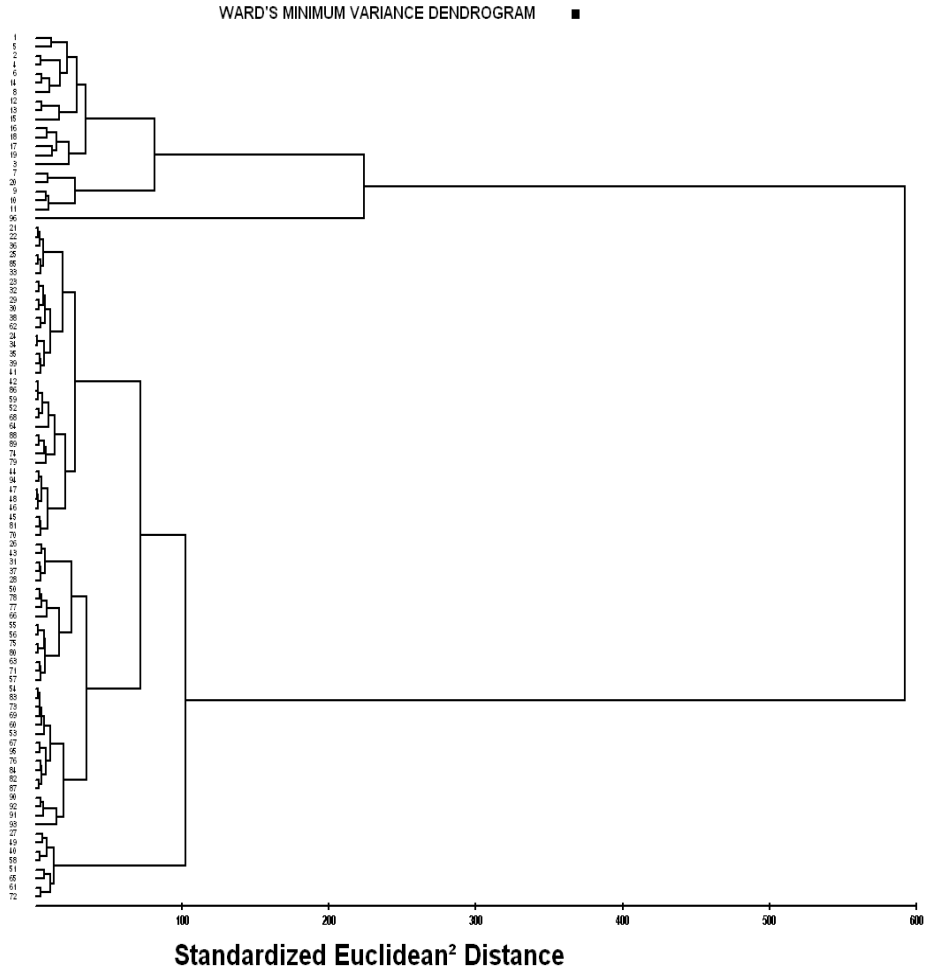

Figure 1: Ward's minimum variance dendrogram for 96 genotypes of sunflower based on standardized Euclidean's distance.

Table 1: Clustering of 96 genotypes of sunflower on the basis of Euclidean minimum distance

Clusters	No. of genotypes	Name of genotypes
Cluster I	15	CMS 11A,CMS 17A,CMS 234A,CMSH 91A,CMS 103A,CMS ARG6A,CMS 207A,CMS ARG2-A,CMS ARG 3-A,CMS DV-10,6D-1,HRHA 4-2,RHA 271,HRHA 5-3,CMS 44A.
Cluster II	5	CMS 148A, RHA 297, CMS 302A, CMS 607A, CMS 852A.
Cluster III	1	HSFH 848
Cluster IV	35	CMS11A x 6D-1,CMS 11A x RHA 271,CMS 148A x RHA 271,CMS 11A x RHA 297, CMS ARG 6A x RHA 297,CMS 44A x HRHA 5-3,CMS 11A x HRHA 4-2,CMS 44A x RHA 271,CMS 17A x HRHA 5-3,CMS 17A x RHA 297,CMS 148A x HRHA 4-2,CMS 607A x RHA 271,CMS 44A x HRHA 5-3,CMS 44A x RHA 297,CMS 148A x HRHA 5-3,CMSH 91A x 6D-1, CMSH 91A x RHA 271, CMS DV-10 x 6D-1, CMS 302A x HRHA 5-3,CMS 234A x HRHA 5-3,CMS 852A x HRHA 4-2,CMS 607A x HRHA 4-2, CMS DV-10 x HRHA 4-2,CMS DV-10 x HRHA 5-3,CMS ARG 3-A x HRHA 5-3,CMSH91 A x HRHA 5-3,CMS 207A x HRHA 5-3,CMS 103A x HRHA 5-3,CMS 103A x HRHA 4-2,CMS 103A x 6D-1, CMSH91 A x RHA 297, CMS ARG 6A x 6D-1, CMS 852A x RHA 297.
Cluster V	32	CMS 17A x 6D-1,CMSH91A x HRHA 4-2,CMS 44A x 6D-1.CMS 148A x RHA 271,CMS 17A x HRHA 4-2,CMS 103A x RHA 297,CMS ARG 3A x HRHA 4-2, CMS ARG 3A x RHA 271, CMS 852A x 6D-1, CMS 234A x RHA 297, CMS 302A x 6D-1, CMS ARG 2A x RHA 297,CMS ARG 3A x RHA 297, CMS 607A x HRHA 4-2, CMS ARG 2A x 6D-1,CMS 302A x RHA 271,CMS 234A x HRHA 5-3, CMS ARG 6A x HRHA 4-2,CMS ARG 2A x HRHA 4-2, CMS 852A x HRHA 5-3,CMS 302A x RHA 297, CMS 234A x HRHA 4-2, CMS 852A x RHA 271, CMS207A x RHA 297, CMS ARG 3A x 6D-1,CMS ARG6A x HRHA 5-3, CMS ARG 6A x RHA 271,CMS DV-10 x RHA 271, CMS DV-10 x RHA 297, CMS 207A x RHA 271,CMS 207A x 6D-1,CMS 207A x HRHA 4-2.
Cluster VI	8	CMS 17A x RHA 271, CMS 103A x HRHA 5-3, CMS 148A x RHA 297, CMS 302A x HRHA 4-2, CMS 234A x 6D-1, CMS 607A x RHA 297, CMS 607A x 6D-1, CMS ARG 2A x RHA 271.

Table 2: Average intra- (diagonal) and inter- (above diagonal) cluster D^2 values for 96 genotypes of sunflower

Clusters	I	II	III	IV	V	VI
I	**5.451**	6.781	16.190	7.694	7.443	8.204
II		**5.099**	14.929	8.591	8.288	7.096
III			**0.000**	16.785	17.040	15.898
IV				**3.444**	4.126	5.299
V					**3.800**	5.240
VI						**3.711**

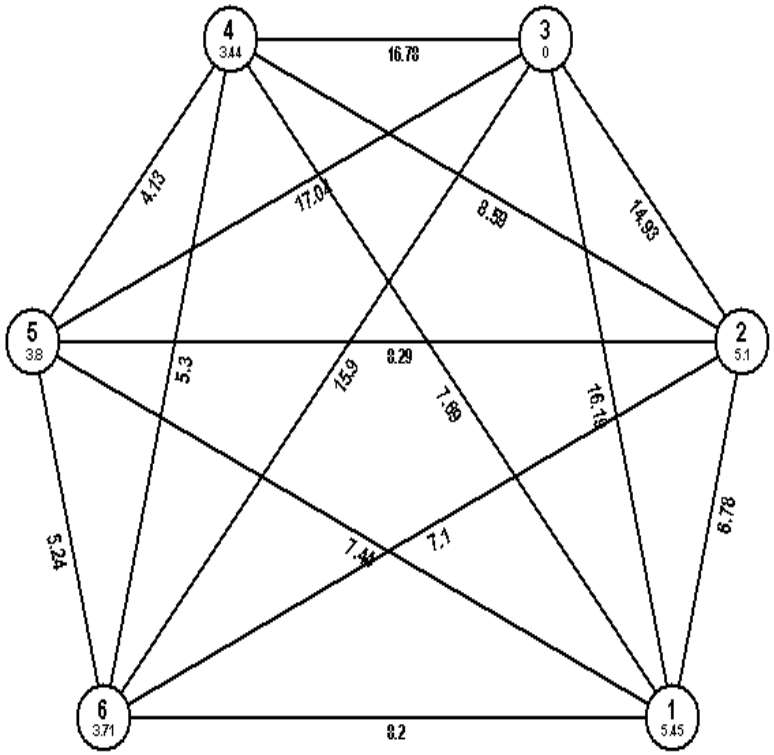

Figure 2: Clustering based on Mahalanobis D^2 analysis (Euclidian method)

Table 3a: Cluster Mean values of seed yield and its component traits in sunflower

Clusters	PH (cm)	HD (cm)	SD (cm)	DF	DM	100 SW (g)	SY (g)	OC (%)	HC (%)	% SF	GERM. (%)	EC ($\mu Scm^{-1}g^{-1}$)
I	120.50	11.55	5.03	62.05	95.53	5.24	23.5	47.17	39.42	55.82	91.20	0.69
II	123.79	10.66	5.07	59.93	94.53	6.23	27.52	48.33	42.15	57.22	67.85	1.16
III	89.96	10.19	5.07	43.33	69.42	4.79	23.68	29.67	33.87	42.72	51.02	0.68
IV	136.14	14.93	7.35	59.40	95.54	7.40	34.65	38.58	50.82	84.88	88.97	0.51
V	134.36	14.55	7.15	59.52	96.49	7.42	34.04	38.73	51.69	85.83	88.98	0.56
VI	136.97	14.95	7.37	59.85	95.26	7.72	35.15	38.48	50.95	76.96	67.74	0.94

Table 3b: Cluster Mean values of seed vigour and fatty acids in sunflower

Clusters	VIAB. (%)	VI I	VI II	PALM (%)	STER. (%)	OLEIC (%)	LINO. (%)
I	86.05	2575.52	26.45	6.83	4.46	41.40	40.77
II	61.50	2591.73	18.84	6.69	3.84	41.95	40.14
III	43.64	1300.48	27.44	4.87	2.67	25.49	25.24
IV	84.94	2685.20	35.93	6.63	4.93	38.02	52.07
V	84.83	2532.72	33.48	7.77	5.37	42.37	46.93
VI	65.08	2496.71	29.08	6.96	5.72	40.08	47.12

PH –Plant height, HD -Head diameter, SD- Stem diameter ,DF- days to flowering, DM- days to maturity,100 Seed weight, SY- Seed yield per plant, OC- Oil content, HC- Hull content, % SF- Percent seed filling, GERM.- Germination ,EC- Electrical conductivity, VIAB. – Viability, VI I -Vigour index I, VI II – Vigour index II, PALM.- Palmitic acid, STER. – Stearic acid, OLEIC- Oleic acid, LINO. - Linolenic acid.

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