



Exploring Genetic Variability and Character Association for Morphological and Yield Traits in Sunflower (*Helianthus annuus* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Investigation of genetic variation and character association has huge potential for the improvement of sunflower. The present study was carried out with 75 diverse inbred lines evaluated for nine yield and their component traits. The presence of significant effects for checks vs varieties was observed for evaluated traits indicating the significant differences between varieties and checks. Based on the least significant increase value comparison over checks and ordination plots developed for seed yield per plant and oil content showed that the genotypes NDI 2, NDI 47, R 64, RHA 1096, NDLB 2, NDI 33, NDI 27 and RHA 172 were promising and could be used for the development of hybrids

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with high yielding and oil content in sunflower. The correlation analysis in combination with the principal component analysis revealed that the traits seed yield per plant and hundred seed weight explain most of the variation among the evaluated traits.

Keywords: Sunflower; augmented design; least significant increase; seed yield per plant.

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the fourth most important oilseed crop after soybean, mustard and groundnut. The global sunflower oilseed production during the year 2023-24 is 51.78 m tonnes (<https://fas.usda.gov/home>). The major sunflower growing countries are Russia, Ukraine, European Union, Argentina and Turkey in the world (<https://fas.usda.gov/home>). The major advantageous characteristics for the cultivation of this crop include photo-insensitivity, thermos-insensitivity, short growth cycle, high yield and superior oil quality. Sunflower oil is a rich source of fatty acids including linoleic acid, oleic acid, palmitic acid and stearic acid (Avni et al., 2016). Further, the sunflower extracts are rich in various bioactive compounds and demonstrate potential applications in the food and pharmaceutical industries. The sunflower meal is a main by-product of sunflower oil extraction and is a valuable protein source for human consumption and animal feed (Vijayakumar et al., 2016).

In India, the early introduced varieties gave a satisfactory performance and then a gradual decrease in yield and oil content due to inherent self-incompatibility (Gandhi et al., 2005). This leads to the shift in the development and cultivation of high yielding hybrids. The success of a hybrid development programme mainly involves the exploitation of a wide range of inbred lines. The selection of inbred lines for different traits to generate potential hybrids requires knowledge of genetics and plant breeding methodologies (Antony et al., 2024). The sunflower hybrids generally outperform open-pollinated varieties, and breeding programs must focus on developing these hybrids through careful selection of inbred lines from a broad genetic base (Tyagi et al., 2018). Further, the identified high seed and oil-yielding inbred lines enable the development of hybrids with high market value (Cvjic et al., 2023). This approach will ensure the long-term success of sunflower breeding.

The understanding of the nature and degree of the link between any two pairs of metric features

is critical for a successful crop improvement programme. From this, it would be possible to bring about genetic enhancement in commercially important characteristics like seed yield through indirect selection of component traits (Gangavati and Kulkarni 2021). The principal component analysis is one of the multivariate techniques that enables the identification of the most contributing variation of traits among the evaluated traits (Reddy et al., 2024). Further, the relationships between yield and their related traits will improve the efficiency of the breeding program by determining appropriate selection criteria (Darvishzadeh et al., 2011). In sunflower, traits like seed yield per plant, hundred seed weight, head diameter and oil content will determine the performance of the hybrid (Sipio et al., 2024). Thus, the present study was designed to assess the variability, character association and identification of genotypes that surpass the check varieties using 75 diverse inbred lines in sunflower.

2. MATERIALS AND METHODS

The experimental material for the present study consists of 75 diverse inbred lines along with four checks namely ARM 243B, CMS 17B, LTRR 341 and RHA 95C-1. All the lines were evaluated in augmented block design in five blocks during Rabi, 2022-23 at Acharya N G Ranga Agricultural University, Regional Agricultural Research Station, Nandyal, India, geographically located at 15°29' north latitude and 78°29' east longitude at an altitude of 211.76 m above mean sea level. In each block, 15 lines were planted randomly along with four checks. Each line was sown in two rows of three-meter length by 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 followed during the crop growth period.

The data recorded from each inbred line for nine quantitative traits viz., days to 50% flowering, days to maturity, plant height (cm), head diameter (cm), hundred seed weight (g), seed yield per plant (g), oil content (%), seed yield (kg ha⁻¹) and oil yield (kg ha⁻¹). The analysis of

variance for augmented design was carried as per the method suggested by Federer (1961). The linear model used was

$$Y_{ij}(k) = \mu + \beta_i + C_j + \tau_k(i) + \varepsilon_{ij}$$

Where $Y_{ij}(k)$ is the value of j^{th} check or k^{th} new entry in i^{th} block, μ is the overall mean, β_i is the

effect of i^{th} block, C_j is the effect of j^{th} check treatment, $\tau_k(i)$ is the effect of the k^{th} new entry in the i^{th} block and ε_{ij} is the random error.

Further, the least significant increase (LSI) values were computed based on the estimation of standard errors (SE) as follows:

List 1. The least significant increase (LSI) values computed based on the estimation of standard errors

Between two check varieties	$SE(1) = \sqrt{\frac{2MSE}{r}}$
Between adjusted means of two test entries in the same block	$SE(2) = \sqrt{2MSE}$
Between adjusted means of two test entries in the different blocks	$SE(3) = \sqrt{2MSE(1 + \frac{1}{c})}$
Between adjusted test entry and check mean	$SE(4) = \sqrt{MSE(1 + \frac{1}{r} + \frac{1}{c} + \frac{1}{cr})}$
Least significant increase	$LSI = t_{\alpha}.SE(4)$

Where MSE is the mean square error, r is the number of blocks, c is checks, t_{α} is the critical t value for a given confidence level.

The ordination plots for the categorization of genotypes based on seed yield per plant and oil content were developed with the MS-EXCEL program. The correlation and principal component analysis (PCA) were carried out using STAR (Statistical Tool for Agricultural Research) 2.1.0 software as suggested by Reddy et al., (2023).

3. RESULTS AND DISCUSSION

The analysis of variance revealed the significant mean sum of squares for all the traits for different sources of variation (Table 1). The block effects (unadjusted) and treatment effects (adjusted and unadjusted) were significant for all the studied traits. Similarly, effects due to checks and varieties were also significant. However, the adjusted block effects were non-significant for all the traits except plant height indicating the homogeneity for evaluation of blocks. Further, the effects due to checks vs varieties were significant for all traits indicating the presence of significant differences between the varieties and checks. Dudhe et al., (2019) employed the augmented design to study the variation in 2149 sunflower germplasm accessions and reported the significant differences among the genotypes for yield and its related traits. Thus, augmented design is the preferred method for the initial evaluation of a large number of germplasm accessions and the identification of appropriate genotypes for specific breeding purposes (Sinana et al., 2023).

The estimation of mean, standard deviation, minimum and maximum values for nine yield and their related traits were studied to assess the variability among 79 sunflower genotypes (Table 2). The seed yield per plant ranges from 11.05 g to 38.87 g with a mean value of 27.85 g. Whereas, the oil content ranges from 27.44 to 38.59% with a mean value of 33.62%. The mean seed yield and oil yield were found to be 1546.81 kg ha⁻¹ and 517.58 kg ha⁻¹, respectively. The presence of significant variability in the germplasm pool is a prerequisite for future breeding programmes to develop the high-yielding sunflower hybrids. Further, the standard error of difference was calculated for all the traits to compare the adjusted means of tested genotypes in the same as well as different blocks along with checks. The least significant increase value was calculated to identify tested genotypes significantly superior to the checks. The study categorised the tested genotypes as significantly superior to the number of checks for nine yield and their related traits. Further, the number of genotypes 12 (days to 50% flowering), 22 (days to maturity), 30 (plant height), 21 (head diameter), 61 (hundred seed weight), 51 (seed yield per plant), 36 (oil content), 51 (seed yield) and 52 (oil yield) were found to be superior compared to the one or checks.

Table 1. Analysis of variance for Augmented design with one way elimination of heterogeneity

Source of variation	df	Mean sum of squares								
		DFF	DM	PH	HD	HSW	SYPP	OC	SY	OY
Blocks (ignoring treatments)	4	570.16**	1457.36**	3364.91**	28.89**	7.57**	151.86**	200.02**	468929.71**	47650.28**
Treatments (eliminating blocks)	77	8.08**	6.24**	720.71**	6.88**	1.94**	67.35**	11.74**	207784.06**	22390.57**
Blocks (eliminating treatments)	4	2.60	1.14	10.67*	0.19	0.24	10.07	0.45	31128.41	4944.34
Treatments (Ignoring blocks)	77	37.57**	81.89**	894.96**	8.37**	2.33**	74.72**	22.10**	230526.98**	24609.06**
Checks	3	18.91**	21.69**	1852.04**	1.06**	3.42**	604.95**	31.21**	1866966.68**	159633.52**
Varieties	73	7.78**	5.65**	620.11**	7.93**	1.15**	39.89**	11.15**	123135.42**	14983.31**
Varieties + Checks vs. Varieties	74	7.64**	5.62**	674.85**	7.11**	1.88**	45.56**	10.95**	140519.90**	16826.67**
Checks Vs Varieties	1	2267.91**	5828.14**	18088.12**	62.17**	84.60**	1025.62**	794.53**	3160791.96**	322215.43**
Error	12	1.25	5.077	2.54	0.147	0.160	6.223	0.264	19206.05	1839.28

*Significant at 5%, ** Significant at 1%

DFF – Days to 50 percent flowering, DM – Days to maturity, PH – Plant Height (cm), HD – Head diameter (cm), HSW – Hundred seed weight (g), SYPP – Seed yield per plant (g), OC – Oil content (%), SY – Seed yield (kg ha⁻¹), OY – Oil yield (kg ha⁻¹), d.f – Degrees of freedom**Table 2. Estimation of descriptive statistics for nine yield and its related traits in 79 sunflower genotypes**

Traits	Mean	SD	Minimum	Maximum	SE (1)	SE (2)	SE (3)	SE (4)	LSI
DFF	58.64	2.82	52.00	65.00	1.54	3.44	3.85	2.98	5.31
DM	93.65	2.82	87.00	100.00	0.89	2.01	2.24	1.74	3.10
PH	116.28	25.07	59.80	159.40	2.19	4.91	5.49	4.25	7.57
HD	9.71	2.73	4.80	18.40	0.52	1.18	1.32	1.02	1.82
HSW	6.36	1.14	2.42	8.40	0.55	1.23	1.38	1.07	1.91
SYPP	27.85	6.66	11.05	38.87	3.43	7.69	8.59	6.66	11.87
OC	33.62	3.30	27.44	38.59	0.71	1.58	1.77	1.37	2.44
SY	1546.81	369.91	613.83	2159.23	190.97	427.03	477.43	369.82	659.02
OY	517.58	125.83	222.45	797.37	59.09	132.15	147.75	114.44	203.93

DFF – Days to 50 percent flowering, DM – Days to maturity, PH – Plant Height (cm), HD – Head diameter (cm), HSW – Hundred seed weight (g), SYPP – Seed yield per plant (g), OC – Oil content (%), SY – Seed yield (kg ha⁻¹), OY – Oil yield (kg ha⁻¹), SD – Standard deviation, SE – Standard Error, LSI – Least significant increase

Table 3. Categorisation of superior genotypes over checks based on the least significant increase value for major important traits

Traits	Superiority over one check	Superiority over two checks	Superiority over three checks	Superiority over four checks
HSW	NDI 18, NDI 31, NDI 53, NDI 58, PM 81, R 102	CMS 302B, CMS 335B, DRM 342, LDMO 2, NDI 26, NDI 33, NDI 40, NDI 41, NDI 54, NDI 57, R 106, RCR 114, RCR 76, RHA 1096, RHA 859, TX 16R	150R, NDI 17, NDI 19, NDI 20, NDI 21, NDI 22, NDI 25, NDI 27, NDI 36, NDI 39, NDI 43, NDI 47, NDI 49, NDI 50, NDI 51, NDI 52, NDI 56, NDI 59, NDLB 2, NDLB 6, NDLB 7, NLR 2, R 853, RCR 21, RCR 33, RHA 1013, RHA 1028, RHA 1051, RHA 1186, RHA 172, RHA 272-1	NDI 23, NDI 28, NDI 30, NDI 45, NDI 55, R 45, RCR 1096, RCR 7
SYPP	DRM 342, NDI 31, NDI 37, NDI 40, NDI 43, NDI 52, NDI 53, RCR 39	150R, LDMO 2, NDI 18, NDI 19, NDI 20, NDI 22, NDI 23, NDI 24, NDI 26, NDI 28, NDI 29, NDI 30, NDI 34, NDI 36, NDI 38, NDI 41, NDI 42, NDI 50, NDI 55, NDLB 6, NDLB 7, NRL 2, PM 81, R 102, R 106, R 45, RCR 33, RCR 76, RHA 272-1, TX 16R	NDI 2, NDI 21, NDI 25, NDI 27, NDI 33, NDI 45, NDI 47, NDLB 2, R 64, RCR 114, RHA 1013, RHA 1096, RHA 172	Nil
OC	CMS 302B, DRM 342, NDI 17, NDI 18, NDI 22, NDI 27, NDI 33, NDI 39, NDI 47, NDI 50, NDI 53, NDI 58, NDLB 2, NDLB 6, PM 81, R 64, RCR 7, RHA 1-1, RHA 6D-1, RHA 1051, RHA 1096, RHA 1163, RHA 172, RHA 272-1	NDI 20, NDI 23, NDI 38, NDI 43, NDI 49, NDI 54, NDLB 7, R 102, RCR 1096, RCR 39, RHA 1186, RHA 859	Nil	Nil

HSW – Hundred seed weight (g), SYPP – Seed yield per plant (g), OC – Oil content (%)

The traits of seed yield per plant and oil content are economically important, which will be the key for research and farmer's point of view on sunflower crop. Interestingly, none of the genotype—is significantly superior over four checks for the traits seed yield per plant and oil content (Table 3). Further, none of the genotypes were significantly superior for the trait oil content over three checks. The genotypes significantly superior over three checks for seed yield per plant were NDI 2, NDI 21, NDI 25, NDI 27, NDI 33, NDI 45, NDI 47, NDLB 2, R 64, RCR 114, RHA 1013, RHA 1096 and RHA 172. The approach of identifying the genotypes that surpassed the best checks based on the least increase value comparison was earlier used in common bean (Saba et al., 2017), rice (Hasan et al., 2020) and wheat (Dukamo et al., 2023). The study showed that the check entry CMS 17B superior for all the traits except days to 50% flowering, days to maturity and oil content. The check entry ARM 243B found superior for the trait oil content. Whereas, the check entry RHA 95C-1 is superior for days to 50% flowering and days to maturity traits. The lines CMS 17B (Singh and Kumar 2018) and RHA 95C-1 (Meena et al., 2013) are good general combiners for yield contributing traits and are being used for the development of hybrids suitable for various agro-climatic zones in India.

The ordination plots were developed to identify the genotypes superior for both seed yield per plant and oil content traits (Fig. 1). This technique categorized the genotypes into four groups based on seed yield per plant and oil content. The genotypes NDI 2, NDI 47, R 64, RHA 1096, NDLB 2, NDI 33, NDI 27, RHA 172, NDLB 7, NDI 23, NDI 22, NDI 18, NDLB 6, NDI

42, NDI 26, RCR 76, RHA 272-1 and NDI 50 are classified in high seed yield per plant and high oil content group. Further, the genotypes RCR 21, NDI 48, CMS 335B, NDI 57, R 853, NDI 51, RHA 1028, NDI 56, RHA 271, NDI 59, NDI 37, NDI 52, NDI 31, LDMO 2 and NDI 40 were categorised in low seed yield per plant and low oil content group. However, based on both supremacy over checks and ordinations plots, the genotypes NDI 2, NDI 47, R 64, RHA 1096, NDLB 2, NDI 33, NDI 27 and RHA 172 are superior for improvement of seed yield and oil content in sunflower breeding programmes. The selected lines with high seed yield per plant and oil content are very useful for future breeding programmes to develop desirable segregants or parental lines for hybrid development in sunflower (Seneviratne et al., 2004, Dudhe et al., 2021, Mariyam et al., 2024).

Pearson's correlation coefficients between nine quantitative traits showed a significant and positive correlation between seed yield (kg ha^{-1}) with hundred seed weight ($r = 0.358$) and seed yield per plant ($r = 1.000$) (Table 4). The trait oil yield also showed a significant and positive correlation with hundred seed weight ($r = 0.308$), seed yield per plant ($r = 0.923$) and seed yield (kg ha^{-1}) ($r = 0.923$). Further, no significant and negative correlations were observed between the tested traits in the study. The principal component analysis was carried out to identify the most contributing traits of variation among the nine quantitative traits. The scree plot of sunflower genotypes revealed the Eigen value of more than one for the first four principal components (Fig. 2). The first four principal components explained the cumulative variance of 84.4% of the total variation (Table 5).

Table 4. Pearson correlation coefficients among nine quantitative traits in sunflower genotypes

Traits	DFF	DM	PH	HD	HSW	SYPP	OC	SY	OY
DFF	1.000								
DM	0.956***	1.000							
PH	0.262*	0.258*	1.000						
HD	0.008	0.007	0.010	1.000					
HSW	0.041	0.038	0.225*	-0.035	1.000				
SYPP	0.117	0.113	0.131	-0.210	0.358***	1.000			
OC	-0.172	-0.168	-0.049	0.263*	-0.155	-0.207	1.000		
SY	0.117	0.112	0.132	-0.210	0.358***	1.000***	-0.207	1.000	
OY	0.058	0.049	0.129	-0.111	0.308**	0.923***	0.178	0.923***	1.000

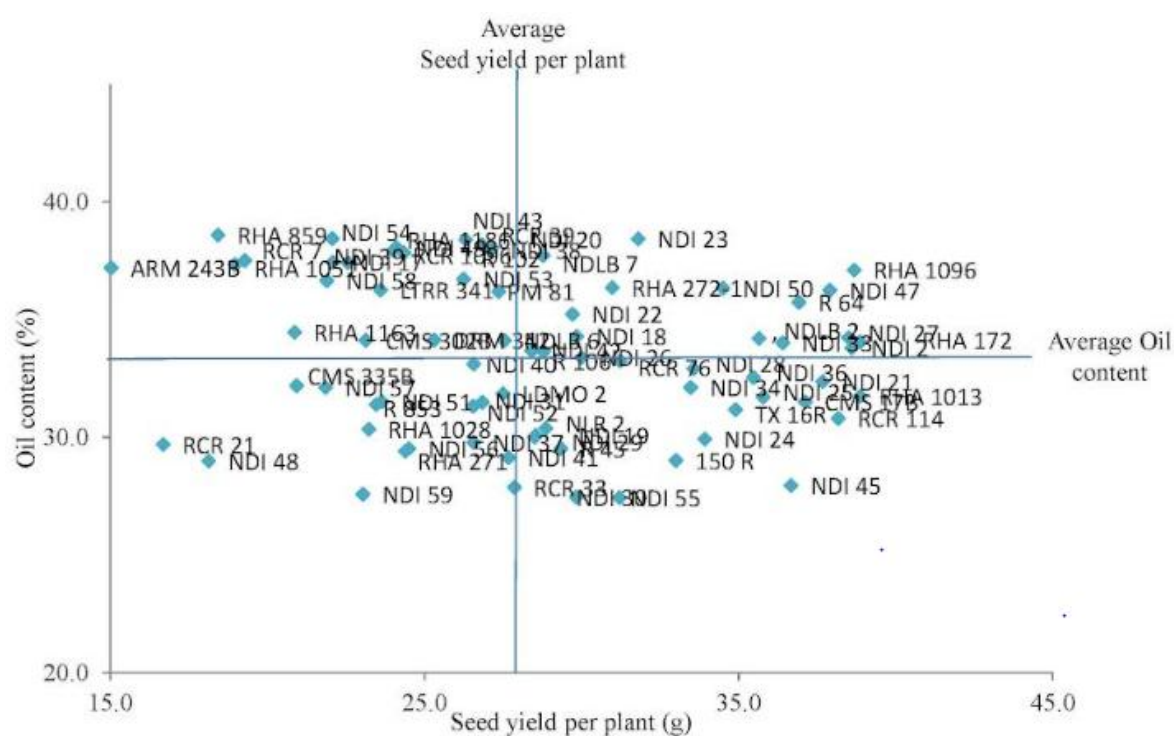
*, ** and *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively

DFF – Days to 50 percent flowering, DM – Days to maturity, PH – Plant Height (cm), HD – Head diameter (cm), HSW – Hundred seed weight (g), SYPP – Seed yield per plant (g), OC – Oil content (%), SY – Seed yield (kg ha^{-1}), OY – Oil yield (kg ha^{-1})

Table 5. Eigen vectors, eigenvalues, percentage variance and cumulative variance of nine quantitative traits in sunflower genotypes

Traits	PC1	PC2	PC3	PC4
Days to 50% flowering	-0.180	-0.635	0.081	-0.186
Days to maturity	-0.176	-0.585	0.078	-0.166
Plant height (cm)	-0.161	-0.246	0.163	0.617
Head diameter (cm)	0.139	-0.055	0.624	0.183
100 Seed weight (g)	-0.269	0.044	-0.027	0.657
Seed yield per plant (g)	-0.531	0.156	-0.004	-0.115
Oil content (%)	0.125	0.172	0.705	-0.172
Seed yield (kg ha ⁻¹)	-0.531	0.156	-0.005	-0.115
Oil yield (kg ha ⁻¹)	-0.487	0.217	0.271	-0.170
Eigen value	3.252	2.069	1.259	1.014
Variance (%)	0.361	0.230	0.140	0.113
Cumulative Variance (%)	0.361	0.591	0.731	0.844
Most contributing traits of variation	SYPP, SY, OY, HSW	DFF, DM	OC, HD	PH, HSW

PC – Principal component, DFF – Days to 50 percent flowering, DM – Days to maturity, PH – Plant Height (cm), HD – Head diameter (cm), HSW – Hundred seed weight (g), SYPP – Seed yield per plant (g), OC – Oil content (%), SY – Seed yield (kg ha⁻¹), OY – Oil yield (kg ha⁻¹)

**Fig. 1. Classification of genotypes based on seed yield per plant and oil content**

The individual variation explained by 1st, 2nd, 3rd and 4th principal components is 36.1%, 23%, 14% and 11.3%, respectively. The study reported that seed yield per plant, seed yield (kg ha⁻¹), oil yield (kg ha⁻¹) and hundred seed weight in the first principal component, days to 50% flowering and days to maturity in the second principal component, oil content and head diameter in third principal component and plant height and

hundred seed weight in fourth principal component are the most contributing traits of variation. In combination with correlation and principal component analysis, the study identified the seed yield per plant and hundred seed weight was sufficient to explain most of the variation in sunflower. Earlier studies also reported that the traits of seed yield per plant and hundred seed weight are important in the selection of

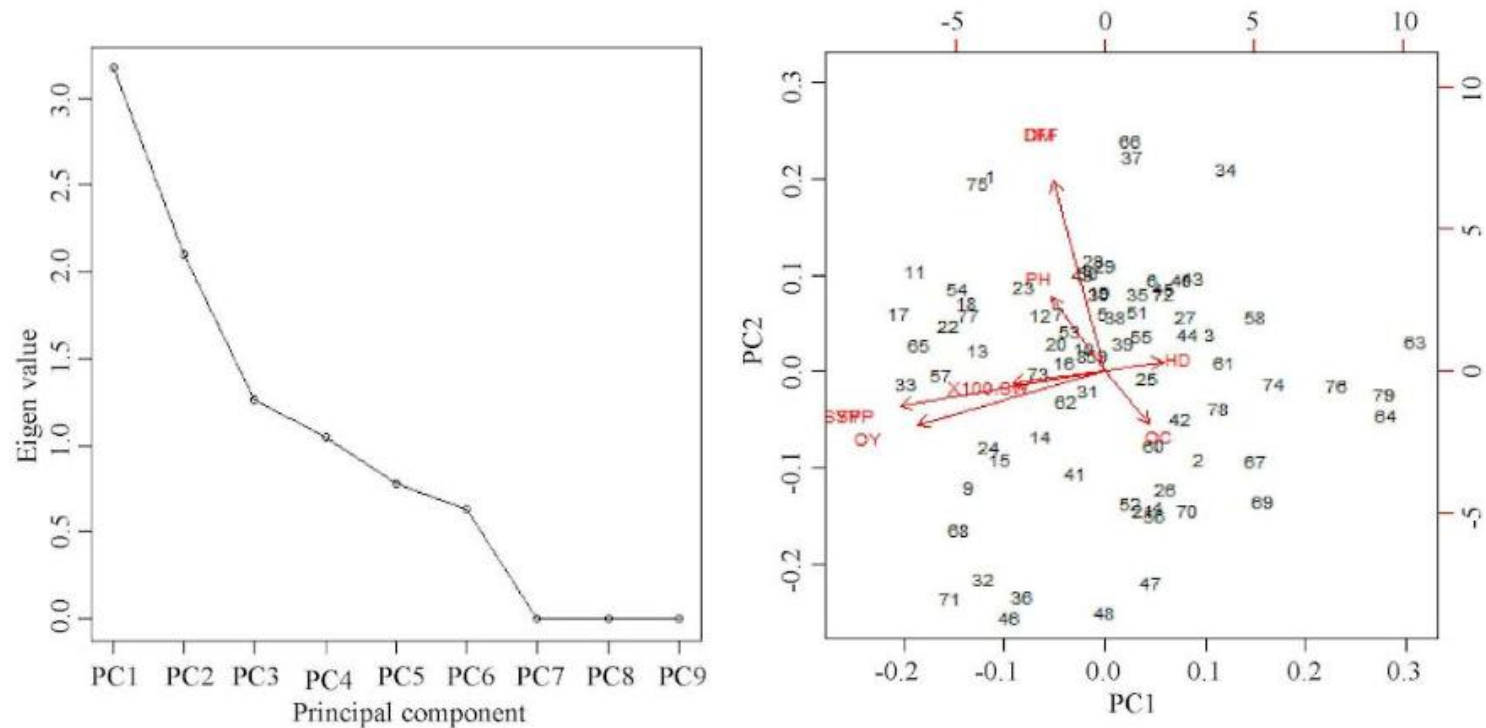


Fig. 2. Principal component analysis showing relation among various morphological traits in sunflower(A) scree plot and (B) Biplot of first two principal components

genotypes in sunflower (Sasikala et al., 2020, Meena et al., 2023). Further, efforts are required to improve these traits independently without affecting each other in sunflower improvement programmes (Loganathan and Gopalan 2006, Varshitha et al., 2023).

4. CONCLUSION

In conclusion, the superiority of tested entries over checks can be better identified by comparing the least significant increase value. The study identified the genotypes NDI 2, NDI 47, R 64, RHA 1096, NDLB 2, NDI 33, NDI 27 and RHA 172 are superior based on superiority over checks as well as ordination plots developed for the traits seed yield per plant and oil content. Further, the correlation and principal component analysis identified the traits of seed yield per plant and hundred seed weight are found enough to explain most of the variation and could be used in identifying the desirable genotypes in sunflower. The identified superior genotypes can be used for the development of desirable segregants or male sterile or restorer line development for further development of superior high-yielding hybrids in sunflower. Thus, the importance of identifying the genotypes for the most desirable traits has become the need of research in sunflower crop for future breeding programmes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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