



## Research Article

### Genetic diversity analysis of linseed (*Linum usitatissimum* L.) genotypes using multivariate techniques

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#### Abstract

Linseed (*Linum usitatissimum* L.) is an important oilseed and fiber crop valued for its nutritional and industrial uses, yet its narrow genetic base limits breeding progress. The present study evaluated 25 linseed genotypes to assess genetic variability, heritability, and trait associations using multivariate techniques. Eleven quantitative traits, including seed yield, plant height, oil content, and yield components, were recorded and analyzed through ANOVA, genetic parameters, Principal Component Analysis (PCA), and hierarchical clustering. Significant genetic variation was observed for all traits, with high heritability and genetic advance estimates for seed yield per plant, 1000-seed weight, and plant height, suggesting strong additive genetic control. PCA identified four principal components explaining 74.84 % of total variation, with seed yield per plant, number of capsules per plant, and plant height as the major contributors to divergence. Cluster analysis grouped the genotypes into five distinct clusters, High-yielding genotypes were identified in Cluster IV, Cluster II included early-flowering and early-maturing types, while high oil content genotypes from Cluster I and V were recorded. These findings highlight the presence of substantial genetic diversity within the studied germplasm and provide valuable insights for selecting parental combinations to enhance yield, adaptability, and early maturity in linseed improvement programs.

**Keywords:** Linseed, Genetic variability, Heritability, Principal Component Analysis (PCA), Cluster analysis

#### INTRODUCTION

Linseed (*Linum usitatissimum* L.) is a self-pollinated annual crop cultivated globally for its high-quality oil and strong bast fiber. Its seeds are rich in  $\alpha$ -linolenic acid, making it nutritionally important and industrially valuable for pharmaceuticals, paints, and bio-lubricants. Due to its adaptability to a wide range of agro-climatic zones, linseed plays a crucial role in low-input sustainable farming systems (Diederichsen and Richards, 2020). In India, where both traditional and improved varieties coexist, enhancing genetic potential is key to improving yield stability, oil content, and stress resilience (Yadav *et al.*, 2022).

Improving such complex traits requires understanding the extent of genetic variability within germplasm collections. Conventional selection methods are limited by their inability to interpret interrelated traits effectively. In this context, multivariate techniques particularly

Principal Component Analysis (PCA) and hierarchical clustering offer robust approaches to dissect trait contributions, quantify diversity, and group genotypes based on multiple traits simultaneously (Jolliffe and Cadima, 2016; Mohammadi and Prasanna, 2003; Meena *et al.*, 2023). These approaches are especially useful in self-pollinating species like linseed, which often suffer from a narrow genetic base due to repeated inbreeding (Kaur *et al.*, 2018).

Although several studies have evaluated linseed germplasm using univariate approaches, comprehensive assessments using multivariate tools in Indian accessions remain scarce. Such analysis is critical for identifying elite genotypes that can serve as parents in future breeding programs. Therefore, the present investigation aimed to (1) estimate genetic variability, heritability, and genetic advance for key agro-morphological traits, (2) identify

principal traits contributing to genetic divergence using PCA, and (3) cluster genotypes to facilitate strategic parent selection in linseed improvement.

## MATERIALS AND METHODS

**Plant Material and Experimental Design:** The experiment was conducted during the 2021-22 and 2022-23 rabi season at the BAC Farm, Bihar Agricultural University, Sabour, Bhagalpur, India (25.23°N, 87.06°E). A total of 25 linseed genotypes were evaluated using a Randomized Block Design (RBD) with three replications. The genotypes were planted with a spacing of 30 × 10 cm. Each plot consisted of a single row that was 5 m long. Eleven quantitative traits were recorded on five randomly selected competitive plants of each genotype in each replication. These traits included technical height (cm), plant height (cm), number of primary branches per plant, number of capsules per plant, capsule diameter (mm), number of seeds per capsule, 1000-seed weight (g), oil content (%), and seed yield per plant (g). For reproductive traits like days to 50% flowering and days to maturity recorded on a plot basis in each replication.

**Statistical Analysis:** The studies utilized various statistical methods, including analysis of variance ((Panse and Sukhatme, 1967), PCV and GCV (Burton, 1952), heritability (Allard, 1960) and genetic advance (Johnson *et al.*, 1955), which were carried out using INDOSTAT 9.2 to assess genotypic differences. The Multivariate analysis, including PCA and hierarchical clustering, was performed in R Studio (version 4.2.1) using the packages factextra, ggbiplot, FactoMineR, ggrepel, and NbClust. PCA was conducted on standardized trait values, retaining components with eigenvalues >1.0 (Jolliffe & Cadima, 2016). Hierarchical clustering was executed using Ward's method based on Euclidean distances to assess genotype similarity.

## RESULTS AND DISCUSSION

**Analysis of variance:** Analysis of variance revealed highly significant ( $p < 0.01$ ) genotypic differences for all 11 quantitative traits in linseed, confirming substantial exploitable genetic variability (Table 1). Days to 50% flowering showed a large genotypic mean square (64.83) with significant genotype × season interaction (53.42), indicating season-dependent shifts in flowering response. Similar patterns were observed for days to maturity, where genotypic (39.23) and interaction (35.83) variances greatly exceeded the error variance (5.21). Plant height exhibited particularly high genetic variability (681.64) and interaction effects (679.15), suggesting strong genotype sensitivity to seasonal conditions. Technical height also showed pronounced genetic (260.91) and interaction (266.80) components relative to the error mean square (9.68). Yield components demonstrated strong genetic control: number of capsules per plant recorded genotypic and interaction mean squares of 223.12 and 195.40, respectively, with a significant seasonal effect (230.39). Capsule diameter, seeds per capsule, and 1000-seed weight all showed highly significant genotypic effects (2.09, 5.46, and 5.69) coupled with notable genotype × season interactions. Oil percentage displayed strong genetic (6.16) and interaction (7.25) variance, indicating environment-dependent oil accumulation. Seed yield per plant showed highly significant effects for genotype (3.28), season (4.50), and interaction (4.19), with comparatively low experimental error (0.30). Across traits, the large ratio of genotypic to error variance confirms high experimental precision and emphasizes that phenotypic differences were primarily genetic rather than random variation. Similar levels of variability in linseed/lentil germplasm have been reported earlier, confirming that these traits are highly responsive to genetic improvement (Adugna and Labuschagne, 2002; Kaur *et al.*, 2018; Sivaraj *et al.*, 2012; Kumar *et al.*, 2020).

**Table 1. Analysis of variance for 11 quantitative traits in linseed**

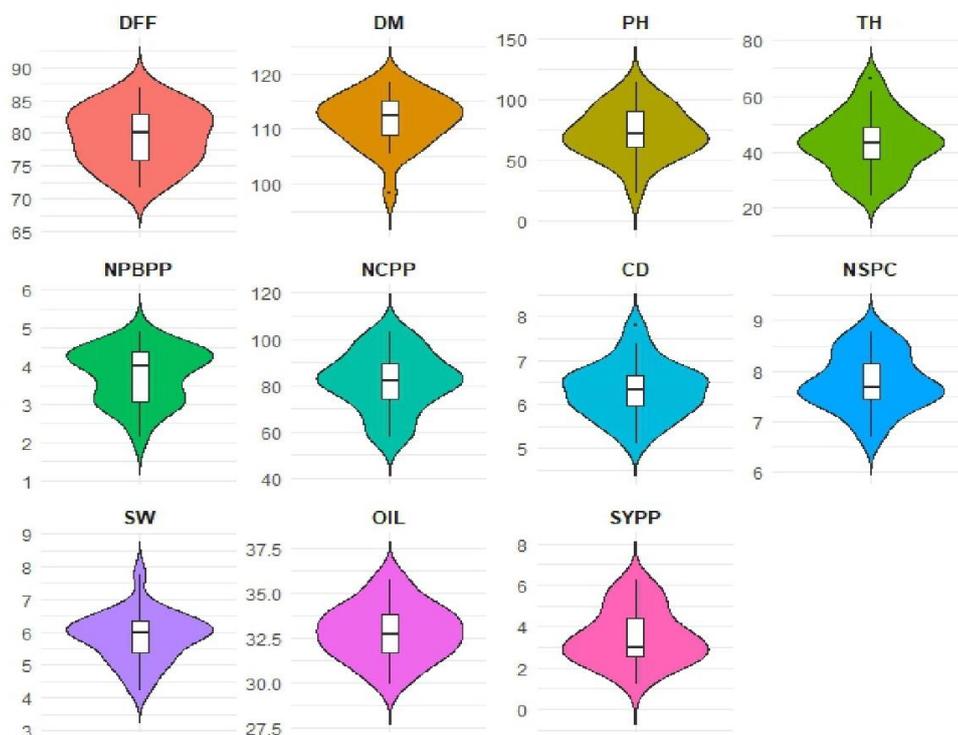
S. No.	Characters	Replication	Genotype	Season	Genotype X Season	Error	Total
	DF	2	24	1	24	98	149
1	Days to 50% flowering	1.262	64.829**	18.727	53.416**	17.466	30.676
2	Days to maturity	23.36	39.232**	11.207	35.832**	5.210	15.906
3	Plant height	15.112	681.644**	58.145	679.154**	27.683	237.989
4	Technical height	2.127	260.908**	0.257	266.797**	9.683	91.398
5	Number of primary branch per plant	0.011	1.711**	0.056	1.576**	0.064	0.572
6	Number of capsule per plant	11.673	223.121**	230.392*	195.401**	35.026	92.153
7	Capsule diameter	0.065	2.085**	0.240	1.786**	0.062	0.667
8	Number of seed per capsule	0.046	5.456**	0.066	2.699**	0.240	1.473
9	1000 seed weight	0.073	5.689**	0.011	3.370**	0.027	1.478
10	Oil percentage	1.445	6.158**	5.944	7.248**	1.521	3.219
11	Seed yield per plant	0.701	3.277**	4.503**	4.187**	0.299	1.438

Note: \*\* & \* indicates significance at  $p < 0.05$  &  $0.01$  respectively.

**Distribution pattern of quantitative traits :** The violin plots illustrate pooled phenotypic distributions of 11 quantitative traits, revealing substantial spread and asymmetry across genotypes (**Fig. 1**). Days to 50% flowering and maturity show relatively narrow, unimodal distributions centered around their means, indicating stabilizing selection and lower environmental plasticity compared with yield components. In contrast, plant height, technical height, and number of capsules per plant exhibit wide density spreads and extended tails, confirming high phenotypic dispersion and the presence of extreme genotypes. Such broad distributions are characteristic of traits governed by additive polygenic control and are consistent with the high GCV and heritability estimates reported in **Table 2**. Yield-related traits, including seed weight and seed yield per plant, display multi-peaked density patterns, suggesting the coexistence of distinct genetic classes within the population. Similar multimodal trait structures have been interpreted as evidence of segregating allelic combinations and exploitable breeding variation (Falconer and Mackay, 1996; Nizar and Mulani 2015). Oil percentage showed comparatively compressed distribution, reflecting tighter genetic regulation and lower phenotypic plasticity.

The violin structure confirms that morphological and yield traits maintain broader phenotypic space than phenological traits, supporting classical expectations that fitness-related traits exhibit greater quantitative variability under selection (Lynch and Walsh, 1998). From a breeding perspective, traits with wider density envelopes represent stronger opportunity for directional selection and transgressive segregation. Comparable trait distribution patterns have been reported in linseed variability studies, where yield components consistently show greater dispersion than phenological traits (Sivaraj *et al.*, 2012; Kumar *et al.*, 2020).

**Genetic variability parameters:** Wide phenotypic ranges across all traits confirmed substantial variability among genotypes (**Table 2**). Plant height (52.51–114.17 cm) and technical height (32.79–63.89 cm) recorded with high genotypic and phenotypic coefficients of variation (GCV 19.11 %, 20.58 %; PCV 20.22 %, 21.57 %, respectively). The close correspondence between GCV and PCV indicates relatively low environmental influence and strong genetic control (Burton, 1952; Falconer and Mackay, 1996). Their high heritability (89.3 % and 91.0 %, respectively) and large genetic advance demonstrate predominance of additive gene action and



**Fig. 1. Violin plot for 11 quantitative traits in Linseed (Pooled for both year)**

Note; Days to 50% Flowering (DFF); Days to maturity; (DM); Plant height (PH); Technical height (TH); Number of primary branches per plant (NPBPP); Number of capsules per plant (NCPP); Capsule diameter (CD); Number of seeds per capsule (NSPC); 1000 seeds weight (SW); Oil content (OIL); Seed yield per plant (SYPP)

**Table 2. Genetic parameters for 11 quantitative traits in linseed**

S. No.	Characters	Range	Mean	Genotypic ( $\sigma^2_g$ )	Phenotypic ( $\sigma^2_p$ )	GCV (%)	PCV (%)	$h^2$ (%)	GA	GA as % of mean
1	Days to 50% flowering	73.50-89.50	4.94	15.72	31.47	4.94	6.98	50.00	5.77	7.19
2	Days to maturity	107.33-119.17	2.70	6.27	15.58	2.22	3.50	40.20	3.27	2.90
3	Plant height (cm)	52.51-114.17	6.61	216.89	242.82	19.11	20.22	89.30	28.67	37.20
4	Technical height (cm)	32.79-63.89	6.48	85.11	93.55	20.58	21.57	91.00	18.13	40.43
5	Number of primary branch per plant	2.79-5.07	7.20	0.51	0.59	18.03	19.41	86.20	1.37	34.48
6	Number of capsule per plant	67.39-99.08	9.23	40.61	94.17	8.04	12.24	43.10	8.62	10.88
7	Capsule diameter (mm)	4.91-8.10	4.07	0.61	0.69	11.70	12.39	89.20	1.52	22.77
8	Number of seed per capsule	5.94-9.95	5.77	1.08	1.30	12.55	13.81	82.60	1.94	23.50
9	1000 seed weight (g)	4.07-8.96	2.58	1.50	1.53	20.54	20.70	98.40	2.50	41.98
10	Oil percentage	31.18-35.96	3.69	1.78	3.26	4.05	5.48	54.60	2.03	6.16
11	Seed yield per plant (g)	3.17-7.85	5.45	0.88	1.04	17.16	18.73	84.00	1.77	38.56

high expected selection efficiency. Comparable trends have been reported in linseed and other oilseed crops, where plant height traits respond strongly to phenotypic selection (Sivaraj *et al.*, 2012; Sivasubramanian and Menon 1973; Dikshit and Sivaraj, 2015; Kumar *et al.*, 2020). Thousand-seed weight exhibited exceptionally high heritability (98.4 %) with nearly identical GCV and PCV (20.54 %; 20.70 %, respectively), confirming minimal environmental interference and strong genetic fixation. Seed yield per plant also showed high heritability (84.00 %), moderate variability (GCV 17.16 %, PCV 18.73%), and substantial genetic advance (32.37 % of mean), indicating reliable improvement through direct selection. Traits combining high heritability and high genetic advance are widely recognized as ideal targets for rapid genetic gain (Johnson *et al.*, 1955; Falconer and Mackay, 1996). Similar observations for yield components have been reported in linseed breeding populations (Sivaraj *et al.*, 2012; Kumar *et al.*, 2020). Primary branches, capsule diameter, and seeds per capsule recorded high heritability (82.60–89.20%) with moderate variability, suggesting effective phenotypic selection. Number of capsules per plant showed moderate heritability (43.10 %) but high genetic advance, indicating both additive genetic and environmental contributions. Days to flowering and maturity exhibited low GCV (4.94 % and 2.22 %, respectively) with moderate heritability (50.00 % and 40.20 %, respectively), reflecting stronger environmental sensitivity. The oil content showed minimal variability and moderate heritability (54.60%), suggesting a delayed response to direct selection. Hybridization is a better tactic than selection alone in certain situations. It is possible to increase recombination and produce transgressive segregants that combine both features by crossing high-yielding genotypes from Cluster IV with high oil content genotypes from Cluster I or V. Efficiency can be increased in separating generations by subsequent selection. For characteristics with complicated inheritance

and moderate heritability, such methods are advised (Falconer and Mackay, 1996; Acquaah, 2012). Across all traits, phenotypic variance exceeded genotypic variance, confirming environmental influence on expression. However, the narrow GCV–PCV gap for yield and its components suggests stable genetic control. Similar variability patterns have consistently been reported in oilseed crops, where yield traits show high exploitable genetic variation and predictable selection response (Falconer and Mackay, 1996; Dikshit and Sivaraj, 2015; Kumar *et al.*, 2020; Singh *et al.*, 2020).

**Multivariate analysis: Principal components and clustering**  
Principal component analysis (PCA) reduced the 11 correlated traits into four principal components with eigenvalues >1, jointly explaining 74.84 % of total variation (**Table 3; Fig. 2**). PC1 alone accounted for 29.96 % of variability and was primarily associated with plant height, technical height, days to maturity, and seeds per capsule, indicating that structural growth and reproductive timing are major axes of divergence. PC2 explained an additional 19.97 % and was strongly influenced by primary branches, capsules per plant, and seed yield per plant, highlighting yield architecture as a second independent dimension of variation. The first two components together captured nearly 50 % of the phenotypic diversity, demonstrating that a limited set of traits drives most inter-genotypic differentiation. The PCA biplot (**Fig. 3**) confirmed that seed yield per plant and number of capsules per plant contributed disproportionately to total variation, as reflected by their longer vector lengths. These traits represent central yield determinants and align with previous multivariate studies identifying capsule number and plant architecture as dominant contributors to linseed productivity (Dikshit and Sivaraj, 2015; Kumar *et al.*, 2020). Genotypes distributed widely across quadrants, indicating broad genetic dispersion and absence of trait redundancy.

Table 3. Principal component analysis for 11 quantitative traits

S. No.	Characteristics	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
1	Eigen Value	3.30	2.20	1.42	1.33	0.96	0.73	0.48	0.26	0.18	0.13	0.03
2	Proportional variation (%)	29.96	19.97	12.86	12.05	8.77	6.63	4.33	2.35	1.59	1.19	0.30
3	Cumulative variation (%)	29.96	49.93	62.80	74.84	83.61	90.24	94.58	96.93	98.52	99.70	100.00
4	Days to 50% Flowering	-0.434	0.147	-0.320	0.047	-0.198	0.084	-0.319	0.351	-0.556	-0.211	0.248
5	Days to maturity	-0.407	0.102	-0.187	0.016	-0.508	0.123	-0.315	-0.297	0.480	0.282	-0.133
6	Plant height	-0.492	0.052	-0.004	0.035	0.395	0.118	0.134	0.106	0.015	-0.178	-0.723
7	Technical height	-0.439	0.020	0.027	0.231	0.422	0.224	0.287	-0.333	0.005	0.237	0.525
8	Number of primary branches per plant	-0.021	0.600	0.033	-0.050	0.256	-0.279	-0.198	0.072	0.449	-0.435	0.239
9	Number of capsules per plant	0.160	0.592	0.061	0.090	0.154	-0.166	-0.183	-0.090	-0.347	0.597	-0.207
10	Capsule diameter	-0.207	0.074	-0.128	-0.670	-0.117	-0.400	0.323	-0.390	-0.224	-0.074	0.002
11	Number of seeds per capsule	-0.346	-0.079	0.446	0.072	-0.198	-0.475	0.220	0.504	0.123	0.281	0.093
12	1000 seeds weight	0.121	0.011	-0.763	-0.039	0.134	-0.092	0.310	0.372	0.245	0.283	0.026
13	Oil content	0.034	0.302	0.243	-0.522	-0.092	0.639	0.184	0.313	0.079	0.122	0.078
14	Seed yield per plant	0.086	0.389	-0.027	0.452	-0.447	0.077	0.586	-0.104	-0.084	-0.251	-0.086

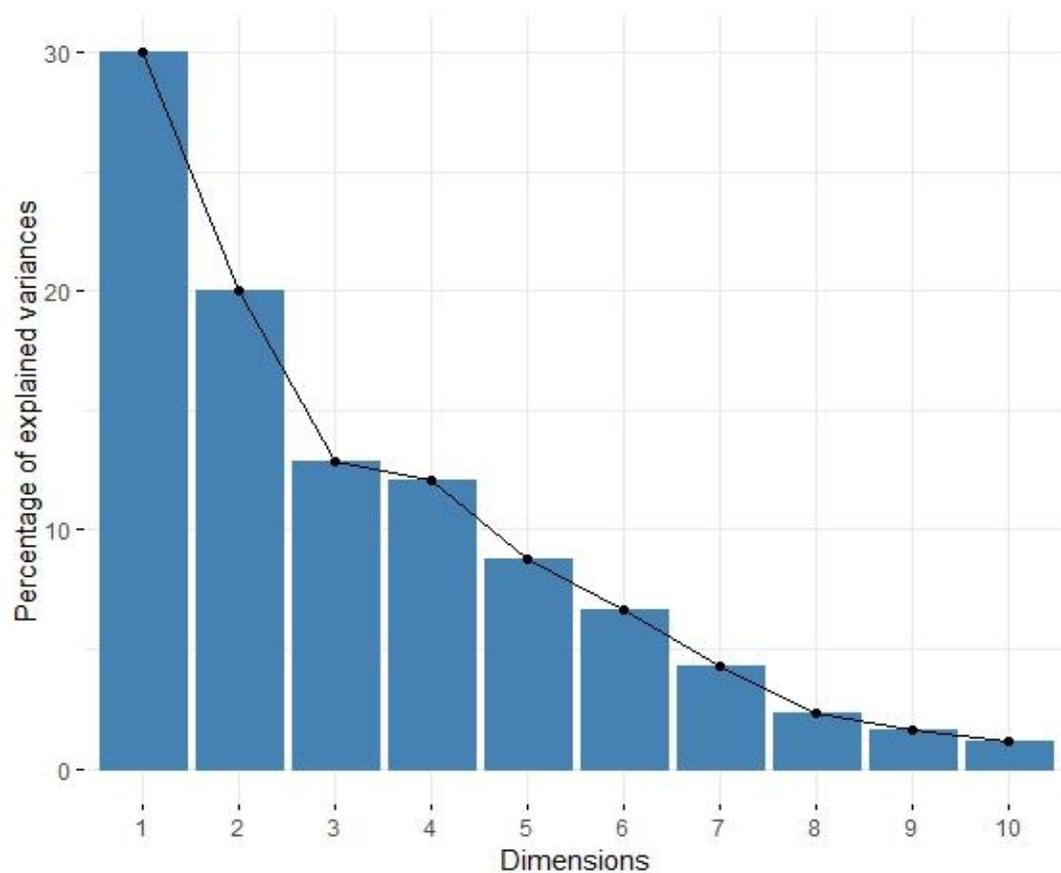
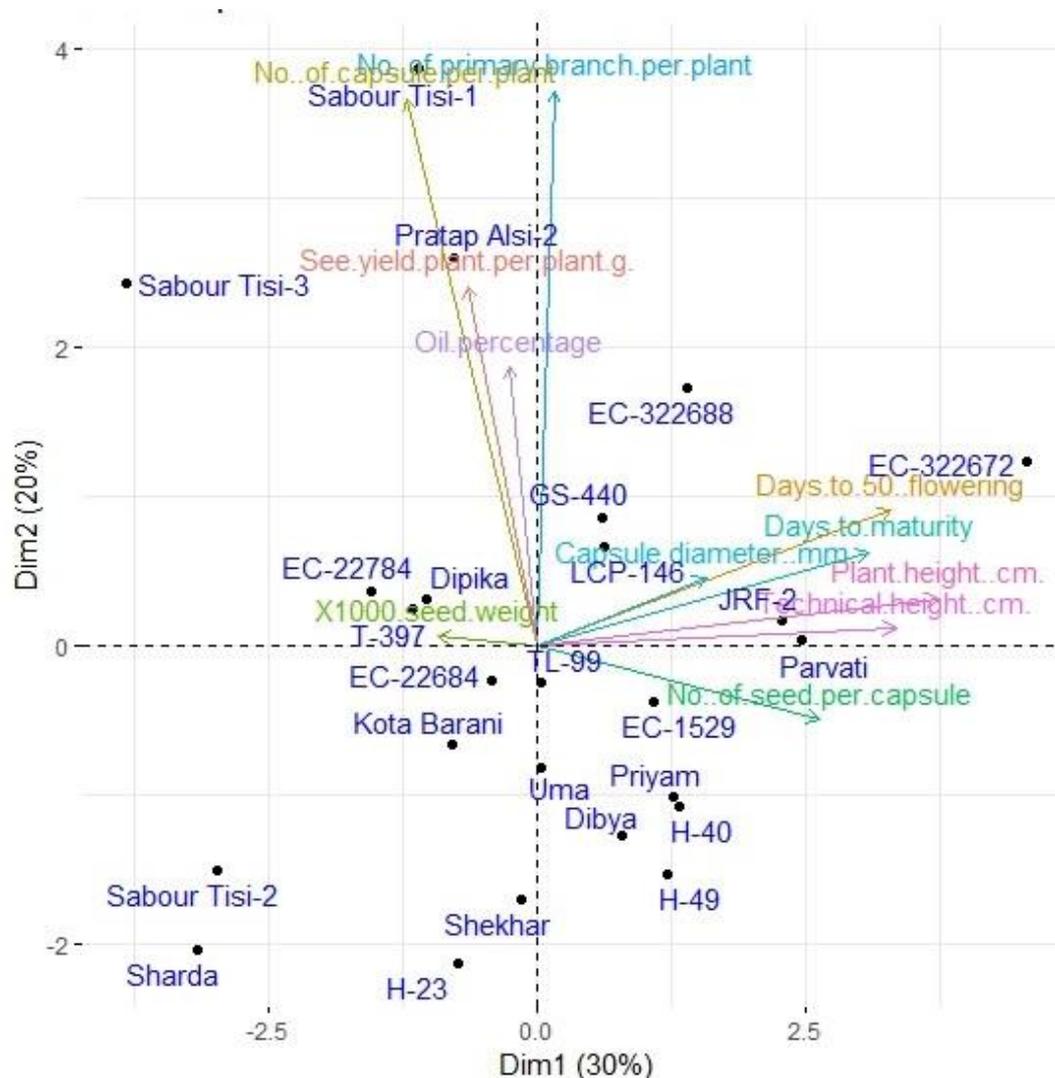


Fig. 2. Scree plot illustrating the eigenvalues and proportional variance explained by each principal component, with an elbow after PC4 indicating retention of four components



**Fig. 3. Biplot depicting the distribution of 25 linseed genotypes and the contribution of 11 traits to PC1 and PC2, with longer vectors for seed yield per plant and capsules per plant indicating their significant role in genetic divergence**

Hierarchical clustering grouped the genotypes into five distinct clusters (Table 4), demonstrating clear genetic structuring. Cluster IV exhibited the highest mean seed yield (4.07 g/plant) and capsule diameter (7.34 mm), while Cluster V showed superior plant and technical height (103.14 cm and 60.13 cm, respectively), suggesting biomass advantage. Cluster II was characterized by early flowering (74.00 days) and maturity (105.22 days), indicating suitability for short-season environments. Cluster differentiation in Table 5 confirms that yield, plant stature, and phenology jointly shape genetic divergence. Crosses between genetically distant clusters particularly Cluster IV (high yield) and Cluster V (vigorous growth) or Cluster II (earliness) are expected to maximize heterosis and produce transgressive segregants. Similar cluster-based parental selection strategies have proven effective in oilseed breeding programs targeting yield

and adaptation (Nachit *et al.*, 1992; Kumar *et al.*, 2020). These results agree with multivariate diversity studies showing that plant architecture and reproductive traits dominate genetic divergence in linseed and other oilseed crops (Kumar *et al.*, 2020; Soto-Cerda *et al.*, 2013; Cai *et al.*, 2014). PCA-driven trait reduction is widely used to identify key yield components and optimize parent selection in breeding programs (Bernardo, 2020; Mohammadi and Prasanna, 2003). Cluster-based parental selection has repeatedly been shown to maximize transgressive segregation in self-pollinated crops (Soto-Cerda *et al.*, 2013; Varshney *et al.*, 2021).

The present study revealed substantial genetic diversity among twenty-five linseed genotypes evaluated across two growing seasons, indicating strong potential for genetic improvement through selection and hybridization.

**Table 4. Composition of genotypes in five clusters**

S. No.	Cluster number	Number of genotypes	Name of genotypes
1	I	3	Parvati, JRF-2, EC-322672
2	II	2	Pratap Alsi-2, EC-322688
3	III	2	Sabour Tisi-1, Sabour Tisi-3
4	IV	8	Shekhar, TL-99, EC-1529, EC-22784, GS-440, H-40, H-49, LCP-146
5	V	10	Sabour Tisi-2, Dipika, Dibya, Priyam, Sharda, T-397, Uma, Kota Barani, EC-22684, H-23

**Table 5. Mean trait values across clusters**

S. No.	Cluster	I	II	III	IV	V
1	Days to 50% Flowering	77.47	74.00	80.17	81.06	86.44
2	Days to maturity	110.80	105.22	113.54	113.28	115.89
3	Plant height (cm)	76.67	56.90	73.27	72.64	103.14
4	Technical height (cm)	45.56	33.63	43.63	39.76	60.13
5	Number of primary branches per plant	3.26	3.76	3.85	4.53	4.38
6	Number of capsules per plant	71.85	83.67	77.72	88.03	81.36
7	Capsule diameter (mm)	6.81	6.00	6.31	7.34	6.62
8	Number of seeds per capsule	8.91	5.86	7.66	7.76	8.44
9	1000 seeds weight (g)	5.14	6.25	6.25	6.32	5.22
10	Oil content (%)	33.20	32.97	31.90	34.54	33.44
11	Seed yield per plant (g)	2.70	2.85	3.72	4.07	2.81

Analysis of variance confirmed significant genotypic differences for all eleven traits. High heritability coupled with high genetic advance for seed yield per plant, 1000-seed weight, plant height, and technical height suggests the predominance of additive gene action, enabling effective improvement through direct selection. Principal Component Analysis identified seed yield, number of capsules per plant, plant height, and maturity traits as major contributors to genetic variability. Cluster analysis grouped genotypes into five distinct clusters, reflecting considerable divergence. High-yielding genotypes were identified in Cluster IV, Cluster II included early-flowering and early-maturing types, while high oil content genotypes from Cluster I and V were recorded. Overall, integrating genetic parameters with multivariate analyses facilitates the identification of superior parents. Hybridization between divergent, high-yielding, and high oil content genotypes is recommended to develop improved linseed cultivars.

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