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FULL-LENGTH RESEARCH ARTICLE

Genetic Variability, Diversity and Identification of Trait-Specific Accessions from the Conserved Sunflower Germplasm for Exploitation in the Breeding Programme

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Abstract The objective of the study is to characterize, evaluate and classify 2149 sunflower germplasm accessions during 2011–2012 and 2012–2013 for identification of trait-specific accessions. The analysis of variance revealed significant differences among the genotypes for almost all characters which favour the selection. High heritability associated with high genetic advance as per cent of mean (GAM) was recorded for days to maturity, indicating lesser environmental influence and role of additive gene action. Moderate heritability coupled with moderate-to-low GAM was observed for seed yield per plant, head diameter and oil content, suggesting the low existence of non-additive genes. Associations among characters exist especially for head diameter, 100-seed weight and plant height in the improvement in seed yield, whereas seed yield per plant had a negative correlation with oil content. Based on cluster analysis, 416 accessions grouped under cluster IV may be considered as the genotypes with high yielding ability along with high oil content and medium maturity. The first principal component accounted for 29.20% of the total variation in the population with more contribution from oil content, while second PC contributed 57.6% to days to maturity and 50% flowering contributed maximum. DUS characters such as pigmentation of seedlings, leaf petiole, disc and stem can be considered as morphological markers to differentiate the germplasm. Ray floret colouration, plant branching, type of branching and pollen colour characters can help the breeder to identify the specific germplasm. The identified trait-specific accessions will help in effective utilization of promising accessions in the breeding.

Keywords Sunflower · Germplasm · Trait-specific germplasm · Variability · Diversity

Introduction

Availability of appropriate genetic resources is a key to any crop improvement programme. The work on collection, evaluation and maintenance of sunflower germplasm was carried out at Germplasm Management Unit (GMU) located at ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad, India. The important objective of sunflower germplasm management unit is to augment, maintain and conserve the genetic variability and to make it available to various researchers. IIOR Gene Bank maintains 3273 sunflower accessions under the GMU. The collection includes exotic collection, augmented germplasm, inbreds, populations, genetic stocks, gene pools for high oil, yield and autogamy, back-cross-derived lines and wild species including their derivatives [18]. The area under sunflower crop in India is declining. The present productivity of sunflower in India is 752 kg/ha, and 4.15 lakh tons of sunflower was produced from 5.52 lakh ha [27].

Augmented block design is used, since large numbers of accessions are evaluated under field conditions. It is essentially important where initially limited seed is available to undertake replicated experiments [22]. This design

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is also used where comparably homogeneous experimental unit which is a basic requirement of field designs is not ensured. Characterization and evaluation of germplasm accessions are a regular activity carried out by germplasm curator to unravel the variability present among the germplasm and to identify the important trait-specific accessions for breeding programme. In any crop, precise phenotyping at field level not only helps to identify useful genes but also provides a material with specific traits with wider adaptability. In order to execute successful breeding programme, the total variability present in the available germplasm pool is important [80]. Heritability (H^2) measures the relative amount of the heritable portion of variability present in the population. It represents the ratio between genetic and all factors (including non-genetic factors) that influence the variability [9, 23]. The performance of the genotype depends upon the genetic potential and environment [14]. In plants, the expression of a character is the consequence of a series of interrelationships between each character, either directly or through other characters. The correlation between characters may be due to genetic linkage or may be due to pleiotropy [30]. Any kind of non-random segregation might cause temporary correlations [38]. Association studies help the breeder in selection and to understand the relations between heritable characters with important economic characters. Indirect selection using yield components may increase seed yield of sunflower crop [24].

In India, sunflower breeding objective is to develop hybrids or varieties with high yield, oil content and oil quality along with disease resistance [19]. To achieve this objective, the breeder has to identify genetically diverse parents with maximum variability for combining desirable characters. Diversity of sunflower germplasm is usually studied to determine the crop variability and to evaluate the existing germplasm for breeding programme or to detect needed variability for morphological and agronomic traits. Therefore, knowledge of the exiting genetic diversity in the germplasm is essential for undertaking recombination breeding [76]. The analysis of genetic diversity in germplasm collections helps the germplasm curator in classification of accessions and in the identification of core accessions or subsets for utilization in specific breeding programme [45]. Based on morphological, physiological and biochemical data, the genetic diversity of sunflower genotypes was estimated [17, 47, 57, 64].

Multivariate analysis refers to statistical technique that is widely used to analyse data which arise from more than one variable. Among the multivariate techniques, cluster analysis, principal component analysis (PCA), principal coordinate analysis (PCoA) and multidimensional scaling (MDS) are commonly employed techniques [23, 28, 41, 45]. Clustering is also used to summarize information on relationships between objects by grouping similar units so that the relationships may be easily understood and communicated. Cluster analysis is a multivariate analysis and widely used to describe genetic diversity based on similarities or differences among genotypes [51]. In the earlier studies, genetic divergence was estimated either in inbreds, parental lines or their hybrid combinations or among working germplasm [3, 5, 15, 20, 59, 61, 74]. As morphological and physiological variations routinely occur in crop species, PCA eliminates redundancy in data sets and gives the pattern of distribution [1]. Principal component analysis has been used for genetic diversity in sunflower [4, 16, 25, 42, 72, 75].

Presently, in India, the area under sunflower is reduced during the recent period. It may be due to the fact that unavailability of the superior genotypes can yield more than three tons/ha, price fluctuations, shift in cropping pattern, profitability of other crops compared to sunflower, withdrawal of private players from sunflower research, bird damage and menace of diseases such as alternaria, powdery mildew, sunflower necrosis and sunflower leaf curl virus disease. In this background, it is important to characterize, evaluate and investigate the variability present among the conserved germplasm. Hence, the objective of the study is to access the magnitude of heritability, coefficient of variation and association of different characters, to classify the 2149 sunflower accessions including three checks based on cluster analysis, to identify those traits which contribute to the major sources of variation within the sunflower germplasm through PCA and to evaluate genetic diversity present in germplasm based on morphological traits.

Materials and Methods

Experimental Material, Site and Design

The experiments were carried out during 2011–2012 and 2012–2013 cropping seasons at the ICAR-Indian Institute of Oilseeds Research, Hyderabad, India, with latitude of 17°22′31″N and longitude 78°28′27″E. This study was conducted to characterize 2146 sunflower germplasm accessions along with three checks, viz. DRSF-113, DRSF-108 and Bhanu which are total of 2149 accessions maintained at Germplasm Management Unit, IIOR, India. The description of the material along with checks is presented in Table 1. The evaluation and characterization of the accessions were carried out during late *kharif* in augmented block design. All the test accessions were divided into 74 blocks along with checks. The number of replications of checks used was 2; hence, the total number of experimental units was 2590. Each accession was grown in a single row

Table 1 Germplasm accessions used for characterization and evaluation

S. no.	Abbreviation	Description	Total no. of accessions
1	GMU	Germplasm management unit number	1055
2	DRSI	Directorate of oilseeds research inbred line	311
3	EC	Exotic collection from France	285
4	PS	Prebred material	166
5	GP	Gene pool material	329
	DRSF-113, 108 and Bhanu	Checks	3
		Total	2149

of 4 m length with a spacing of 60 cm × 30 cm. In each row, 13 plants were maintained. Observations were recorded from five randomly selected plants in each accession for seven quantitative characters such as days to 50% flowering (DF), days to maturity (MD), plant height (PH), head diameter (HD), 100-seed weight (SW) and seed yield per plant (Y/P). The germplasm accessions were characterized for 25 morphological descriptors as per the DUS traits of sunflower. The 25 descriptors are hypocotyl pigmentation, leaf size, leaf shape, leaf colour, leaf blistering, leaf serration, leaf base, orientation of leaf blade, leaf petiole pigmentation, stem pigmentation, ray floret number, ray floret shape, ray floret colour, disc floret colour, disc floret pigmentation, pollen colour, bract shape, bract pigmentation, head attitude, head shape, plant branching, type of branching, seed length, seed shape and seed stripes.

Statistical Analysis

By adopting the standard methods in the present investigation, the major descriptive statistics were worked out [49]. Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated as per the following formula [67].

$$\text{PCV } (\bar{x})\times = 100/\sigma_p$$
$$\text{GCV } (\bar{x})\times = 100/\sigma_g$$

where σ_p, σ_g and $\bar{x}$ are the phenotypic, genotypic standard deviation and grand mean of the traits, respectively.

Heritability in the broad sense (H^2) was estimated as described previously [2].

$$\text{Heritability } (H^2) = \sigma_g^2/\sigma_p^2$$

Expected genetic advance (GA) and percentage of GA were calculated previously [66]. Expected genetic advance (GA) = $i\ \sigma ph^2$

$$\text{GA } (\%) = (\text{GA}/\bar{x}) \times 100$$

where i is standardized selection differential, a constant (2.06), and σ_p phenotypic standard deviation.

Phenotypic correlation coefficients were calculated using the formula as suggested and described [33]. Cluster analysis (CA) was used to group the sunflower accessions based on their quantitative traits, and principal component analysis (PCA) was used to characterize germplasm. To identify the patterns of morphological variations and also importance of characters in each component, PCA was conducted through correlation matrix. Clustering of genotypes into similarity groups was performed using Ward's method based on squared Euclidean distances as a measure of similarity. Prior to squared Euclidean distance calculation, the data were normalized.

R version 3.1.3 package was used for determining the diversity and PCA analysis [56]. The biplot was generated by using the 'FactoMineR' (factor analysis and data mining with R) package [31] with function 'biplot'.

The germplasm was characterized using morpho-agronomic descriptors [53]. Frequency distributions for all the morphological traits were computed in excel.

Results

Variance Analysis, GCV, PCV, Heritability and Genetic Advance

The analysis of variance indicated a significant variation ($P < 0.05$), among blocks, treatments, test entries and checks versus test entry (Table 2), whereas highly significant differences among the test entries were observed for days to maturity, plant height, head diameter, 100-seed weight and seed yield per plant. For 50% flowering, a significant variation was present among the test entries. A significant variation was observed in checks versus test entry for seed yield per plant. A highly significant variation among the blocks was recorded. The block differences were higher for all the traits, which was due to incorporation of more number of treatments under evaluation. As the number of treatments was more, the number of blocks was also large. To compare the variation among various

Table 2 Analysis of variance for seven traits in 2149 accessions

Source	Blocks	Treatments	Checks	Test entry	Checks versus test	Error
Days to 50% flowering						
DF	73	2148	2	2145	1	3
SS	3831.416	46,909.89	120.33	46,600.76	188.79	45.00
MSS	52.48	21.83	60.16	21.72	188.79	15.00
F cal.	3.49**	1.4559	4.0111	1.4484*	12.5863	
Days to maturity						
DF	73	2148	2	2145	1	3
SS	5121.06	71,966.81	30.33	71,773.28	163.19	64.50
MSS	70.15	33.50	15.16	33.46	163.19	21.50
F cal.	3.26**	1.5583	0.7054	1.55**	7.5905	
Plant height						
DF	73	2148	2	2145	1	3
SS	40,830.49	1,773,248.43	1204.00	1,767,325.40	4719.03	517.50
MSS	559.32	825.53	602.00	823.92	4719.03	172.50
F cal.	3.24**	4.7857*	3.48	4.77**	27.35	
Head diameter						
DF	73	2148	2	2145	1	3
SS	534.20	22,399.40	4.00	22,378.24	17.15	6.00
MSS	7.31	10.42	2.00	10.43	17.15	2.00
F cal.	3.65**	5.21	1.00	5.2164**	8.57	
100-Seed weight						
DF	73	2148	2	2145	1	3
SS	261.72	5329.96	0.33	5318.99	10.62	1.00
MSS	3.58	2.48	0.16	2.47	10.62	0.33
F cal.	10.84**	7.44**	0.50	7.43**	31.88	
Seed yield/plant						
DF	73	2148	2	2145	1	3
SS	10,572.01	143,954.90	32.33	142,004.34	1918.22	22.50
MSS	144.82	67.01	16.16	66.20	1918.22	7.50
F cal.	19.30*	8.93**	2.15	8.82**	255.76*	
Oil content						
DF	73	2148	2	2145	1	3
SS	1096.58	11,003.32	4.33	10,968.92	30.06	4.50
MSS	9753.61	5.12	2.16	5.11	30.06	1.50
F cal.	6502.00	3.41	1.44	3.40**	20.04	

*, **Significant at 0.05 and 0.01 probability level

genotypes, phenotypic (PCV) and genotypic coefficient of variability (GCV), broad sense of heritability (H^2) and genetic advance were calculated (Table 3). The magnitude of PCV values for all the traits was marginally higher than the corresponding GCV values. Phenotypic coefficients of variability ranged from 7.0 to 72.5%, and the highest PCV was noticed for seed yield per plant and the lowest for plant height. The highest genotypic coefficient of variability was recorded for seed yield per plant (46.4), whereas the lowest GCV was recorded for plant height (2.1). Broad-sense heritability estimates were maximum for days to maturity (92.1), whereas they were moderate for days to 50% flowering (77.8) and oil content (55.8). Genetic advance as per cent of mean (GAM) was highest for seed yield per plant (61.2%) followed by days to maturity (38.3%), and the other traits showed a moderate-to-low genetic advance.

Association Between Seed Yield and Yield Components

The association between characters was estimated by correlation analysis (Table 4). Character association indicated

Table 3 Range of variation and estimates of genetic parameters for seed yield and yield components

S. no.	Character	Min	Max	σ_p^2	σ_g^2	PCV (%)	GCV (%)	H^2	GA	GAM (%)	Mean ± SE	CV (%)
1	Days to 50% flowering	44	75	22.2	17.3	8.1	7.1	77.8	7.5	13.0	57.9 ± 1.5	3.8
2	Plant height (cm)	54.2	207.4	39.7	3.7	7.0	2.1	9.4	1.2	1.3	88.8 ± 4.2	6.7
3	Days to maturity	74	105.0	785.1	723.2	20.2	19.3	92.1	53.1	38.3	138.6 ± 5.5	5.6
4	Head diameter (cm)	3.3	23.0	13.2	6.4	25.4	17.7	48.5	3.6	25.4	14.3 ± 1.8	18.2
5	100-Seed weight (g)	1.0	9.6	2.9	0.15	34.6	7.8	5.0	0.1	3.61	4.9 ± 1.1	33.7
6	Seed yield/plant (g)	1.5	39.1	88.3	36.2	72.5	46.4	40.9	7.9	61.2	12.9 ± 5.1	55.7
7	Oil content (%)	26.7	43	6.5	3.6	7.3	5.4	55.8	2.9	8.4	34.8 ± 1.2	4.8

σ_p^2 and σ_g^2 are phenotypic and genotypic variance, respectively

PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; H^2, broad-sense heritability; GA, genetic advance, GAM%, genetic advance as per cent of mean; SE, standard error; CV, coefficient of variation

Table 4 Phenotypic correlation coefficients for seven traits in 2149 sunflower accessions

Character	Days to 50% flowering	Plant height	Days to maturity	Head diameter	100-Seed weight	Seed yield/plant	Oil content
Days to 50% flowering	1.00	0.23**	0.88**	− 0.06**	− 0.16	0.01	− 0.04*
Plant height (cm)		1.00	0.21**	0.52**	0.25**	0.31**	− 0.05*
Days to maturity			1.00	− 0.08**	− 0.16**	− 0.08	− 0.03
Head diameter (cm)				1.00	0.39**	0.22**	− 0.10**
100-Seed weight (g)					1.00	0.16**	− 0.06**
Seed yield/plant (g)						1.00	− 0.29**
Oil content (%)							1.00

*, **Significant at 0.05, 0.01 probability level

that among the seven traits studied, seed yield per plant had a highly significant positive association with plant height (0.31), head diameter (0.22) and 100-seed weight (0.16) at $P = 0.01$. Plant height had significant positive correlations with days to maturity (0.21), head diameter (0.52) and 100-seed weight (0.31) and however a significant negative association with oil content. A significant negative association of days to 50% flowering was observed with head diameter (− 0.06) and oil content (− 0.04). Seed yield per plant and head diameter had a significant negative correlation with oil content. 100-seed weight had a significant positive correlation with seed yield per plant (0.16) and a significant negative correlation with oil content (− 0.06).

Clustering Pattern of the Sunflower Accessions

The cluster means for seven characters of 2149 entries including checks are presented in Table 5, and a large range of mean values among the 14 clusters was recorded for different traits. Cluster VI recorded the highest mean values for plant height (201.5), whereas the lowest cluster mean was recorded for 100-seed weight in cluster IX (3.7). The genotypes with the highest value for seed yield were recorded in cluster IV (24.2) and the lowest in cluster I (6.6). Clusters XII and VI exhibited the highest cluster mean values for days to maturity, i.e. 94 and 93 days, respectively, indicating the inclusion of late maturing accessions. Genotypes in cluster VI recorded the highest value for oil content (36.0), while the least was recorded by genotypes in the cluster II (33.0). The 2149 genotypes were grouped into 14 clusters, out of which the Ist cluster was the largest having 580 genotypes (Table 5). Similarly, cluster II is comprised of 529 genotypes, cluster IV with 416 genotypes and cluster III with 299 accessions, indicating genetic similarity among the genotypes. Clusters XII and XIV are solitary clusters containing one genotype each. Clusters V, VI, VII, VIII, IX, X, XI and XIII grouped 91, 160, 3, 48, 5, 2, 12 and 2 genotypes, respectively. The accessions grouped in each cluster are presented in Table 10 (Supplementary Table).

Principal Component Analysis

The PCA resulted in seven independent principal components (PCs) that had a cumulative explained variance of 100% (Table 6). The importance of a character towards the PC could be seen from the corresponding eigenvalues. The results of PCA revealed that the first four components with

Table 5 Grouping of 2149 genotypes and cluster means based on seven characters

Cluster no.	Number of genotypes	Characters						
		FPF	PH	MD	HD	SW	SY	OC
Cluster I	580	62.0	114.9	92.0	11.4	4.0	6.6	34.0
Cluster II	529	58.0	94.7	89.0	11.5	4.3	8.1	33.0
Cluster III	299	59.0	162.9	88.0	16.1	5.5	22.5	35.0
Cluster IV	416	57.0	146.0	88.0	15.0	5.2	24.2	35.0
Cluster V	91	57.0	128.0	87.0	14.1	4.9	8.8	35.0
Cluster VI	160	63.0	201.5	93.0	17.1	5.4	18.7	36.0
Cluster VII	3	61.0	189.5	92.0	13.7	4.6	12.1	35.0
Cluster VIII	48	57.0	125.0	87.0	14.2	4.8	22.3	35.0
Cluster IX	5	57.0	168.4	86.0	9.2	3.7	8.2	34.0
Cluster X	2	60.0	159.9	90.0	15.5	5.1	9.5	35.0
Cluster XI	12	61.0	176.0	90.0	15.8	5.2	13.8	35.0
Cluster XII	1	65.0	140.7	94.0	13.8	4.4	8.9	35.0
Cluster XIII	2	56.0	145.5	86.0	15.2	5.4	9.8	35.0
Cluster XIV	1	54.0	109.9	84.0	12.9	4.7	11.1	34.0

FPF, days to 50% flowering; PH, plant height; MD, days to maturity; HD, head diameter; SW, 100-seed weight; SY, seed yield per plant; OC, oil content

Table 6 Principal components showing the eigenvalues and proportion of variation

	Principal component axis						
	1	2	3	4	5	6	7
Eigen values	2.044	1.989	1.105	0.727	0.633	0.389	0.110
Proportion of variance (%)	29.20	28.44	15.78	10.39	9.00	0.05	0.01
Cumulative variation (%)	29.20	57.67	73.46	83.88	92.81	98.40	100.00

eigenvalue of greater than 0.72 accounted for about 83.8% of total variability in 2149 genotypes involving all the seven traits (Table 7). More than 75% of the variation observed is accountable by the first four PCs. The first principal component accounted for 29.20% of the total variation in the population. Oil content (0.19) contributed more to the variation in PC1, whereas characters such as 50% flowering (− 0.47), plant height (− 0.52), days to maturity (− 0.46), head diameter (− 0.34) and seed yield per plant (− 0.31) contributed negative to the first component (Table 7). The second principal component accounted for 57.6% of the total variation, and characters which contributed maximum variation to PC2 are days to maturity (0.48) and 50% cent flowering (0.47). The third principal component accounted for 73.4% of the total variation in the population. Oil content (0.76) contributed

Table 7 Principal component analysis for seed yield and yield components—non-rotated loadings

Character	PC1	PC2	PC3	PC4	PC5
FPF	− 0.47	0.47	− 0.12	0.12	0.71
PH	− 0.52	− 0.22	0.24	0.29	0.22
MD	− 0.46	0.48	− 0.13	− 0.10	0.13
HD	− 0.34	− 0.44	0.26	0.46	0.63
SW	− 0.17	− 0.44	0.23	− 0.60	− 0.58
SYP	− 0.31	− 0.27	− 0.47	0.54	− 0.49
OC	0.19	0.13	0.76	0.35	− 0.35

FPF, days to 50% flowering; PH, plant height; DM, days to maturity; HD, head diameter, SW, 100-seed weight; SY, seed yield per plant; OC, oil content

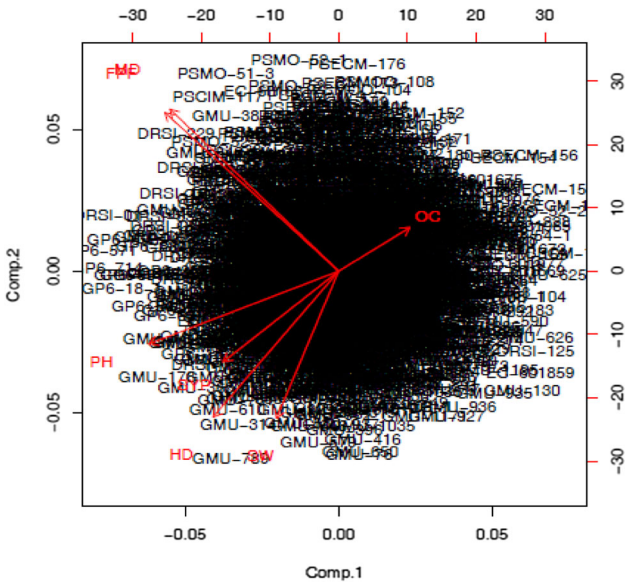

Fig. 1 Biplot between PCs 1 and 2 showing contribution of various traits to variation

maximum to PC3. Likewise, the fourth principal component accounted for 83.8% of the total variation. The major characters that contributed highly to the variation include seed yield (0.54) and head diameter (0.46), while 50% flowering (0.12) contributed least to the variation. The important consideration of the biplot diagram is the angles of vectors. In Fig. 1, four vectors of traits, viz. seed yield, head diameter, 100-seed weight and plant height, had a small angle with each other; similarly, two traits, i.e. 50% flowering and days to maturity, had small angle of vector. On the other hand, oil content had a completely opposite direction vector with all the traits.

Morphological Characterization as Per DUS Guidelines

Twenty-five DUS descriptors were used to characterize 2149 germplasm accessions and three checks. Morphological characterization of the sunflower germplasm revealed wide variability among the genotypes and for all the traits (Table 8). Hypocotyl pigmentation was recorded medium in 1037, while it was strong among 208 accessions. Medium leaf size (1364), medium leaf blistering (2009) and medium leaf serration (1570) were dominant among the accessions being characterized. Triangular leaf shape was observed in more than 50 per cent accessions (1222). Leaf petiole pigmentation was present in 285 accessions, while stem pigmentation was present in 20 accessions. Disc floret pigmentation was strong in 16 accessions. Six white pollen accessions were observed, whereas 2143 accessions had yellow pollen. Bract pigmentation was present in only two accessions. Plant

Table 8 Characterization of accessions based on DUS descriptors

Character	Frequency	Character	Frequency
1. Hypocotyl pigmentation		14. Disc floret colour	
(a) Absent	904	(a) Orange	1229
(b) Medium	1037	(b) Yellow	906
(c) Strong	208	(c) Purple	14
2. Leaf size		15. Disc floret pigmentation	
(a) Small	226	(a) Absent	2053
(b) Medium	1364	(b) Medium	80
(c) Large	559	(c) Strong	16
3. Leaf shape		16. Pollen colour	
(a) Lanceolate	194	(a) Yellow	2143
(b) Triangular	1222	(b) White	6
(c) Cordate	733	17. Bract shape	
4. Leaf colour		(a) Round	354
(a) Green	1981	(b) Elongated	1795
(b) Light green	165	18. Bract pigmentation	
5. Leaf blistering		(a) Absent	2147
(a) Absent	59	(b) Present	2
(b) Medium	2012	19. Head attitude	
(c) Strong	78	(a) Vertical	1154
6. Leaf serration		(b) Half-turned down	545
(a) Fine	278	(c) Turned down	50
(b) Medium	1573	20. Head shape	
(c) Coarse	298	(a) Flat	1641
7. Leaf base		(b) Concave	191
(a) Obtuse	593	(c) Convex	317
(b) Acute	1556	21. Plant branching	
8. Orientation of leaf blade		(a) Present	283
(a) Drooping	2073	(b) Absent	1866
(b) Erect	76	22. Type of branching	
9. Leaf petiole pigmentation		(a) Basal	74
(a) Present	285	(b) Overall	199
(b) Absent	1864	(c) Apical	16
10. Stem pigmentation		23. Seed length	
(a) Absent	2126	(a) Short	841
(b) Present	23	(b) Medium	1237
11. Ray floret number		(c) Long	71
(a) Few (< 30)	46	24. Seed shape	
(b) Medium (30–40)	1426	(a) Elongated	190
(c) Many (> 40)	677	(b) Ovoid elongated	1321

Table 8 continued

Character	Frequency	Character	Frequency
12. Ray floret shape		(c) Ovoid wide	638
(a) Ovate	699	25. Seed stripes	
(b) Elongated	1271	(a) Absent	489
(c) Round	179	(b) Present	1660
13. Ray floret colour			
(a) Orange	850		
(b) Yellow	1299		

branching was present in 283 accessions with overall branching as dominant (193). Seed length (> 1.5 cm) was long among 71 accessions, while seed stripes were absent among 489 accessions.

Frequency distribution of germplasm was performed for seed yield, oil content and days to maturity (Figs. 2, 3, 4). Out of 2149 accessions, 2095 accessions were identified as medium duration (75–100 days) and 54 (> 120 days) as late maturing; 360 accessions were identified as high yielding (25–35 g) and 62 accessions identified as very high yielding (> 35 g). Seed oil content was high (40–43%) in 16 genotypes. Limited accessions are available with high oil content, and hence, there is a need to generate variability through systematic breeding or by augmenting the high oil accessions. Germplasm accessions identified for very high seed yield include 62 accessions (> 35 g/plant), high seed oil per cent 16 accessions (40–43%), seed length long 71 accessions (> 1.5 cm) and very high 100-seed weight five accessions (> 9 g) which are represented in Table 9 and may be considered as superior trait-specific accessions. Promising trait-specific germplasm accessions can serve as a base material for the genetic improvement in sunflower for yield component traits.

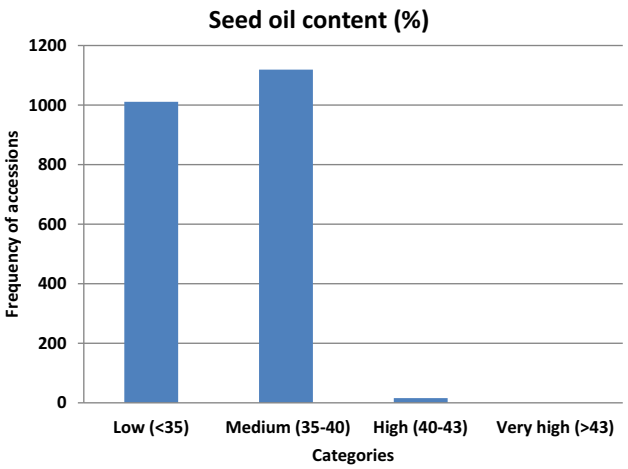

Fig. 2 Frequency distribution of 2149 sunflower accessions for seed oil content (%)

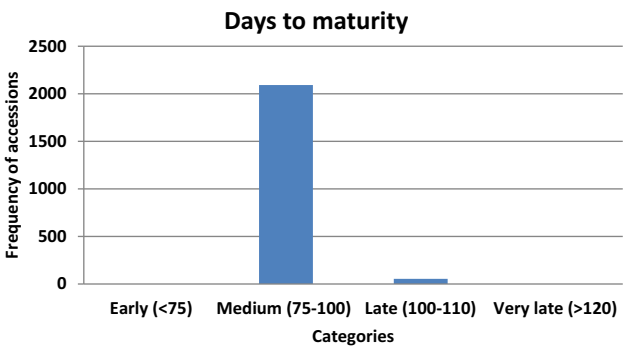

Fig. 3 Frequency distribution of 2149 sunflower accessions for days to maturity

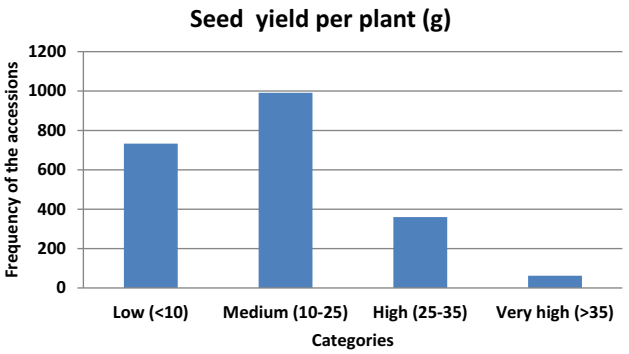

Fig. 4 Frequency distribution of 2149 sunflower accessions for seed yield per plant (g)

Discussion

Genetic Variability

When a large set of germplasm accessions are to be evaluated to select appropriate genotypes for specific breeding purposes, augmented block design [22] is a most preferred method for initial evaluation. Large number of test entries is evaluated along with standard checks, with the checks being replicated randomly in all blocks. The data from checks are used to adjust mean values of test entries to make them comparable and also to provide an estimate of experimental error [63]. The analysis of variance revealed significant differences among the 2149 genotypes for almost all the characters which may favour selection for further utilization in recombination breeding programmes.

Greater variability in the initial germplasm is a prerequisite which ensures better chances of producing desired material for future breeding programmes. Similarly, selection pressure is effective when magnitude of variability in the material is appropriate. Hence, to fasten the crop breeding programme the variation present in the native gene pool assumes prime importance. In the present

Table 9 Promising accessions identified for different characters of sunflower

Sr. no	Character	Best check performance	No. of accessions identified and per cent contribution	No. of accessions identified	Accessions
1	High seed yield (> 35 g/plant)	DRSF-113	62 (~ 3%)	62	GMU189, GMU211, GMU-335, GMU-383, GMU-385, GMU-405, GMU-409, GMU440, GMU 477GMU-520, GMU-540, GMU-598, GMU-635, GMU-736, GMU753, GMU786, GMU798, GMU-802, GMU-804, GMU-806, GMU-821, GMU 935, GMU-909, GMU1025, GMU1034, GMU1079, GMU1116, GMU1119, EC-601609, EC-601747, EC-601760, EC-601765, EC-601962, EC-602045, EC-602046, EC-623009, DRSI-1, DRSI-3, DRSI-9, DRSI-10, DRSI-100, DRSI-133, DRSI-144, DRSI-160, DRSI-182, DRSI-224, DRSI-256, DRSI-305, GP_2 745, GP21217, GP4745, GP4-2884, GP4-2902, GP4-2927, GP6211, GP6271, GP6286, GP_6 951, GP6-976, PSMO-53, PSCIM-181, PSCIM199
		35 g			
2	High oil content (40-43)	DRSF-113	16 (> 1%)	16	EC-601960, GP6-217, GP6-339, GP6-418, GP6-564, GP6-566, GP6-1035, GP6-1076, GP6-1088, GP6-1233, GP6-1407, GP6-1431, GP6-1436, GP4-392
		37%			
3.	Seed length long (> 1.5 cm)	DRSF-113	71 (~ 3%)	71	GMU-04, GMU-16, GMU-46, GMU-68, GMU-71, GMU-81, GMU-96, GMU-106, GMU-155, GMU-178, GMU-208, GMU-222, GMU-232, GMU-234, GMU-255, GMU-273, GMU-310, GMU-425, GMU-434, GMU-464, GMU-534, GMU-539, GMU-600, GMU-602, GMU-618, GMU-624, GMU-648, GMU-711, GMU-743, GMU-761, GMU-776, GMU-837, GMU-872, GMU-892, GMU-919, GMU-952, GMU-959, GMU-1054, GP6-18-1, GP6-211, GP6-217, GP6-356, GP6-358, GP6-714, GP6-832, GP6-1114, GP6-1227, GP6-1254, GP6-1350, GP6-1436, GP6-1550, DRSI-34, DRSI-63, DRSI-68, DRSI-77, DRSI-78, DRSI-81, DRSI-104, DRSI-112, DRSI-122, DRSI-151, DRSI-171, DRSI-172, DRSI-173, DRSI-232, DRSI-253, PSMO-51-1, PSMO-53-1, PSMO-55-1, PSECO-95, PSECO-97
		Medium			
4	Pollen colour (White)	DRSF-113	6 (> 1%)	6	GMU-370, GMU-511, GMU-647, GMU-702, GMU-888, PSMO-53-1
		Yellow			
5	100-Seed weight (very high > 9 g)	DRSF-113	5 (> 1%)	5	GMU-186, GMU-359, GMU-400, PSMO-113, PSMO-54
		6.2 g			
6	Type of branching (apical)	DRSF-113	16 (> 1%)	16	GMU-201, GMU-486, GMU-554, GMU-714, GMU-719, GMU-740, GMU-772, GMU-1089, GMU-1186, EC-601778, EC-601807, EC-601848, EC-601994, EC-602016, EC-602023, EC-602045
		No branching			
7	Stem pigmentation (present)	DRSF-113	23 (~ 1%)	23	GMU-70, GMU-327, GMU-328, GMU-334, GMU-411, GMU-479, GMU-596, GMU-616, GMU-637, GMU-726, GMU-772, GMU-841, GMU-905, GMU-1195, EC-601845, GP6-1554, GP4-459, DRSI-52, DRSI-107, DRSI-278, PSCRM-129, PSCIM-184-1, PSCIM-197
		No pigmentation			
8	Disc floret colour (purple)	DRSF-113	14 (> 1%)	14	GMU-104, GMU-126, GMU-130, GMU-479, GMU-817, GMU-851, GMU-1074, GMU-1104-1, EC-601654, DRSI-15, DRSI-107, DRSI-184, DRSI-229, PSCIM-184-1
		Yellow			

study, the phenotypic coefficient of variation values for all the characters were marginally higher than the corresponding genotypic coefficient of variation values, indicating the least influence of environment. Seed yield, 100-seed weight and plant height were mostly influenced by environment compared with the other characters. The GCV and PCV were high for seed yield, which indicated the presence of additive genes for this character [50, 70, 79]. The genotypic coefficient of variation is not always true to reflect the amount of actual variation which is heritable. The heritable variation cannot be estimated through genetic coefficient of variation [11]. Also the genotypic coefficient of variation along with heritability would give the reliable information on the magnitude of genetic advance to be expected from selection. The heritability in broad sense is described as the ratio of genotypic variance to the total variance in the non-segregating populations [29]. Further, it indicates whether there is sufficient genetic variation present in a population which will respond to selection pressure [43]. Selection of the genotype based on specific character with high broad-sense heritability will lead to faster and increased gains in the offspring than selecting for specific character with low heritability [10]. Broad-sense heritability estimate was maximum for days to maturity (92.1), while that for days to 50% flowering (77.8) and oil content (55.8) was moderate. Hence, higher heritability estimates for these traits indicated that environmental factors did not greatly affect phenotypic variation of these characters.

Heritability in broad sense includes both additive and non-additive gene action; hence, heritability values alone cannot provide any indication of the amount of progress that would result from selection. High heritability estimates together with highly expected genetic advance indicate an additive gene effect [33]. Therefore, in sunflower high heritability estimates in broad sense would be a reliable tool for selection if accompanied by high genetic advance as per cent of mean [20, 58, 65]. High heritability associated with high genetic advance as per cent of mean was recorded for days to maturity, indicating lesser environmental influence on days to maturity and a role of additive gene action. High heritability estimates for days to maturity were reported [3, 60]. Moderate heritability coupled with moderate-to-low GAM was noted for seed yield, head diameter and oil content, suggesting the non-additive gene action in the expression of these traits, and hence may be exploited better in recombination breeding. Earlier moderate heritability for oil content was reported in sunflower [24, 36]. In the present study, 100-seed weight and plant height recorded low heritability, low genetic advance and low expected genetic advance as per cent of mean. In contrast to our study, high heritability with low genetic advance for 100-seed weight, oil content, days to 50% flowering, days to maturity, volume weight and head diameter suggested that these characters cannot be effectively improved by selection [71]. Low expected genetic advance irrespective of high or low heritability leads to non-additive gene action [48], and the improvement in that trait by simple selection may not be rewarding. Earlier in sunflower many workers reported moderate heritability for seed yield per plant and number of seeds per head [26]. Non-additive gene action for days to maturity and oil content indicated the low GA and moderate heritability [6].

Association Analysis

Seed yield is a complex character which is influenced by a number of other component characters and greatly influenced by the environment. Correlation between seed yield and other component traits and among components helps in improving the selection efficiency. The association between characters also depends upon the association of loci or major QTLs, or group of QTLs governing variability for different characters located on same chromosome. A highly significant negative correlation was observed for oil content and seed yield. Oil content had a negative correlation for 100-seed weight and head diameter which indicated an increase in one character would lead to a decrease in another character. Similarly, oil content had a significant negative association with days to 50% flowering and plant height. Lack of strong association between these characters indicates that selection made for early types will not affect oil content. Correlation between seed yield and oil content was not significant in previous study [3], whereas weak correlation of seed yield with lateness was also reported [12]. Oil content was negatively associated with all growth and yield components in sunflower [50]. Correlation analysis indicated that strong associations among desirable components exist for head diameter, 100-seed weight and plant height for the improvement in seed yield in sunflower. The significant positive correlation observed between plant height and seed yield is justifiable. Many researchers reported a positive association of seed yield with head diameter, 100-seed weight and plant height [13, 34, 37, 52, 54, 78]. Generally, taller sunflower plant has many leaves which could increase total biomass production through increased carbon fixation and ultimately yield to reproductive organ formation. Based on these findings, a high yielding ideal sunflower plant is tall and is capable of maintaining maximum leaf area and bear large head to hold as many filled seeds as possible. Hence, selection criteria based on these component traits along with seed yield will be more useful than simply based on seed yield.

Cluster Analysis

The present study focuses on the identification of accessions with high yield coupled with high oil through cluster analysis. The 2149 genotypes grown in two seasons were subjected to hierarchical clustering (HCA). Cluster analysis is a statistical method which is used to identify groups or clusters of similar items in multidimensional space which is based on similarity between items. Based on cluster means, a wide range of genetic variation for seed yield per plant and its components was observed in the sunflower accessions. Based on cluster mean values, it is always rewarding to select genotypes with more than one desirable trait and however belonging to different clusters. Therefore, it is suggested that 416 accessions grouped under cluster IV can be considered as the genotypes with high yielding ability along with high oil content and medium maturity. These genotypes can serve as the genetic base for development of high yielding and high oil content varieties to develop inbred lines which can be subsequently utilized in the crossing programme, whereas one genotype, i.e. grouped under Cluster XV, can be considered as early maturing genotype based on maturity. If the breeding objective is to increase seed weight, the genotypes grouped under clusters III and VI can serve as a base material to initiate the breeding. Genotypes that are grouped under cluster VI can serve as donors for high oil content. The well-known fact is the use of genetically diverse parents in hybrid programme, to have better chances of obtaining high heterotic hybrids and broad spectrum of variability in the segregating generations [46]. It has been observed that the most productive hybrids may come from high yielding parents with high genetic diversity. Based on cluster mean values for a given character, we can select highly divergent genotypes from the respective clusters to be used in crossing work. Earlier 177 sunflower genotypes were classified into ten clusters based on morphological and agronomic traits based on the standardized Euclidean distance measure [8]. Diversity for 178 genotypes using hierarchical cluster analysis with Euclidean distance was calculated and identified lines with high oil content [40]. Unique clusters which were having only a single genotype were reported with specific trait and genotype [74].

Principal Component Analysis

Principal component analysis (PCA) was followed to determine the traits that contribute to the major sources of variation within the germplasm panel. The in-depth information about PCA analysis has been given [68, 81]. PCA is one of the most useful methods used in taxonomic research since the degree of relatedness among individuals between or among genotypes or within a breeding population or within species can be studied with principal components. PCA can differentiate the groups of morphologically similar phenotypes when plotted against principal components. PCA is used in multivariate analysis to derive statistical inference, with a large number of independent variables with a number of dependent outcomes without enough observations for the analysis. This technique is used for genetic diversity analysis and grouping of genotypes through biplot diagram [72]. It identifies the traits which contribute maximum to the most observed variation within a large group of genotypes and employed to reduce the complexity of the data while minimizing the variation within the data and increasing interpretability [62]. In PCA, the variance explained by each principal component is depicted by the eigenvalues and decreases monotonically from the first principal component to the last. In this study, the eigenvalues and variance associated with each principal component decreased gradually. Oil content contributed higher to the variation and had the highest loading in PC1, indicating the significant importance of the trait for this component. Five characters, viz. 50% flowering, plant height, days to maturity, head diameter and seed yield, contributed negative to the first component and showed a negative association, which implies that genotypes with negative values of PC1 have reduced height, less seed yield, early flowering and maturity. The traits which accounted for maximum variation of the total variation in second component, i.e. PC2, are days to maturity and days to 50% flowering. It is generally assumed that in sunflower, genotypes with reduced height flower early and produce less seed yield than the taller genotypes. Seed yield and oil content had a negative association in sunflower. Oil content contributed the most to PC3, while seed yield accounted for the most negative value. Earlier the characters which contributed to variability in PC1 were reported for days to flower initiation, full flowering days and plant height, while studying sunflower hybrids and inbred lines [7].

A PC biplot in Fig. 1 showed that variables and genotypes are superimposed on the plot as vectors. The distance of each variable with respect to PC1 and PC2 showed the contribution of these variables in the variation of the genotypes used. Genotypes scattered around the vectors in the biplot diagram lead to distinct groups of genotypes, which, however, failed be detect individual accession due to more number of genotypes. The angle of vectors shows correlations with vectors and therefore, among the traits [35]. Seed yield, head diameter, 100-seed weight and plant height had a small angle with each other, which indicates that they had positive correlations. Similarly, 50% flowering and days to maturity had positive correlations. On the other hand, oil content had a completely opposite direction vector with all the traits, indicating a negative correlation. Therefore, the smaller angle among vectors indicates the

greater positive correlation among related traits and vice versa. Earlier in sunflower, [16] used PCA based on specific combining ability to show the structure of sunflower populations by country of origin. [16] used PCA for revealing two-dimensional structures among genotypes and their environments based on the interactions. They reported the effectiveness of PCA for revealing genotype × environment interactions. Previous researcher based on the biplot diagram reported three distinct groups of the sunflower hybrids and identified the best performing hybrids [72]. The use of principal component analysis method for selection of superior three-way cross hybrids in sunflower reported [25].

Morphological Characterization

Plant breeders are interested to understand genetic diversity in germplasm based on quantitative and morphological traits. Evaluation of morphological traits does not require sophisticated techniques and expensive equipments. Based on DUS characters among 2149 accessions, a wide range of variation for various traits is indicated. The results were in conformity to previous report. Earlier report indicated wide variation for all the characters among sunflower accessions [39]. Morphological variation for studied characters in sunflower varied among the genotypes [73]. In the present study, 208 accessions were identified with strong hypocotyl pigmentation at seedling stage which can serve as morphological markers to identify the accessions. The 285 accessions for leaf petiole pigmentation, 20 accessions with stem pigmentation and 20 and 16 accessions with disc floret pigmentation can be used to identify the accessions as well as to differentiate with other accessions. Most of the accessions showed triangular leaf shape. Seed stripes are not preferred by Indian farmer. Black colour seeds without stripes are favoured due to attractive and shiny look, and 489 accessions were identified with the absence of stripes. Earlier in sunflower, [44, 69, 79] have reported the frequent occurrence of cordate leaf shape in the studies. Previously dominant character for leaf was identified as medium green; anthocyanin pigmentation was absent in most of the accessions on stem and seedlings, leaf size was medium, and no branching pattern was observed for more than 80% accessions [44]. Light green leaf colour, convex head shape and black seed colour accessions as dominant were identified in sunflower [69]. They also recorded medium serration, yellow ray floret colour and maximum number of accessions as non-branching accessions which are in agreement with the present study. Categorization of the 3126 sunflower accessions as per DUS guidelines was published and identified trait-specific accessions [21]. Earlier [55] characterized the sunflower accessions as per the UPOV guidelines and germplasm database of the IPGRI to conduct DUS test [32, 77]. Their results indicated that sunflower accessions have higher variability based on the morphological characters.

Conclusions

While selecting appropriate sunflower germplasm, the breeder looks for genetically diverse and superior genotypes which could be utilized in population and heterosis breeding. The present study exhibited very high differences among the genotypes for seed yield almost all yield component characters which may favour the selection and its further utilization in recombination breeding programmes. The genetically diverse sunflower germplasm identified could be utilized in development of diverse inbreds which may be utilized in heterosis breeding. Promising trait-specific superior sunflower germplasm accessions identified will serve as donors for the development of trait-specific heterotic gene pools which can be further exploited in sunflower improvement, for seed yield, oil content and plant type besides biotic and abiotic resistance under diverse agro ecological situations.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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