

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

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SUMMARY

A study was conducted to determine the extent of genetic divergence with respect to 16 quantitative characters in 144 sunflower genotypes consisting of 66 germplasm accessions, 75 inbred lines and three checks at the University of Agricultural Sciences (UAS), Bangalore, India. Univariate and multivariate analyses of variance confirmed the presence of significant differences among the genotypes. Mahalanobis' D^2 statistics indicated the presence of substantial genetic diversity. Higher D^2 values were observed among the inbred lines than in the germplasm accessions. Clustering of the genotypes resulted in the formation of seven and 14 clusters in the germplasm accessions and inbred lines, respectively. Some clusters were unique having only a single genotype, while clusters with up to 55 genotypes were also formed. Factors other than geographic origin appeared to be a potent source of genetic diversity. The inter-cluster distance showed that clusters II and V among the germplasm accessions and clusters XI and XIII among the inbred lines were most divergent. Clusters were also demarcated with respect to characters they excel and/or for which they were inferior.

Key words: D^2 , genetic divergence, germplasm, inbred lines, multivariate analyses, sunflower (*Helianthus annuus*)

INTRODUCTION

In a situation where a number of high yielding varieties are continuously being released, the task of breeding varieties better than the existing ones, in terms of quantity and quality, requires much effort. In the quest to develop genotypes with desirable attributes, the breeder would like to choose genetically distant parents for hybridization because it has been previously established in different crop species that the larger the divergence between genotypes, the higher the heterosis as genetic

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diversity between populations/genotypes indicates differences in gene frequencies. Several measures of genetic distances have been proposed. Mahalanobis' generalized distance (D^2) is most widely used in plant breeding (1936). It has been considered by several authors (Murthy and Arunachalam, 1966; Bhatt, 1973; Joshi and Singh, 1979) as a powerful tool for estimating genetic diversity and for selecting diverse parents for hybridization.

Mahalanobis' D^2 considers the variation produced by each character and the consequent effect that it bears on other characters and resolves genetic divergence at inter-varietal and sub-species level in classifying crop plants (Rao, 1960). This is possible by clustering the entries based on D^2 values, since it represents the index of genetic diversity among genotypes and clusters.

Although this technique has been used frequently in many crop species, few reports are available in the literature regarding its application in sunflower. In addition, the few genetic studies carried out so far involve only a limited number of genotypes with narrow geographic diversity. Therefore, this study was initiated using genotypes introduced from 24 countries to determine the genetic divergence of 16 quantitative characters.

MATERIALS AND METHODS

The materials for the present investigation consisted of 144 sunflower genotypes of which 66 were germplasm accessions, 75 inbred lines (maintainer and restorer lines and non-converted inbred lines) and three checks introduced from 24 countries (Tables 1 and 2).

Table 1: Cluster number with their respective accessions number and source for the 66 germplasm accessions and three checks

Cluster number	Number of genotypes	Accession number/ name and their origin (source)
I	55	Acc. no 222 (Argentina); *391, 391, 21 (Australia); 410, 405 (Bulgaria); 450, Morden (Canada); 135 (Colombia); 194 (Denmark); 848 (Egypt); 44 (France); 471 (Germany); 1266, 600, 82 (Hungary); 1039, 689, MSFH-17 (India); 1273 (Iran); 690 (Israel); 244, 456, 158, 525, 715 (Italy); 212 (Poland); 127 (Romania); 133 (Switzerland); 1185, 1187, 1184, 1178, 1211, 257 (Turkey); 338, 367, 347, 358, 337 (UK); 1135 (Uruguay); 786, 1483, 873, 1381 (USA); 109, 35, 400, 42, 99, 15, 226, 128, 1876, 901 (USSR); 1288 (Zimbabwe)
II	4	Acc. no 222 1175 (Turkey); 1172, 88, EC 68414 (USSR)
III	5	Acc. no 222 1143, 1263 (Turkey); 918 (Bulgaria); 1279 (Ethiopia); 1630 (Kenya)
IV	2	Acc. no 222 1156 (Turkey); 356 (USSR)
V	1	Acc. no 222 1647 (Argentina)
VI	1	Acc. no 222 220 (Poland)
VII	1	Acc. no 222 1147 (Turkey)

* Numbers refer to Accession number (Acc. no) unless stated.

The experiment was conducted at experimental plots of the University of Agricultural Sciences, GKVK, Bangalore, India, during the rainy season of 1997 in a 12 x 12 simple lattice design. The genotypes were raised in two-row plots of 3 m length and 60 and 30 cm inter- and intra-row spacing, respectively. All agronomic practices recommended for the region were followed to raise a good crop.

Table 2: Cluster number with their respective accessions number and source for the 75 inbred lines and three checks

Cluster number	Number of genotypes	Name of inbred line/code and origine (source)
I	35	RHA 273, RHA 345, RHA 586, RHA 265, RHA 856, RHA 298, RHA 346, RHA 859, RHA 272, RHA 297, RHA 587, RHA 801, RHA 278, RHA 354, RHA P-356, RHA 344 and 300 B, 352 B, 853 B, 852 B (USA); RHA RR-1, RHA MR-1, RHA 118, RHA 133, RHA IB, MOR R-127, IB-19-1R (India) and HAM-162, HAM-183, HAM-165, HAM-182, HAM-6R (India); RHA 83-R6 (France); 234 B (Australia); 589 B, 608 B (Canada)
II	16	MOR-144 (India), HAM-187 (India), ARM-36-3-4-2-6-6, ARM-36-3-2-8-4-4, ARM-36-3-2-8-4-5, ARM-36-3-5-2-8-4, ARM-36-3-1-5-7-11, ARM-36-3-2-3-9, ARM-36-3-5-2-8-11, ARM-36-3-1-5-7-15, ARM-BC-11-19-2-1 (India); 597 B (Canada); 336 B, 343 B, 207 B (USA)
III	5	349 B (USA); HAM-168, HAM-177, HAM-195, HAM-196 (India)
IV	4	RHA-6-D-1 (India); 89 B, 62 B, 335 B (USA)
V	3	IB-28, IB-4, MSFH-17 (India)
VI	3	RHA-274, RHA-17, 851-B (USA)
VII	2	RHA-PZ-8R (France); 302 B (USA)
VIII	2	350 B, 351 B (USA)
IX	2	338(C) B (Canada); No IV-55-NB (India)
X	2	RHA-334 (USA); EC 68414 (USSR)
XI	1	339 B (USA)
XII	1	369 B (USA)
XIII	1	Morden (Canada)
XIV	1	HS-6-1-3-1-7 (India)

From each genotype, five plants were randomly selected and covered with cloth bags on the same day the first ray florets opened and remained covered until harvest to observe percent seed set, which was used later to calculate percent autogamy. All other characters were recorded from other five plants left uncovered for open pollination. The characters considered in the study were days to 50 percent flowering, number of leaves, leaf area, days to maturity, plant height, stem girth, head diameter, seed yield per plant, 100 seed weight, number of filled seeds, seed filling percent, autogamy percent, harvest index, grain filling period, oil content and oil yield per plant. Seed set and percent autogamy were calculated using the formula given by George and Shein (1980). Leaf area determination was carried out according to Nanja Reddy *et al.* (1994), which was a non-destructive and rapid estimation method. Oil content, expressed as percent, was determined with a nuclear magnetic resonance (NMR) spectrometer.

Mean values of each character from the five sample plants in each replication were analyzed using MSTATC and SPAR1 programs. Statistical analysis was carried out for the germplasm accessions and inbred lines (including three checks in each case) separately. Univariate analysis of variance following Cochran and Cox (1957) and simultaneous test of significance of differences between genotypes using Wilks' lambda criterion (Wilks, 1932) were performed. The genetic diversity existing between genotypes with respect to a set of the 16 characters was estimated using Mahalanobis' D^2 statistics (Mahalanobis, 1936). Treating D^2 as a generalized statistical distance, the criterion used by Tocher (Rao, 1952) was applied for determining the group constellation. The character-wise rank totals have been used to calculate the percent contribution of each character to the total divergence. Average intra- and inter-cluster distances were determined following the method described by Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

Univariate analysis of variance for each of the 16 characters studied showed highly significant differences among the genotypes ($P < 0.001$). The simultaneous testing of significance of difference in mean value between genotypes based on Wilks' lambda criterion revealed highly significant differences ($\chi^2 = 2202.298$ with 1088 df for the germplasm accessions and $\chi^2 = 2997.851$ with 1232 df for the inbred lines) among the genotypes for the aggregate of the 16 characters considered (ANOVA and MANOVA not presented).

The percent contribution of each character to total divergence varied between 4.36 to 8.03 and 4.11 to 7.78 (for days to 50 percent flowering and duration of grain filling) in the germplasm accession and inbred lines, respectively. Relatively higher contribution was recorded for duration of grain filling, head diameter, days to maturity, stem diameter, and number of seeds per plant. In sunflower, Anand and Chandra (1980) reported that days to 50 percent flowering was an important character contributing to total genetic divergence, whereas Sankarapandian *et al.* (1996) considered seed yield as the most important trait.

Grouping the genotypes into clusters resulted in the formation of seven and 14 clusters for the germplasm accessions and inbred lines, respectively. As indicated in Table 1, it is interesting to note that out of 69 germplasm accessions introduced from 24 countries, 55 accessions representing 22 countries were grouped in cluster I. Genotypes from North and South America were found to belong to the same group with genotypes from Europe, Africa, Asia and Australia. This trend was repeated in four of the seven clusters. The situation was the same with the inbred lines (Table 2). This indicates an absence of relationships between genetic diversity and geographic diversity. Similarly, Anand and Chandra (1980), Yadava *et al.* (1988), Haile (1994) and Sankarapandian *et al.* (1996) observed no relationship between geographic and genetic diversity in sunflower. It is likely that the genetic

material from different countries has a common gene pool with respect to some economic traits such as oil content, seed yield and other attributes. Therefore, based on the genetic distance (D^2) few genotypes that represent genetic diversity could be selected to form a core collection.

Murthy and Arunachalam (1966) stated that genetic drift, selection pressure and environment could cause greater diversity than geographic origin. The results of this study support their finding. It can be seen from the clustering that genotypes introduced from the same country belonged to different clusters. For example, genotypes introduced from Turkey were grouped in 5 of the 7 clusters for the germplasm accessions, whereas genotypes from USA were grouped in 10 of the 14 clusters for the inbred lines. This indicates that factors other than geographic origin are responsible for the observed genetic diversity. The impact of selection pressure in increasing genetic diversity was demonstrated in grouping the inbred lines of the same parentage but representing different selections. This was well illustrated by grouping the four inbred lines developed from variety Morden in two clusters, while their common parent formed a solitary cluster (XIII). A similar situation was observed for the lines developed by crossing *Helianthus argophyllus* and *Helianthus annuus* variety Morden (HAM) which were distributed into three cluster (clusters I, II and III).

Clustering pattern based on D^2 statistics grouped most of the restorer (R) lines into one cluster. Nineteen restorer lines out of 24 included in the study were clustered in Cluster I. This shows the lack of genetic variability within the restorer lines. The other five restorer lines including RHA-6-D-1 and RHA-274 were grouped into four different clusters. The clustering pattern confirmed more divergence in the maintainer (B) lines. They were dispersed in 10 of the 14 clusters, although more than one maintainer occurred in the same cluster.

Restorer and maintainer lines were observed to be sorted in the same cluster. This was observed in four out of the five clusters where the restorer lines were found. This suggests that these inbred lines have similar genetic background except they are treated as sterile and maintainer lines. Crossing such parents to realize heterosis will, therefore, be a futile exercise.

The formation of seven clusters in the case of the germplasm accessions as compared with 14 for the inbred lines coupled with higher inter-cluster D^2 value suggested the presence of greater diversity in the later. Entries included in the former could share common genetic background, although they are introduced from different countries. The release of genetic variability tied up in the heterozygote form during inbreeding and/or the efficiency of selecting divergent lines by the breeder to realize maximum heterosis could be the other reasons to justify the greater genetic diversity observed in the inbred lines.

Three and four solitary-entry clusters were formed with the germplasm accessions and inbred lines, respectively. Earlier studies associated such phenomenon with geographic barriers preventing gene flow or intensive natural and human selec-

tion for adaptive gene complexes (Murthy and Arunachalam, 1966; Bhatt, 1973; Joshi and Singh, 1979). The later two reasons seem more appropriate in this study. Morden as a population formed a solitary entry when it was grouped with inbred lines but not with germplasm accessions. The inbred lines that go through rigorous selection as compared with the germplasm accession remained distant to Morden. The impact of human selection in genetic divergence can further be substantiated by the formation of cluster XIV as a single-entry cluster (Table 2). HS-6-1-3-1-7, the sole inbred line placed under cluster XIV was developed by Project Coordinating (PC) Unit, Bangalore and was selected for its high oil content (42.0%) and high number of seeds which made it distinct from other inbred lines.

Table 3: Intra- (bold) and inter-cluster D^2 values for seven clusters formed by 66 germplasm accessions and three checks

Cluster	I	II	III	IV	V	VI	VII
I	62.16	155.30	104.10	176.08	190.27	121.03	243.08
II		74.00	247.75	328.00	476.14	255.12	471.74
III			79.38	256.10	166.60	115.43	327.99
IV				65.35	258.99	261.72	351.02
V					0.00	194.14	245.84
VI						0.00	378.58
VII							0.00

Among the seven clusters formed in the germplasm accessions, cluster III showed the maximum intra-cluster D^2 value of 79.38 followed by cluster II with 74.00. Clusters I and IV had intra-cluster D^2 values of 62.16 and 65.35, respectively. Since they contained solitary entries, clusters V, VI and VII had a D^2 value of zero. The inter-cluster D^2 values varied from 204.10 (between clusters I and III) to 476.14 (between clusters II and V) (Table 3). In the case of the inbred lines, cluster X containing only two entries showed the highest intra-cluster D^2 value (83.59) followed by cluster IX with 83.05. The inter-cluster D^2 values varied from 204.10 (between clusters I and III) to 476.14 (between clusters II and V) (Table 4).

Genotypes grouped into the same cluster presumably diverge little from one another as the aggregate characters are measured. In this context, as the inter-cluster distance was high between clusters II and V followed by II and VII in the case of the germplasm accessions and between clusters XI and XIII followed by XI and IX in case of inbred lines, genotypes from these clusters could be selected for a hybridization program as they are expected to produce highly heterotic crosses.

The value of genetic distance to predict heterosis can be discussed with respect to the clustering pattern of the parents of hybrids BSH-1 and KBSH-1. These are hybrids released by UAS, Bangalore. KBSH-1 is a superior and more recent release than BSH-1. Both of them have the same female parent, 234 B. But, BSH-1 has RHA-274 as its male parent, while KBSH-1 has RHA-6-D-1. The average genetic distance between the clusters that contain the female and male parents of KBSH-1 has been found to be greater (154.81, between clusters I and IV) than that of BSH-1

Table 5: Mean value of 16 quantitative character for seven cluster for the germplasm accessions and inbred lines

Cluster No.	DF	NL	LA	DM	PH	SG	HD	SY	HSW	NFS	SFP	HI	AP	GFP	OC	OY
Germplasm																
I	55.5	26.3	7591.5	87.6	158.5	2.1	14.7	41.3	5.3	728.9	77.7	38.5	18.37	32.1	32.9	3.7
II	55.9	23.5	6828.1	86.1	147.9	1.9	13.1	28.3	4.5	597.1	78.6	40.0	34.14	30.3	32.0	8.7
III	57.0	27.5	10003.7	89.4	171.8	2.4	16.0	58.6	5.8	874.0	74.3	38.8	3.95	32.4	27.4	15.7
IV	61.8	31.0	7403.0	93.8	171.8	2.2	14.5	29.1	4.7	647.3	81.5	33.1	51.16	32.0	28.1	8.5
V	63.0	29.9	11731.5	111.0	222.9	2.3	16.0	60.4	7.4	791.5	72.6	44.2	44.75	48.0	29.3	17.8
VI	60.0	26.2	90.85.5	94.0	172.1	2.5	17.0	62.2	4.6	989.5	79.3	32.8	59.42	29.0	34.1	21.2
VII	56.0	26.8	7772.0	84.0	142.7	2.0	12.6	36.3	5.6	647.0	69.0	40.1	64.35	28.0	24.8	9.0
Inbred lines																
I	55.4	21.9	4224.8	85.3	114.3	1.6	10.0	11.8	3.0	384.6	73.9	28.4	63.71	29.9	32.8	3.8
II	62.7	29.2	7186.2	93.5	161.4	1.9	12.3	22.1	3.9	539.7	78.3	27.4	72.48	30.8	35.6	8.0
III	54.0	21.8	6882.6	85.7	122.7	2.1	14.3	28.1	4.2	641.2	74.7	34.1	38.21	31.7	30.3	8.8
IV	67.9	24.9	4665.9	93.9	122.0	1.8	9.1	6.0	2.1	239.1	63.3	17.4	68.51	26.0	33.7	1.9
V	59.3	29.3	7709.5	90.0	168.8	2.1	14.2	48.3	4.9	784.2	80.6	38.5	73.82	30.7	34.9	16.8
VI	55.8	28.3	4342.2	86.8	133.9	1.6	9.9	18.1	2.9	622.3	81.1	35.4	52.37	31.0	34.7	6.3
VII	52.3	24.9	5872.0	85.8	140.7	1.8	11.3	22.0	5.0	426.8	78.7	33.7	0.0	33.5	35.2	7.8
VIII	55.3	22.8	6198.5	84.8	119.9	2.1	13.8	8.9	6.1	129.3	37.0	21.9	54.37	29.5	24.0	2.1
IX	45.5	17.1	2839.8	76.3	95.0	1.6	11.7	20.0	4.4	402.8	74.7	29.9	56.07	30.8	34.0	6.8
X	60.3	26.2	10078.5	94.5	167.5	2.1	14.7	26.4	4.4	573.5	77.7	29.7	44.98	33.5	33.1	8.9
XI	72.5	36.0	8550.0	99.0	168.8	2.0	10.3	15.4	2.5	619.5	78.1	37.8	72.13	26.5	31.8	4.9
XII	58.5	28.3	10183.5	86.0	116.7	1.8	13.7	22.9	4.3	530.5	72.5	35.7	30.27	29.5	29.6	6.7
XIII	46.5	19.1	5888.5	80.0	97.0	1.8	14.1	33.0	4.9	690.5	83.5	46.8	0.31	33.5	38.0	12.5
XIV	61.0	23.7	6754.0	90.0	153.9	2.1	12.4	23.6	2.9	864.0	84.8	27.6	51.18	29.0	42.4	10.0

DF = Days to flowering; NL = Number of leaves; LA = Leaf area; DM = Days to maturity; PH = Plant height

(105.13, between clusters I and VI) (Table 4). Here genetic distance and heterosis are positively correlated. But this does not always hold true. For instance, the cluster which contained RHA-6-D-1 is more distant from the cluster which contain 338(C) B (with a D^2 value of 390.46) but failed to produce a superior heterotic hybrid than KBSH-1 which was obtained by crossing 234-B and RHA-6-D-1 with a D^2 value of 154.81. The same is true for RHA-274. Clusters that contain RHA-274 and 338(C) B were more divergent ($D^2=228.54$) than clusters containing RHA-274 and 234 B (Table 4). This indicates that there exists an optimum level of parental divergence for the occurrence of superior heterosis. Arunachalam (1981) indicated that too high a divergence may not produce the highest frequency of heterotic crosses. According to Falconer (1981), heterosis is a direct function of the square of gene frequency difference between parental population and directional dominance. In spite of the presence of sufficient genetic divergence, thus, internal cancellation of dominance effect at various loci is expected to reduce the heterosis that could be realized.

Among the germplasm accessions, cluster VI containing the single entry Acc. No 220 (introduced from Poland) had the highest seed and oil yields, bearing the highest number of seeds, largest head, thickest stem and highest oil content, but had the lowest harvest index. Cluster VII containing Acc. No 1147 from Turkey manifested the highest autogamy coupled with early maturity and dwarf stature. However, it had a smaller head diameter, short duration of grain filling, low oil content and low seed filling percent. The single-entry cluster V, with the genotype Acc. No 1647 from Argentina, had the second highest seed yield with highest 100 seed weight but, was latest in flowering and maturity. Other clusters had medium to low mean values (Table 5).

In the case of the inbred lines, cluster XIV (containing HS-6-1-3-1-7) for its highest oil content, number of filled seed and stem girth; genotypes of cluster V for their superb autogamy percent, seed and oil yields and stem diameter could be earmarked as potential clusters for improving the respective characters. Moreover, cluster XII showed the highest leaf area while Morden forming cluster XII independently had the highest harvest index.

It is worthy to note that in calculating cluster mean the superiority of a particular genotype with respect to a given character could get diluted by other genotypes that are grouped in the same cluster but are inferior or intermediate for the character in question. Hence, apart from selecting genotypes from the clusters, which have higher inter-cluster distance for hybridization, one can also think of selecting parents based on the extent of divergence with respect to a character of interest.

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ESTUDIO SOBRE LA DIVERGENCIA GENÉTICA DEL GIRASOL (*Helianthus annuus* L.)

RESUMEN

El estudio ha sido emprendido para determinar la amplitud de la divergencia genética de dieciséis características cuantitativas de 144 genotipos de girasol, que incluyeron 66 números del banco de plasma germinal, 75 líneas inbred y tres controles. La investigación ha sido hecha en la Universidad de Ciencia Agrícolas (UAS) en Bangalore, India. Los análisis de la variancia con una y muchas variaciones confirmaron la presencia de diferencias considerables entre los genotipos investigados. La estadística D^2 de Mahalanobis ha indicado la presencia de la divergencia genética considerable. Los valores D^2 eran más altos en las líneas inbred que en los números del banco de plasma germinal. Los números del banco de plasma germinal han formado siete grupos, las líneas inbred 14 grupos. Algunos grupos incluyeron solo un representante, mientras los otros incluyeron hasta 55 genotipos cada uno. Además del origen geográfico, también otros factores eran las fuentes importantes de la

divergencia genética. Las distancias dentro de los grupos mostraron que la divergencia existía en los grupos II y V según los números del banco de plasma germinal y en los grupos XI y XII en las líneas inbred. Los grupos se diferenciaban también por las características a las cuales eran superiores y las características a las cuales eran inferiores.

ÉTUDE DE LA DIVERGENCE GÉNÉTIQUE DANS LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Cette étude a été faite dans le but d'établir l'étendue de la divergence génétique pour ce qui concerne 16 caractéristiques quantitatives de 144 génotypes de tournesol consistant en 66 nombres de la banque de germesplasmés, 75 lignes inbred et trois contrôles. L'étude a été effectuée à l'université de sciences agricoles (UA), Bangalore, Inde. Des analyses univariées et multivariées des variances ont confirmé la présence de différences significatives entre les génotypes observés. Les statistiques de Mahalanobis D^2 ont montré la présence de divergences génétiques importantes. Les valeurs D^2 dans les nombres de la banque de germesplasmés. Les nombres de la banque de germesplasmés formaient sept groupes, les lignes inbred 14 groupes. Quelques groupes comprenaient un seul représentant, alors que d'autres comprenaient jusqu'à 55 génotypes. D'autres facteurs, en plus des facteurs géographiques, se sont montrés d'importantes sources de divergence génétique. Les intervalles à l'intérieur des groupes ont montré que la divergence existe dans les groupes II et V dans les nombres de la banque de germesplasmés et dans les groupes XI et XIII dans les lignes inbred. Les groupes se différenciaient aussi par les caractéristiques où ils étaient excellents ou inférieurs.

