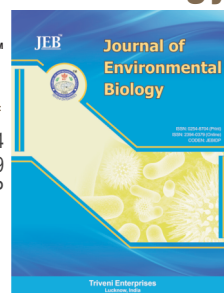
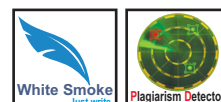


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Characterization, evaluation and multivariate analysis of sunflower germplasm under semi-arid environments of three locations in two sunflower growing states in India



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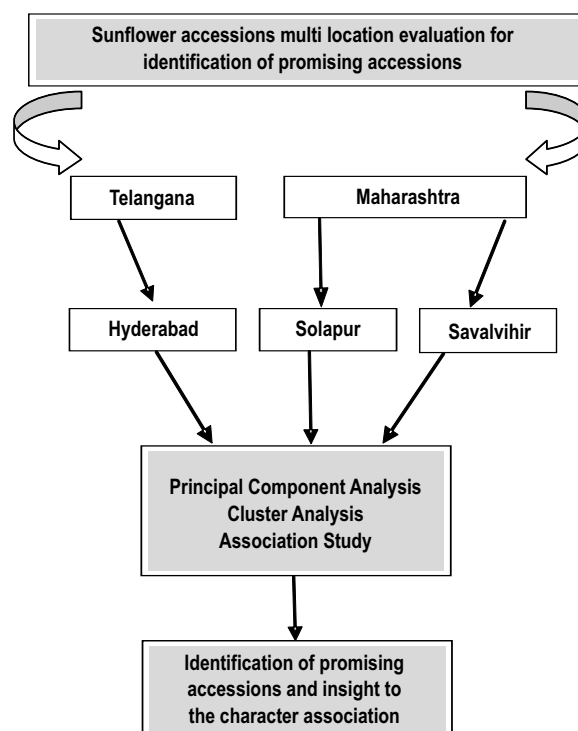
Abstract

Aim : The present study aimed at identifying high yielding genotypes through principal component analysis and cluster analysis by utilizing multilocation data generated by growing 32 germplasm accessions under semi-arid environments of Telangana and Maharashtra states.

Methodology : The evaluation trial was conducted in two Indian states Telangana (Hyderabad) and Maharashtra (Solapur and Savalvihiir). R software 3.1.3 package was used to determine the major descriptive statistics, cluster analysis, principal component analysis (PCA) and correlations among the yield contributing traits.

Results : Wide range of genetic variation for seed yield and its components were recorded in the accessions studied. Principal component analysis disclosed that the first four components with eigen values greater than 0.43 contributed about 93.3% of total variability in the studied germplasm involving all the seven characters. The genotypes analysed were grouped into eleven clusters, out of which cluster IX was the largest having six genotypes indicating genetic similarity among them. Character association indicated that among the seven agromorphological traits, five traits viz., days to flowering, days to maturity, head diameter, 100-seed weight and volume weight had significant and positive correlation with seed yield.

Interpretation : Based on PCA and cluster analysis, eight accessions GMU189, GMU799, GP₂1217, GP₆271, GMU440, GP₆211, GP₆286 and DRSF113 were identified as high yielding which can be widely utilised for the development of new diverse varieties/ hybrids for enhanced seed yield under semi-arid conditions.



Introduction

Sunflower is one of the important oilseed crops in India with productivity of 752 kg ha⁻¹ (GOI, 2016). Semi-arid regions are characterized by a climate with low or insufficient rainfall to sustain agricultural production. In India, almost 53.4 per cent land area comprises arid and semi-arid regions (GOI, 2004). The extent of genetic variability present in the germplasm for desired traits is the key for success of any crop improvement programmes aimed at development of new superior inbreds, varieties and hybrids (Dudhe *et al.*, 2017). Bringing improvement over existing crop varieties and hybrids is a continuous process. To achieve this objective, the breeder has to identify diverse promising accessions from working germplasm.

Therefore, knowledge on the extent of diversity for the desired traits is essential for efficient germplasm conservation, characterisation and determining the relationships among germplasm accessions to enhance the efficiency of its management and genetic improvement (Geleta *et al.*, 2005). Data generated through multilocation evaluation provides better indication about the buffering capacity and actual worth of the genotypes under diverse conditions. Multivariate statistical techniques like principal component analysis (PCA) and cluster analysis (CA) which analyze multiple measurements based on morphological or molecular markers proved to be successful in selecting appropriate genotypes that meets the objectives of the breeding programme (Mohammadi and Prasanna, 2003). Cluster analysis is commonly used to study genetic diversity and for forming core subset for grouping accessions with similar characteristics into one homogenous category.

Clustering is also used to summarize information on relationships between objects by grouping similar units so that the relationships may be easily communicated. For analysing genetic diversity in sunflower, multivariate techniques are frequently employed (Muppidathi *et al.*, 1995; Mohan and Seetharam, 2005; Kholghi *et al.*, 2011 and Reddy *et al.*, 2012). PCA is used to reveal patterns and eliminate redundancy in data sets (Adams, 1995) as morphological and physiological variations routinely occur in crop species. PCA technique is effective in finding the structure of the sets, grouping of genotypes and estimation of diversity of breeding materials. PCA has previously been used in sunflower either for grouping genotypes or for estimation of genetic diversity (Maruthi Sankar *et al.*, 1999; Dong *et al.*, 2007; Tabrizi *et al.*, 2011). PCA is used to obtain a small number of linear combinations, which accounts for most of the variability in the evaluated germplasm. Hence, by considering the above aspects, this investigation was undertaken to study the variability of germplasm accessions by means of descriptive statistics, to understand the association of various characters, to identify high yielding accessions through PCA and cluster analysis by utilizing multilocation evaluation data generated by growing the germplasm under semi-arid environments of Telangana and Maharashtra states of India.

Materials and Methods

Experimental material and location : This study was conducted to characterize 32 sunflower accessions along with three checks viz., DRSF-113, Morden and Bhanu. Evaluation was carried out during *khari*f 2013 at three semi-arid locations which included one location in Telangana state (IIOR, Hyderabad) and two locations at Maharashtra (ZARS, Solapur; ARS, Savalvihi). The experimental design was a randomized complete block design (RCBD) with two replications. The accessions were planted at row to row plant spacing of 60 x 30 cm on ridges and normal management practices were followed. Observations were recorded on five randomly selected plants per replication and included days to 50% flowering (FPF); days to maturity (DM), plant height in cm (PH), head diameter in cm (HD), seed yield per hectare in kg (YPH), 100-seed weight (SW) and volume weight (VW) in g. Seed yield per plant was converted to seed yield ha⁻¹ by the formula:

Seed yield ha⁻¹ = Seed yield per plant x One hectare plant population.

Statistical analysis : The major descriptive statistics such as mean, range, standard deviation and coefficient of variation were computed using standard methods as described by Panse and Sukhatme (1964). Phenotypic correlation coefficients were calculated by the formula given by Johnson *et al.* (1955). Principal Component Analysis (PCA) was worked through correlation matrix. The Ward's method was used for clustering of genotypes into similarity groups. Cluster analysis and PCA were used to characterize germplasm and to group them based on their traits. R version 3.1.3 package was used for determining the diversity and PCA analysis (R Core Team, 2013). Command 'hclust' command was used to draw dendrogram. The biplot was generated by using the 'FactoMineR' (Factor analysis and data mining with R) package (Husson *et al.*, 2009) with command 'biplot'.

Results and Discussion

In any crop improvement programme, the main prerequisite for response to selection is the genetic variability in the population. The analysis of variance showed highly significant differences among the genotypes for all characters (Table 1). This indicated that germplasm under evaluation was genetically diverse and considerable amount of variability existed in the material, hence there is an opportunity for selection to develop new improved inbred lines. Earlier significant variation in sunflower inbreds was reported by Sujatha *et al.* (2002).

The wide range was exhibited for seed yield and the genotype GP₂1217 (1912 kg ha⁻¹) had the highest value while GP6714 (699 kg ha⁻¹) recorded the lowest. The high yielding check, DRSF-113 recorded 1547 kg ha⁻¹. The genotypes GMU601 (87 days) recorded the minimum, while two genotypes GP6951 and GP61227 (103 days) recorded the maximum days to maturity (Table 2). The short duration check variety, Morden also

Table 1 : Pooled ANOVA for seed yield and its component traits in sunflower

Traits	Mean sum of squares		
	Replications (d.f.=2)	Treatments (d.f.=31)	Error (d.f.=62)
Seed yield	140582.5	542002.8**	8094.596
50% percent flowering	101.5729	67.13441**	14.66969
Days to maturity	21.125	68.12601**	3.254032
Plant height	251.4254	1138.957**	104.3114
Head diameter	0.605	16.38278*	0.388871
100-Seed weight	1.088189	2.695874**	0.186122
Volume weight	5.917247	106.7118*	4.053228

**and *significant at 0.01 and 0.05 level of significance. d.f.= Degrees of freedom

recorded duration of 87 days. GP61227 recorded the maximum head diameter (17.3 cm) and 100-seed weight (5.8 g). Earlier Sudrik et al. (2014) reported similar results in sunflower.

The association of morphological traits was estimated by correlation analysis (Table 3). Character association indicated that among the seven agro-morphological traits, head diameter (0.82), 100-seed weight (0.8) and volume weight (0.42) had significant and positive correlations with seed yield ($P = 0.01$ level). Significant negative association of seed yield was observed with 50% flowering (-0.33) and days for maturity (-0.26). For the simultaneous improvement of traits in sunflower, Mogali and Virupakshappa (1994) studied the association of seed yield with yield related traits. Greater positive association observed for most of the characters with seed yield indicated that these

Table 2 : Mean of seed yield and its components of sunflower evaluated at 3 locations and 2 states

Entry	YPH (kg ha ⁻¹)	FPF	DM	PH (cm)	HD (cm)	SW (g)	VW (g)
GMU168	1419	56	89	147	14.2	4.4	41.3
GMU189	1863	65	97	145	16.4	5.0	40.6
GMU229	1445	59	90	102	14.8	4.5	38.4
GMU258	923	64	94	113	12.3	4.7	35.8
GMU440	1679	61	93	113	14.3	4.8	40.5
GMU601	1063	55	86	119	10.5	3.7	38.3
GMU616	1447	58	90	132	14.9	4.0	40.2
GMU713	1163	54	87	127	13.6	3.7	36.2
GMU776	1203	58	91	144	13.4	4.0	35.8
GMU753	1453	55	87	116	13.1	4.3	36.6
GMU786	1247	58	91	149	13.7	4.6	36.0
GMU787	1091	57	90	148	12.9	4.5	35.0
GMU799	1880	55	88	138	16.5	5.5	40.9
GMU798	1345	55	87	138	14.8	4.8	41.0
GMU834	1200	56	89	126	12.7	4.8	36.2
GMU889	1240	57	93	117	13.2	4.6	40.7
GMU897	1131	56	90	131	13.1	4.5	42.6
GP ₂ 211	1678	67	99	156	14.8	5.7	39.3
GP ₂ 271	1601	58	92	156	15.9	5.3	42.1
GP ₂ 286	1750	60	95	147	16.4	5.0	35.0
GP ₂ 571	1686	67	100	177	15.9	4.5	43.6
GP ₂ 644	822	67	99	167	11.9	3.3	36.4
GMU797	1235	53	87	119	11.3	4.4	38.8
GP ₂ 951	1116	70	103	165	11.6	4.2	37.3
GP ₂ 1217	1912	53	87	126	17.2	5.7	35.9
GP ₂ 1227	1006	70	103	174	12.1	3.6	36.4
GP ₂ 1475	901	67	99	135	10.9	3.4	38.5
GP ₂ 1334-3	967	69	99	127	14.1	3.0	32.9
GP ₂ 714	699	69	99	163	10.3	2.9	37.6
DRSF-113 ©	1547	58	90	155	13.6	5.2	41.8
Morden ©	1233	52	87	117	12.5	4.9	37.2
Bhanu ©	1717	55	90	130	12.6	5.3	38.9
Mean	1333	59.81	92.53	138.1	13.65	4.508	38.37
Min.	699	52.00	86.00	102.0	10.33	2.990	32.85
Max.	1912	70.00	103.00	177.0	17.23	5.790	43.55
SD	323.18	5.61	5.09	19.15	1.79	0.72	2.57
CV (%)	24.24	9.39	5.50	13.87	13.13	16.06	6.71
S.E. (±)	57.13	0.99	0.90	3.39	0.32	0.13	0.46

YPH : Yield per hectare, FPF : Days to 50 % flowering; DM: Days to maturity; PH : Plant height; HD : Head diameter, SW : 100-seed weight and VW : Volume weight

Table 3 : Phenotypic correlation coefficients for different traits in germplasm accessions from Telangana and Maharashtra

	FPF	DM	PH	HD	SW	VW
YPH	-0.33**	-0.26**	-0.05	0.82**	0.80**	0.42**
FPF		0.96**	0.56**	-0.17**	-0.44**	-0.13**
DM			0.63**	-0.13**	-0.36**	-0.09
PH				0.03	-0.12**	0.13**
HD					0.61**	0.27**
SW						0.36**

YPH : Seed yield per hectare; FPF : Days to 50 % flowering; DM : Days to maturity; PH : Plant height; HD : Head diameter; SW : 100-seed weight and VW : Volume weight

characters could be simultaneously improved and further recommend that increase in any one would lead to improvement of other characters.

Correlation with 50% flowering was significantly negative for head diameter (-0.17), 100-seed weight (-0.44) and volume weight (-0.13). Lack of strong association between these characters indicates that selection made for early types will not influence these characters. Perusal on correlation among component characters revealed that strong associations among desirable component characters exist particularly for head diameter, 100-seed weight and volume weight for the improvement of seed yield in sunflower. Hence, selection criteria based on these component traits along with seed yield will be more useful than based solely on seed yield. Simultaneously, improvement in seed yield along with other component traits in sunflower is important as reported earlier (Kaya *et al.*, 2009; Anandhan *et al.*, 2010).

The principal component analysis has practical application in selection of superior genotypes for breeding (Tabrizi *et al.*, 2011). The results of PCA revealed that the first four components with eigen value of greater than 0.43 contributed about 93.3% of total variability in 32 genotypes involving all the seven traits (Table 4). The importance of traits towards the principal components could be seen from the corresponding eigen values which are presented in Table 4. Eigen values are the variance explained by each PC and to repeat, are constrained to decrease monotonically from the first PC to the last. In this case it decreased from 3.27 (PC 1) to 0.02 (last PC). The eigen values plotted on a screen plot show a decreasing trend which explains

the variance by additional principal components (Legendre and Legendre, 1998). The eigen values and the variance associated with each principal component decreased gradually. The first principal component accounted for 46.7% of the total variation in the material studied. Hussain *et al.* (2017) on evaluation of sunflower hybrids assessed for yield related traits, the first two principal components contributed 73.7% variability among the hybrids while the remaining 26.3% variability was due to other components. Among the traits, seed yield (0.45) contributed maximum to the variation and divergence followed by head diameter, 100-seed weight, volume weight indicating the importance of these components as selection indices. Hussain *et al.* (2017) also reported greatest variation due to seed yield in PC 1 which is in agreement with the present study. Three characters viz., 50% flowering (-0.42), days to maturity (-0.403) and plant height (-0.23) showed negative contribution to PC 1 indicating that genotypes with negative values of PC 1 had reduced height, early flowering and maturity.

The PC 2 contributed 28.78% of the total variation and the characters whose contribution was negative in PC 2 showed negative associations among the traits. The PC 3 accounted for 11.56% of the total variation in the accessions studied. Among the traits that accounted for this variation head diameter contributed the highest (0.39) followed by seed yield (0.17) and days to maturity with minimal variation. The fourth principal component (PC 4) contributed 6.2% of the total variation. The characters that contributed to this variation include head diameter (0.58), seed yield (0.79), 100-seed weight (0.44) and days to maturity (0.28). The observations are in agreement with those of Tabrizi *et al.*, (2011) who evaluated the contribution of each trait to the total variation while evaluating sunflower hybrids at germination and early seedling growth stage.

A PC biplot (Fig. 1) showed that variables and genotypes are super imposed on the plot as vectors. The biplot showed that head diameter, 100-seed weight, volume weight and seed yield, as a whole contributed maximum towards the variability in the germplasm. For example, the accessions GP₆ 1217, 286, 271, 211, GMU799, 440, 189 and DRSF113 were scattered around the vectors of head diameter, 100-seed weight, volume weight and seed yield. Likewise, GP₆ 1227 and GP₆ 951 were scattered around the vectors for 50% flowering and days to maturity. This is explained through the mean values of these genotypes for seed yield and days to maturity (Table 2). Hence, genotypes GP₆ 1217, 286, 271, 211, GMU799, 440, 189 and DRSF113 can be

Table 4 : Principal components showing the eigen values and proportion of variation

	Principal component axis						
	1	2	3	4	5	6	7
Eigen values	3.27	2.01	0.80	0.43	0.33	0.10	0.02
Proportion of Variance (%)	46.731	28.787	11.568	6.268	0.047	0.017	0.003
Cumulative Variation (%)	46.73	75.51	87.08	93.34	98.11	99.65	100.00

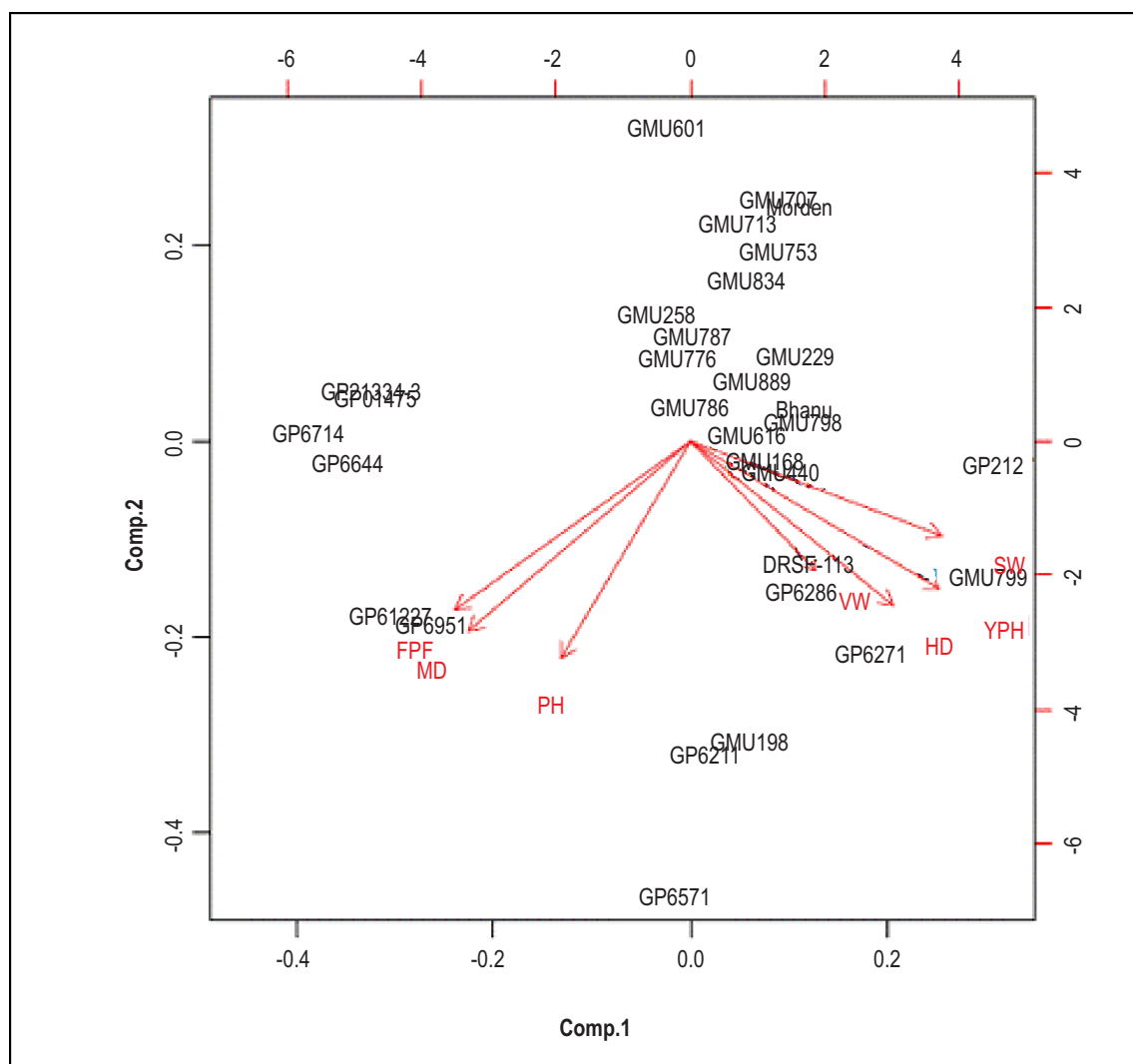


Fig. 1 : Biplot between PCs 1 and 2 showing contribution of various traits. YPH : Seed yield per hectare, FPF : Days to 50 % flowering; DM : Days to maturity; PH : Plant height; HD : Head diameter, SW : 100-seed weight and VW : Volume weight.

considered as high yielding and GP₁₂₂₇ and GP₉₅₁ can be regarded as long duration genotypes under semi-arid environments. For semi-arid environments, sunflower growing farmers prefer medium duration genotypes. Hence, selection for any of these traits should be accompanied by the associated traits, as it provides an opportunity to exert multi-trait selections in sunflower breeding as indicated by Ghaffari *et al.* (2011). Kroonenberg (1995) concluded that the angle of vectors shows correlations among the traits. In Fig. 1, there were some vectors for different traits, viz., head diameter, 100-seed weight, volume weight and seed yield which had a small angle with each other indicating the existence of positive associations among these traits. Likewise, plant height, days to 50% flowering, days to maturity showed positive correlations which are in agreement with the studies of Tabrizi *et al.* (2011) who recorded similar type of observations based on angle of vectors while evaluating

sunflower hybrids during germination and early seedling growth.

In the present study, the objective was to identify high yielding accessions through cluster analysis regardless of the trait expression. In this investigation, 32 genotypes were grown across three test sites and subjected to hierarchical clustering (HCA) and the dendrogram is presented (Fig. 2). The 32 genotypes were grouped into 11 clusters, out of which cluster IX was the largest having 6 genotypes indicating genetic similarity among them (Table 6, Fig. 2). Cluster VII is a solitary cluster containing only one genotype indicating the uniqueness of the genotype when compared to other genotypes. The GP lines used in this study were derived from systematic breeding for several generations for high yield under different genetic backgrounds. The same trend of pattern *i.e.* grouping of the germplasm and

Table 5 : Principal component analysis for sunflower accessions – non rotated loadings

Character	PC1	PC2	PC3	PC4
YPH	0.451	-0.342	0.178	0.798
FPF	-0.428	-0.391	0.135	0.381
DM	-0.403	-0.441	0.127	0.289
PH	-0.235	-0.503	-0.159	-0.808
HD	0.368	-0.381	0.390	0.586
SW	0.456	-0.219	-0.116	-0.762
VW	0.228	-0.300	-0.864	0.298

YPH : Seed yield per hectare; FPF: Days to 50 % flowering; DM : Days to maturity; PH : Plant height; HD : Head diameter; SW : 100-seed weight and VW : Volume weight

Table 6 : Constituents of 11 clusters of 32 sunflower accessions

Cluster	No. accessions	Accessions
Cluster I	3	GMU189, GMU799, GP21217
Cluster II	2	GP6271, DRSF-113 ©
Cluster III	5	GMU440, GP6211, GP6286, GP6571, Bhanu©
Cluster IV	2	GMU258, GP61475
Cluster V	2	GP61227, GP21334-3
Cluster VI	2	GP6644, GP6714
Cluster VII	1	GMU798
Cluster VIII	4	GMU168, GMU229, GMU616, GMU753
Cluster IX	6	GMU776, GMU786, GMU834, GMU889, GMU797, Morden©
Cluster X	2	GMU713, GMU897
Cluster XI	3	GMU601, GMU787, GP6951

Table 7 : Cluster means of 11 clusters of 32 sunflower accessions from Telangana and Maharashtra

	YPH	FPF	DM	PH	HD	SW	VW
Cluster I	1441.0	57.0	89.0	124.2	14.3	4.3	39.1
Cluster II	1188.6	56.0	89.0	132.3	13.3	4.2	36.0
Cluster III	1702.0	62.0	95.4	144.6	14.8	5.0	39.4
Cluster IV	1345.0	55.0	87.0	138.0	14.8	4.8	41.0
Cluster V	1100.2	59.5	92.2	140.7	12.0	4.2	38.3
Cluster VI	1574.0	58.0	91.0	155.5	14.8	5.2	41.9
Cluster VII	1885.0	57.6	90.6	136.3	16.7	5.4	39.1
Cluster VIII	949.2	67.5	98.7	137.2	12.4	3.7	35.8
Cluster IX	1238.7	55.0	89.5	125.5	12.7	4.6	38.1
Cluster X	699.0	69.0	99.0	163.0	10.3	2.9	37.6
Cluster XI	822.0	67.0	99.0	167.0	11.9	3.3	36.4

YPH: Seed yield per hectare; FPF : Days to 50 % flowering; DM : Days to maturity; PH: Plant height; HD : Head diameter; SW: 100-seed weight and VW: Volume weight

gene pool lines in different clusters can be seen in Fig. 2 viz., clusters V and VI comprised of GP lines whereas clusters VIII, IX and X comprised of GMU accessions. Hence, the genotypes within the same clusters may have originated from similar genetic

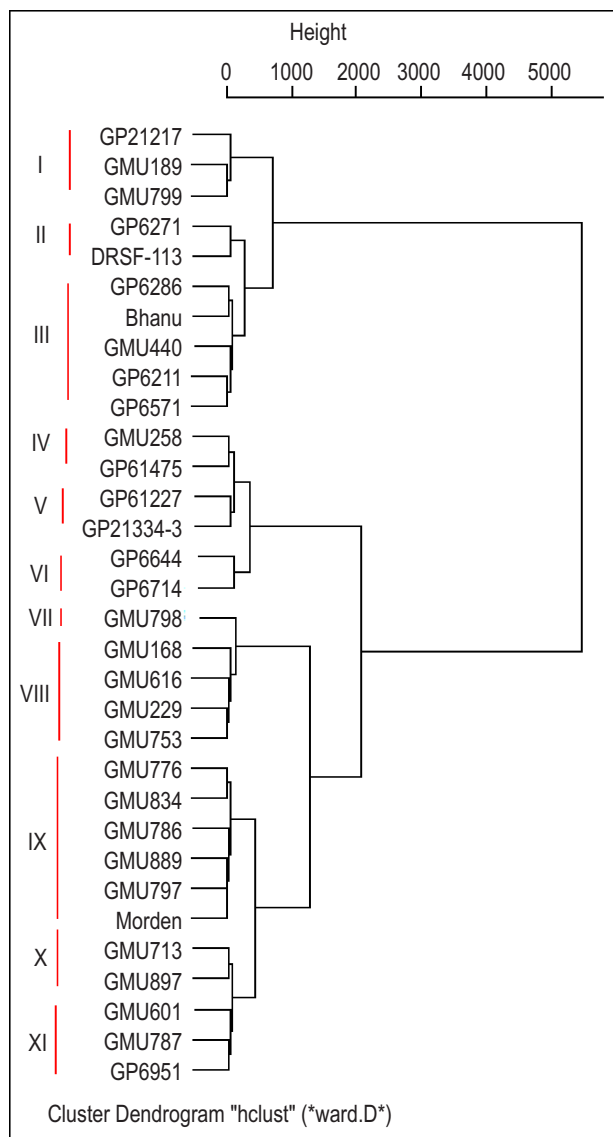


Fig. 2 : Dendrogram for 32 sunflower accessions produced by Ward's clusters analysis (scale: Squared Euclidean distance).

backgrounds. Reddy *et al.* (2012) grouped 64 sunflower accessions into nine clusters and observed that genotypes of different origin were clustered together, indicating absence of relationship between genetic and geographic diversity. Clusters II, I and III comprised of 2, 3 and 5 genotypes, respectively and based on their mean performance these genotypes were found to be high yielding with seed yield in the range of 1500-1900 kg ha⁻¹. Hence, these clusters can be considered as germplasm accessions with high seed yield potential and could be exploited in breeding programme. All the other germplasm accessions are moderate yielders and were grouped under IV to XI clusters. Srinivas *et al.* (2006) identified the high yielding breeding material based on cluster analysis which confirms the present study.

As shown in Table 7, the cluster VII recorded highest

mean values for seed yield (1885 kg ha⁻¹) whereas cluster X recorded the lowest cluster mean for 100-seed weight. The genotypes with lowest seed yield are included in cluster X as indicated by lowest cluster mean value of 699 kg ha⁻¹. Clusters X and XI exhibited highest cluster mean values for days to maturity (99.0) indicating the inclusion of late maturing accessions in that cluster. Genotypes in cluster VI recorded highest volume weight (41.9 g) while the least was recorded by cluster VIII (35.8 g). Earlier, sunflower genetic divergence among parental lines, inbreds and working germplasm was studied to group the germplasm accessions based on cluster means of yield and yield related traits (Ananda Kumar *et al.*, 2007; Reddy *et al.*, 2012). Based on the diversity recorded in seed yield and yield related traits, Reddy *et al.* (2012) emphasized the use of diverse parents in crossing programme for combining desirable genes and for obtaining novel and greater recombination.

It is concluded that substantial amount of variability is present among the accessions. The study led to identification of promising accessions across the diverse environmental conditions through multi-location evaluation. Eight promising accessions identified for high seed yield could be utilised for the development of high yielding genotypes as well as inbred lines for enhanced seed yield under semi-arid conditions.

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