

## Original Research Article

### Study on Genetic Diversity in 45 elite Sunflower Inbred lines (*Helianthus annuus* L.)

#### ABSTRACT

The present investigation aimed to study the genetic diversity of 45 sunflower inbred lines to identify genetically distinct parents for hybridization, facilitating the development of high yielding hybrid cultivars. The field experiment was carried out during the Rabi 2023-2024 at the Regional Agricultural Research Station, Nandyal, Andhra Pradesh, India. Findings revealed significant differences among inbred lines for the studied traits. The inbred lines were grouped into six clusters using Tocher's method. Cluster I is the largest cluster with 34 inbred lines. Maximum intra cluster distance of 15.17 is associated with cluster I whereas maximum inter cluster distance of 79.75 is found between cluster II and cluster V. Cluster VI recorded maximum means for six traits. Among the studied traits, seed yield (g/plant) (50.81) contributed maximum towards total diversity. Based on the significant genetic diversity observed among the 45 sunflower inbred lines, the study successfully identified genetically distinct parental genotypes for hybridization. This provides a foundation for developing superior hybrid cultivars with enhanced seed yield potential.

**KEY WORDS:** Sunflower, genetic diversity and  $D^2$  analysis

#### INTRODUCTION

Sunflower belongs to family Compositae (*Asteraceae*) and is diploid with chromosome number  $2n = 34$ . It is one of the four most widely grown and consumed oil crops in the world (Li *et al.*, 2024). Presently, it is cultivated in an area of 20.00 million hectares globally with production of 30.00 million tonnes and productivity of 1,500 kg ha<sup>-1</sup> (Kumar *et al.*, 2024). Sunflower seed oil is widely acclaimed in several countries compared to other vegetable oils owing to its easy availability and several health benefits including less serum cholesterol, low-density lipoprotein levels, antioxidants, regulating blood pressure, anti-inflammatory, skin protection, and pain relief (Holgado *et al.*, 2021). Sunflower seed oil is characterized by a relatively high proportion of unsaturated fatty acids, especially linoleic acid and oleic acid, conferring high nutritional value (Yang *et al.*, 2022). Furthermore, the residual meal from sunflower seeds after oil extraction contains rich protein (40 to 50%), with a high nutritional value and balanced amino acid composition, thus rendering it a high-quality plant protein resource (Subasi *et al.*, 2022). A substantial decline in sunflower cultivated area has been observed in recent years, primarily attributed to a limited availability of high-quality hybrid

cultivars. This scarcity underscores the significant potential for harnessing sunflower genetic diversity to identify superior accessions (Reddy *et al.*, 2024). These elite accessions can serve as valuable resources for the development of advanced hybrid cultivars, thereby enhancing sunflower production and yield. Biometrical techniques like Mahalanobis's  $D^2$  statistic offer a quantitative approach to assess genetic divergence among breeding lines and provides a valuable index of genetic diversity, allowing the grouping of genotypes based on  $D^2$  values. The genetic diversity analysis in the studied inbred lines help in identifying most diverse and distantly related inbred lines which can be further crossed ensuring better manifestation of heterosis (Tomkowiak *et al.*, 2020). Present study aimed to significantly contribute to the identification of genetically diverse sunflower inbred lines, thereby facilitating the development of superior hybrids with enhanced yield potential. This research addresses the critical challenge of limited availability of high-quality hybrid cultivars.

## MATERIAL AND METHODS

The experimental material comprised of 45 sunflower inbred lines, including five checks. The inbred lines used in the study were listed in Table 1. An Alpha Lattice Design with three replications is employed for cultivation of 45 sunflower inbred lines. The field experiment was carried out during the Rabi 2023-2024 at the Regional Agricultural Research Station, Nandyal, Andhra Pradesh, India, geographically located at 15<sup>0</sup>29' north latitude and 78<sup>0</sup>29' east longitude at an altitude of 211.76 m above mean sea level. Each genotype was cultivated in two rows with a row length of 3m, with plot size of 3.0 × 1.8 m<sup>2</sup> per genotype maintaining a row spacing of 60 cm and plant spacing of 30 cm. All the agronomic practices recommended by Acharya N.G. Ranga Agricultural University were carried to raise a healthy crop. Data were collected on 11 traits, among which days to 50% flowering, days to maturity and final plant stand were recorded on plot basis. Plant height (cm), head diameter (cm), volume weight (g 100ml<sup>-1</sup>), 100-seed weight (g), seed yield (g/plant), seed yield (kg ha<sup>-1</sup>), oil content (%), and oil yield (kg ha<sup>-1</sup>) were recorded from five randomly selected plants per genotype in all replications.

Seed yield (kg ha<sup>-1</sup>) and oil yield (kg ha<sup>-1</sup>) were calculated as per the following formula.

$$\text{Seed yield (kg ha}^{-1}\text{)} = \frac{\text{Seed yield (kg/plot)} \times 10000}{\text{Net plot area (m}^2\text{)}}$$

$$\text{Oil yield (kg ha}^{-1}\text{)} = \frac{\text{Seed yield (kg/ha)} \times \text{Oil content (\%)}}{100}$$

Analysis of variance (ANOVA) was conducted on the Alpha Lattice Design using the Variability package in R Studio. The collected data is subjected to statistical analysis employing Mahalanobis  $D^2$  distance (Mahalanobis, 1928) and Tocher's method (Rao, 1952) to identify group clusters. Statistical analysis was calculated using INDOSTAT 9.2 software and R Studio.

## RESULTS

In the present study, genetic diversity among 45 sunflower inbred lines was assessed using  $D^2$  statistics of Mahalanobis (1928) followed by clustering of genotypes using tocher's method. The mean values of 45 inbred lines were subjected to analysis of variance (ANOVA) and the mean sum of square for each trait was calculated and represented in Table 2.. The analysis of dispersion based on the Wilk's criterion presented in Table 3 revealed highly significant difference ( $P= 0.00349 **$ ) among inbred lines for 11 traits. Group constellation was carried out following tocher's method (Rao, 1952) and grouped 45 inbred lines into six clusters. The cluster composition is represented in Table 4 and Fig.1. Cluster I was the largest comprising of maximum number of 34 genotypes followed by cluster II with seven genotypes the remaining clusters are monogenic with single genotype. Lakshman *et al.* (2021) and Kumar *et al.* (2024) also observed similar clustering pattern of genotypes among cluster as some clusters were unique having only single genotype.

The intra and inter cluster distance were displayed in Table 5 and Fig.2. Maximum intra cluster value was observed for cluster I ( $D^2=15.17$ ) followed by cluster II ( $D^2=15.13$ ). Cluster III, IV, V and VI has zero intra cluster distance since they are monogenic clusters. The maximum inter cluster distance was found between cluster V and cluster II ( $D^2=79.75$ ) followed by cluster IV and cluster II ( $D^2=70.78$ ) and cluster VI and cluster V ( $D^2=57.87$ ). Crossing between inbred lines of these clusters will result in higher hybrid vigour. Inter cluster distance was found minimum between cluster V and cluster III ( $D^2=9.39$ ) suggesting close relation between them and low level of diversity. The inter cluster distances were greater than intra cluster except in some clusters and same is reported by Lakshman *et al.* (2021) and Kumar *et al.* (2024).

A perusal of cluster means for 11 traits per cluster was presented in the Table 6. The Cluster VI reported maximum cluster means for the traits *viz.*, days to 50% flowering (56.67), plant height (117.87), head diameter (13.00), 100 seed weight (4.50), seed yield (g/plant) (26.87), seed yield ( $\text{kg ha}^{-1}$ ) (1493.57). Highest cluster mean values for final plant stand (18.66) and volumetric weight (47.00) was displayed in cluster II. Cluster IV recorded desired highest cluster mean for oil yield ( $\text{kg ha}^{-1}$ ). The desired values for days to 50 % flowering (52.67), days

to maturity (86.33) *i.e.*, earliness was reported in cluster III and the same cluster exhibited higher value for oil content (37.80). Considering the mean performance of the clusters, the cluster VI had highest mean value for days to 50% flowering, plant height, head diameter, 100 seed weight, seed yield (g/plant), seed yield (kg ha<sup>-1</sup>). Therefore genotypes from this cluster used as one of parents to get higher yield. Genotypes of this cluster can be used for generating variability.

Relative contribution of different traits in the present study is represented in the Table 7 and Fig.3. Among the traits studied, seed yield (g/plant) (50.81%), contributed maximum to the total variation followed by seed yield (kg ha<sup>-1</sup>) (12.02%) and head diameter (11.92 %). The traits that contributed least to the genetic diversity were final plant stand (0.2 %) and days to maturity (0.3 %). Rani *et al.* (2016) reported that seed yield (g/plant) contributed maximum towards diversity in their study. Lakshman *et al.* (2021) reported that head diameter contributed maximum towards diversity in their study.

## DISCUSSION

The significance of mean sum of squares across all studied traits indicates presence of variability in these traits and also suggests a focus on these traits for further improvement (Mariyam *et al.*, 2024). Malik *et al.* (2016) in their study identified cluster VII and cluster VIII as most diverse ( $D^2= 156.01$ ) and suggested for hybridization between these two groups to harness high magnitude of heterosis. Their finding supported this study, where similar conclusions were drawn for cluster V and cluster II based on inter cluster distance ( $D^2=79.75$ ). Revanth *et al.* (2021) in their study obtained high mean value for head diameter, 100 seed weight, seed yield per plant and oil content in cluster VI. They suggested for selection of genotypes belonging to cluster VI for use of parents in hybridization programmes aimed to increase seed yield. Similar kind of parent selection from cluster VI based on cluster mean is recommended in this study. Neelima *et al.* (2016) in their study identified 100 seed weight contributed maximum towards total divergence. In order to select genetically diverse parents, they suggested classification of material based on traits like 100 seed weight, plant height, days to maturity and seed yield per plant. This study emphasised more on traits seed yield (g/plant), seed yield (kg ha<sup>-1</sup>) and head diameter for selection of most diverse parents.

## CONCLUSIONS

The present study exhibited high differences among the genotypes for seed yield and almost all the yield components which may favour selection and its utilization in recombination breeding programme. Cluster analysis revealed that clusters V and II were most diverse

therefore, more emphasis should be given on cluster V and II in selecting inbreds for development of high-quality hybrid cultivars. Cluster VI exhibited superior mean values for most traits. Genotype (RHA-1013) from cluster VI can be a promising parent for increasing yield and generating genetic variability. Seed yield (g/plant), seed yield (kg ha<sup>-1</sup>) and head diameter were considered to be important traits contributing toward genetic divergence. Further research and validation of these findings across diverse environments and genotypes will be crucial for the successful implementation of breeding programs aimed at enhancing sunflower productivity.

### **FUTURE LINE OF WORK**

- Genotypes from the most genetically distinct clusters, V (R-106) and II (ARM-243B, PM-81, R-64, NDI-20, RP-10, CMS-243B, and CMS-302B), could serve as promising parental candidates for hybridization programs aimed at exploiting heterosis.
- The genotype RHA-1013, a member of cluster VI, could serve as a valuable parental source for enhancing yield and genetic diversity in breeding programs

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### **CONFLICT OF INTEREST**

No conflict of interest

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**Table 1. List of 45 inbred lines used in the study**

S. No	Entry	Source	S. No	Entry	Source
1	NDSI-2	RARS, Nandyal.	24	RP-10	RARS, Nandyal.
2	NDLB-5	RARS, Nandyal.	25	CMS-249B	IIOR, Hyderabad.
3	NDSI-3	RARS, Nandyal.	26	NDI-47	RARS, Nandyal.
4	RP-16	RARS, Nandyal.	27	CMS 302B	PDKV, Akola.
5	OPH-74	PAU, ludhiana	28	RCR-39	RARS, Nandyal.
6	CMS- 30B	RARS, Nandyal.	29	PB-120	IIOR, Hyderabad.
7	CMS- 104B	UAS, Bangalore.	30	IC-502039	VNMKV, Lathur.
8	RHA-1096	UAS, Bangalore.	31	NDI-20	RARS, Nandyal.
9	R-106	UAS, Raichur.	32	NDLR-27	RARS, Nandyal.
10	NDLR40	RARS, Nandyal.	33	RHA 859	RARS, Nandyal.
11	RSFH 5	RARS, Nandyal.	34	PS2023B	IIOR, Hyderabad.
12	PB-110	IIOR, Hyderabad.	35	R X R -2-38	RARS, Nandyal.
13	NDLR1	RARS, Nandyal.	36	NDLR4	RARS, Nandyal.
14	RHA-172	RARS, Nandyal.	37	NDI-50	RARS, Nandyal.
15	NDLR-36	RARS, Nandyal.	38	RHA-1013	UAS, Bangalore.
16	NDI-55	RARS, Nandyal.	39	ARM-243-B	IIOR, Hyderabad.
17	COSF-6B	TNAU, Coimbatore.	<b>Checks</b>		
18	COSF-7B	TNAU, Coimbatore.	40	R-64	UAS, Raichur.
19	EC-601829	IIOR, Hyderabad.	41	LTRR-341	UAS, Bangalore.
20	RSFH-11	RARS, Nandyal.	42	RHA-95C-1	TNAU, Coimbatore.
21	NDI-43	RARS, Nandyal.	43	RHA 6D-1 (br)	UAS, Bangalore.
22	EC-601768	IIOR, Hyderabad.	44	PM-81	UAS, Raichur.
23	NDLR-32	RARS, Nandyal.	45	KBSH-44	UAS, Bangalore.

**Table 2. Analysis of variance for yield and yield attributing traits in sunflower**

S. No	Traits	Mean squares			
		Replications (df:2)	Genotypes (df:44)	Blocks (df:24)	Error (df:64)
1	Days to 50% flowering	1.49	9.60**	1.98	2.63
2	Days to maturity	0.59	14.82**	5.97	6.04
3	Final plant stand	0.77	5.27**	3.28	2.64
4	Plant height (cm)	1.20	606.00**	57.80	75.40
5	Head diameter (cm)	1.15	8.67**	0.90	0.82
6	Volumetric weight (g 100ml <sup>-1</sup> )	2.55	92.19**	2.02	2.50
7	100 seed weight(g)	0.06	1.22**	0.15	0.14
8	Seed yield (g/plant)	0.29	30.96**	5.27	3.03
9	Seed yield (kg ha <sup>-1</sup> )	3947.00	109698.00**	26871.00	15457.00
10	Oil content (%)	2.53	8.17**	2.94	2.30
11	Oil yield (kg ha <sup>-1</sup> )	680.00	13364.00**	2724.00	1410.00

\*, \*\* Significant at 1% and 5% levels, respectively

**Table 3 . Analysis of variance for dispersion in sunflower genotypes**

Source of Variations	DF	Mean Squares	Probability
Varieties	44	3.69**	0.00349 **
Error	87	1.87	
Total	131	0.048	

\*\* Significant at 5% level

**Table 4. Cluster composition of sunflower genotypes based on Tocher's method**

Cluster Group	No. of Genotypes	List of Genotypes
Cluster I	34	NDLR-36,NDLR-4,NDSI-3,NDI-47,RHA-1096,COSF-7B,RCR-39,PB-120,EC-601768,NDLR-27,OPH-74,RSFH-11,RHA-172,EC-601829,PS2023B,RHA-95C-1,NDI-50,NDSI-2,IC-502039,RHA-6D-1,NDLR-40,RP-16,PB-110,NDI-55,NDLB-5,RHA-859,CMS-30B,NDLR-32,NDLR-1,COSF-6B,KBSH-44,LTRR-341,NDI-43 and CMS-104B

Cluster II	7	ARM-243B, PM-81, R-64, NDI-20, RP-10, CMS-249B and CMS-302B
Cluster III	1	RSFH-5
Cluster IV	1	R X R-2-38
Cluster V	1	R-106
Cluster VI	1	RHA-1013

**Table 5. Average intra and inter cluster distances for the sunflower genotypes**

Cluster Distances						
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	15.17					
Cluster II	30.51	15.13				
Cluster III	26.33	54.73	0.00			
Cluster IV	27.72	70.78	35.39	0.00		
Cluster V	33.84	79.75	9.39	25.19	0.00	
Cluster VI	24.10	41.92	53.60	36.61	57.87	0.00

**Table 6. Cluster means with respect to yield and its attributes among sunflower**

**genotypes**

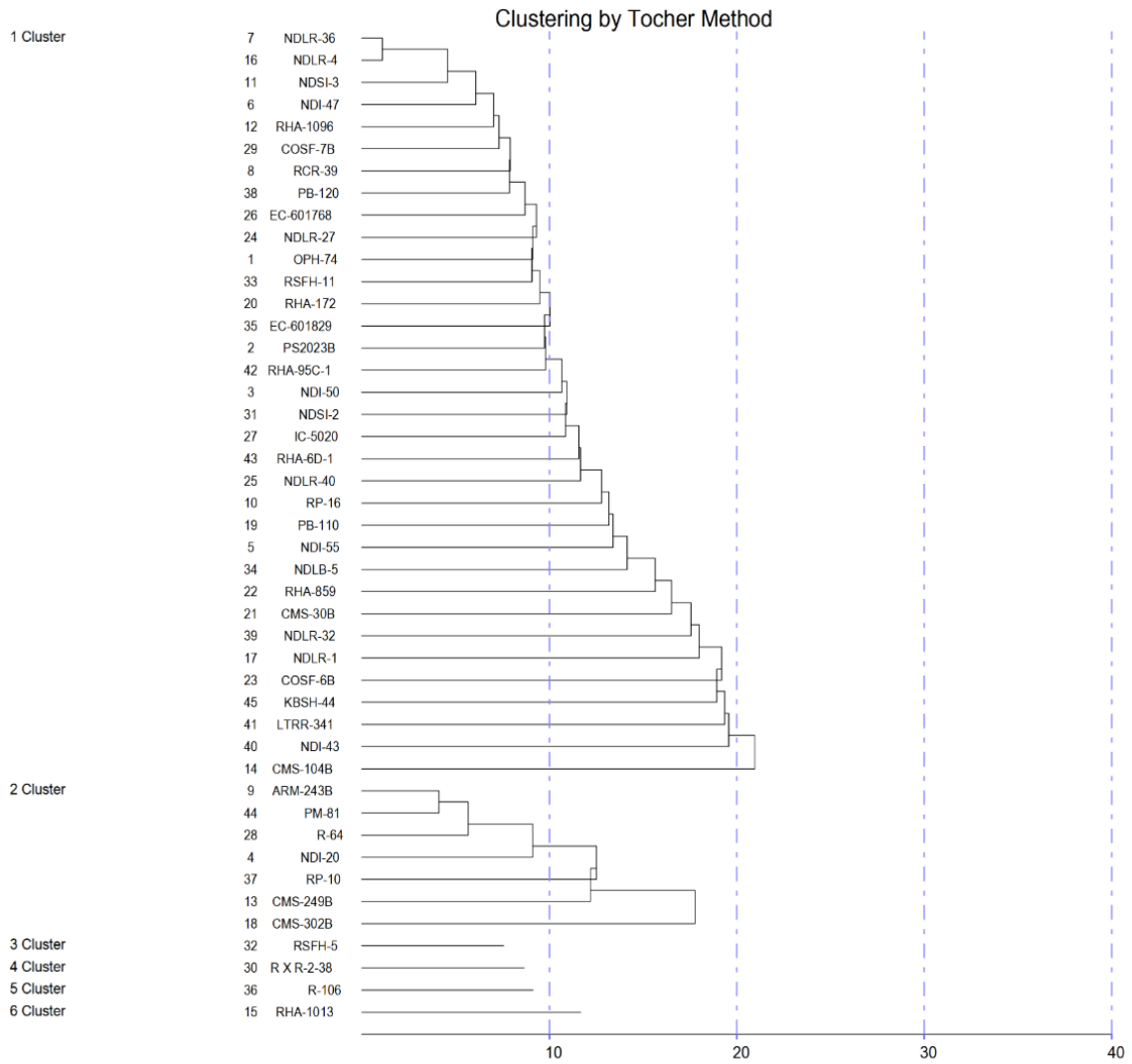
S. No.	Character	Cluster means					
		I	II	III	IV	V	VI
1.	Days to 50% flowering	53.78	54.57	52.67	56.33	54.67	56.67
2.	Days to maturity	88.08	87.19	86.33	92.00	87.00	91.33
3.	Final plant stand	18.10	18.66	17.94	18.00	18.54	17.86
4.	Plant height (cm)	94.66	97.18	70.07	103.40	72.53	117.87
5.	Head diameter (cm)	9.76	9.51	6.38	9.27	5.43	13.00
6.	Volumetric weight (g 100ml <sup>-1</sup> )	37.03	47.00	33.60	29.67	27.57	34.93
7.	100 seed weight (g)	3.78	4.03	2.97	2.70	3.63	4.50
8.	Seed yield (g/plant)	22.61	23.24	14.77	25.80	18.73	26.87
9.	Seed yield (kg ha <sup>-1</sup> )	1256.03	1290.54	820.43	1433.37	1040.67	1493.57
10.	Oil content (%)	35.52	36.46	37.80	37.53	37.37	30.73
11.	Oil yield (kg ha <sup>-1</sup> )	446.54	469.78	310.00	539.33	388.27	459.80

**Table 7. Contribution of yield and its attributes towards total diversity in sunflower genotypes**

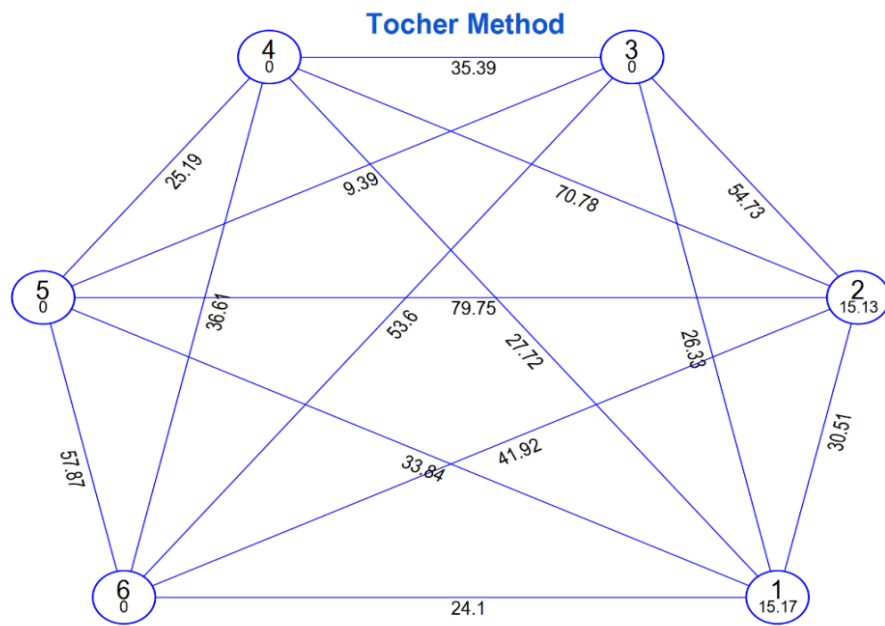
S. No.	Source	Contribution %	Times ranked 1st
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1	Days to 50% flowering	3.84	38
2	Days to maturity	0.30	3
3	Final Plant Stand	0.20	2
4	Plant height (cm)	7.58	75
5	Head Diameter (cm)	11.92	118
6	Volumetric weight (g 100ml <sup>-1</sup> )	9.09	90
7	100 seed weight (g)	0.51	5
8	Seed yield (g/plant)	50.81	503
9	Seed yield (kg ha <sup>-1</sup> )	12.02	119
10	Oil content (%)	2.42	24
11	Oil yield (kg ha <sup>-1</sup> )	1.31	13

UNDER PEER REVIEW

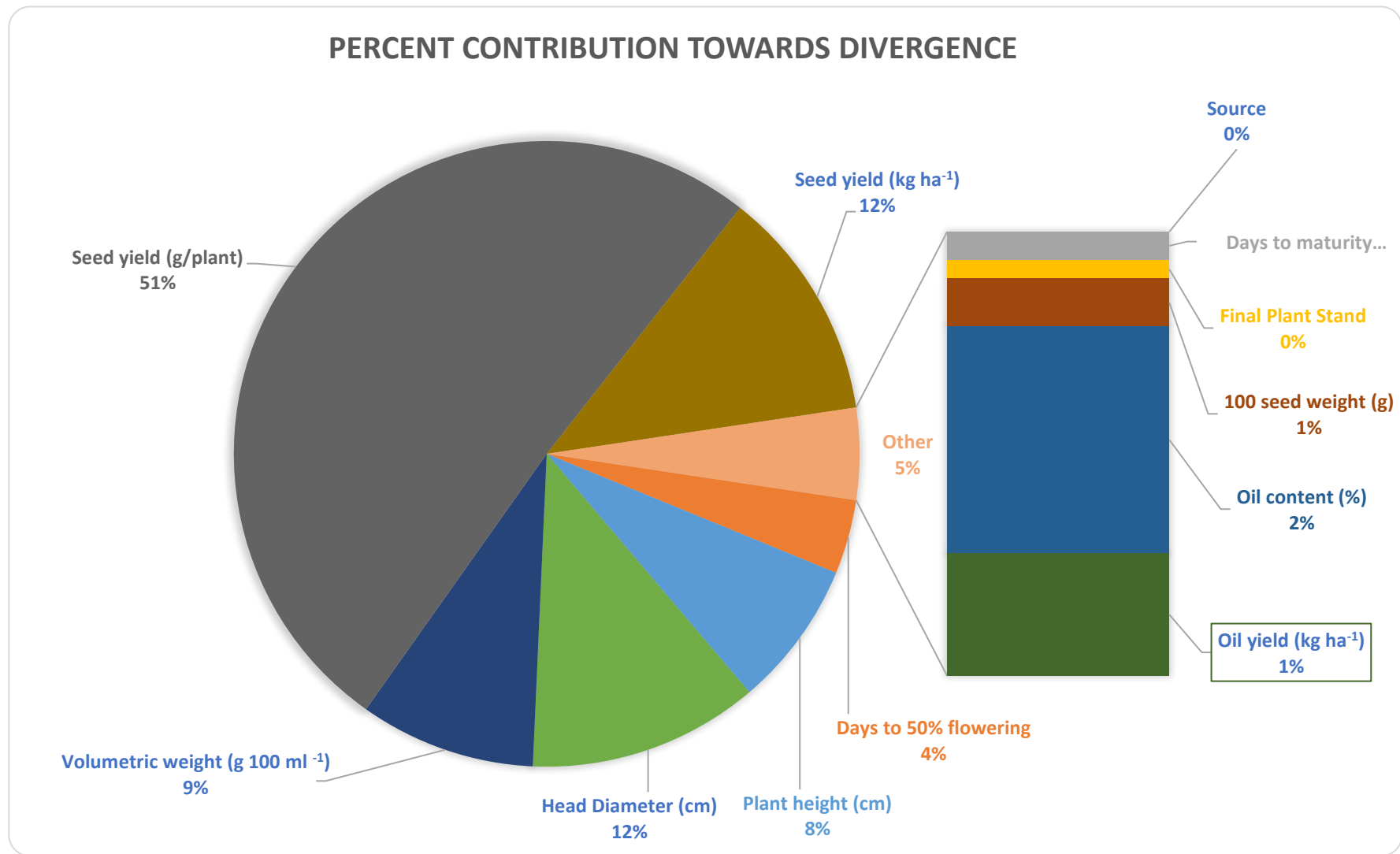


**Fig.1. Dendrogram showing relationship among sunflower genotypes in six clusters based on  $D^2$  values**



**Fig.2. Cluster diagram showing average intra and inter cluster distance for yield and its attributes of sunflower genotypes**

UNDER PEER REVIEW



**Fig. 3. Relative contribution of 11 traits to total genetic diversity in sunflower genotypes**