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RESEARCH ARTICLE

Phenotypic characterization and genetic analysis for identification of trait-specific germplasm in Sunflower (*Helianthus annuus* L.)

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Abstract

An experiment conducted at TNAU, Coimbatore, during the rabi season of 2023-2024, evaluated 105 sunflower germplasm lines using ARBD. The ANOVA indicated significant differences among twelve traits, with high genetic variability observed in oil yield per plant, seed yield per plant and hundred seed weight. Heritability and genetic advance were notably high for plant height, seed yield per plant and oil yield per plant. Correlation analysis showed that seed yield per plant was positively correlated with plant height, head diameter, hundred seed weight, oil content and oil yield per plant but negatively with leaf size. Path analysis revealed that oil yield per plant had the most substantial positive direct effect on seed yield, while oil content had the most significant negative direct effect. Plant height, head diameter and hundred seed weight positively influenced yield indirectly through oil yield per plant but negatively via oil content. Principal Component Analysis (PCA) identified four principal components with Eigen values greater than 1, explaining 62.91% of the variability. Notably, diverse genotypes included CB-GMU1189, CB-GMU-400 and CB-IB-62. Mahalanobis D^2 statistics identified seven clusters, with Cluster 1 containing the most genotypes and Clusters 6 and 7 showing the highest inter-cluster distance, indicating significant genetic diversity. Cluster 5 had the highest mean for several traits. The sunflower germplasm accessions exhibited considerable variability in morphological characteristics such as hypocotyl pigmentation, leaf blistering, leaf serration, petiole pigmentation, stem pigmentation, ray floret shape, stigma pigmentation, seed shape, seed stripes and seed stripe colour. Therefore, the accessions identified for superior traits include 14 genotypes for very high yield (>35 g/plant), five for high seed oil content (40-43%) and 22 for high 100-seed weight (>6 g) and can be considered as superior trait-specific accessions in sunflower.

Keywords

characterization; correlation; diversity; germplasm; PCA; sunflower; variability

Introduction

Oilseed crops are vital to Indias' agricultural economy, driven by the increasing demand for vegetable oil for both edible and industrial purposes. Major oilseeds, such as soybeans, sunflower seeds, rapeseed (canola), cottonseed and peanuts, are cultivated for their high oil content, which ranges from about 20% in soybeans to over 40% in sunflowers and rapeseed. These crops are essential for producing edible oils, animal feed and various industrial products. They play a significant role in global agriculture and trade, providing vital nutrients and energy sources. Sunflower (*Helianthus annuus* L.), known as Surajmukhi in India, is a significant oilseed crop from the Compositae family with a chromosomal number of 2n = 34. The detailed taxonomic classification of sunflower is given in Table 1.

Table 1. Taxonomic classification of sunflower

Taxonomic Rank	Classification
Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Order	Asterales
Family	Asteraceae
Genus	Helianthus
Species	*Helianthus annuus*

Sunflower is the second most cultivated oilseed crop in the world, after soybeans and it is also notable that sunflower meal ranks as the third most consumed oilseed meal (1). Sunflowers are believed to have originated in Mexico or the southwestern United States and were introduced to India by the USSR in 1969 (2). Sunflowers have been widely adopted for their high seed and oil yield, good agronomic qualities and versatility. Sunflower seeds, containing 40% oil, are highly valued for cooking due to their light-yellow colour, bland flavour, high smoke point and beneficial fatty acid profile, which includes high linoleic acid and low oleic acid (3). It is considered a premium edible oil beneficial for heart health due to its low cholesterol and anticholesterol properties. The oil cake, containing 17-20% high-quality proteins, is an excellent feed for livestock and poultry. Sunflower hulls produce furfural, ethyl alcohol, roughage in livestock feed, and fuel for power generation. Sunflower kernels can be consumed raw or roasted, and the oil can be hydrogenated. In India, sunflower oil is a significant source of edible oil, valued for its high polyunsaturated fatty acid content, which helps lower heart disease risk. It contains 55-60% linoleic acid and 25-30% oleic acid (4).

Due to their lower yield potential, Indias' cultivation area for edible oilseed crops is smaller than pulses and cereals. As the largest importer of edible oils globally, after the USA and China, India's limited sunflower production is due to its narrow genetic base. This underscores the importance of studying variability and diversity to enhance seed and oil yield. To enhance sunflower yield and oil content, creating high-performing hybrids is crucial to ensuring ample genetic variability within the germplasm. This variability, influenced by environmental and genotypic factors, is critical to improving yield components. Understanding genetic parameters like GCV, PCV, heritability and genetic advancement helps to estimate genetic gains in breeding programs (5). Correlation and path coefficient analyses are valuable for understanding the trait relationships and their direct and indirect effects. Selecting parents with significant genetic divergence, using techniques like Mahalanobiss' D^2, is essential for creating high-yielding hybrids and achieving broad-spectrum variability in subsequent generations (6). Further, the Distinctiveness, Uniformity and Stability (DUS) characterization determines if the variety or germplasm under consideration varies from other varieties in the same species. Therefore, the present study aims to assess the genetic variability, association of traits, genetic diversity among the germplasm, principal component analysis, characterization and evaluation of different phenotypic traits to identify diverse inbred lines with desirable characteristics for future breeding programs.

Materials and Methods

The current experiment was conducted in the rabi season of 2023-2024 at the research fields of the Department of Oilseeds, TNAU, Coimbatore, India, with latitude of 11.0168°N and longitude 76.9558° E. This research aimed to characterize 100 sunflower germplasm accessions and five checks *viz.,* IR 6, PM 81, COSF 10B, COSF 15B and CSFI 99. Table S1. shows the checks and germplasms used for the study.

The experiment was set up using an augmented block design. The checks and all test accessions were organized into four blocks. The checks were replicated 4 times. Each accession was grown in a 4-meter-long row with a plant spacing of 60 cm × 30 cm. Each row included a total of twelve plants. All recommended agricultural techniques were followed to keep the plants healthy and productive. Each of the sunflower germplasm collections has five randomly chosen plants that were tagged and measured for twelve distinct quantitative parameters *viz.,* days to 50 % flowering, days to maturity, plant height (cm), head diameter (cm), leaf size (cm), seed length (cm), 100 seed weight (g), volume weight (g/100 ml), single plant yield (g), oil yield gram per plant (g), oil content (%) and hull content (%) were recorded.

The germplasm accessions were evaluated for 25 morphological characteristics following the distinctiveness, uniformity and stability (DUS) criteria for sunflowers. These characteristics include hypocotyl pigmentation, leaf shape, leaf colour, leaf blistering, leaf serration, leaf angle of lateral veins, the orientation of blade, leaf petiole pigmentation, stem pigmentation, ray floret shape, ray floret colour, disk floret colour, pollen colour, bract shape, bract pigmentation, plant natural position of closest lateral head to the central head, head attitude, shape of grain side, plant branching, plant type of branching, seed shape, seed coat base colour, seed coat stripes and colour of seed coat stripes.

Statistical analysis

The mean values of quantitative traits for all 100 sunflower genotypes and five check entries were computed. Analysis of variance, followed by an augmented design, was performed to partition the total variation. Genetic variability and correlation coefficients were calculated using the method and path coefficient analysis was employed to estimate the direct and indirect effects of twelve component traits on seed yield per plant (7-8). Tochers' method was followed for cluster formation and PCA (9-10). Statistical analyses were conducted using the INDOSTAT version 9.3 software program.

Results and Discussion

The results of the variance analysis showed notable variations between blocks, treatments, checks and check versus treatment (Table 2.). Highly significant variations were observed among the treatments for plant height, volume weight, seed length, single plant yield, oil content, oil yield per plant and hull content. For head diameter, significant variation was found among treatments. A highly significant difference

Table 2. Analysis of variance

Sr. No.	Source of variation	Block	Treatment	Checks	Checks + Var vs. Var.	Error
	DF	3	104	4	100	12
1	Days to 50 % flowering	51.24**	11.43	40.86**	10.3	7.37
2	Days to maturity	20.60	11.38	30.93*	10.60	7.79
3	Leaf size	15.74*	14.15*	96.48**	10.86*	4.25
4	Plant height	523.64**	593.72**	1996.09**	537.62**	33.28
5	Head diameter	25.05**	7.6 *	21.13**	7.13*	2.31
6	100-seed weight	2.98	2.66*	2.17	2.68*	1.13
7	Volume weight	7.37	35.51**	56.36**	34.68**	8.50
8	Seed length	0.01	0.014**	0.00	0.01**	0.00
9	Seed yield per plant	185.95**	150.02**	962.96**	117.50**	7.41
10	Oil content	20.35**	26.89**	31.59**	26.70**	2.21
11	Oil yield gram per plant	27.47**	20.32**	126.17**	16.09**	1.22
12	Hull content	21.74*	17.72*	13.62	17.89*	5.27

was observed in check versus treatment for single plant yield, oil content, oil yield per plant, leaf size, 100 seed weight, volume weight and seed length. Additionally, blocks showed highly significant variation for traits such as days to 50% flowering, plant height, head diameter, single plant yield, oil content and oil yield per plant.

Genetic variability

Genetic variability is essential for crop improvement as it provides the basis for selecting appropriate parents and genotypes in breeding operations. Understanding the various aspects of genetic variability is critical for practical breeding. In this context, variability was assessed using several metrics such as genotypic variance, phenotypic variance, heritability, genotypic coefficient of variation (GCV), the phenotypic coefficient of variation (PCV) and genetic advance as a percentage of the mean. These metrics are instrumental in identifying quantitative traits that need improvement (Table 3).

The PCV was consistently higher than the GCV for all the traits examined in the present study. (Fig. 1). The consistently higher PCV compared to GCV indicates that environmental factors contribute more to the observed trait variability than genetic factors, reflecting substantial environmental influence on trait expression. High estimates of PCV and GCV (>20%) were found for the key traits such as oil yield per plant (50.35 and 47.97%, respectively), seed yield per plant (43.85 and 42.18%) and 100-seed weight (31.95 and 22.72%). Similar results were observed in earlier studies (11), highlighting the significant potential for improvement by selecting these traits due to their high variability. Moderate PCV and GCV (10-20%) values were recorded for the traits head diameter (20.00 and 16.73%), plant height (18.05 and 17.41%), volume weight (16.88 and 13.94%), leaf size (15.53 and 10.85%), oil content (14.61 and 13.81%) and hull content (13.36 and 11.03 %) indicating a reasonable level of variability and potential for genetic gains. In contrast, low estimates of PCV and GCV (<10%) were observed for seed length (9.12 and 7.84%), days to 50% flowering (5.72 and 3.29%) and days to maturity (3.57 and 1.76%), suggesting limited variability and a lower potential for improvement in these traits. These findings are consistent with previous studies, which revealed that for every variable, the phenotypic coefficient of variation (PCV) was often higher than the genotypic coefficient of variation (GCV), suggesting that the environment had an impact on variance in addition to genotype (12-13).

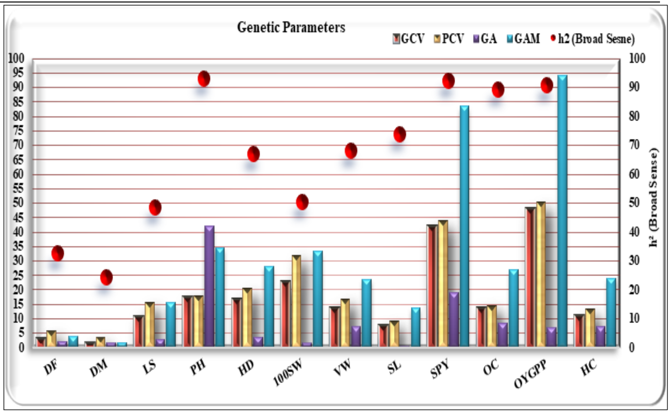

Fig.1. Estimation of genetic variability parameters of different biometrical traits in sunflower

Heritability and genetic advance

The observed heritability and genetic advance values offer valuable insights into trait improvement through breeding programs. Traits such as plant height (93.07%), seed yield per plant (92.52%), oil yield per plant (90.78%), oil content (89.33%), seed length (73.86%), volume weight (68.19%) and head diameter (67.33%) had high heritability (more than 60%), indicating that their variation is predominantly due to genetic factors. This suggests that these traits can be effectively

Table 3. Estimation of genetic variability among the 12 different traits in sunflower

Sr. No.	Characters	Mean	Range	PCV	GCV	H^2	GA	GAM
1	Days to 50 % flowering	57.93	51.00-65.00	5.72	3.29	32.92	2.25	3.88
2	Days to maturity	89.86	83.00-97.00	3.57	1.76	24.36	1.61	1.79
3	Leaf size	18.80	13.00-31.80	15.53	10.85	48.84	2.90	15.62
4	Plant height	121.59	55.50-166.50	18.05	17.41	93.07	42.01	34.60
5	Head diameter	13.10	6.00-20.00	20.00	16.73	67.33	3.70	28.28
6	100- seed weight	4.75	1.52-9.57	31.95	22.72	50.56	1.58	33.28
7	Volume weight	30.87	14.97-40.99	16.88	13.94	68.19	7.27	23.72
8	Seed length	1.06	0.80-1.33	9.12	7.84	73.86	0.15	13.88
9	Seed yield per plant	23.09	4.27-53.85	43.85	42.18	92.52	18.98	83.57
10	Oil content	31.31	24.10-43.10	14.61	13.81	89.33	8.38	26.88
11	Oil yield gram per plant	7.38	1.06-21.63	50.35	47.97	90.78	6.79	94.15
12	Hull content	30.51	17.54-36.93	13.36	11.03	30.00	7.32	24.04

enhanced through selection, as the genetic contributions will likely be passed on to offspring, which coincides with the earlier reports (14-16). In contrast, leaf size (48.84%) and 100-seed weight (50.56%) showed moderate heritability (30-60%), which means that both genetic and environmental factors are involved. Improvements in these traits are possible but may require additional selection cycles and consideration of environmental influences. Hull content (30.00%) and days to maturity (24.36%) had low heritability, suggesting that these traits are more influenced by environmental conditions, making them harder to improve through traditional selection methods.

The majority of the traits showed substantial genetic advance as a percentage of the mean (>20%), with oil yield per plant showing the highest level of genetic advance (94.15%) and seed yield per plant (83.57%) demonstrating high potential for significant gains through continued selection. Leaf size and seed length showed moderate genetic advance (10-20%), indicating they may be less pronounced while improvements are achievable. Meanwhile, days to 50 percent flowering (3.88%) and days to maturity (1.79%) exhibited low genetic advance, suggesting minimal potential for improvement even with effective selection. Overall, the traits with high heritability and genetic advance, namely oil yield gram per plant and seed yield per plant, are the prime traits for focused breeding efforts. In contrast, traits with lower values may require alternative strategies. The present results are based on the earlier observations (17-21).

Correlation analysis

The multidisciplinary nature of seed yield is significantly impacted by environmental conditions and is governed by several component traits. Understanding the correlations between seed yield and its component traits, as well as among these components, is crucial for improving selection efficiency. The relationships between traits are also shaped by the interaction of loci or significant quantitative trait loci, which govern the variability of traits located on the same chromosome. The strong positive correlation between single plant yield and traits such as plant height, head diameter, 100 seed weight, oil content and oil yield per plant is highly significant (Table 4.). This suggests that improving these traits could boost overall yield. Plant height and head diameter are critical for supporting larger seed heads, directly impacting yield. Furthermore, the positive relationship with 100 seed weight underscores the role of seed size in yield potential, with larger seeds typically indicating higher yield prospects. The significant positive correlation between plant height and traits such as days to 50% flowering, head diameter, volume weight and single plant yield highlights its importance in overall plant productivity.

Taller plants often possess more extensive vegetative structures that can accommodate larger heads, potentially leading to higher yields. There was a negative correlation between plant height and hull content, suggesting that taller plants might produce seeds with lower hull content, which could indicate better seed quality and higher oil content. A positive correlation between days to 50% flowering and days to maturity is expected, as plants with extended flowering periods usually require more time to mature. This information is crucial for understanding the growth cycle of sunflowers and can assist in selecting varieties suited to specific growing conditions. The negative correlation between head diameter and hull content and between 100 seed weight and hull content suggests that larger heads and heavier seeds are associated with reduced hull content. This can be beneficial, as seeds with lower hull content often have higher quality, which may lead to better oil extraction efficiency and increased seed value. These findings align with the results of previous research, highlighting the importance of these characteristics in determining seed yield in sunflowers. Oil content significantly correlated with the seed yield per plant (22-27). Similarly, the oil yield per plant also correlated significantly with the seed yield per plant (28-31).

Table 4. Phenotypic and Genotypic correlation for seed yield and associated traits in sunflower

Characters		DFF	DM	LS	PH	HD	100SW	VW	SL	OC	OYGPP	HC	SPY
DFF	**P**	1	0.84**	0.06	0.21*	0.08	0.0	0.02	-0.01	0.051	0.17	-0.087	0.18
	G	1	0.71**	-0.39**	0.43**	0.59**	-0.65**	-0.38**	-0.03	0.28*	0.52**	-0.43**	0.45**
DM	**P**		1	0.01	0.11	0.03	0.06	0.03	-0.04	0.06	0.17	-0.08	0.17
	G		1	-0.39**	0.30*	0.61**	-0.40**	-0.40**	-0.11	0.21*	0.56**	-0.54**	0.55**
LS	**P**			1	-0.10	0.08	-0.01	-0.08	0.07	-0.07	-0.14	-0.06	-0.14
	G			1	-0.16	0.02	-0.67**	-0.29*	0.05	0.19*	-0.13	-0.15	-0.21*
PH	**P**				1	0.34**	0.16	0.18	0.13	0.28*	0.31*	-0.21*	0.27*
	G				1	0.42**	0.19	0.23*	0.19	0.30*	0.36**	-0.21*	0.31*
HD	**P**					1	0.30*	-0.04	0.05	0.29*	0.55**	-0.24*	0.54**
	G					1	0.74**	-0.01	0.05	0.44**	0.59**	-0.25*	0.61**
100SW	**P**						1	0.15	0.21*	0.20*	0.24*	-0.22*	0.22*
	G						1	0.01	0.50**	0.47**	0.49**	-0.37**	0.39**
VW	**P**							1	-0.01	0.21*	0.08	-0.2	0.00
	G							1	-0.14	0.35**	0.09	-0.40**	-0.04
SL	**P**								1	0.17	0.18	-0.06	0.16
	G								1	0.24*	0.23*	-0.42**	0.18
OC	**P**									1	0.54**	-0.13	0.26*
	G									1	0.58**	-0.18	0.30*
OYGPP	**P**										1	-0.07	0.94**
	G										1	-0.05	0.96**
HC	**P**											1	-0.03
	G											1	-0.03
SPY	**P**												1
	G												1

DFF=Days to 50% flowering, DM=Days to maturity, LS=Leaf size, PH=Plant height, HD=Head diameter, 100SW=100-seed weight, VW=Volume weight, SL=Seed length, OC=Oil content, OYGPP=Oil yield gram per plant, HC=Hull content, SPY=Seed yield per plant, P=Phenotypic correlation, G=Genotypic correlation

Path analysis

Direct effects on seed yield per plant

According to the critical estimation of path coefficient analysis (Table 5.), the trait oil yield gram per plant (0.84 and 0.95) had the most direct and positive effect on seed yield per plant, followed by head diameter (0.02 and 0.43). The diagonal values in this study showed the direct impact at phenotypic and genotypic levels, respectively. Similarly, on the other hand, oil content (-0.34) and leaf size (-0.02) had the highest negative direct effect on seed yield per plant at the phenotypic level. Oil content (-0.48) and 100-seed weight (-0.18) had the highest negative direct effect on seed yield per plant. These results suggest that enhancing oil yield and head diameter could effectively improve seed yield while reducing oil content, which may also be necessary, supporting the earlier findings (32-33).

Indirect effects on seed yield per plant

Traits such as days to 50% flowering, days to maturity, plant height, head diameter,100-seed weight and oil content through oil yield per gram positively influenced seed yield per plant indirectly. Additionally, oil content indirectly affects seed yield via volume weight. In contrast, leaf size and hull content show positive indirect effects through oil content and adverse indirect effects through oil yield per gram. This highlights the complex interplay of traits influencing seed yield in sunflower breeding programs. These traits are, therefore, crucial for improving oil yield. Significant associations between oil and seed yield were reported earlier (34-35).

Principal component analysis

The top four components, which had more significance than one eigenvalue, accounted for 62.91% of the dataset's total variability through principal component analysis, underscoring their substantial role in explaining the data's structure (Table 6.). Eigenvalues of less than one for principal components were considered non-significant. With essential features including oil output per plant (0.49), seed yield per plant (0.45), head diameter (0.40), oil content (0.33) and plant height (0.31), the first principal component (PC1) explained 27.33% of the total variation explaining the maximum variance and demonstrating positive loadings.

The second principal component (PC2) explained 15.65% of the variance, primarily driven by days to 50% flowering (0.64) and days to maturity (0.66), which exhibit a high positive contribution in this component. PC3, the third component, accounted for 10.79% of the variation, with volume weight (0.52) and hundred seed weight (0.27) being the primary traits with significant loadings. The fourth component (PC4) accounted for 9.13% of the variance, where leaf size (0.76) and seed length (0.46) had the highest positive scores. Collectively, these principal components provide a comprehensive view of the genetic diversity and trait relationships within the population, highlighting the importance of various traits in understanding the dataset's variability and differentiation.

Table S2. illustrates the results of computing the PCA outcomes for 105 sunflower genotypes in the first three principal components, which were regarded as three axes: X, Y and Z. The square distances of every genotype through these three axes were also determined. The three-dimensional diagram (Fig. 2) was created by plotting all these PCA scores for 105 genotypes. These findings showed that genotype 61(CB-GMU1189) differed significantly from the other genotypes examined in this study. Additionally, the most diverse genotypes identified in the data were CB-IB-62 and CB-GMU-400, respectively.

Table 5. Phenotypic and Genotypic path coefficient analysis on seed yield in sunflower

Characters		DFF	DM	LS	PH	HD	100SW	VW	SL	OC	OYGPP	HC	SPY
DFF	**P**	0.02	0.01	0.00	0.00	0.00	0.00	0.00	-0.00	0.00	0.00	-0.00	0.18
	G	-0.14	-0.1	0.06	-0.06	-0.08	0.09	0.05	0.01	-0.04	-0.07	0.06	0.45**
DM	**P**	-0.01	-0.01	-0.00	-0.00	-0.00	-0.00	-0.00	0.00	-0.00	-0.00	0.00	0.17
	G	0.19	0.26	-0.10	0.08	0.16	-0.11	-0.11	-0.03	0.06	0.15	-0.14	0.55**
LS	**P**	-0.00	-0.00	-0.02	0.00	-0.00	0.00	0.00	-0.00	0.00	0.00	0.00	-0.14
	G	-0.01	-0.01	0.03	-0.00	0.00	-0.02	-0.01	0.00	0.01	-0.00	-0.00	-0.20*
PH	**P**	0.00	0.00	-0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	-0.00	0.27*
	G	-0.06	-0.04	0.02	-0.13	-0.06	-0.03	-0.03	-0.03	-0.04	-0.05	0.03	0.31*
HD	**P**	0.00	0.00	0.00	0.01	0.02	0.01	-0.00	0.00	0.01	0.01	-0.01	0.54**
	G	0.25	0.26	0.01	0.18	0.43	0.32	-0.01	0.02	0.19	0.25	-0.11	0.61**
100SW	**P**	0.00	0.00	-0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	-0.00	0.22*
	G	0.17	0.07	0.12	-0.03	-0.13	-0.18	-0.00	-0.09	-0.08	-0.09	0.07	0.39**
VW	**P**	-0.00	-0.00	0.00	-0.00	0.00	-0.00	-0.02	0.00	-0.00	-0.00	0.00	0.00
	G	-0.12	-0.13	-0.09	0.07	-0.00	0.00	0.31	-0.04	0.11	0.03	-0.13	-0.04
SL	**P**	-0.00	-0.00	0.00	0.00	0.00	0.00	-0.00	0.02	0.00	0.00	-0.00	0.16
	G	-0.01	-0.04	0.02	0.07	0.02	0.18	-0.05	0.36	0.08	0.08	-0.15	0.18
OC	**P**	-0.02	-0.02	0.02	-0.09	-0.10	-0.07	-0.07	-0.06	-0.34	-0.18	0.05	0.26*
	G	-0.13	-0.10	-0.09	-0.14	-0.21	-0.23	-0.17	-0.11	-0.48	-0.28	0.09	0.30*
OYGPP	**P**	0.19	0.19	-0.15	0.34	0.61	0.26	0.09	0.2	0.59	0.84	-0.08	0.94**
	G	0.50	0.53	-0.12	0.34	0.56	0.46	0.09	0.22	0.55	0.95	-0.04	0.96**
HC	**P**	-0.00	-0.00	-0.00	-0.00	-0.00	-0.00	-0.00	-0.00	-0.00	-0.00	0.01	-0.03
	G	-0.13	-0.16	-0.05	-0.06	-0.08	-0.11	-0.12	-0.13	-0.05	-0.01	0.3	-0.03
SPY	**P**	0.18	0.17	-0.14	0.27*	0.54**	0.22*	0.00	0.16	0.26*	0.94**	-0.03	
	G	0.45**	0.55**	-0.21*	0.31*	0.61**	0.39**	-0.04	0.18	0.30*	0.96**	-0.03	

DFF=Days to 50% flowering, DM=Days to maturity, LS=Leaf size, PH=Plant height, HD=Head diameter, 100SW=100-seed weight, VW=Volume weight, SL=Seed length, OC=Oil content, OYGPP=Oil yield gram per plant, HC=Hull content, SPY=Seed yield per plant, P=Phenotypic Path, G=Genotypic Path

Table 6. Eigenvalues, proportion of the total variance by the principal components, cumulative per cent variance for different traits in sunflower

Sl. No.	Particulars	PC1	PC2	PC3	PC4	PC5
	Eigen Value (Root)	3.28	1.87	1.29	1.09	0.98
	% Var. Exp.	27.33	15.65	10.79	9.13	8.16
	Cum. Var. Exp.	27.33	42.99	53.78	62.91	71.07
1	Days to 50 % flowering	0.15	0.64	0.04	0.17	0.13
2	Days to maturity	0.12	0.66	0.04	0.11	0.10
3	Leaf size	0.01	-0.17	0.01	0.76	0.09
4	Plant height	0.31	0.03	0.20	-0.12	-0.03
5	Head diameter	0.40	-0.08	-0.11	0.03	-0.44
6	100-seed weight	0.26	-0.12	0.27	0.04	-0.25
7	Volume weight	0.12	-0.12	0.52	-0.29	0.45
8	Seed length	0.18	-0.23	0.07	0.46	0.36
9	Seed yield per plant	0.45	-0.03	-0.38	-0.05	-0.01
10	Oil content	0.33	-0.09	0.13	-0.14	0.35
11	Oil yield gram per plant	0.49	-0.05	-0.29	-0.08	0.11
12	Hull content	-0.15	-0.05	-0.58	-0.14	0.46

Table 7. Clustering of genotypes based on D^2 analysis in sunflower

Cluster Group	No. of Genotypes	List of Genotypes
Cluster 1	61	CB-GMU-322, CB-GMU-475, CB-IB-07, CB-NO.179/6-1, CB-ACC 99-1, CB-CSFI 5295, CB-GMU-1019, CB-CSFI 5377, CB-GP 103-1, CB-GMU-1025, CB-CSFI 5077, CB-CSFI 5334, CB-GP 13, CB-GP 118-1, CB-ACC 142-1, CB-GMU-430, CB-ID-4093-1, CB-GP 145-2, CB-GMU434, CB-NW20, CB-GP155, CB-IHE250-1, CB-RCB-GP38, CB-GP151-1, CB-ID-2033, CB-GP85, CB-GMU-568, CB-GP6I-2, CB-GMJ-4 01, CB-CSF15095, PM81, CB-CSF15280, CB-C-F15239, CB-TNAUSUF6/7-2, COSF15B, CB-CSF15082, CB-GP271, COSF10B, CB-CSF15281, CB-GMU463,CB-IB-18, IR6, CB-GP145-1, CB-NW28, CB-GMJ-487, CB-CSF15090, CB-1149-2-1, CB-GMJ-301, CB-GP280-1, CB-GMJ-433, CB-GMU360, CB-IB-03, CB-GMU1058, CB-ACC216, CB-GMU-488, CB-ID-4036, CB-IR-1-1, CB-GP11-1, CB-GMU-451, CB-CO1/10 and CB-GMU1130
Cluster 2	18	CB-TX16R, CB-ID-5021, CB-IV55NB2, CB-GP155-1, CB-GMU-1120, CB-NW34-2, CB-IHE269-1, CB-RCB-GP104, CB-CSF15231, CB-ACC230-3, CB-GMU-479, CB-LDM-2, CB-C-F15125, CB-IB119, CB-GMU-1074, CB-AHM10-2, CB-EC421078-1 and CB-PS1049-1
Cluster 3	22	CB-CSF15133, CB-GMU-38 6, CB-RHA274-2, CB-ID-5004-3, CB-RHA83:6, CSF15205, CB-CSF15232, CB-GMU1032, CB-GMU300, CB-GMU-558, CB-GMU320, CB-IB-62, CB-GMU-429, CB-GMU-4 0 0, CB-GMU-498, CB-GMU-47 0, CB-CSF15121, CB-IB-61, CB-GMU438, CB-NO.88-8, CB-GMU-517 and CB-GMU1189
Cluster 4	1	CB-IHE269-1
Cluster 5	1	CB-GMU-452
Cluster 6	1	CSFI99
Cluster 7	1	CB-GMU423

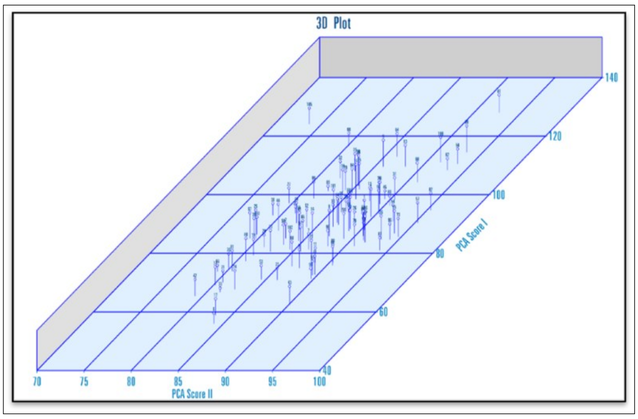

Fig. 2. PCA scores for 105 genotypes were plotted in a graph to get the three-dimensional diagram.

Principal component analysis (PCA) performed on sunflower genotypes explained that the first four components had contributed to the total variability (36-40), emphasizing the importance of oil content and shedding light on trait associations in sunflower germplasm. These findings highlight the vast genetic distance in the germplasm, making these sunflower lines valuable for breeding programs to enhance existing cultivars. Other sunflower researchers have also recognized the importance of PCA in conventional breeding for genetic divergence, germplasm assortment and cultivar development. Overall, PCA provides valuable insights into the genetic architecture of sunflower traits, facilitating targeted breeding efforts.

Diversity analysis

Estimating genetic divergence using Mahalanobis D^2 statistics classified 105 sunflower genotypes into seven distinct clusters (Table 8.) (34). Cluster 1 was the largest, encompassing 61 genotypes (Fig. 3), followed by Clusters 2 and 3, having 18 and 22 genotypes, respectively, while Clusters 4, 5, 6 and 7 each had single accession. This classification highlights the substantial genetic variability among the sunflower genotypes, with particular clusters exhibiting distinct trait characteristics. The significant contributions of economic traits such as plant height, 100-seed weight, oil content, volume weight and seed yield per plant to genetic diversity align with previous studies in sunflowers (41-46). Genotypes exhibiting high divergence can be selected from these clusters for hybridization to select superior progenies in sunflower breeding programs.

Intra and Inter-Cluster Distances

The intra Cluster distance among the seven Clusters revealed that maximum intra-cluster distance was recorded in Cluster 3 (738.74) followed by Cluster 1 (578.90), Cluster 2 (501.38) and there is no intra Cluster distance (0.00) among the clusters 4, 5, 6 and 7 (Table 9.). The maximum inter-cluster distance was observed between Cluster 6 and Cluster 7 (12984.14), followed by Cluster 3 and Cluster 7 (9803.67), Cluster 2 and Cluster 6 (8030.04), Cluster 2 and Cluster 3 (5095.25), Cluster 1 and Cluster 7 (4692.63) indicated the existence of high genetic diversity among genotypes in these clusters. Whereas the minimum inter-cluster distance was observed between Cluster 4 and Cluster 5 (410.09), Cluster 1 and Cluster 4 (1021.77), Cluster 2 and Cluster 7 (1279.2), Cluster 1 and Cluster 5 (1306.56), suggesting closeness and similarities among the genotypes in these clusters for most of the traits.

Table 8. Intra (diagonal) and inter-cluster distance in sunflower

	Cluster Distances						
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**	**Cluster 7**
Cluster 1	**578.90**	1789.81	1654.22	1021.77	1306.56	3589.41	4692.63
Cluster 2		**501.38**	5095.25	1846.97	2709.41	8030.04	1279.20
Cluster 3			**738.74**	2274.94	2058.12	1554.21	9803.67
Cluster 4				**0.00**	410.09	3156.06	3802.38
Cluster 5					**0.00**	2655.29	5066.72
Cluster 6						**0.00**	12984.14
Cluster 7							**0.00**

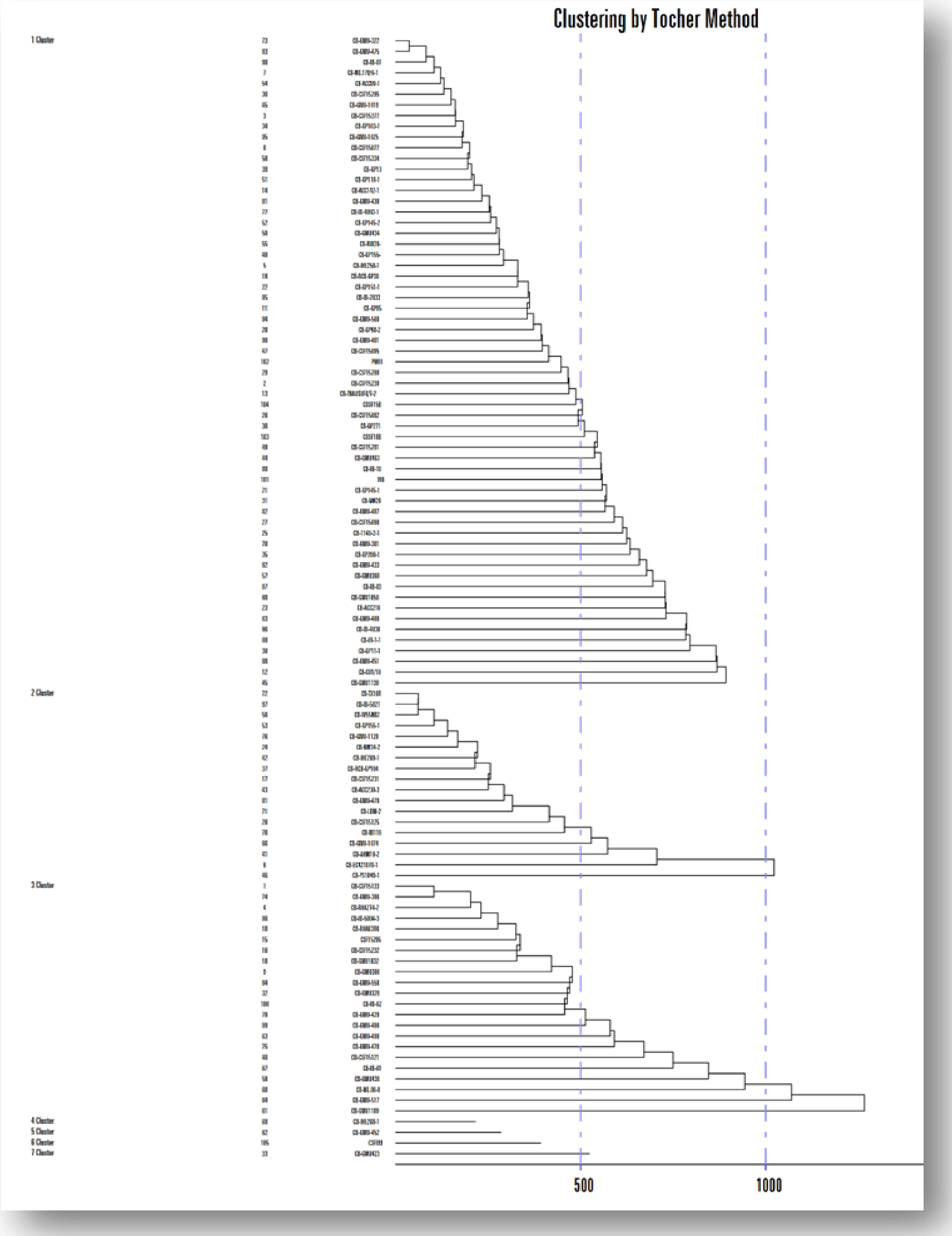

Fig. 3. Grouping of sunflower genotypes

Table 9. Phenotypic characterization of sunflower genotypes

No.	Characteristics	Category	No. of genotypes	Percentage
1	Hypocotyl: Anthocyanin Pigmentation	Absent	28	26.67
		Medium	19	18.10
		Strong	58	55.24
2	Leaf: Shape	Lanceolate	2	1.91
		Triangular	0	0.00
		Cordate	103	98.10
		Rounded	0	0.00
3	Leaf: Color	Light green	0	0.00
		Green	105	100.00
		Dark green	0	0.00
4	Leaf: Blistering	Absent	2	1.90
		Medium	33	31.43
		Strong	70	66.67
5	Leaf: Serration	Fine	1	0.95
		Medium	14	13.33
		Coarse	90	85.71
6	Leaf: Angle of lateral veins	Acute (<90)	98	93.33
		Obtuse (>90)	7	6.67
7	Leaf: Orientation of blade	Erect	0	0.00
		Drooping	105	100.00
8	Leaf: Petiole anthocyanin pigmentation	Absent	65	61.90
		Present	40	38.09
9	Stem: Pigmentation	Absent	65	61.90
		Present	40	38.09
10	Ray floret: Shape	Elongated	87	82.86
		Ovate	18	17.15
		Rounded	0	0.00
11	Ray floret: Color	Light yellow	0	0.00
		Yellow	105	100.00
		Orange	0	0.00
		Purple	0	0.00
12	Disk floret: Color	Yellow	105	100.00
		Orange	0	0.00
		Purple	0	0.00
13	Disk floret: Anthocyanin pigmentation of stigma	Absent	52	49.53
		Medium	45	42.86
		Strong	8	7.62
14	Disk floret: Pollen colour	White	0	0.00
		Yellow	105	100.00
15	Bract: Shape	Elongated	0	0.00
		Rounded	105	100.00
16	Bract: Anthocyanin pigmentation	Absent	105	100.00
		Present	0	0.00
17	Plant: Natural position of closest lateral head to the central head (end of flowering- branched)	Above	0	0.00
		Below	24	22.86
18	Head: Attitude	Inclined	0	0.00
		Vertical	0	0.00
		Half turned down	0	0.00
		Turned down	105	100.00
19	Head: Shape of the grain side	Concave	10	9.53
		Flat	6	5.72
		Convex	56	53.34
		Irregular	33	31.43
20	Plant: Branching	Absent	81	77.15
		Present	24	22.86
21	Plant: Type of branching	Basal	6	5.72
		Overall	6	5.72
		Apical	12	11.43
22	Seed: Shape	Elongated	25	23.81
		Ovoid elongated	60	57.15
		Ovoid wide	20	19.05
23	Seed coat: Base colour	White	0	0.00
		Grey	24	22.86
		Brown	30	28.58
		Black	51	48.58
24	Seed coat: Stripes	Absent	41	39.05
		Present	64	60.96
25	Seed coat: Color of Stripes	White	4	3.81
		Grey	29	27.62
		Brown	24	22.86
		Black	8	7.62

Phenotypic characterization

The distinctiveness, uniformity and stability (DUS) study evaluated 25 morphological traits to determine their variation among the 105 genotypes taken for analysis (Table 10.). At the seedling stage, 58 genotypes exhibited intense hypocotyl anthocyanin pigmentation; 19 had medium pigmentation and the rest showed no pigmentation. Leaf shapes mainly were cordate (103 genotypes), with 2 having lanceolate leaves and none with round or triangular leaves. All leaves were green and leaf blistering was strong in 70 genotypes, medium in 33 and absent in 2. Leaf serrations were mostly coarse (90 genotypes), with 14 having medium serrations and 1 having fine serrations. Acute leaf angles were observed in 98 genotypes, while 7 had obtuse angles. All genotypes had drooping leaves and petiole anthocyanin pigmentation was present in 40 genotypes but absent in 65 accessions. Stem anthocyanin pigmentation appeared as red streaks in 40 genotypes and was lacking in 65 genotypes. The ray florets were elongated in 87 genotypes and ovate in 18, with all genotypes displaying yellow ray and disc florets. Stigma anthocyanin pigment was absent in 52 genotypes, medium in 45 and strong in 8, while pollen colour was uniformly yellow. Bracts were round in all genotypes, with no pigmentation noted.

All 24 genotypes with lateral heads had the main head positioned below the lateral head. Head shapes included convex (56 genotypes), irregular (33 genotypes), concave (10 genotypes) and flat (6 genotypes). Of the 105 genotypes, 24 were branching types (6 with basal, 6 with overall and 12 with apical branching) and 81 with non-branching. Seed shapes were predominantly ovoid elongated (60 genotypes), with 20 having ovoid broad seeds and 25 having elongated seeds. Seed coat colours varied, with 24 genotypes having grey seeds, 30 having brown seeds and 51 having black seeds. Striped seeds appeared in 64 genotypes, with different stripe colours: grey (29), brown (24), white (4) and black (8), while the remaining seeds were unstriped. The study of 105 genotypes revealed significant diversity in traits such as leaf shape, colour, blistering, serration and hypocotyl anthocyanin pigmentation. Differences in seed and oil yield mainly caused genetic divergence. Floral traits, including ray floret shape, colour and seed coat features, also contributed to the overall diversity. These findings provide valuable insights for breeding programs aimed at enhancing crop productivity. The findings corroborated by earlier research that categorized sunflower accessions based on their physical traits (47-50). Pie charts of germplasm were created to display data on oil content and single plant yield (Fig 4-5). Of the 105 accessions, 14 were classified as having a very high yield (>35g) and 23 as having a high yield (25 to 35g). Five germplasm accessions exhibited high seed oil content (>40%). Therefore, the accessions identified for superior traits include 14 genotypes for very high yield (>35 g/plant), five for high seed oil content (40-43%) and 22 for high 100-seed weight (>6g) and can be considered as superior trait-specific accessions (Table 12). Such promising trait-specific germplasm accessions can serve as foundational material for the genetic improvement of sunflower yield components.

Conclusion

The present study resulted in the identification of four promising sunflower germplasm viz., CSFI 99, CB-GMU 1189, CB-GMU 498 and CB-IB 119 with significant variability for most of the yield traits, with CSFI 99 and CB-GMU 1189 notable for its exceptional high oil content and seed yield. The characterization of qualitative traits also revealed considerable diversity among the germplasm for various morphological characteristics, providing valuable insights into selecting germplasm with specific trait profiles. These trait-specific genotypes can be better utilized to achieve particular breeding goals in sunflowers.

Table 10. Promising sunflower germplasm for various economic traits

Characters	Germplasm accessions
Single plant yield (>35g)	CB-ACC 216, CB-GP 11-1, CB-GMU 1130, CB-PS 1049-1, COSF15B, CB-GMU 438, CB-GMU 1189, CB-GMU-452, CB-GMU-517, CB-NO.88-8, CB-IHE 269-1, CB-GMU-470, CB-GMU-488, CSFI99
Seed oil content (>40 %)	CB-RHA 83R6, CB-GMU 1189, CB-GMU-498, IR 6, CSFI99
100-Seed weight(>6g)	CB-GMU 300, CB-CO1/10, CB-TNAUSUF 6/7-2, CB-CSF1 5090, CB-NW 28, CB-GMU 320, CB-GP 103-1, CB-CSF1 5295, CB-GP 11-1, CB-GP 13, CB-AHM 10-2, CB-GP 118-1, CB-NW 20, CB-GMU 1189, CB-GMU-498, CB-GMU-517, CB-GMU-301, CB-GMU-429, CB-GMU-479, CB-IB-62, PM81, CSFI99

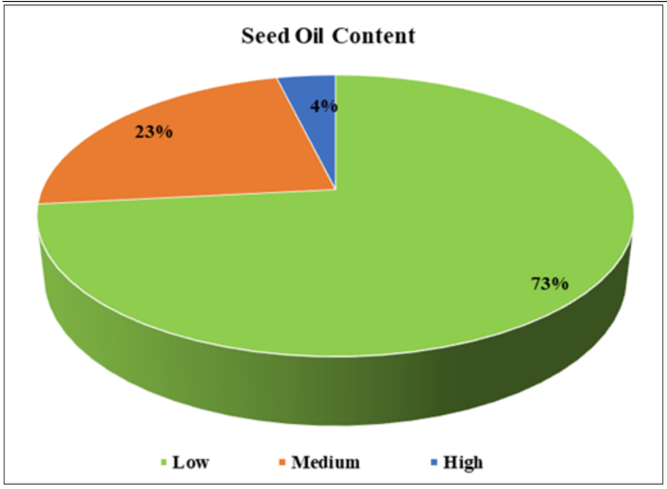

Fig. 4. Sunflower accessions for high, medium and low seed oil content (%)

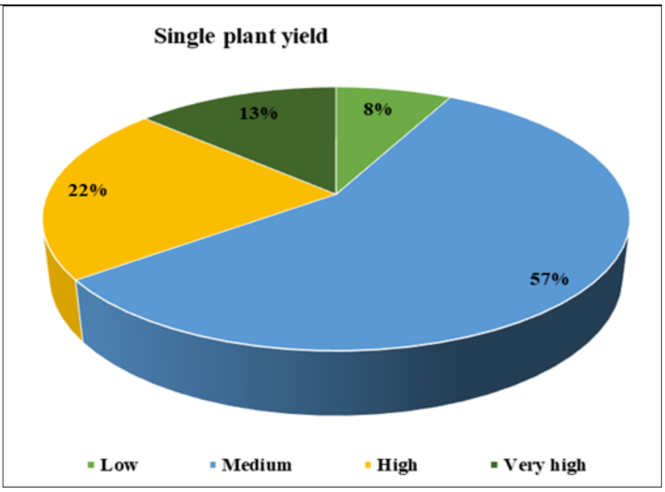

Fig 5. Sunflower accessions for high seed yield per plant

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Authors' contributions

NN carried out the experiment, took observations, and analyzed the data. PRR, RS, SR and RC participated in summarizing the results and guided the preparation of the manuscript. DM guided the research work, contributed ideas in formulating the research concept, provided research funds and finally edited the final manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of the work, the author(s) used chaptgpt-4 and Grammarly to improve the language not for the content creation. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the publication's content.

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