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GENETIC VARIABILITY, TRAIT ASSOCIATIONS, AND MULTIVARIATE DIVERGENCE FOR FATTY ACID COMPOSITION AND SENSORY QUALITY IN CONFECTIONERY GROUNDNUT BREEDING LINES

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ABSTRACT

Confectionery groundnut requires kernels that combine a favourable fatty acid composition, which determines oxidative stability and shelf life, with high sensory acceptance, yet information on the genetic variability and association of these traits in breeding lines is limited. Thirty-six advanced confectionery groundnut breeding lines were evaluated in a randomized complete block design with three replications during the summer season of 2023 at the University of Agricultural Sciences, Dharwad, India. Data were recorded for oleic acid, linoleic acid, palmitic acid, stearic acid, oleic-to-linoleic acid ratio, appearance, colour, flavour, taste, texture, and overall acceptability. Oleic acid content ranged from 41.35 to 69.98%, linoleic acid from 13.28 to 37.25%, palmitic acid from 5.20 to 12.52%, stearic acid from 0.93 to 3.66%, and the oleic-to-linoleic acid ratio from 0.31 to 4.78, while the mean scores of the sensory traits were 7.22 to 7.63 on a nine-point scale. Oleic acid content was strongly correlated with the oleic-to-linoleic acid ratio, linoleic acid was strongly correlated with palmitic acid, and palmitic acid with stearic acid. All fatty acid traits were positively associated with the sensory scores, and the sensory traits were very strongly intercorrelated. The first five principal components explained 91.28% of the total variation, with the first principal component representing a fatty acid composition axis and the second a sensory quality axis, and K-means clustering grouped the genotypes into three clusters of 11, 11, and 14 lines. Cluster I was characterized by high oleic acid content and a high oleic-to-linoleic acid ratio, Cluster II by high linoleic and palmitic acid contents, and Cluster III by superior sensory scores. Hybridization between divergent parents from Clusters I and III offers scope to combine high oil quality with strong sensory acceptance in confectionery groundnut cultivars.

***Key words*:** confectionery groundnut; fatty acid composition; oleic-to-linoleic acid ratio; sensory quality; principal component analysis; K-means clustering

Introduction

Groundnut (*Arachis hypogaea* L.), a self-pollinated allotetraploid legume of the family Fabaceae believed to have originated in Bolivia, is regarded as the “king of oilseeds” because its kernels contain 40 to 60% oil, 20 to 40% protein, and 10 to 20% carbohydrate, together with minerals, vitamins, phytosterols, phenolics, and antioxidants (Arya *et al*., 2016). The crop is grown on about 30.92 million hectares worldwide, with a production of about 54.27 million tonnes and a productivity of 1.76 t ha^{-1}, and India ranks second after China (FAOSTAT, 2023). Although most of the produce is crushed for edible oil, a growing share is consumed directly as roasted, salted, or coated kernels, for which market value depends on kernel quality and consumer acceptance, and therefore on the genetic improvement of confectionery types.

The quality of confectionery groundnut is determined by physical traits (seed mass, shape, and testa integrity), sensory traits (appearance, colour, flavour, taste, and texture), and biochemical traits (oil, protein, and fatty acid composition) (Dwivedi *et al.*, 1996). Among the biochemical traits, fatty acid composition links nutritional value to shelf life. Groundnut oil comprises roughly 50% monounsaturated, 33% polyunsaturated, and 14% saturated fatty acids. Oleic acid (OLA) and linoleic acid (LNA) together account for 75 to 80% of the total fatty acids, and with palmitic acid they make up about 90%. Stearic acid and the other minor fatty acids constitute the remaining 1 to 3% (Akcura *et al.*, 2021; Abadya *et al.*, 2021).

Because of this dominance, the relative proportion of OLA and LNA, expressed as the oleic-to-linoleic acid ratio, is the main determinant of the oxidative stability of the kernels. Polyunsaturated fatty acids are readily oxidized, leading to rancidity and off-flavours, and LNA oxidizes about ten times faster than OLA (O'Keefe *et al.*, 1993). Genotypes with a high O/L ratio therefore have a substantially longer shelf life than those with a normal ratio. Dietary OLA has also been associated with lower low-density lipoprotein levels, inhibition of tumorigenesis, and relief of inflammatory conditions (Mesa-Garcia *et al.*, 2006). These advantages have directed breeding programmes towards improving fatty acid composition. In confectionery types, however, a gain in oil quality is useful only if the sensory attributes that determine consumer acceptance are retained, so both groups of traits must be improved together.

The response to selection for such multiple traits depends on the magnitude of the genetic variability in the breeding population and on the associations among the traits (Bhargavi *et al.*, 2026; Hemanth and Patil, 2023). The genotypic and phenotypic coefficients of variation (GCV and PCV), broad-sense heritability (h^2bs), and genetic advance as percent of mean (GAM) indicate the extent of variability, the proportion of it that is heritable, and the genetic gain expected from phenotypic selection (Hemanth *et al.*, 2026). Correlation analysis reveals the interrelationships among traits and the scope for simultaneous or indirect selection (Hemanth *et al.*, 2022; Bhargavi *et al.*, 2024). Principal component analysis (PCA) and cluster analysis resolve the multivariate structure of the population and group genotypes according to their overall trait profiles, which helps in identifying genetically divergent parents for hybridization and the recovery of transgressive segregants (Hemanth *et al.*, 2024; Hemanth *et al.*, 2025). Despite their value, studies of genetic variability and trait associations for fatty acid composition in groundnut are limited, and those that consider fatty acid and sensory traits together in confectionery breeding lines are more limited still.

The present study therefore evaluated 36 advanced confectionery groundnut breeding lines to (i) estimate the genetic variability, heritability, and genetic advance for fatty acid composition and sensory quality, (ii) determine the interrelationships among these traits, and (iii) characterize the multivariate structure of the panel by PCA and K-means clustering, so as to identify groups of genotypes that can serve as parents for developing confectionery cultivars that combine superior oil quality with high sensory acceptance.

Materials and Methods

Plant material and experimental layout

The study material consisted of 36 advanced-generation breeding lines of confectionery groundnut (Table 1). The lines were phenotyped in the summer season of 2023 at the University of Agricultural Sciences, Dharwad, Karnataka, India, located at 15°25' N latitude and 75°25' E longitude at an elevation of 678 m above mean sea level, where the mean annual rainfall is about 800 mm. The field trial followed a randomized complete block design with three replications, so that environmental heterogeneity among blocks could be separated from genotypic effects. Each genotype was grown in a single-bed plot of 3 m length with six rows, at an inter-row spacing of 45 cm and an intra-row spacing of 15 cm. All plots received uniform recommended agronomic practices and plant protection measures.

Fatty acid profiling and sensory evaluation of kernels

The kernels of each genotype were profiled for fatty acid composition and evaluated for sensory quality. The fatty acid traits were oleic acid (OLA), linoleic acid (LNA), palmitic acid (PLA), and stearic acid (STA), each expressed as a percentage of total fatty acids, together with the oleic-to-linoleic acid ratio (O/L). The sensory traits were appearance (APP), colour (COL), flavour (FLA), taste (TAS), texture (TEX), and overall acceptability (OAA). Fatty acid composition was first determined by gas chromatography, and these reference values were used to develop and calibrate near-infrared spectroscopy prediction equations, which were then applied to estimate OLA, LNA, PLA, and STA in the genotypes. The O/L ratio was computed by dividing OLA by LNA. Sensory attributes were scored on a nine-point hedonic scale, from 1 (dislike extremely) through 5 (neither like nor dislike) to 9 (like extremely), following established procedures for peanut sensory evaluation

Table 1: Description of 36 confectionery groundnut genotypes.

Sr. No.	Genotypes	Pedigree
1	CG-1	TG - 76 × ICGV 86699
2	CG-2	TG - 76 × ICGV 86699
3	CG-3	TG - 76 × ICGV 86699
4	CG-4	TG - 76 × ICGV 86699
5	CG-5	TG - 76 × ICGV 86699
6	CG-6	TG - 76 × ICGV 86699
7	CG-7	TG - 76 × ICGV 86699
8	CG-8	TG - 76 × ICGV 86699
9	CG-9	TG - 76 × ICGV 86699
10	CG-10	TG - 76 × ICGV 86699
11	CG-11	TG - 76 × ICGV 86699
12	CG-12	TG - 76 × ICGV 86699
13	CG-13	TG - 76 × ICGV 86699
14	CG-14	TGLPS 3 × GPBD-5
15	CG-15	TGLPS 3 × GPBD-5
16	CG-16	TGLPS 3 × GPBD-5
17	CG-17	TGLPS 3 × GPBD-5
18	CG-18	TGLPS 3 × ICGV 86699
19	CG-19	TGLPS 3 × ICGV 86699
20	CG-20	TGLPS 3 × ICGV 86699
21	CG-21	TG - 76 × GPBD-5
22	CG-22	TG - 76 × ICGV 86699
23	CG-23	TG - 76 × ICGV 86699
24	CG-24	TG - 76 × ICGV 86699
25	CG-28	ICGV 06189 × DH 245-10
26	CG-29	ICGV 06189 × AGL 2766-8
27	CG-30	ICGV 06189 × ICGV 08025-1
28	CG-31	JSP-39 × GM6-1SSD-5
29	CG-32	JSP-39 × GM6-1- SSD-25
30	CG-33	ICGV 06189 × GM 6000-24-5
31	CG-34	ICGV 06189 × 6000-65-3
32	ICGV 86699	Parental lines
33	TG76	Parental lines
34	GPBD-5	Parental lines
35	TGLPS 3	Parental lines
36	ICGV 06189	Check

(Young *et al*., 2005; Nepote *et al*., 2009). The mean score of each genotype was used for statistical analysis.

Statistical analysis

Phenotypic data were recorded on all three replications, and the replication means were used for the analysis. All analysis was performed in RStudio with R version 4.4.2. A randomized complete block design analysis of variance (ANOVA) was carried out with the *metan* package to test genotypic differences for each trait. The genetic variability parameters were estimated from the expected mean squares with the *variability* package. These were the genotypic and phenotypic variances, the GCV and PCV, h^2bs, and GAM. Pearson's phenotypic correlation coefficients were computed to assess the interrelationships among traits and were visualized with the *corrplot* package (Wei *et al*., 2021). The multivariate structure of the trait variation was resolved by PCA with the *FactoMineR* package (Kassambara, 2020), and the trait loadings on the principal components were displayed as Circos plots to visualize trait associations. The optimum number of clusters was determined by the elbow criterion, based on the within-cluster sum of squares, and the genotypes were then partitioned into groups by K-means clustering.

Results and Discussion

Mean performance and genetic variability parameters

The ANOVA showed highly significant mean squares due to genotypes for all fatty acid composition and sensory quality traits among the 36 confectionery groundnut genotypes (Table 2), indicating substantial genetic variability and scope for selection in the panel. The coefficient of variation (CV), an index of experimental error, was low (0.65% for OLA to 8.07% for O/L), indicating good precision of the trial. Significant genotypic differences for PLA, STA, OLA, and LNA have also been reported in groundnut (Mukhthambica *et al*., 2026), confirming that the germplasm holds enough heritable variation for the improvement of nutritional and industrial quality (Table 2).

OLA was the predominant fatty acid, with a mean performance of 55.57% and a range of 41.35 to 69.98%. It showed low GCV and PCV, very high h^2bs (99.83%), and moderate GAM (10.60%). LNA (mean 26.15%; range 13.28 to 37.25%) showed moderate GCV and PCV,

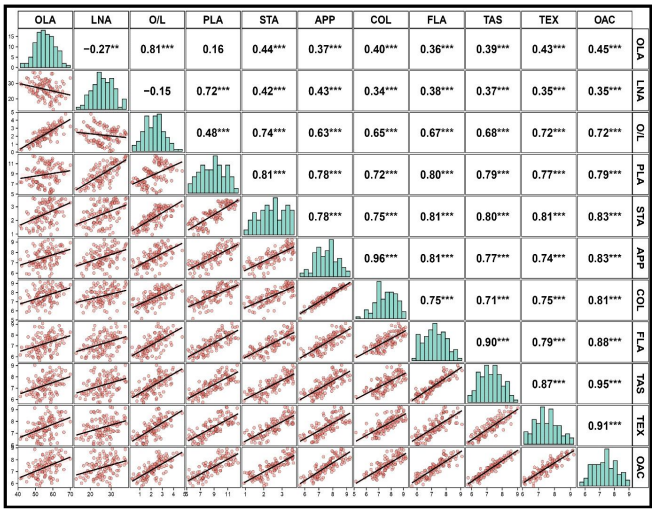


Fig. 1: Correlation and distribution analysis of fatty acid composition and sensory quality traits across 36 confectionery groundnut genotypes.

Table 2: Analysis of variance of 36 advanced confectionery groundnut breeding lines for fatty acid composition and sensory quality traits.

SV	DF	OLA	LNA	O/L	PLA	STA	APP	COL	FLA	TAS	TEX	OAC
REP	2	380.45	380.45	25.87	165.64	25.87	26.61	26.61	26.61	24.85	24.85	24.85
GEN	35	79.63 ***	64.56 ***	1.08 ***	1.52 ***	0.19 ***	0.49 ***	0.71 ***	0.4 ***	0.33 ***	0.32 ***	0.26 ***
Error	70	0.13	0.13	0.03	0.16	0.03	0.04	0.04	0.04	0.05	0.05	0.05
Total	107	33.25	28.32	0.86	3.7	0.57	0.69	0.76	0.66	0.61	0.6	0.58
CV		0.65	1.39	8.07	4.51	7.34	2.77	2.74	2.89	3.22	3.12	3.18

OLA: Oleic acid; LNA: Linoleic acid; O/L: Ratio of oleic to linoleic acid; PLA: Palmitic acid; STA: Stearic acid; APP- Appearance; COL- Colour; FLA- Flavour; TAS- Taste; TEX- Texture; OAA- Overall acceptability

h²bs of 99.79%, and low GAM (9.54%). O/L showed the widest relative variation (mean 2.25; range 0.31 to 4.78), with high GCV and PCV, h²bs of 96.96%, and low GAM (1.20%). The saturated fatty acids were less variable. PLA (mean 8.84%; range 5.20 to 12.52%) showed low GCV and PCV, h²bs of 89.52%, and low GAM (1.31%), whereas STA (mean 2.47%; range 0.93 to 3.66%) showed low GCV, moderate PCV, h²bs of 82.33%, and the lowest GAM (0.42%) among the fatty acid traits. The six sensory traits showed a uniform genetic pattern. Their mean performance was 7.22 to 7.63 on the nine-point hedonic scale, and the genotypic means ranged from 5.15 (COL) to 9.24 (APP). APP (5.65 to 9.24; mean 7.54), COL (5.15 to 9.21; mean 7.63), FLA (5.65 to 9.09; mean 7.22), TAS (5.75 to 9.03; mean 7.27), TEX (6.08 to 9.21; mean 7.50), and OAA (5.78 to 8.91; mean 7.36) all showed low GCV and PCV, high h²bs (78.86 to 93.86%), and low GAM (0.48 to 0.94%).

The order of abundance of the fatty acids (OLA > LNA > PLA > STA) is the same as in Mukhthambica *et al*., (2026), where oleic acid predominated in the kernel oil (mean 55.35%), followed by linoleic acid (23.39%), palmitic acid (12.80%), and the minor component stearic acid (2.82%). The high h²bs of OLA (99.83%), LNA (99.79%), PLA (89.52%), and STA (82.33%) agrees with earlier reports of high heritability (about 74 to 97%) for these fatty acids (Zekeria *et al*., 2019; Deepthi *et al*., 2022; Mukhthambica *et al*., 2026), and it shows that most of the phenotypic variance was genotypic, with little environmental influence. Because h²bs includes both additive and non-additive genetic variance, it should be interpreted together with GAM. High h²bs with high GAM indicates predominantly additive gene action, whereas high h²bs with low GAM, as found for STA, indicates the involvement of non-additive gene action (dominance and epistasis) in addition to additive effects. Phenotypic selection for the minor saturated fatty acids may therefore be less effective than direct selection for the major unsaturated fractions (Mukhthambica *et al*., 2026).

Trait correlation spectrum, interrelationships, and potential trade-offs

Pearson's phenotypic correlation coefficients (r) among the fatty acid composition and sensory quality traits (Fig. 1) revealed distinct patterns of association. OLA was strongly and positively correlated with O/L (r = 0.81) and positively associated with STA (0.44), APP (0.37), COL (0.40), FLA (0.36), TAS (0.39), TEX (0.43), and OAA (0.45), but showed weak associations with LNA (-

Table 3: Mean performance of 36 advanced confectionery groundnut breeding lines for fatty acid composition and sensory quality traits.

Trait	Mean	Minimum	Maximum	GCV	PCV	Heritability	GAM
OLA	55.57	41.35	69.98	9.26	9.27	99.83	10.6
LNA	26.15	13.28	37.25	17.72	17.74	99.79	9.54
O/L	2.25	0.31	4.78	26.32	26.73	96.96	1.2
PLA	8.84	5.2	12.52	7.6	8.04	89.52	1.31
STA	2.47	0.93	3.66	9.14	10.08	82.33	0.42
APP	7.54	5.65	9.24	5.13	5.38	91.16	0.76
COL	7.63	5.15	9.21	6.18	6.38	93.86	0.94
FLA	7.22	5.65	9.09	4.77	5.06	89.09	0.67
TAS	7.27	5.75	9.03	4.17	4.56	83.44	0.57
TEX	7.5	6.08	9.21	3.93	4.33	82.68	0.55
OAA	7.36	5.78	8.91	3.54	3.99	78.86	0.48

OLA: Oleic acid; LNA: Linoleic acid; O/L: Ratio of oleic to linoleic acid; PLA: Palmitic acid; STA: Stearic acid; APP- Appearance; COL- Colour; FLA- Flavour; TAS- Taste; TEX- Texture; OAA- Overall acceptability

0.27) and PLA (0.16). LNA was strongly and positively correlated with PLA (0.72) and positively associated with STA (0.42), APP (0.43), COL (0.34), FLA (0.38), TAS (0.37), TEX (0.35), and OAA (0.35), but showed a weak negative association with O/L (-0.15). In groundnut oil biosynthesis, oleic acid is converted into linoleic acid by oleate desaturase, which is encoded by the *AhFAD2* gene family, so a negative relationship between the two fatty acids is expected (Bandyopadhyay and Desai, 2000; Cholin *et al.*, 2010; Deshmukh *et al.*, 2020; Mukhthambica *et al.*, 2026). Reduced activity or mutation of *AhFAD2* blocks this conversion and leads to the accumulation of oleic acid at the expense of linoleic acid (Deshmukh *et al.*, 2020; Mukhthambica *et al.*, 2026). The strong positive correlations of LNA with PLA (0.72) and of STA with PLA (0.81) are consistent with the positive associations among saturated and polyunsaturated fatty acids reported by Mukhthambica *et al.*, (2026) and Yol *et al.*, (2017). O/L was positively associated with PLA (0.48), STA (0.74), APP (0.63), COL (0.65), FLA (0.67), TAS (0.68), TEX (0.72), and OAA (0.72). PLA was strongly and positively correlated with STA (0.81), APP (0.78), COL (0.72), FLA (0.80), TAS (0.79), TEX (0.77), and OAA (0.79), and STA was strongly and positively associated with APP (0.78), COL (0.75), FLA (0.81), TAS (0.80), TEX (0.81), and OAA (0.83). Among the sensory traits, APP was very strongly correlated with COL (0.96) and positively associated with FLA (0.81), TAS (0.77), TEX (0.74), and OAA (0.83). COL was positively associated with FLA (0.75), TAS (0.71), TEX (0.75), and OAA (0.81), FLA was very strongly correlated with TAS (0.90) and positively associated with TEX (0.79) and OAA (0.88), and TAS was very strongly correlated with TEX (0.87) and OAA (0.95). TEX was very strongly correlated with OAA (0.91).

Principal component analysis and cluster structure

Variance structure and principal component contribution:

Principal component analysis showed that the first five principal components (PCs) together accounted for 91.28% of the total phenotypic variation among the 36 confectionery groundnut genotypes (Fig. 2A). PC1 explained the largest share of the variation (34.38%), followed by PC2 (24.40%), PC3 (15.38%), PC4 (9.10%), and PC5 (8.02%). PC1 and PC2 together explained 58.78% of the total variation (Table 4), indicating that more than half of the variability among the genotypes was captured in the first two dimensions.

Table 4: Principal component analysis of 36 advanced confectionery groundnut breeding lines for fatty acid composition and sensory quality traits.

Variables	PC1	PC2	PC3	PC4	PC5
OLA	0.76	-0.43	0.11	-0.17	-0.11
LNA	-0.91	0.21	0.03	0.05	0.04
O/L	0.92	-0.32	0.02	-0.12	-0.01
PLA	-0.86	0.40	-0.02	-0.06	0.06
STA	0.19	-0.07	-0.11	0.94	-0.23
APP	0.15	0.60	0.73	-0.01	-0.20
COL	0.28	0.49	0.79	0.07	0.07
FLA	0.27	0.70	-0.50	-0.12	-0.23
TAS	0.36	0.69	-0.50	-0.08	-0.12
TEX	0.41	0.27	-0.12	0.19	0.84
OAA	0.54	0.74	-0.07	0.04	0.00
Eigen value	3.78	2.68	1.69	1.00	0.88
Proportion of variance (%)	34.38	24.40	15.38	9.10	8.02
Cumulative variance (%)	34.38	58.78	74.16	83.26	91.28

OLA: Oleic acid; LNA: Linoleic acid; O/L: Ratio of oleic to linoleic acid; PLA: Palmitic acid; STA: Stearic acid; APP- Appearance; COL- Colour; FLA- Flavour; TAS- Taste; TEX- Texture; OAA- Overall acceptability

Multivariate trait relationships:

The PCA variable plot revealed distinct patterns of association among the fatty acid composition and sensory quality traits (Fig. 2B). PC1 was positively associated with O/L (0.92) and OLA (0.76) and strongly negatively associated with LNA (-0.91) and PLA (-0.86), and can therefore be interpreted as a fatty acid composition axis contrasting oleic-rich with linoleic- and palmitic-rich genotypes. PC2 was positively associated with OAA (0.74), FLA (0.70), TAS (0.69), and APP (0.60), and thus represents a sensory quality axis. COL (0.79) and APP (0.73) loaded strongly and positively on PC3, whereas FLA and TAS loaded negatively (-0.50 each). STA had the highest positive loading on PC4 (0.94), and TEX the highest on PC5 (0.84). These loading patterns indicate that the major axes of variation were shaped by fatty acid composition and sensory quality traits. This separation agrees with Mukhthambica *et al.* (2026), where PCA identified PLA, OLA, and LNA as having the highest loadings on PC1, with the oleic acid vector forming an angle greater than 90° with those of LNA, PLA, and STA. The PCA biplot separated the genotypes into three groups on the basis of their multivariate trait profiles (Fig. 2C), and the Circos plot illustrated the contribution of individual traits to the PCs (Fig. 2D).

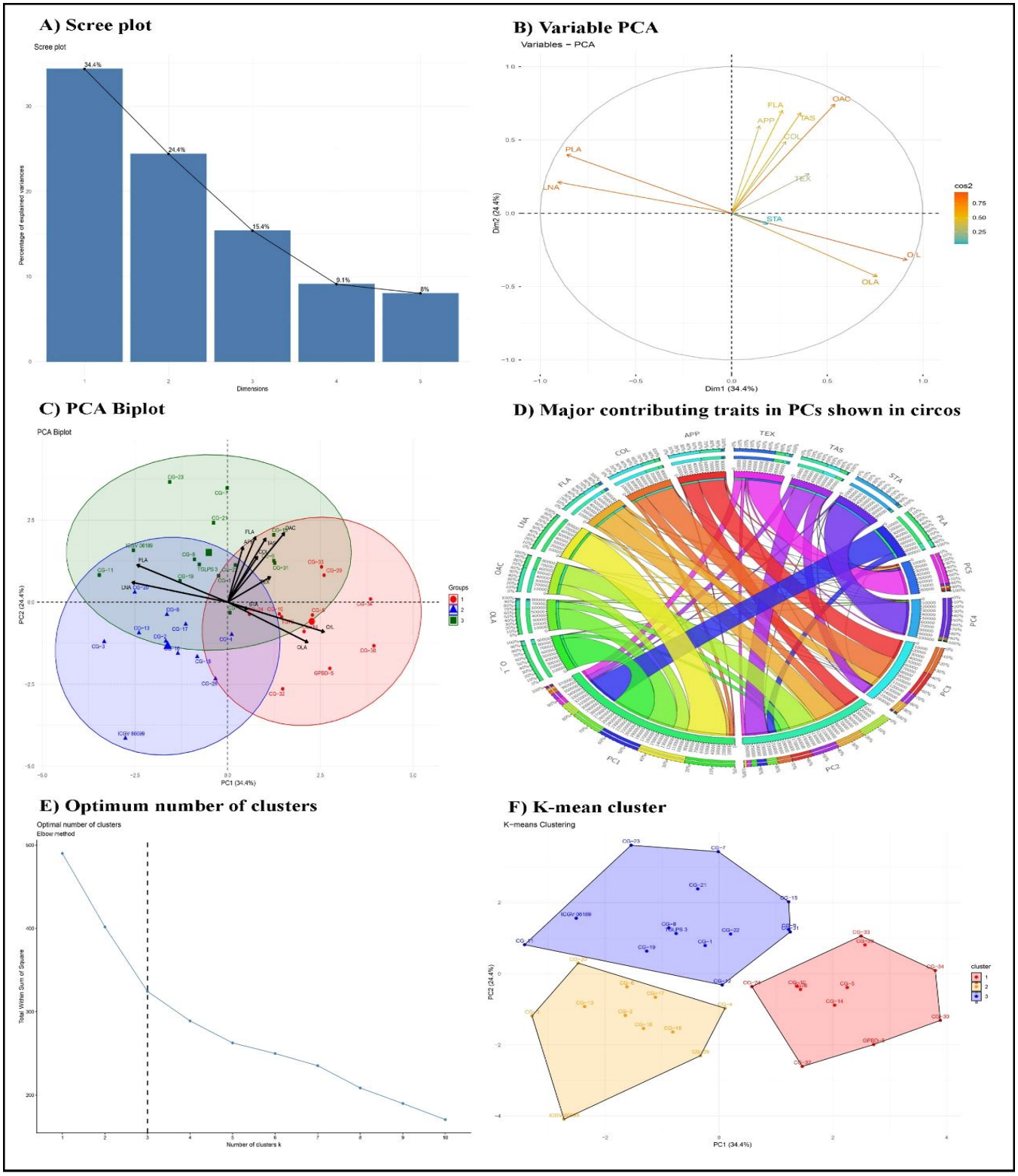


Fig. 2: Multivariate analysis and cluster structure of 36 confectionery groundnut genotypes based on fatty acid composition and sensory quality traits. (A) Scree plot showing eigenvalues and the proportion of total variance explained by each principal component. (B) PCA variable plot showing the contribution and association of fatty acid composition and sensory quality traits across the principal components. (C) PCA biplot showing the distribution of genotypes based on their multivariate trait profiles. (D) Circos plot illustrating the contribution of individual traits to the principal components. (E) Determination of the optimal number of clusters based on the Elbow method. (F) K-means cluster analysis showing the grouping of 36 confectionery groundnut genotypes into three clusters based on fatty acid composition and sensory quality traits.

Cluster structure and genotypic grouping:

The elbow criterion identified three clusters (k = 3) as the optimum, indicating three distinct groups within the 36-genotype panel (Fig. 2E). K-means clustering grouped the genotypes into Cluster I (11 genotypes), Cluster II (11 genotypes), and Cluster III (14 genotypes) (Fig. 2F). Cluster I was located predominantly in the positive PC1 and negative PC2 region and was characterized by high OLA and O/L. Cluster II was located predominantly towards negative PC1 and was characterized by high LNA and PLA. Cluster III was located predominantly in the negative PC1 and positive PC2 region and was characterized by high APP, FLA, TAS, and OAA. A similar grouping was documented by Mukhthambica *et al*., (2026), who resolved groundnut genotypes into high-oleic clusters (high OLA and O/L with low LNA and PLA), suitable as donor parents for quality improvement, and separate clusters with elevated polyunsaturated and saturated fatty acids. In confectionery breeding, selecting parents from Cluster I (high O/L, indicating greater potential oxidative stability) and crossing them with genotypes from Cluster III (superior sensory scores) offers a promising hybridization strategy to combine long shelf life with enhanced consumer acceptance.

Conclusions

This study provides practical guidance for breeding confectionery groundnut cultivars that combine good oil quality with high sensory acceptance. The 36 advanced breeding lines differed widely in fatty acid composition and sensory quality, and the high broad-sense heritability of all traits indicates that most of the observed differences were genetic, so lines can be selected on their mean performance, although this should be confirmed across seasons and locations. Oleic acid content and the oleic-to-linoleic acid ratio varied enough to allow selection for improved oil quality. Oleic acid content can serve as a simple selection criterion, since it was strongly correlated with oleic-to-linoleic acid ratio and positively associated with all sensory scores, which suggests that selection for it is unlikely to lower sensory acceptance. The six sensory traits were very strongly intercorrelated, so breeders could screen with fewer attributes, such as overall acceptability, saving panel time and cost. Multivariate analysis grouped the lines into three clusters of 11, 11, and 14 lines. Lines of Cluster I (high oleic acid content and O/L) and Cluster III (superior appearance, flavour, taste, and overall acceptability) are recommended as parents, and crosses between them offer the best prospect of recovering progeny that combine improved oil quality with strong sensory acceptance.

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