

Genetic Divergence and Identification of Promising Parents for Seed Yield and Nutritional Quality Traits in Linseed

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Research Article

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Abstract

Identification of genetically divergent parents is essential for broadening the genetic base and enhancing breeding efficiency in linseed. Therefore, a panel of 51 linseed (*Linum usitatissimum* L.) entries, comprising 45 genotypes and six check cultivars, was evaluated using Mahalanobis D² statistics to characterize genetic diversity and identify superior parental combinations for seed yield and nutritional quality improvement. Ten agronomic and seed quality traits, including days to 50% flowering, days to maturity, plant height, branches per plant, capsules per plant, 1000-seed weight, seed yield per plant, omega-6, omega-3 fatty acid, and secoisolariciresinoldiglucoside (SDG) content, were recorded. The genotypes were grouped into eight distinct clusters, indicating substantial genetic diversity. Cluster VIII exhibited the highest intra-cluster distance (0.697), whereas the greatest inter-cluster divergence was observed between Clusters V and VI (17.056), followed by Clusters V and VIII (16.486). Cluster VII recorded the highest omega-3 fatty acid (55.65%) and SDG (10.94 mg g⁻¹) contents, while Cluster I exhibited the highest seed yield per plant (4.56 g) and Cluster VI the highest 1000-seed weight (6.83 g). The integration of genetic divergence with cluster mean performance enabled the identification of promising parental combinations for simultaneous improvement of seed yield and nutritional quality. These findings provide a practical framework for parent selection in linseed breeding programmes, although multi-environment validation will be essential to confirm the stability of the identified genotypes.

INTRODUCTION

Linseed (*Linum usitatissimum* L.) is one of the oldest domesticated oilseed crops, cultivated worldwide for its seed oil, dietary fibre, and industrial applications. In recent years, it has gained considerable attention as a functional food owing to its exceptionally high content of α -linolenic acid (ALA; omega-3 fatty acid) and lignans, particularly secoisolariciresinoldiglucoside (SDG), which possess antioxidant, anti-inflammatory, cardioprotective, and anticancer properties (Kajla et al., 2015; Goyal et al., 2014; Noreen et al., 2023). Consequently, the development of nutritionally superior linseed cultivars has become an important objective in modern breeding programmes.

India is one of the major linseed-producing countries, where the crop is cultivated predominantly under rainfed conditions. However, productivity remains relatively low because of a narrow genetic base, environmental fluctuations, and limited use of genetically diverse parents in breeding programmes (FAOSTAT, 2024; Directorate of Economics and Statistics, 2024). Therefore, identifying genetically diverse parents with superior agronomic performance and enhanced nutritional quality is essential for broadening the breeding pool and accelerating genetic gain.

Genetic diversity is the cornerstone of crop improvement because it determines the likelihood of recovering superior recombinants following hybridization. The magnitude of genetic divergence among parents has been reported to influence heterosis and transgressive segregation in segregating generations (Arunachalam, 1981). Among multivariate approaches, Mahalanobis D² statistics provide a robust method for assessing genetic divergence using multiple quantitative traits simultaneously, while Tocher's clustering facilitates the classification of genotypes into genetically homogeneous groups and assists in the identification of suitable parents for hybridization (Mahalanobis, 1936; Rao, 1952).

Although several studies have employed Mahalanobis D² analysis to evaluate genetic diversity in linseed based on agronomic traits, relatively few have integrated **nutritional quality traits**, particularly omega-3 fatty acid and SDG, with conventional yield attributes for parental selection. Considering the increasing demand for nutritionally enriched functional foods, breeding programmes should simultaneously target high seed yield and enhanced nutritional quality. Integrating agronomic and nutraceutical traits within a genetic divergence framework offers a more comprehensive strategy for identifying elite parents capable of improving both productivity and seed quality.

Therefore, the present study evaluated 51 released linseed varieties, including six standard check cultivars, for agronomic and seed quality traits. Genetic divergence was estimated using Mahalanobis D² statistics, followed by Tocher's cluster analysis and cluster mean evaluation to identify genetically diverse and nutritionally superior parental combinations. The

findings provide a practical framework for selecting parents for the simultaneous improvement of seed yield, omega-3 fatty acid, and SDG content, thereby supporting the development of high-yielding and nutritionally superior linseed cultivars.

MATERIALS AND METHODS

Plant Materials

The experimental material consisted of 51 linseed (*Linum usitatissimum* L.) entries comprising 45 released varieties and six standard check cultivars. The germplasm was obtained from the Chandra Shekhar Azad University of Agriculture and Technology (CSAUAT), Kanpur, Uttar Pradesh, India. The entries represented diverse breeding programmes and agro-climatic regions of India. The 45 released varieties were Neelam, Himalini, R-552, Chambal, LC-54, Neela, Pusa-2, Pusa-3, Jawahar-23, Garima, Sweta, Jawahar-1, Jawahar-7, LC-185, Mukta, Janki, Surabhi, S-36, Jeevan, Nagarkot, Shikha, Suyog, Binwa (KL-210), Baner (KL-224), Laxmi-27, Heera, LC-2030, LC-2063, RLC-6, Sheela, JLS-9, Shekhar, Indira als-i-32 (RLC-78), Deepika (RLC-78), Him als-i-1, Him als-i-2, Parvati, Shubhra, C-429, RLC-914, Gaurav, Sharda (LMS-4-27), K-2, RLC-92 and Rashmi. The six standard check cultivars were NL-97, PKV-NL-260, Padmini, Kartika, Kiran and T-397. The field experiment was conducted during the Rabi 2025–26 season at the Research Farm, College of Agriculture, Nagpur, Maharashtra, India. Mature seeds harvested from the evaluated entries were subsequently submitted to the National Agriculture and Food Analysis and Research Institute (NAFARI), Pune, Maharashtra, India, for estimation of omega-3 fatty acid, omega-6 fatty acid and secoisolariciresinol diglucoside content.

Table 1
Summary of plant materials

Category	Description
Released varieties	Neelam, Himalini, R-552, Chambal, LC-54, Neela, Pusa-2, Pusa-3, Jawahar-23, Garima, Sweta, Jawahar-1, Jawahar-7, LC-185, Mukta, Janki, Surabhi, S-36, Jeevan, Nagarkot, Shikha, Suyog, Binwa (KL-210), Baner (KL-224), Laxmi-27, Heera, LC-2030, LC-2063, RLC-6, Sheela, JLS-9, Shekhar, Indira als-i-32 (RLC-78), Deepika (RLC-78), Him als-i-1, Him als-i-2, Parvati, Shubhra, C-429, RLC-914, Gaurav, Sharda (LMS-4-27), K-2, RLC-92 and Rashmi.
Check cultivars	Standard checks: NL-97, PKV-NL-260, Padmini, Kartika, Kiran and T-397
Total entries evaluated	51 (45 released varieties + 6 check cultivars).

Experimental Details

The experiment was conducted in a single environment during *Rabi* 2025–26 seasons at the Research Farm, College of Agriculture, Nagpur, Maharashtra, India using an Augmented Block Design (ABD) as described by Federer (1961). A total of 51 released linseed varieties, comprising 45 released varieties and six standard check varieties, were evaluated in four augmented blocks. The check varieties were replicated across all blocks to estimate block effects and obtain adjusted means for the released varieties. Mature seeds harvested from each genotype were used for laboratory estimation of Omega-3 fatty acid, Omega-6 fatty acid, and secoisolariciresinoldiglucoside (SDG) at the National Agriculture and Food Analysis and Research Institute (NAFARI), Pune, India.

Phenotypic Evaluation of Yield and Seed Quality Traits

Phenotypic evaluation of the released linseed varieties was carried out based on seven agronomic and three seed quality traits. Agronomic observations were recorded for days to 50% flowering, days to maturity, plant height (cm), number of branches per plant, number of capsules per plant, 1000-seed weight (g), and seed yield per plant (g). Days to 50% flowering

and days to maturity were recorded on a plot basis, while the remaining agronomic traits were measured on five randomly selected competitive plants from each genotype, and their mean values were used for statistical analysis.

Mature seed samples from each genotype were analysed at the National Agriculture and Food Analysis and Research Institute (NAFARI), Pune, Maharashtra, India, to determine three nutritionally important seed quality traits, namely Omega-3 fatty acid (α -linolenic acid), Omega-6 fatty acid (linoleic acid), and secoisolariciresinoldiglucoside (SDG). Fatty acid composition was expressed as the percentage of total fatty acids, whereas SDG content was expressed as mg g⁻¹ seed. The analytical procedures were carried out according to the standard laboratory protocols followed at NAFARI.

Table 2
Phenotypic traits evaluated

Phenotypic trait	Unit	Recording/Expression
Days to 50% flowering	Days	Plot basis
Days to maturity	Days	Plot basis
Plant height	cm	Mean of five plants
Branches per plant	Number	Mean of five plants
Capsules per plant	Number	Mean of five plants
1000-seed weight	g	Mean seed weight
Seed yield per plant	g	Mean of five plants
Omega-3 fatty acid (α -linolenic acid)	%	Percentage of total fatty acids
Omega-6 fatty acid (linoleic acid)	%	Percentage of total fatty acids
Secoisolariciresinoldiglucoside (SDG)	mg g ⁻¹	Seed concentration

Figure 1 illustrates the overall experimental workflow adopted in the present investigation. Fifty-one linseed genotypes (45 released varieties and six checks) were evaluated under field conditions for agronomic and seed quality traits. The adjusted trait means were subjected to Mahalanobis D² analysis followed by Tocher clustering, estimation of intra- and inter-cluster distances, and cluster mean analysis. The combined multivariate approach facilitated the identification of genetically divergent parental combinations for simultaneous improvement of seed yield and nutritional quality.

Statistical Analysis

Adjusted means of seed quality traits were estimated using the augmented block design procedure. Genetic divergence among released varieties was estimated using Mahalanobis D² statistics (Mahalanobis, 1936). Based on the D² values, genotypes were grouped into clusters using Tocher's method (Rao, 1952). Average intra- and inter-cluster distances were computed to assess genetic divergence. Canonical vector analysis was performed to identify the relative contribution of seed quality traits toward total genetic divergence, while cluster means were used to identify superior parents for quality improvement.

Table 3
Statistical analyses employed for seed quality traits

Analysis	Objective	Reference
Adjusted mean analysis	Estimation of adjusted trait means	Federer (1961)
Mahalanobis D ² statistics	Assessment of genetic divergence	Mahalanobis (1936)
Tocher's clustering	Classification of genotypes	Rao (1952)
Intra- and inter-cluster distance	Estimation of genetic divergence among clusters	Rao (1952)
Canonical vector analysis	Determination of trait contribution to divergence	Rao (1952)
Cluster mean analysis	Identification of superior clusters	Rao (1952)

RESULTS

Average Intra- and Inter-cluster Distance

The average intra- and inter-cluster distances estimated using Mahalanobis D² statistics are presented in Table 4. The intra-cluster distance ranged from 0.000 to 0.697, indicating varying levels of genetic variability among genotypes within clusters. Cluster VIII exhibited the highest intra-cluster distance (0.697), suggesting comparatively greater genetic diversity among its constituent genotypes, whereas Cluster V, represented by a single genotype (LC-185), recorded an intra-cluster distance of zero. Cluster I also exhibited relatively high intra-cluster variability (0.612), while Clusters II, III, IV, VI, and VII showed comparatively lower intra-cluster distances, indicating greater homogeneity among their constituent genotypes.

Inter-cluster distances were consistently higher than the corresponding intra-cluster distances, demonstrating substantial genetic divergence among clusters. The maximum inter-cluster distance was observed between Clusters V and VI (17.056), followed by Clusters V and VIII (16.486) and Clusters V and VII (11.300). Relatively high inter-cluster distances were also recorded between Clusters III and VIII (8.936), IV and VIII (8.395), and IV and VI (8.343), indicating considerable genetic divergence among these cluster pairs. In contrast, the minimum inter-cluster distance (1.035) was observed between Clusters VI and VII, suggesting close genetic similarity. The overall mean statistical distance ($D^2 = 4.030$) indicated that cluster combinations with inter-cluster distances exceeding this value could be considered promising for hybridization to maximize genetic divergence and increase the probability of recovering superior transgressive segregants.

Table 4
Average intra- and inter-cluster distances among eight clusters

Cluster	I	II	III	IV	V	VI	VII	VIII
I	0.612	1.242	1.586	2.034	7.185	3.107	1.408	4.252
II		0.420	1.814	1.129	4.999	6.299	3.345	6.510
III			0.278	1.118	4.050	6.464	4.065	8.936
IV				0.227	1.956	8.343	4.524	8.395
V					0.000	17.056	11.300	16.486
VI						0.274	1.035	2.047
VII							0.274	1.431
VIII								0.697

Mean statistical distance (D) = 4.030.

PCA biplot

Figure 2 presents the principal component analysis (PCA) biplot illustrating the distribution of the evaluated linseed genotypes based on agronomic and seed quality traits. The the first two principal components accounted for 57.60% of the total phenotypic variation (replace with actual values), indicating that these components adequately summarized the observed variability. The PCA clearly separated the genotypes into distinct groups that were largely consistent with the Tocher clusters obtained from Mahalanobis D² analysis, confirming the existence of substantial genetic diversity. Trait vectors indicated the relative contribution of agronomic and nutritional traits to genotype discrimination and highlighted genotypes possessing favourable combinations of seed yield, omega-3 fatty acid, and SDG content. The close agreement between PCA and cluster analysis further validated the genetic relationships among the evaluated genotypes.

Cluster Means

The mean performance of the eight clusters for agronomic and seed quality traits is presented in Table 5. Considerable variation among clusters for all evaluated traits reflected substantial genetic diversity.

Cluster VII recorded the highest SDG content (10.94 mg g⁻¹) together with the highest omega-3 fatty acid content (55.65%), indicating its potential as an excellent donor for improving seed nutritional quality. Cluster III also exhibited high omega-3 fatty acid content (55.30%), followed closely by Cluster VIII (54.96%). Cluster VI recorded the highest 1000-seed weight (6.83 g) and was the earliest maturing cluster, whereas Cluster I exhibited the highest seed yield per plant (4.56 g). The earliest flowering was observed in Cluster VIII. The complementary distribution of favourable agronomic and nutritional traits among different clusters provides opportunities for selecting genetically divergent parents for the simultaneous improvement of productivity and nutritional quality.

Table 5
Cluster means for agronomic and seed quality traits

Cluster	50% flowering	Days to Maturity	Plant height (cm)	Branches plant ⁻¹	Capsules plant ⁻¹	1000-seed weight (g)	Seed yield plant ⁻¹ (g)	Omega-6 (%)	Omega-3 (%)	SDG (mg g ⁻¹)
I	75.52	115.07	56.37	7.64	98.28	5.73	4.56	11.28	53.02	9.17
II	79.41	118.34	64.07	7.57	84.24	5.53	3.61	9.12	51.59	6.56
III	80.77	113.58	53.64	7.30	83.07	4.94	2.95	9.22	55.30	7.23
IV	81.97	118.45	53.90	7.43	88.50	4.39	3.00	10.51	50.84	9.94
V	88.50	120.75	47.83	7.72	32.72	2.76	0.80	10.05	51.21	7.49
VI	66.17	111.08	48.39	5.56	72.53	6.83	3.95	11.15	53.68	8.85
VII	69.89	114.56	48.61	6.26	93.22	5.97	4.25	11.99	55.65	10.94
VIII	65.43	116.92	46.43	6.52	87.53	6.23	3.98	9.66	54.96	7.49

Correlation analysis

Figure 3 presents the Pearson correlation heatmap illustrating the relationships among agronomic and seed quality traits. The heatmap provides a comprehensive visualization of positive and negative trait associations, facilitating the identification of traits that can be simultaneously improved through selection. Positive correlations indicate the possibility of concurrent genetic improvement, whereas negative correlations suggest potential trade-offs that should be considered

during parent selection. These relationships provide valuable information for developing breeding strategies aimed at improving both seed yield and nutritional quality.

Identification of Promising Parental Combinations

Promising parental combinations were identified by considering inter-cluster distances greater than the overall mean ($D^2 = 4.030$) together with superior cluster mean performance for seed yield, omega-3 fatty acid content, and SDG concentration. Based on these criteria, seven highly divergent cluster combinations, comprising twelve promising parental crosses, were identified for hybridization (Table 6). Among these, PKV NL-260 × LC-185, Kartika × LC-185, NL-97 × Shekhar, Jawahar-1 × Shekhar, PKV NL-260 × Shekhar, Surabhi × PKV NL-260, and Padmini × Him Als-1 emerged as the most promising crosses for the simultaneous improvement of seed yield and nutritional quality traits. In particular, the exceptionally high inter-cluster distance between Clusters V and VI ($D^2 = 17.056$), together with their complementary cluster mean performance, indicates that crosses involving these clusters are likely to generate broad genetic variability and enhance the probability of recovering superior transgressive segregants in subsequent generations.

Table 6
Average inter-cluster distances and promising parental combinations

Cluster combination	Inter-cluster distance (D^2)	Promising cross	Target trait(s)
V × VI	17.056	PKV NL-260 × LC-185	Omega-3, SDG
		T-397 × LC-185	Seed yield, SDG
I × V	7.185	Kartika × LC-185	Seed yield, Omega-3
II × VIII	6.510	NL-97 × Shekhar	SDG, Seed yield
		Jawahar-1 × Shekhar	SDG, Seed yield, Omega-3
II × VI	6.299	NL-97 × Jawahar-23	SDG
		Sharda × Jawahar-23	SDG, Seed yield, Omega-3
II × V	4.999	NL-97 × LC-185	SDG, Omega-3
IV × VII	4.524	Surabhi × PKV NL-260	Omega-3, SDG, Seed yield
		Padmini × Him Als-1	SDG, Omega-3, Seed yield
I × VIII	4.252	PKV NL-260 × Shekhar	Omega-3, SDG
		Kartika × Shekhar	Seed yield, SDG

DISCUSSION

The substantial inter-cluster divergence observed in the present study indicates considerable genetic variability among the evaluated linseed varieties, providing valuable opportunities for parent selection. Crosses between genetically divergent parents are more likely to generate broad segregation and transgressive recombinants, thereby increasing the probability of recovering superior genotypes. Integrating genetic divergence with cluster mean performance enabled the identification of parental combinations possessing complementary agronomic and nutritional attributes, making them particularly valuable for the simultaneous improvement of seed yield, omega-3 fatty acid, and SDG content.

The relatively high intra-cluster distance recorded for Cluster VIII indicates greater genetic heterogeneity among its constituent genotypes, whereas the zero intra-cluster distance of Cluster V reflects its representation by a single genotype (LC-185). Mono-genotypic clusters are generally considered genetically distinct and may harbour unique allelic

combinations that can be exploited to broaden the genetic base of breeding populations. Similar findings have been reported by Kumar et al. (2018), Patel et al. (2025), and Shankar et al. (2026), who emphasized the importance of solitary clusters as reservoirs of unique genetic variability.

The highest inter-cluster distance was observed between Clusters V and VI, followed by Clusters V and VIII and Clusters V and VII, indicating wide genetic divergence among these cluster pairs. According to Arunachalam (1981), hybridization between genetically divergent parents increases the likelihood of exploiting heterosis and recovering superior transgressive segregants. Similar recommendations have been reported by Tewari et al. (2023), Patel et al. (2025), Mandal et al. (2025), and Shankar et al. (2026), who advocated the use of highly divergent parents for broadening the genetic base and improving breeding efficiency.

The PCA biplot further supported the genetic divergence revealed by Mahalanobis D^2 analysis by clearly separating the evaluated genotypes into distinct groups. The close agreement between PCA and cluster analysis confirms the reliability of the identified genetic relationships. Moreover, the orientation of trait vectors facilitated the identification of genotypes possessing desirable agronomic and nutritional characteristics, thereby strengthening the selection of suitable parental combinations.

Cluster mean analysis demonstrated that favourable agronomic and nutritional traits were distributed across different clusters. Cluster VII exhibited the highest omega-3 fatty acid and SDG contents, identifying it as an important donor for improving seed nutritional quality, whereas Cluster I recorded the highest seed yield per plant and Cluster VI combined higher 1000-seed weight with early maturity. The complementary distribution of these desirable traits indicates that strategic hybridization among genetically divergent parents could facilitate the simultaneous improvement of productivity and nutritional quality. Similar breeding strategies integrating genetic divergence with cluster mean performance have been reported by Kumari et al. (2018), Tewari et al. (2023), Patel et al. (2025), and Shubham et al. (2026).

Correlation analysis further revealed the relationships among agronomic and nutritional traits, providing valuable information for indirect selection. Traits exhibiting positive associations with seed yield can be effectively utilized as selection criteria in breeding programmes, whereas negatively associated traits should be carefully considered to minimize undesirable correlated responses. The integration of correlation analysis with genetic divergence provides a more comprehensive basis for selecting superior parents and designing efficient breeding strategies.

Integrating genetic divergence with cluster mean performance enabled the identification of several promising parental combinations. Among these, PKV NL-260 × LC-185, Kartika × LC-185, NL-97 × Shekhar, Jawahar-1 × Shekhar, Surabhi × PKV NL-260, Padmini × Him Alsi-1, and PKV NL-260 × Shekhar combined wide genetic divergence with complementary agronomic and nutritional attributes. In particular, PKV NL-260 × LC-185, involving the most divergent cluster combination (V × VI), represents a promising cross for enhancing omega-3 fatty acid and SDG content while generating broad genetic variability. Similar breeding strategies have been advocated by Patel et al. (2025), Mandal et al. (2025), and Shankar et al. (2026) for developing high-yielding and nutritionally superior linseed cultivars.

Study limitations and future perspectives

The present study was conducted under a single-environment condition; therefore, the observed genetic divergence and cluster composition may have been influenced by environmental effects. Genotype × environment (G×E) interactions can substantially affect the expression of agronomic and seed quality traits, potentially influencing estimates of genetic divergence and parental selection. Consequently, the promising parental combinations identified in this study should be validated across multiple environments and years to confirm their stability and broad adaptability. Future studies integrating multi-environment phenotyping with molecular marker-based diversity analysis, genome-wide association studies (GWAS), and genomic selection will further improve the precision of parent selection and accelerate the development of high-yielding, nutritionally superior linseed cultivars.

CONCLUSION

The present study demonstrated substantial genetic diversity among the evaluated linseed varieties and identified several highly divergent parental combinations with complementary agronomic and nutritional attributes. Integrating Mahalanobis D² analysis with cluster mean performance proved to be an effective strategy for selecting parents for the simultaneous improvement of seed yield, omega-3 fatty acid, and SDG content. Validation of these parental combinations under multi-environment conditions will further enhance their utility in developing nutritionally superior and high-yielding linseed cultivars.

Declarations

Funding

The authors received no specific funding for this study.

Competing Interests

The authors declare no competing interests.

Author Contributions

Amit T. Adsul conceived and designed the study, performed the experiments, analyzed the data, interpreted the results, and wrote, reviewed, and edited the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study involved cultivated linseed (*Linum usitatissimum* L.) germplasm and did not involve human participants or animals. The plant material consisted of released linseed varieties and standard check cultivars obtained from the Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India. The cultivated germplasm was used in accordance with applicable institutional and national guidelines. No wild, endangered, or protected plant material was involved in the study.

Permissions to Collect the Plants/Plant Parts

Not applicable. The experimental seed material was obtained from an established cultivated institutional germplasm source and was not collected from wild populations, forests, protected areas, government-owned land, or private farmland. Therefore, no field collection permit or licence was required.

Consent for Publication

Not applicable.

Clinical Trial Registration

Not applicable. This study is not a clinical trial.

Declaration of Generative AI Use

During the preparation of this manuscript, the author used generative AI tools solely for language editing, grammar correction, and improvement of readability. The author reviewed and edited the output and takes full responsibility for the accuracy, originality, and integrity of the manuscript.

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References

1. Arunachalam V. Genetic distance in plant breeding. *Indian J Genet Plant Breed*. 1981;41:226–36.
2. Directorate of Economics and Statistics. *Agricultural Statistics at a Glance 2024*. Department of Agriculture and Farmers Welfare, Government of India; 2024.
3. FAOSTAT. *FAOSTAT Statistical Database*. Rome: Food and Agriculture Organization of the United Nations; 2024.
4. Federer WT. Augmented designs with one-way elimination of heterogeneity. *Biometrics*. 1961;17(3):447–73.
5. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: An ancient medicine and modern functional food. *J Food Sci Technol*. 2014;51(9):1633–53.
6. Kajla P, Sharma A, Sood DR. Flaxseed—A potential functional food source. *J Food Sci Technol*. 2015;52(4):1857–71.
7. Kumari S, Rani N, Pal AK, & Nirala RBP. Genetic divergence study in linseed (*Linum usitatissimum* L.) through D² and principal component analysis. *J Oilseeds Res*. 2018;35(4):264–9. 10.56739/jor.v35i4.137544.
8. Mahalanobis PC. (1936). On the generalized distance in statistics. *Proceedings of the National Institute of Sciences of India*, 2: 49–55.
9. Mandal RK, Mallikarjun, Suresh K, et al. Studying genetic diversity in genotypes of linseed (*Linum usitatissimum* L.) using agro-morphological traits for rainfed conditions. *J Adv Biology Biotechnol*. 2025;28(2):411–8.
10. Patel AY, Wadikar PB, Kulkarni PD, Nakhate VM. Genetic divergence in linseed (*Linum usitatissimum* L.) germplasm. *Int J Plant Soil Sci*. 2025;37(7):540–8.
11. Rao CR. *Advanced Statistical Methods in Biometric Research*. New York: Wiley; 1952.
12. Shankar K, Tiwari N, Singh A, Kumar CVS, Suresh G. Evaluation of genetic diversity in linseed (*Linum usitatissimum* L.) germplasm through D² cluster analysis and PCA. *Indian J Agricultural Res*. 2026;60(2):166–74. <https://doi.org/10.18805/IJARE.A-6385>.
13. Shubham, Singh D, Choudhary R, Verma K, Srivastav R, Ali T. Assessment of genetic variability divergence, biochemical influence on growth and yield parameter in linseed (*Linum usitatissimum* L.). *J Adv Biology Biotechnol*. 2026;29(5):865–78. <https://doi.org/10.9734/JABB/2026/v29i53963>.
14. Noreen S, Tufail T, Ain U, H. B., & Awuchi CG. Pharmacological, nutraceutical, and nutritional properties of flaxseed (*Linum usitatissimum*): An insight into its functionality and disease mitigation. *Food Sci Nutr*. 2023;11(11):6820–9. <https://doi.org/10.1002/fsn3.3662>.
15. Singh RK, Chaudhary BD. *Biometrical Methods in Quantitative Genetic Analysis*. New Delhi: Kalyani; 1985.

Figures

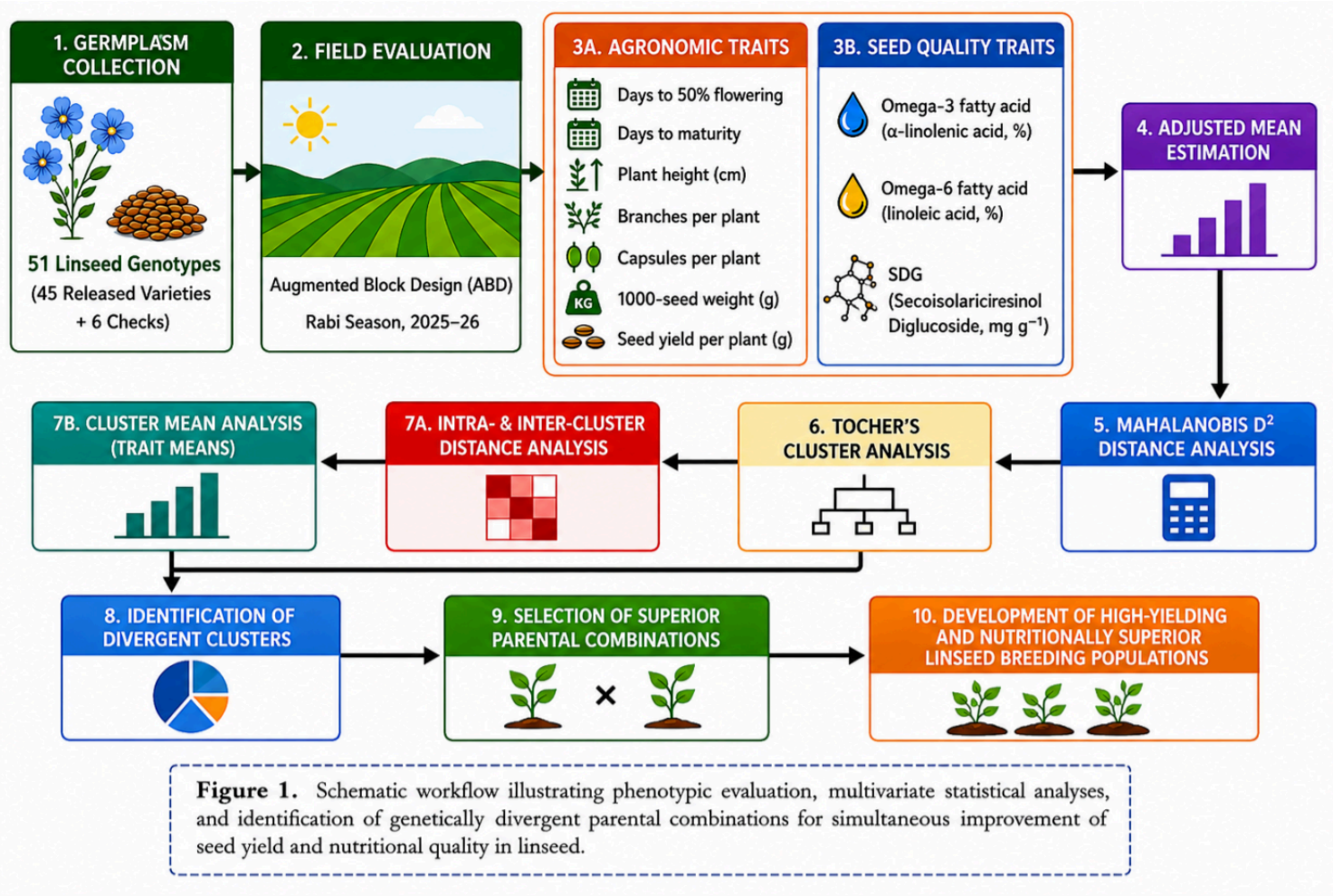
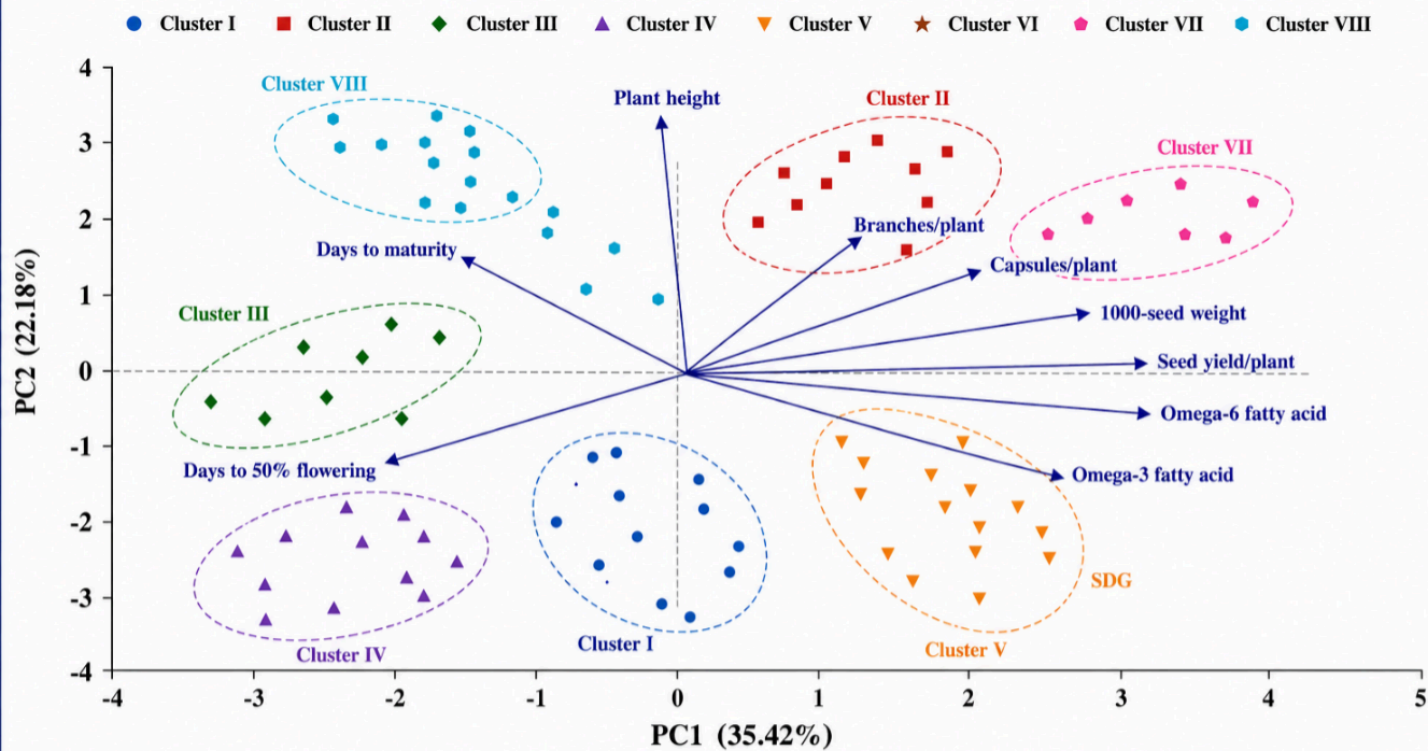


Figure 1

See image above for figure legend

Figure 2. PCA biplot of 51 linseed genotypes based on 10 traits



PC1 and PC2 together explained 57.60% of the total variation.

Cluster VII genotypes are associated with higher omega-3 fatty acid and SDG content, whereas Cluster I genotypes are associated with higher seed yield and earliness.

Figure 2

See image above for figure legend

Figure 3. Correlation heatmap among 10 traits



- Red color indicates positive correlation, blue indicates negative correlation and white indicates near zero correlation.
- Seed yield per plant showed positive correlation with 1000-seed weight (0.34), omega-3 fatty acid (0.36) and SDG (0.31), and negative correlation with days to 50% flowering (-0.31) and days to maturity (-0.25).

Figure 3

See image above for figure legend