

DIVERGENCE AND ASSOCIATION STUDIES IN SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

In the present study we report the genetic diversity among 18 sunflower inbred lines involving alloplasmic *cms* lines, conventional *cms* and restorer lines (*petiolaris* source) using twenty traits. Efforts were also made to correlate various morphological and physiological traits with seed yield and oil content. The study was performed at Oil Seed Section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, India. The analysis of variance recorded presence of significant variability among the gamplasm lines. Mahalanobis D^2 statistic indicated presence of substantial genetic diversity and genotypes were grouped into five clusters. ARG-3A and *cms*-XA were designated as the most diverse *cms* sources. Grain yield an important character showed highly significant positive correlation with days to 50% flowering, days to maturity, plant height, chlorophyll content, oil content, and biological yield at both genotypic and phenotypic levels. Path coefficient analysis revealed direct positive effect of no. of leaves per plant, 100 seed weight, chlorophyll content, leaf area, leaf area index, oil content, biological yield and harvest index on grain yield. Plant height, seed size, number of leaves per plant, chlorophyll content, leaf traits, and oil content were identified as important traits for selection criteria to improve seed yield in sunflower. It was also observed that different cytoplasmic sources used in the present study did not show any deviations from the previously established correlations between important morphophysiological and seed yield traits and can be exploited in heterosis breeding programme.

Key words: sunflower, alloplasmic *cms* lines, correlation, path coefficient, D^2 analysis

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oilseed crop widely adopted and accepted for its high quality edible oil. Seed yield an important economic character in most of the crops is a complex trait and its inheritance depends upon a

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number of traits which are often polygenic in nature and highly affected by environmental factors (Nadarajan and Gunasekaran, 2005). Studies on genetic divergence are important to guide breeding programs aiming to obtain hybrid cultivars, so that crosses are made among genetically divergent lines that have contrasting and complementary traits (Luciene *et al.*, 2010). The central component of sunflower hybrid development is cytoplasmic male sterility (*cms*). Single source of cytoplasm PET-1 has been exploited extensively for hybrid production so far (Gauri Shankar *et al.*, 2006). There is a need to diversify the cytoplasmic base to safeguard sunflower crop from future biotic and abiotic threats, if the cytoplasm becomes susceptible. Recently, several *cms* backgrounds have been developed by interspecific and intraspecific crosses which resulted in more than 70 *cms* sources (Series, 2002). Since these *cms* sources were identified, several experiments to estimate the influence of the cytoplasmic effect on important agronomic traits have been developed before their introgression into commercial breeding programmes. At PAU we developed a set of ten alloplasmic *cms* lines from different sources using NC41B as common maintainer for all these sources through backcross method. Now we were interested to study the performance of these sources with respect to morphophysiological, yield and quality traits and level of diversity between these sources as well as from our previously developed *cms* and restorer lines (based on conventional *Petiolaris* source) in order to identify diverse genotypes for use in hybrid breeding programme. Information on nature and magnitude of variability present in a population due to genetic and non genetic components is also an important prerequisite for systematic breeding programme. Correlation coefficient analysis measures the mutual relationship among various plant traits and determines the component traits on which selection can be based for improvement in yield. Similarly path coefficient analysis is a powerful statistical technique which provides means to quantify the interrelationship of different yield components and also indicates whether the influence is directly reflected in seed yield or takes some other pathway for ultimate effects, so that the contribution of each contributory variable to the resultant variable can be estimated (Llahi *et al.*, 2009). Keeping all these points into consideration the present study was planned to evaluate the available sources for different traits, to study diversity among these sources and determine the association between various morpho-physiological and yield traits, to ensure a better understanding of the *cms* lines, maintainer and restorers which may help to develop better performing hybrids.

MATERIALS AND METHODS

Plant materials

The study involved germplasm lines involving nine alloplasmic *cms* lines from different cytoplasmic background viz: *cms*-XA, E002-91, PKU-2 (*H. annuus*), ARG-2, ARG-3, ARG-6A (*H. argophyllus*), DV-10A (*H. debilis* spp. *vestitus*), PHIR-27A

(*H. praecox* spp. *hirtus*) and PRUN-29A (*H. praecox* spp. *runyonic*) developed at PAU Ludhiana having a common maintainer line (NC-41B), along with four *cms* lines (40A, 42A, 234A and 38A), four restorers (P69R, P124R, P100R and RCR8297) from commercial *Petiolearis* source. The experiment was conducted during spring season 2011 and 2012 in randomized block design with three replications in a plot size of 0.6×3.0 m in the experimental area of Oilseed section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India.

Data recording

The data for morphological, seed yield, physiological and quality traits were recorded as per the procedure described below:

i) Morphological and yield traits

Days to 50% flowering was recorded from the date of sowing till when approximately 50% of the flower buds per plot opened its flowers in each replication. Days to maturity were counted from sowing to full maturity when the backside of the heads turned brown. Number of leaves per plant was determined by counting number of leaves in five randomly selected plants in each replication at the time of complete flowering. Plant height was measured in centimeters from ground level to the attachment of head from five randomly selected plants per plot at the time of physiological maturity. Head diameter was measured in centimeters from one end of head to other at maturity from five randomly chosen plants in each replication. In order to obtain the precision, diameter of each head was measured from two cross sections and their arithmetic means was worked out. Hundred seed weight was recorded from 100 seeds counted from random sample of open pollinated seeds from each genotype in each replication. Biological yield was recorded as weight of total biomass of five random plants in each replication from each genotype. Grain yield per plant was recorded from five open pollinated plants in each replication and then average was calculated.

ii) Physiological traits

Leaf area per unit dry weight method was used to measure leaf area. For this leaf area of one plant from one genotype was calculated using graph sheet. The total leaves from the same plant were then dried in the oven and their dry weight was recorded to calculate area per unit of weight (1 g dry wt. = 0.021 m²). Dry weight of all the genotypes were recorded and multiplied with this common factor *i.e.*, 0.021 to obtain leaf area for all the genotypes.

Leaf area index (LAI) defined as leaf area per unit soil area (m²) was calculated as follows (Watson, 1958).

$$\text{LAI} = (A \times N) / 10,000$$

Where A is leaf area (cm²) and N is number of plants per m².

Specific leaf weight (SLW) was calculated using the following formula

$$\text{Specific leaf weight (g)} = \frac{\text{Dry matter of total leaves per plant (g)}}{\text{Total number of leaves per plant}}$$

Leaf water potential (Mega-Pascal's - (Mpa)) was recorded by leaf water potential meter (Water Potential System – WESCOR – 4357526011) on five plants in each replication for all genotypes.

Relative leaf water content (RLWC): for this 100 mg leaf discs (fresh weight), from each genotype, were submerged in distilled water in test tubes till saturation. After 6 h the leaf discs were removed from test tubes. Surface water of the discs was blotted off without putting any pressure and then they were weighed to obtain saturated weight. Then after drying the discs at 70°C for 72 h their dry weight was determined. From these data RLWC was calculated (Weatherly, 1950) as follows:

$$\text{RLWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}} \times 100$$

Chlorophyll content was recorded using SPAD in five intact plants (third-fourth leaf from top of plant) for all genotypes in each replication.

Quality traits

Oil content: To determine oil percent in seeds, the wide line nuclear magnetic resonance (NMR) instrument Newport Analyzer MK 111 A was used. The NMR was standardized by the use of 4 g seed of known oil content. The clean seed samples were first dried for 3 h in an oven at 11°C. A representative sample (2 g) was used for estimating oil content. The instrument was operated keeping the following calibrations.

- a) Gate width = 1.5 gauss
- b) Integration time = 32 sec
- c) R.F. level = 100

$$\text{Oil content (\%)} = \text{NMR reading of 2 g seed} \times 2$$

Fatty acid composition: Gas Liquid Chromatography (GLC) was used for fatty acid estimation. Fatty acids were first converted to their ethyl esters by standard method of transesterification developed at the Liquid Chemical Laboratory, Svalof, Sweden.

The mean data recorded for all the traits was subjected to analysis of variance. D² analysis was performed according to Mahalanobis (1936). Correlations were worked out and path coefficient analysis was done.

Table 1.: Mean and range for morphophysiological, biochemical traits and yield.

S.N.	Genotype	Days to 50% flowering	Days to maturity	No. of leaves/plant	100 Seed wt. (g)	Plant height (cm)	Head diameter (cm)	Chlorophyll cont. (SPAD Reading)	Relative leaf water cont. (%)	Leaf dry wt. (g)	Leaf area (m ²)	leaf area Index	Specific leaf wt./plant (g)	Oil cont. (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Grain Yield/plant (g)	Biological Yield/plant (g)	H. I (%)
1	CMS-XA	68.00	95.33	22.67	3.60	82.00	17.50	37.44	74.10	115.55	2.43	13.35	5.11	26.00	6.10	4.00	51.40	36.10	0.80	13.67	335.33	4.06
2	E002-91	69.00	98.00	18.00	4.90	88.33	23.83	37.07	64.68	66.74	1.40	7.71	3.71	27.22	5.50	1.00	58.00	32.80	0.70	16.33	648.33	2.52
3	PKU2-A	71.33	94.00	16.33	4.33	60.67	20.33	35.85	88.26	28.57	0.60	3.30	1.75	29.63	5.70	3.40	51.90	36.10	0.80	18.00	291.33	6.17
4	ARG-2	72.33	93.33	18.67	4.00	70.00	15.17	37.15	69.87	22.35	0.47	2.58	1.20	28.40	5.40	4.90	52.00	34.60	0.70	35.33	630.00	5.60
5	ARG-3	68.67	97.00	22.33	4.87	110.00	24.17	40.41	70.32	58.79	1.24	6.79	2.63	32.00	5.10	3.80	56.60	32.80	0.90	42.67	840.00	5.08
6	ARG-6A	68.00	92.33	20.67	4.40	66.67	18.00	37.81	75.65	22.57	0.47	2.61	1.10	27.50	6.20	5.60	51.20	36.80	0.60	24.00	335.33	7.13
7	DV-10A	79.00	100.67	21.00	3.73	87.33	15.33	40.22	61.84	49.57	1.04	5.73	2.37	33.60	5.60	4.00	55.70	29.00	1.00	34.47	581.67	5.92
8	PHIR-27A	75.67	99.00	17.00	3.10	63.33	16.33	41.14	56.83	17.00	0.36	1.96	1.00	25.20	6.90	4.40	40.80	45.30	1.00	29.50	226.67	13.02
9	PRUN-29A	69.00	100.33	29.00	5.83	122.00	23.33	36.46	63.65	38.58	0.81	4.46	1.33	35.40	5.60	2.80	57.60	31.70	1.00	20.33	681.67	2.98
10	NC-41B	71.00	96.67	17.33	4.50	51.67	9.17	32.26	78.52	14.77	0.60	1.71	0.82	24.00	5.50	4.40	47.70	39.90	0.90	12.00	53.33	22.33
11	40A	69.00	92.67	21.33	4.13	100.00	12.00	37.44	51.05	28.38	0.61	3.28	1.33	29.60	6.40	3.80	41.60	46.40	1.10	27.53	98.00	28.11
12	42A	69.00	94.67	31.67	6.57	138.33	13.67	32.48	56.34	29.18	0.79	3.37	0.92	32.00	6.60	3.10	50.70	37.00	0.70	30.00	94.00	32.01
13	234A	70.33	101.00	30.00	4.50	145.00	11.67	39.74	62.94	37.83	0.84	4.37	1.26	25.20	7.60	5.80	53.00	30.00	1.00	50.07	543.33	9.21
14	38A	71.00	96.67	25.67	6.43	116.33	11.50	37.48	66.63	39.74	0.56	4.59	1.55	33.60	5.80	2.50	53.40	35.20	1.50	38.53	546.67	7.04
15	P69R	78.33	93.33	26.67	6.00	65.00	11.00	32.85	64.59	26.62	0.61	3.08	1.00	24.00	6.70	4.00	50.80	36.20	1.20	18.20	131.33	14.14
16	P124R	73.00	94.33	22.67	4.90	78.33	10.83	34.07	59.07	29.00	0.31	3.35	1.28	26.00	6.60	3.80	41.10	46.10	1.00	13.25	293.00	4.53
17	P100R	71.00	96.33	24.00	5.50	104.33	11.00	33.14	68.87	14.89	0.27	1.72	0.62	32.02	5.20	3.60	60.80	28.70	1.50	21.13	284.33	7.44
18	RCR-8297	69.00	92.00	28.67	4.20	116.67	8.67	37.15	62.04	13.03	0.31	1.51	0.45	28.00	6.90	4.40	37.50	48.10	1.10	15.00	152.33	9.86
	Range	68.00	92.00	16.33	3.10	51.67	8.67	32.26	51.05	13.03	0.27	1.51	0.45	24.00	5.10	1.00	37.50	28.70	0.60	12.00	53.33	2.52
	Max.	79.00	101.00	31.67	6.57	145.00	24.17	41.14	88.26	115.55	2.43	13.35	5.11	35.40	7.60	5.80	60.80	48.10	1.50	50.07	840.00	32.01
	Mean	71.26	95.98	22.98	4.75	92.56	15.19	36.68	66.40	36.29	0.76	4.19	1.64	28.85	6.08	3.85	50.66	36.82	0.97	25.56	375.93	10.40
	LSD _{5%}	1.65	1.45	2.36	0.49	13.86	2.51	1.38	4.43	12.33	0.26	1.42	0.58	1.77	0.35	0.55	3.27	3.02	0.13	5.54	118.55	4.28
	LSD _{1%}	2.27	1.99	3.24	0.67	19.04	3.45	1.90	6.09	16.94	0.36	1.96	0.80	2.43	0.48	0.76	4.49	4.15	0.17	7.61	162.83	5.88

RESULTS

To widen the genetic base of the germplasm and the hybrids, the evaluation and utilization of other *cms* sources in the hybrid breeding is of utmost importance. Therefore it is desirable to develop and evaluate *cms* lines from variable sources for morpho-physiological and biochemical parameters. Mean and range for different characters are given in Table 1. The data reveals a wide range of values for all the characters.

Genetic divergence: The genetic divergence among 18 sunflower inbred lines comprising alloplasmic *cms* lines, euplasmic *cms* lines and restorer lines was studied by D^2 statistics of Mahalanobis (1936) followed by clustering of genotypes by Tocher's method. These analyses were carried out to know the extent of divergence in the breeding material to identify superior genotypes for further utilization in the hybrid breeding programme. The analysis of variance recorded significant differences among the germplasm lines for all the traits (Table 2). Significance of these statistics suggested considerable diversity and justified further evaluation by D^2 analysis.

Table 2: Analysis of variance for morphophysiological, quality traits and seed yield

SOURCE	d.f.	1*	2	3	4	5	6	7	8	9	10
Replicates	2	0.29	5.12	0.01	0.31	31.71	0.48	19.55	23.58	7.54	0.00
Treatments	17	33.19**	25.46**	67.58**	2.86**	2329.05**	76.60**	23.17**	238.38**	1843.63**	0.81**
Error	34	0.80	0.75	1.41	0.08	30.23	2.704	0.49	0.68	0.77	0.00

SOURCE	d.f.	11	12	13	14	15	16	17	18	19	20	21
Replicates	2	0.09	0.02	1.94	0.13	0.06	0.17	0.99	0.00	28.37	1051.75	3.05
Treatments	17	24.60**	4.11**	37.84**	1.45**	3.67**	129.76**	110.76**	0.19**	372.29**	170420.10**	221.89**
Error	34	0.01	0.01	0.83	0.02	0.05	1.50	0.83	0.01	5.29	130.52	1.48

* 1. Days to 50 % flowering, 2. Days to maturity, 3. No. of leaves/ plant, 4. 100 Seed wt. (g), 5. Plant height (cm), 6. Head diameter (cm), 7. Chlorophyll cont. (%), 8. Relative leaf water cont. (%), 9. Leaf dry wt. (g), 10. Leaf area (m²), 11. Leaf area index, 12. Specific leaf wt./plant, 13. Oil content, 14. Palmitic acid, 15. Stearic acid, 16. Oleic acid, 17. Linoleic acid, 18. Linolenic acid, 19. Biological yield, 20. Harvest index and 21. Grain yield

** Significant at P=0,01

The data presented in Table 3 indicates a wide range of D^2 values from 33.87 to 791.23. Among the alloplasmic *cms* lines ARG-3 cytoplasm was observed to be most divergent from NC-41B with D^2 value of 791.23. It also recorded high level of diversity from *cms*-XA (509.64), ARG-6A (508.35), PHIR-27A (617.37), 40A (743.86), 42A (748.08), P69R (711.67), P124R (550.28), P100R (558.12) and RCR-8297 (690.54). The second most diverse alloplasmic *cms* line was E002-91 having D^2 values of 599.28 (NC41B), 553.28 (40A), 559.26 (42A), 519.68 (P69R) and 500.86 (RCR-8297).

Cluster composition: D^2 analysis assigned the test accessions into five clusters (Table 4, Figure 1). indicating the presence of enough genetic diversity in the mate-

rial. Cluster I contained maximum number of accessions *ie.*, six (*cms* E002-91, *cms* ARG-2, *cms* DV-10A, *cms* PRUN-29A, *cms* 234A and *cms* 38A), followed by cluster III and cluster V comprising of five genotypes each. Cluster III comprised of *cms* PKU-2, P124R, *cms* ARG-6A, P100R and *cms* PHIR-27A, while *cms* 40A, *cms* 42A, P69R, RCR-8297 and NC-41B fell in cluster V. Cluster II and IV had only one genotype each *i.e.*, *cms* ARG-3A and *cms*-XA respectively which shows that ARG3A and *cms* XA are the most diverse sources among all.

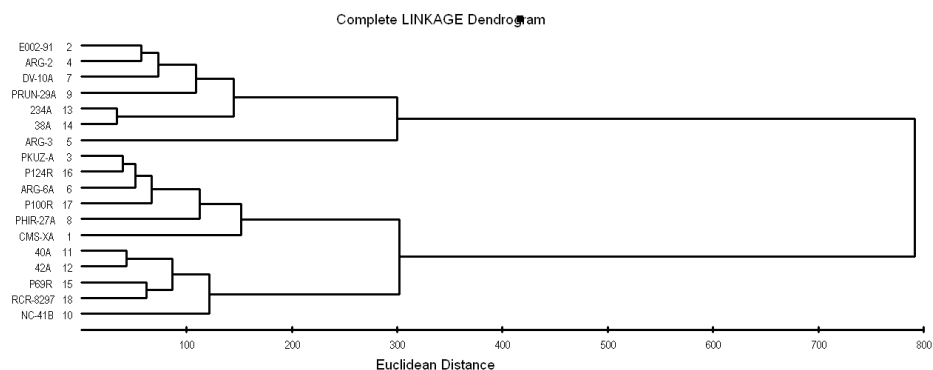


Figure 1: Dendrogram of sunflower genotypes based on morphological traits

Table 4: Cluster composition with genotypes / sources

Clusters	No of lines	Designation of lines
1 Cluster	6	E002-91, ARG-2, DV-10A, PRUN-29A, 234A and 38A
2 Cluster	1	ARG-3
3 Cluster	5	PKU-2, P124R, ARG-6A, P100R and PHIR-27A
4 Cluster	1	<i>cms</i> -XA
5 Cluster	5	40A, 42A, P69R, RCR-8297 and NC-41B

The mean performance of clusters is presented in (Table 5). The cluster I was characterized as having highest mean values for days to maturity, plant height and oil content. Cluster II had highest mean values for head diameter, chlorophyll content, oleic acid, grain yield and biological yield. Similarly lowest value for days to 50% flowering and plant height; and highest values for relative leaf water content, leaf dry weight, leaf area, leaf area index and specific leaf weight were recorded in cluster IV. Cluster V was characterized as having lowest value for days to maturity and maximum values for number of leaves per plant, 100 seed weight, palmitic acid, linoleic acid and linolenic acid.

Euclidian cluster analysis was also used to identify the diverse and desirable accessions in terms of inter cluster distance and mean performance of traits in each cluster, respectively. The important points considered while selecting genotypes were: (1) Choices of the clusters which are separated by maximum inter cluster distance. (2) Selection of particular accessions that showed good performance in the

Table 5: Cluster means values

	Days to 50 % flowering	Days to maturity	No. of leaves/plant	100 Seed weight (g.)	Plant height (cm)	Head diameter (cm)	Chlorophyll content (%)	Relative leaf water content (%)	Leaf dry weight (g)	Leaf area (m ²)
1 Cluster	71.778	98.333	23.722	4.9	104.833	16.806	38.02	64.936	42.469	0.853
2 Cluster	68.667	97	22.333	4.867	110	24.167	40.413	70.32	58.793	1.237
3 Cluster	71.8	95.2	20.133	4.447	74.667	15.3	36.4	69.734	22.407	0.403
4 Cluster	68	95.333	22.667	3.6	82	17.5	37.44	74.1	115.55	2.427
5 Cluster	71.267	93.867	25.133	5.08	94.333	10.9	34.435	62.508	22.397	0.585

	Leaf area index	Specific leaf weight/plant	Oil content (%)	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Grain yield	Biological yield	Harvest index
1 Cluster	4.907	1.904	30.56	5.917	3.5	54.95	32.21	0.983	32.511	605.27	5.54
2 Cluster	6.793	2.633	32	5.1	3.8	56.6	32.8	0.9	42.667	840	5.07
3 Cluster	2.589	1.151	28.07	6.12	4.16	49.161	38.6	0.98	21.177	286.13	7.65
4 Cluster	13.35	5.117	26	6.1	4	51.4	36.1	0.8	13.667	335.33	4.06
5 Cluster	2.588	0.905	27.52	6.42	3.94	45.66	41.52	1.0	20.547	105.	21.29

Table 6: Inter and intra cluster distances

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster
1 Cluster	88.71	238.24	324.57	284.33	503.12
2 Cluster		0.00	557.36	509.64	737.07
3 Cluster			64.09	113.19	188.13
4 Cluster				0.00	252.67
5 Cluster					76.81

Table 7: Correlations among different morphophysiological, seed yield and quality parameters

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1																						
2	0.197																					
3	0.104	0.118																				
4	0.382**	0.850**	0.477**																			
5	0.430**	0.830**	0.430**	0.477**																		
6	0.438**	0.252	0.420**	0.438**	0.478**																	
7	0.438**	0.252	0.420**	0.438**	0.478**	0.478**																
8	0.125	0.237	0.187	0.187	0.237	0.187	0.125															
9	0.120	0.157	0.120	0.157	0.120	0.157	0.120	0.121														
10	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**													
11	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**												
12	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**											
13	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**										
14	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**									
15	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**								
16	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**							
17	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**						
18	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**					
19	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**				
20	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**			
21	0.968**	1.000**	0.968**	0.968**	1.000**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**		

Below diagonal values are genotypic correlations and above phenotypic correlations, Critical value of r at 5%=0.2631 and that at 1%=0.3415
 1. Days to 50% flowering, 2. Days to maturity, 3. No. of leaves/plant, 4. 100 seed wt. (g.), 5. Plant height (cm), 6. Head diameter (cm), 7. Chlorophyll cont. (%), 8. Relative leaf water cont. (%), 9. Leaf dry wt. (g.), 10. Leaf area (m²), 11. Leaf area Index, 12. Specific leaf wt./plant, 13. Oil content, 14. Palmitic acid, 15. Stearic acid, 16. Oleic acid, 17. Linoleic acid, 18. Linolenic acid, 19. Biological yield, 20. Harvest index and 21. Grain yield

selected clusters. The intra cluster distance (Table 6, Figure 2) ranged from 0 for cluster II and cluster IV (as these have single genotype each) to 88.71 (cluster I) indicating that accessions in cluster I (E002-91, ARG-2, DV-10A, PRUN-29A, 234A and 38A) were more diverse with respect to morphological features and yield performance than other clusters. Low levels of intra-cluster distance are indicative of narrow genetic variation within a cluster and the results indicate that PKU-2, ARG-6A, PHIR-27A, P124R and P100R members of cluster III show less genetic variation among themselves. The members of cluster V and II exhibited maximum divergence (inter-cluster distance 737.07) followed by the members of cluster III and II (inter-cluster distance 557.36), cluster IV and II (inter-cluster distance 509.64), cluster V and I (inter-cluster distance 503.12). The members of cluster IV and III were least divergent (inter cluster distance 113.19).

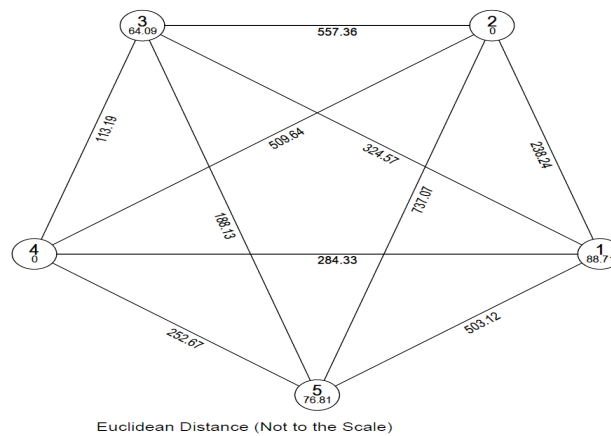


Figure 2: Clustering based on Mahalanobis D^2 analysis

Character association studies: The perusal of the table 7 revealed that grain yield an important character showed significant positive association with days to maturity, plant height, chlorophyll content, oil content and biological yield at both genotypic and phenotypic levels. In the present study it was observed that *cms* ARG3 which had maximum plant height, and head diameter; highest chlorophyll content, and highest biological yield, also recorded highest seed yield. Highly positive and significant correlations were observed between leaf dry weight with leaf area and specific leaf weight per plant; leaf area index with specific leaf weight per plant followed by number of leaves per plant with plant height. Other traits having moderate association were days to maturity with plant height, chlorophyll content, oil content, biological yield and grain yield. Head diameter showed positive correlation with biological yield, oil content, and physiological parameters *i.e.*, chlorophyll content, relative leaf water content, leaf dry weight, leaf area, leaf area index and specific leaf weight.

Table 8: Direct and indirect effects of yield components on yield

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	r value	
1	GP	-1.845	-0.017	-0.044	-0.020	1.714	0.669	0.034	0.062	10.664	-1.014	-9.784	0.037	-0.332	0.320	-0.105	-0.049	0.035	0.062	-0.282	-0.036	0.067
	PP	0.164	0.025	-0.101	-0.005	0.038	0.029	0.009	-0.057	171.924	0.164	-171.651	-0.385	-0.029	0.022	0.041	0.002	-0.014	0.032	-0.099	-0.035	0.073
2	GP	-0.413	-0.075	0.029	-0.001	-1.528	-0.620	0.261	0.053	-11.780	1.035	10.808	-0.039	0.612	-0.081	0.163	0.310	0.268	0.056	1.558	-0.220	0.395
	PP	0.032	0.128	0.060	-0.002	-0.034	-0.029	0.097	-0.048	-187.607	-0.168	187.298	0.403	0.050	0.000	-0.068	-0.012	-0.103	0.035	0.547	-0.225	0.365
3	GP	0.330	-0.009	0.247	0.095	-3.772	0.863	-0.210	0.154	-0.448	0.035	0.416	0.043	0.698	1.734	0.016	-0.029	0.011	0.114	-0.235	0.161	0.212
	PP	-0.029	0.013	0.575	0.026	-0.084	0.040	-0.081	-0.1399	-7.785	-0.006	7.836	-0.474	0.061	0.111	-0.009	0.001	-0.005	0.064	-0.085	0.155	0.185
4	GP	0.240	0.001	0.153	0.154	-2.119	0.249	-0.446	0.040	5.998	-0.628	-5.494	0.048	0.959	-0.221	0.412	0.273	0.139	0.097	0.028	0.151	0.034
	PP	-0.019	-0.005	0.335	0.044	-0.045	0.013	-0.176	-0.036	94.110	0.102	-93.852	-0.050	0.080	-0.017	-0.161	-0.011	-0.057	0.048	0.007	0.159	0.023
5	GP	0.713	-0.026	0.210	0.074	-4.434	0.321	0.003	0.166	-5.604	0.419	5.144	0.014	1.201	1.138	0.166	0.062	0.063	0.110	0.618	0.099	0.453
	PP	-0.060	0.041	0.463	0.019	-0.105	0.014	0.002	-0.148	-90.832	-0.069	90.771	-0.137	0.100	0.078	-0.062	-0.003	-0.026	0.065	0.225	0.104	0.442
6	GP	0.482	-0.018	-0.083	-0.015	0.556	-2.559	0.373	-0.097	-19.627	1.677	18.001	-0.099	0.658	-1.798	0.283	0.450	0.271	-1.157	2.173	-0.372	0.097
	PP	-0.038	0.030	-0.183	-0.005	0.012	-0.124	0.140	0.085	-313.475	-0.271	312.880	1.030	0.051	-0.114	-0.104	-0.018	-0.103	-0.089	0.757	-0.375	0.086
7	GP	-0.080	-0.025	-0.067	-0.088	-0.015	-1.225	0.780	0.047	-10.783	0.729	9.888	-0.055	0.171	-0.141	-0.229	0.083	0.115	-0.085	1.873	-0.305	0.586
	PP	0.005	0.039	-0.146	-0.025	-0.001	-0.055	0.319	-0.042	-176.725	-0.122	176.390	0.581	0.014	-0.009	0.086	-0.004	-0.045	-0.050	0.665	-0.320	0.556
8	GP	0.316	0.011	-0.105	-0.017	2.026	-0.687	-0.102	-0.362	-5.400	0.613	4.954	-0.038	-0.239	-1.549	-0.094	0.279	0.181	-0.063	0.345	-0.310	-0.238
	PP	-0.028	-0.018	-0.239	-0.005	0.046	-0.031	-0.040	0.337	-89.139	-0.102	88.992	0.407	-0.022	-0.105	0.039	-0.012	-0.073	-0.035	0.127	-0.325	-0.227
9	GP	0.440	-0.020	0.003	-0.021	-0.556	-1.123	0.188	-0.044	-44.725	3.741	41.030	-0.177	0.136	-0.446	0.270	0.235	0.154	-0.035	1.232	-0.297	-0.015
	PP	-0.038	0.032	0.006	-0.006	1.904	0.012	-0.030	0.040	-746.170	-0.630	744.915	1.904	0.012	-0.030	-0.106	-0.010	-0.062	-0.021	0.451	-0.314	-0.017
10	GP	0.485	-0.020	0.002	-0.025	-0.482	-1.112	0.147	-0.058	-43.355	3.859	39.774	-0.172	0.006	-0.440	0.224	0.239	0.162	-0.057	0.965	-0.183	-0.041
	PP	-0.041	0.033	0.005	-0.007	-0.011	-0.052	0.060	0.053	-722.970	-0.650	721.761	1.848	0.001	-0.029	-0.088	-0.010	-0.065	-0.034	0.353	-0.195	-0.039
11	GP	0.440	-0.020	0.003	-0.021	-0.556	-1.123	0.188	-0.044	-44.725	3.741	41.030	-0.177	0.136	-0.446	0.270	0.235	0.154	-0.035	1.232	-0.297	-0.015
	PP	-0.038	0.032	0.006	-0.006	-0.013	-0.052	0.075	0.040	-746.170	-0.630	744.916	1.904	0.012	-0.030	-0.106	-0.010	-0.062	-0.021	0.451	-0.314	-0.016

Table 8: Direct and indirect effects of yield components on yield

12	GP	0.369	-0.016	-0.057	-0.040	0.326	-1.373	0.234	-0.074	-43.104	3.611	39.541	-0.184	-0.027	-0.891	0.306	0.253	0.156	-0.072	1.290	-0.335	-0.088
	PP	-0.032	0.026	-0.137	-0.011	0.007	-0.065	0.093	0.069	-716.379	-6.006	715.143	1.983	-0.003	-0.059	-0.118	-0.011	-0.062	-0.042	0.471	-0.351	-0.084
13	GP	0.269	-0.020	0.076	0.065	-2.343	-0.741	0.059	0.038	-2.674	0.009	2.453	0.002	2.272	-1.419	0.377	0.337	0.205	0.077	1.350	-0.090	0.303
	PP	-0.024	0.031	0.172	0.017	-0.051	-0.031	0.022	-0.036	-43.829	-0.003	43.754	-0.033	0.204	-0.097	-0.144	-0.014	-0.079	0.046	0.479	-0.103	0.280
14	GP	-0.174	0.002	0.126	-0.010	-1.488	1.357	-0.033	0.166	5.883	-0.501	-5.397	0.048	-0.951	3.391	-0.289	-0.540	-0.313	0.066	-1.525	0.251	0.071
	PP	0.015	0.000	0.267	-0.003	-0.034	0.060	-0.013	-0.148	94.698	0.080	-94.523	-0.489	-0.083	0.239	0.112	0.022	0.125	0.038	-0.543	0.267	0.081
15	GP	-0.225	0.014	-0.004	-0.073	0.851	0.836	0.206	-0.039	13.941	-1.000	-12.784	0.065	-0.990	1.133	-0.865	-0.203	-0.039	-0.062	-0.549	0.069	0.284
	PP	0.019	-0.021	-0.014	-0.021	0.019	0.037	0.079	0.038	227.860	0.164	-227.413	-0.674	-0.085	0.077	0.348	0.009	0.016	-0.034	-0.194	0.074	0.283
16	GP	0.112	-0.029	-0.009	0.052	-0.339	-1.431	0.081	-0.126	-13.086	1.149	12.013	-0.058	0.953	2.277	0.218	0.804	0.567	-0.052	2.042	-0.315	0.269
	PP	-0.009	0.046	-0.024	0.014	-0.008	-0.066	0.032	0.115	-214.068	-0.190	213.849	0.613	0.080	-0.154	-0.085	-0.035	-0.226	-0.029	0.737	-0.326	0.269
17	GP	0.118	0.034	-0.005	-0.037	0.478	1.187	-0.153	0.113	11.770	-1.071	-10.806	0.049	-0.797	1.818	-0.057	-0.781	-0.584	0.055	-2.037	0.311	-0.400
	PP	-0.010	-0.055	-0.012	-0.011	0.011	0.054	-0.060	-0.104	193.510	0.178	-193.339	-0.517	-0.068	0.125	0.024	0.033	0.238	0.032	-0.737	0.327	-0.379
18	GP	-0.412	-0.015	0.101	0.054	-1.763	1.457	-0.240	0.083	5.651	-0.796	-5.180	0.048	0.636	0.812	0.194	-0.151	-0.117	0.276	-0.607	0.001	0.032
	PP	0.030	0.026	0.212	0.013	-0.039	0.064	-0.092	-0.069	92.108	0.129	-91.917	-0.483	0.054	0.053	-0.069	0.006	0.044	0.173	-0.211	-0.003	0.026
19	GP	0.164	-0.037	-0.018	0.001	-0.862	-1.749	0.459	-0.039	-17.329	1.171	15.898	-0.075	0.965	-1.626	0.149	0.516	0.374	-0.053	3.179	-0.567	0.520
	PP	-0.014	0.060	-0.042	0.000	-0.020	-0.081	0.182	0.037	-288.342	-0.197	287.855	0.800	0.084	-0.111	-0.058	-0.022	-0.151	-0.031	1.317	-0.601	0.514
20	GP	0.082	0.020	0.049	0.028	-0.535	1.163	-0.291	0.137	16.226	-0.866	-14.882	0.075	-0.251	1.039	-0.073	-0.310	-0.222	0.000	-2.205	0.818	0.004
	PP	-0.007	-0.033	0.102	0.008	-0.012	0.053	-0.116	-0.125	268.135	0.145	-267.590	-0.795	-0.024	0.073	0.029	0.013	0.089	-0.001	-0.801	0.875	0.018

Direct effects (diagonal bold values) and indirect effects

1. Days to 50% flowering, 2. Days to maturity, 3. No. of leaves/plant, 4. 100 Seed wt. (g.), 5. Plant height (cm), 6. Head diameter (cm), 7. Chlorophyll cont. (%), 8. Relative leaf water cont. (%), 9. Leaf dry wt. (g), 10. Leaf area (m²), 11. Leaf area index, 12. Specific leaf wt./plant, 13. Oil content, 14. Palmitic acid, 15. Stearic acid, 16. Oleic acid, 17. Linoleic acid, 18. Linolenic acid, 19. Biological yield, 20. Harvest index and 21. Grain yield

Oil content was positively associated with plant height, 100 seed weight and no. of leaves per plant. There was a positive association of no. of leaves per plant with 100 seed weight, plant height and oil content. Significant negative association was observed for days to 50% flowering with plant height and grain yield; days to maturity with biological yield; no. of leaves per plant with head diameter, chlorophyll content with relative leaf water content; plant height with relative leaf water content and head diameter with harvest index. Reductions in seed yield upon reducing number of days to flowering and plant height in sunflower has been well documented. Highly significant negative association was recorded between oleic acid and linolenic acid.

Path analysis: Simple correlations between yield components are not very informative with respect to determining the functional relation between components from diverse hierarchy. The analytical method of path coefficients analysis permits the decomposition of the correlations between two variables (X and Y) in a sum of the direct effect of X on Y, and the indirect effects of X on Y *via* other independent variables. Genotypic correlations were partitioned into direct and indirect effects through various yield contributing characters to investigate the selection criteria in sunflower breeding programme (Table 8). Number of leaves per plant, 100 seed weight, chlorophyll content, leaf area, leaf area index, oil content, biological yield and harvest index had positive direct effects on grain yield. The highest direct effect (41.03) was exhibited by leaf area index, followed by leaf area (3.85), biological yield (3.17) and oil content (2.27). Other traits also had positive direct effects on yield but these were quite low. The leaf area index was recorded to have highest positive direct effect on yield both at genotypic and phenotypic level (41.03 and 744.91, respectively). Earlier, Farratullah *et al.* (2006), Madhavalatha *et al.* (2004) and Muhammad *et al.* (2007) had also observed same results working on sunflower. Patil *et al.* (1996) reported significant positive direct effects of 100-seed weight on seed yield in sunflower. The direct effect of head diameter on seed yield was negative. Many researchers have also reported these (Alba *et al.*, 1979; Marinković, 1992; Habib *et al.*, 2006).

DISCUSSION

The mean values as recorded for various traits indicate a lot of variation for the studied traits in the present material. Days to maturity a very important trait for development of hybrids with different maturity groups, ranged from 92.0 to 101 days. *Cms* DV-10A, PRUN-29A and P100R were the late maturing lines hence suitable for developing late maturing hybrids. In Punjab where sunflower is grown in spring season, late maturing hybrids perform well and give high yields when sown early in the month of December. Early maturing lines *i.e.*, ARG-2A, ARG-3A, ARG-6A and *cms*-XA can be crossed with early maturing restorers *i.e.*, P69R, P124R and RCR-8297 to develop early/medium maturing hybrids which can fit well under

late sown conditions in Punjab where most of the land is vacated late in the months of February / March after the harvest of potato crop. It was observed that different cytoplasmic sources significantly influenced biochemical traits. Large variation was reported regarding oil content (24.0-34.4%), *cms* line PRUN-29A and P100R have been identified as the best genotypes for improvement in this character. Oleic acid is considered to be an important fatty acid for any oil as it gives oxidative stability to the oil. The alloplasmic line E002-91A (58.0%) from wild *H. annuus* source and P100R (60.8%) from conventional source were identified as promising genotypes for use in heterosis breeding programme. Hybridization between these two genotypes is expected to give F₁ hybrid with high oleic acid content. Similarly P100R having oil content of 32.0% might be crossed with PRUN-29A (oil content, 34.4%) to exploit heterosis for oil content.

Intercrossing lines from different clusters may generate large variability and would produce transgressive segregants for yield and yield attributes in population improvement programmes. In this study the highest inter cluster distance was observed between clusters II and V followed by clusters II and III, clusters II and IV and clusters I and V.

Minimum diversity was observed between the members of cluster IV and III. The inter-cluster distances were larger than intra-cluster distances indicating wider genetic diversity between genotypes of the clusters with respect to the traits considered. According to Murty and Arunachalam (1966) and Ananda *et al.* (2008) combinations with high heterotic response and superior recombinants may be obtained through hybridizations between genotypes across the clusters. Studies conducted by Punitha *et al.* (2010) also indicated that the major contribution of seed yield towards genetic divergence and suggested selection of lines from diverse clusters to get maximum heterosis. It is pertinent to mention here that although NC-41B (Morden) is the common maintainer of the alloplasmic *cms* lines and has same genetic makeup as all alloplasmic *cms* lines but this was not grouped with any of these sources which indicates the uniqueness of NC-41B cytoplasm (*petiolaris*) and reveals diversity from other cytoplasmic sources used in the present study. Further, grouping of NC-41B (Morden) with the *cms* lines 40A, 42A, P69R, RCR-8297 (developed from *Petiolaris* source) in cluster V reveals close similarity among these *cms* lines and restorers, which can be attributed to common cytoplasmic background. The results further indicate that *cms* lines grouped in cluster I (E002-91, ARG-2A, DV-10A, PRUN-29A, 234A, 38A), cluster II (ARG- 3A) and cluster IV (*cms*-XA) can give more heterotic combination with restorer lines present in cluster III and V (P69R, RCR-8297, P124R and P100R). These results suggest that ARG-3A the sole member of cluster II can give more heterotic combinations upon hybridization with the R lines *viz.* P69R and RCR-8297 grouped in cluster V, ARG-3A can also be crossed with P124R and P100R (cluster III) to get good hybrids. Since ARG-3A was also characterized as having highest grain yield therefore it is expected that the hybrids resulting from these cross combinations would be high yielding.

Character association studies revealed that genotypic correlation coefficients were higher as compared to phenotypic correlations for most of the traits indicating high reliability of results. Significant positive association of seed yield with plant height, chlorophyll content, biological yield, days to maturity and oil content was recorded. Positive correlation between yield and plant height; number of leaves per plant and seed yield have earlier been reported by (Sujatha and Nandini 2002) and Kholghi et al (2011) respectively. Plant height is an agronomic trait involved in plant productivity. It is polygenically controlled and short plant height is controlled by recessive dwarfing genes. Chlorophyll content also an important component character of plant productivity having positive correlation with head diameter, 100 seed weight and seed yield has been reported by Behradfar *et al.* (2009). Head diameter being an important yield component, several researchers have suggested significant positive correlations between head diameter and seed yield and thus concluded that increased head diameter could lead to higher seed yield. However, in the present study we did not observe any significant positive correlation between head diameter and seed yield, while head diameter was positively associated with biological yield. These findings suggest the involvement of some other factors like seed filling in the head and seed size as important parameters besides head diameter in determining the seed yield. Oil content was positively associated with plant height, 100 seed weight and no. of leaves per plant. Positive association of oil content with days to 50% flowering (Khan *et al.*, 2003) and with seed yield per plant (Kaya *et al.*, 2007) has been discussed earlier also. Significant negative association for days to 50% flowering with plant height and grain yield; days to maturity with biological yield; no. of leaves per plant with head diameter, chlorophyll content and relative leaf water content; plant height with relative leaf water content; head diameter with harvest index were observed in the present study. Reductions in seed yield upon reducing number of days to flowering and plant height in sunflower has been well documented. A high or low correlation coefficient between two variables may be due to the effect of a third variable or group of variables (Singh and Chaudhary, 1977; Cruz and Regazzi, 1997; Vencovsky and Barriga, 1992). In order to identify a trait as an indirect selection criterion for seed yield through path coefficient, the trait should have positive direct effect on seed yield as well as significant positive correlation with seed yield (Das and Taliaferro, 2009). The results of this study suggest that leaf area index, leaf area and biological yield are the main seed yield components. Highly significant negative association recorded between oleic acid and linolenic acid has been well established in a number of studies. It is important to mention here that different cytoplasmic sources used in the present study do not show any impact on the already established correlations between important traits and can be exploited in heterosis breeding programme.

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