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Assessment of genetic diversity among linseed (*Linum usitatissimum* L.) germplasm based on morphological traits

Ronika Thakur, Satish Paul, Garima Thakur, Arjun Kumar and Ruchika Dogra

Abstract

Variability and genetic diversity assessment is foremost activity to be embarked before any crop improvement programme. Divergence analysis is helpful in identifying suitable and diverse parents for realizing high heterotic effects with transgressive segregants in later generations. Thus, a field experiment was conducted using 30 genotypes of linseed germplasm in randomized block design with three replications during *rabi* 2018-19 to assess the extent of genetic diversity. As per the results obtained, cluster analysis grouped whole germplasm into VI clusters. Cluster II was the largest with 10 linseed accessions and cluster I was smallest with single accession. The inter-cluster distance was the farthest between cluster I and cluster VI ($D^2=7.143$) followed by cluster I and III ($D^2=7.134$) depicting greater genetic divergence among clusters. The intra-cluster distance ranged from 2.066 in cluster II to 2.776 in cluster IV. The contribution of days to maturity (11.08%), technical height (10.63%) and seeds per capsule (9.66%) towards genetic divergence were higher. During PCA analysis, seven principal components (PC1 to PC7) had eigen values greater than one and accounted for 99.70% of the total variance while the first principal component (PC1) alone explained about 50.30% of the entire variability. The major contributing characters from the first PC, were days to 50% flowering, plant height, technical height, primary and secondary branches per plant, capsules per plant along with aerial biomass and seed yield per plant.

Keywords: cluster analysis, genetic diversity, linseed, principal component analysis

1. Introduction

Linseed (*Linum usitatissimum*.) is an ancient oilseed crop belonging to family Linaceae having 14 genera, out of which *Linum usitatissimum* L. (diploid, $2n=2x=30$) is the only cultivated species of genus *Linum*. Linseed is believed to be originated in the region east of the Mediterranean Sea (Simmonds, 1976; Zohary, 1999) [21, 24]. On the global scenario, India ranks fifth in terms of area and production after Kazakhstan, Canada, Russian federation and China with 0.32 million ha area, contributing 0.17 million tons with the productivity of 543.80 kg per ha (FAOSTAT 2018) [4]. Linseed is a versatile crop grown for both oil and fibre with a varying oil content of about 33-47% in different varieties. However, in India it is mainly grown as an industrial oil seed crop in marginal soils under rainfed conditions.

Linseed is an underutilized crop with varying health benefits. It is the richest vegetarian source of essential polyunsaturated fatty acids (PUFA) i.e. omega-3 linolenic acid (35-66%) and omega-6 linoleic acid (7-18%) (McGregor *et al.*, 1966; Gill, 1987) [12, 5] which cannot be synthesized by human body and have to be ingested through food. PUFA are useful to reduce coronary heart diseases while anti-cancerous properties of lignans adds to the health benefits of linseed. Due to the presence of mucilage fibres, linseed is also ingested for laxative properties. Also its high linolenic acid content has made it an important ingredient for manufacture of paints, varnish, oil- cloth, printing ink, stains, polymer linoleum (Rowland, 1998) [18] etc. because of the rapid drying property that it imparts. Besides its oil, the linseed fibres (phloem fibre) are used by industries for producing high quality linen fabrics having high strength and durability, pulp, biofuel (Diederichsen and Ulrich, 2009) [3], raw materials of thermal insulations (Kymalainen and Sjoberg, 2008) [10], bioplastics (Kwiatkowska *et al.*, 2009) [9], etc.

Ascribed to its both edible and non-edible uses, it has an increasing demand in agro based industries but its average productivity in India is very low as compared to other countries, due to lack of high yielding varieties, cultivation of crop in marginal lands coupled with disease, insect-pest problems, biotic and abiotic stresses. Moreover, the high level of linolenic acid in the oil makes it unsuitable for use in edible products due to undesirable odour and flavour

resulted from the auto-oxidation of the unsaturated fatty acid (Green, 1986) [6]. To tackle these problems varieties need to be developed which are more suited to given agricultural conditions of India. Therefore, variability need to be assessed and genetic diversity needs to be studied as it is the foremost activity to be embarked before any crop improvement programme. Genetic diversity arises due to either geographical separation, crossability barriers or due to different evolutionary pattern. Divergence analysis is attempted to identify suitable parents for realizing heterosis and recombination in breeding programmes. The more diverse parents with in overall criterion of fitness is supposed to give higher amount of heterotic effects along with desirable transgressive segregants and variability in later segregating generations. Different scientists have highlighted the value of parental diversity in optimum magnitude to obtain superior genotypes in the segregating generations (Srivastava *et al.*, 2009 and Tyagi *et al.*, 2015) [22, 23].

Considering the need for assessment of variability and genetic divergence in linseed the present investigation was carried out to identify the divergent genotypes of linseed germplasm using Mahalanobis D² method. The method is based on multivariate analysis of quantitative traits which is a powerful

tool to measure the degree of divergence among genotypes based on multiple characters and for selecting efficient parents for hybridization programme in out-breeding and self-pollinated crops (Rao, 1952; Murty and Anunachalam, 1966) [17, 13].

2. Materials and Methods

The experiment was conducted at Experimental Farm of Department of Genetics and Plant Breeding, CSKHPKV, Palampur, Himachal Pradesh, India (32°8' N, 76°3' E) during *rabi* 2018-2019. The material consisted of 30 linseed genotypes grown in randomized block design with line to line and plant to plant distance of 30 cm × 10 cm, respectively (Table 2.1). All the recommended cultural practices were followed to raise a good crop. Five competitive plants were taken randomly from each plot for recording the observations of various characters namely; plant height (cm), technical height (cm), primary branches per plant, secondary branches per plant, capsules per plant, 1000-seed weight (g), seeds per capsule, aerial biomass (g), harvest index (%) and seed yield per plant (g) whereas, days to 50% flowering and days to 75% maturity were recorded on plot basis.

2.1 List of linseed genotypes under study

S. No.	Genotype	Pedigree/ Source	Sr.no.	Genotype	Pedigree/ Source
1	KL-305	TL-27 × Nagarkot	16	KL-320	(Gaurav × Nagarkot) × Nagarkot
2	KL-306	Nagarkot × T-397	17	KL-321	TL-43 × Binwa
3	KL-307	Him Alsi-2 × Nagarkot	18	KL-322	(TL-43 × Binwa) × TL-43
4	KL-308	T-397 × Nagarkot	19	KL-323	(KL-178 × Ariane) × KL-178
5	KL-309	Canada × Nagarkot	20	KL-324	TL-11 × Him Alsi-2
6	KL-310	Giza-8 × Nagarkot	21	KL-325	TL-37-2 × Him Alsi-2
7	KL-311	Giza-6 × Nagarkot	22	KL-326	Binwa × Him Alsi-2
8	KL-312	Giza-7 × Nagarkot	23	KL-327	(Janki × TL-43) × Janki
9	KL-313	Faiking × Nagarkot	24	KL-284	Rajeena × Him-Alsi-2
10	KL-314	Belinka-60 × Nagarkot	25	Belinka	Exotic collection
11	KL-315	TL-27 × Flake-1	26	K 1 Raja	Exotic collection
12	KL-316	Him Alsi-2 × Binwa	27	Ayogi	Exotic collection
13	KL-317	Him Alsi-1 × Binwa	28	JRF-4	CRIJAF, (Barrackpore)
14	KL-318	Him Alsi-2 × TL-11	29	Nagarkot	New River × LC-216
15	KL-319	(KL-243 × Janki) × KL-243	30	Him Palam Alsi-2	Him-Alsi-2 × Baner

2.2 Statistical analysis: The genetic divergence among the linseed genotypes were calculated by Tocher's method. The D² values were calculated by using the method described by Mahalanobis (1936) [11] and Rao (1952) [17].

3. Results and Discussion

3.1 Cluster Analysis

1. Contribution of each trait towards divergence: Out of twelve traits, 75% maturity (11.08%) and technical height (10.63%) have maximum contribution towards the divergence in linseed germplasm (Table 1). The contribution of seeds per capsule (9.66%), secondary branches per plant (9.46%), plant height (7.95%), capsules per plant (7.89%), 1000-seed weight (7.57%), aerial biomass (7.40%), harvest index (7.36%), days to 50% flowering (6.67%), seed yield per plant (6.37%) were next in order. Hence, these traits must be recorded invariably while evaluating germplasm.

2. Clustering of genotypes: By calculating the estimated D² values as the square of the generalized distance and by following Tocher's method of clustering, 30 linseed genotypes were grouped into 6 clusters (Table 2). Kumar *et al.* (2017) [8] observed six clusters too while analyzing

35 genotypes. The grouping of genotypes from same geographical origin into different clusters may be due to the different genetic backgrounds. Different genetic background is perhaps due to the free exchange of materials among different regions of country for breeding purpose and selection in different environments could be the contributing to the divergence Kasana *et al.* (2018) [7]. In this case, cluster II is the largest with 10 linseed genotypes while cluster I is the smallest with only one genotype. Cluster III and IV contains 6 linseed genotypes each whereas cluster V and VI have 3 and 4 genotypes, respectively. So, such a large and diverse clustering pattern is an indication of existence of significant large amount of variability and diversity in the linseed germplasm.

3. Inter and Intra-cluster distance: The cluster distances have practical implications in plant breeding programmes as they are indicator of genetic diversity and variability. Greater the distance between clusters and within clusters more the divergence among genotypes. The divergence among the genotypes belonging to the same group might be the diverse grouping of genotypes in same cluster with different origins might be due to unidirectional selection

pressure practiced by the breeders during the development programme of the promising genotypes (Raina *et al.* 2015)^[16]. The results revealed that the intra-cluster distance ranged from 2.066 in cluster II to 2.776 in cluster VI (Table 3). The inter-cluster distance was the farthest between cluster I and cluster VI ($D^2=7.143$) and cluster I and III ($D^2=7.134$) (Table 3) depicting greater genetic divergence hence maximizing the chances of obtaining transgressive segregants and recombinants. Sharma *et al.* (2018)^[19] reported the same results, suggesting that crosses should be attempted between the genotypes belonging to clusters separated by large inter-cluster distance. Therefore, crosses between the genotypes belonging to cluster I and cluster VI are likely to produce more desirable transgressive segregants. However, minimum inter-cluster distance was observed between cluster II and cluster IV ($D^2=2.469$) indicating that the genotypes belonging to these clusters are genetically similar. Cluster II has maximum linseed genotypes (Table 2) *viz.* KL-308, KL-310, KL-311, KL-312, KL-316, KL-319, KL-320, KL-326, KL-327 and Nagarkot which have originated in India. Cluster III (KL-314, KL-315, KL-317, KL-322, KL-323 and KL-325) and cluster IV (KL-306, KL-307, KL-309, KL-318, KL-321 and KL-324) have six genotypes each while cluster V contains three genotypes namely, KL- 284, JRF-4 and KL-263. There are four linseed genotypes under cluster VI namely; KL-313, Belinka, K 1 Raja and Ayogi, while cluster I have single genotype KL-305 making it solitary cluster.

In cluster II, KL-312 represented a combination of higher seed yield (2.82g/ plant) with 1000-seed weight (7.08 g) and days to 50% flowering (127days), other genotype KL-326 has relatively higher plant height (68.06cm) and higher 1000-seed weight (7.25g) along with KL-327 and Nagarkot having seed yield (3.05g/ plant, 3.27g/ plant) and 1000-seed weight (6.71g, 7.39g), respectively. Thus, genotypes KL-312, KL-326, KL-327 and Nagarkot are of good choice for plant breeding programmes which could be exploited for seed as well as for flax. Out of six genotypes in cluster III, KL-314 is early with seed yield of 4.12 g per plant. While, KL-315 and KL-322 have higher seed yield (6.73g/ plant, 3.56g/ plant) and 1000-seed weight (7.54g, 7.65g) along with 50% flowering (128 days, 125 days) respectively. For dual purpose linseed breeding programmes, KL-321 (PH=70.48cm; seed yield=3.08g/plant) of cluster IV and Belinka (PH=95.86 cm; seed yield 3.64g/plant) and Ayogi (PH=85.11cm; seed yield 3.18g/plant) of cluster VI would be desirable for better results. While for fibre purpose both KL-284 and JRF-4 of cluster V could be exploited (PH=94.68cm and 76.85cm; 50% flowering 114 days and 124 days, respectively).

4. Cluster means: The cluster means (Table 4) represent the uniqueness of a particular genotype in that cluster and is the basis for choosing parental line from other clusters as well. In present investigation, cluster III is showing highest mean value for seed yield (3.97 g/plant) as well as for 1000-seed weight (7.26g). Cluster V is showing

earliness as genotype attained 50% flowering in 121 days. If one desire to breed fibre type flax then genotypes from cluster VI having highest mean height (88.27cm) could be used for breeding programmes. With the help of cluster means it is possible to select high value genotypes from the formed clusters for direct exploitation as commercial varieties or for use as parents in different hybridization programmes.

3.2 Principal component analysis

Principal component analysis (PCA) is most oftenly used multivariate statistical technique (Crossa, 1990)^[2] which indicates the importance of the largest contributor (trait) to the total variation at each axis for differentiation (Sharma, 1998)^[20]. It is used to transform the data from one set of coordinate axis to another, which preserves, as much as possible, the original configuration of the set of points and concentrates most of the data structure in the first principal component axis. The eigenvalues in PCA are often used to determine how many factors to retain and the thumb rule is to retain those factors whose eigen values are greater than one when PCA is run on correlations (Chaudhary *et al.*, 2016)^[1]. Principal component analysis was conducted in the present study and it grouped the twelve variables into 12 components which contributed for the entire variability present among the tested genotypes. The score plot shows (fig. 1) that seven principal components (PC1 to PC7) had eigenvalues greater than one and eigen values start to form a straight line after seventh Principal Component. These components accounted for 99.70% of the total variance in the data indicating its significance (Table 5). The first three accounted for a cumulative value of about 89.20% whereas first principal component (PC1) alone explained about 50.30% of the entire variability. Important contribution of first PC in total variability was also explained by Paul *et al.* 2017^[15] and Patial *et al.* 2019^[14] in linseed. The analysis revealed that the major contributing characters from the first PC, was days to 50% flowering, plant height, technical height, primary and secondary branches per plant, capsules per plant along with aerial biomass and seed yield per plant. The first main component (PC 1) was positively correlated with only plant height and was negatively correlated with days to 75% maturity, 1000-seed weight, seeds per capsule and harvest index. Second Principal Component however showed a positive correlation with aerial biomass and a strong negative correlation with capsules per plant followed by plant height. This correlation can also possibly be seen in Fig. 2 (biplot) which shows the dispersion of characteristics according to score between the first two PCs. The present finding revealed that most of the traits which are mainly associated with high seed yield i.e. primary branches per plant, secondary branches per plant, capsules per plant, seeds per capsule, 1000-seed weight showed negative correlation or negligible positive correlation with the Principal Components. This means that selecting the genotypes for high yield in hybridization programmes on the basis of these traits is not favorable.

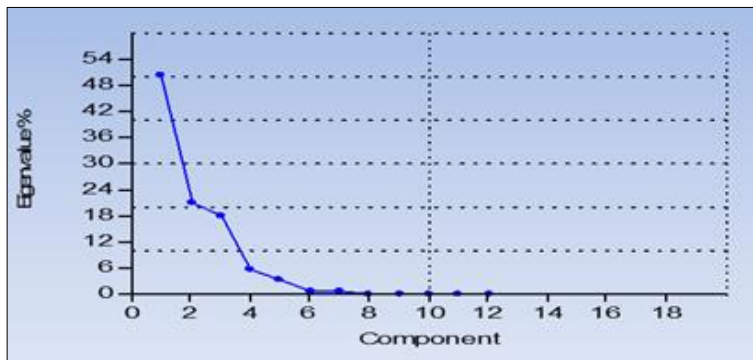


Fig 1: A score plot representing relation between components

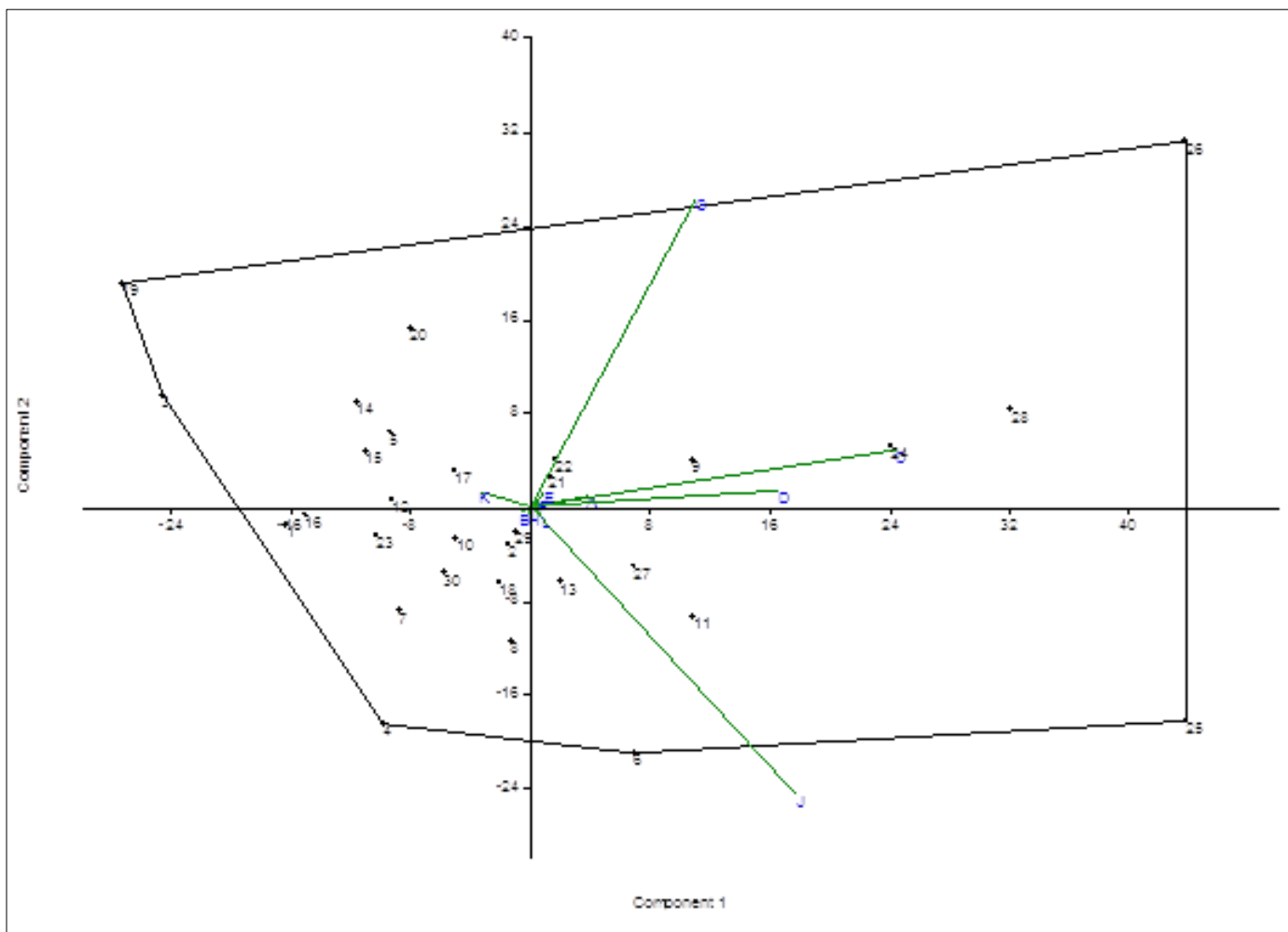


Fig 2: Principal Eigenvalues and different components

Table 1: Percent contribution of 12 characters towards total divergence

S. No.	Character	% Contribution
1.	Days to 50% flowering	6.67
2.	Days to 75% maturity	11.08
3.	Plant height (cm)	7.95
4.	Technical height (cm)	10.63
5.	Primary branches/ plant	7.96
6.	Secondary branches/ plant	9.46
7.	Capsules/ plant	7.89
8.	1000-seed weight (g)	7.57
9.	Seeds/ capsule	9.66
10.	Aerial biomass (g)	7.40
11.	Harvest index (%)	7.36
12.	Seed yield/ plant (g)	6.37

Table 2: Clustering pattern of 30 linseed genotypes by Tocher's method for 12 characters

Clusters	No. of genotypes	Genotypes
I	1	KL-305
II	10	KL-308, KL-310, KL-311, KL-312, KL-316, KL-319, KL-320, KL-326, KL-327, Nagarkot
III	6	KL-314, KL-315, KL-317, KL-322, KL-323, KL-325
IV	6	KL-306, KL-307, KL-309, KL-318, KL-321, KL-324
V	3	KL- 284, JRF-4, KL-263
VI	4	KL-313, Belinka, K 1 Raja, Ayogi

Table 3: Average D² value depicting inter-cluster distances (off-diagonal values) and intra-cluster distances (diagonal values)

Clusters	I	II	III	IV	V	VI
I	2.125					
II	5.203	2.066				
III	7.134	3.342	2.766			
IV	5.058	2.469	3.184	2.397		
V	4.958	3.317	4.601	4.194	2.070	
VI	7.143	4.288	4.824	4.507	4.257	2.776

Table 4: Cluster mean of 6 clusters for different characters in linseed

Characters	Days to 50% flowering	Days to 75% maturity	Plant height (cm)	Technical height (cm)	Primary branches / plant	Secondary branches/ plant	Capsules / plant	1000- seed weight (g)	Seeds / capsule	Aerial biomass (g)	Harvest index (%)	Seed yield plant (g)
I	130.67	181.67	58.29*	35.64	5.47*	4.07*	35.47*	3.76*	7.97	46.06*	26.39**	2.34
II	130.43	183.50	62.93	37.95	8.59	7.60	38.46	7.12	7.79	56.06	19.92	2.32*
III	127.94	184.28**	59.39	32.19*	11.31**	9.21**	53.07	7.26**	7.89	63.57	25.55	3.97**
IV	133.28	184.11	62.65	34.86	9.59	7.65	46.00	6.16	8.42**	46.17	24.40	2.62
V	120.67*	181.56*	77.94	45.27	9.27	7.20	42.04	5.68	7.69*	57.49	19.75	3.11
VI	138.17**	182.92	88.27**	53.84**	10.63	8.60	61.01**	6.09	7.95	68.89**	16.52*	2.70
CV %	1.27	0.60	6.68	10.45	11.95	11.98	12.25	7.56	4.23	12.36	11.61	11.67

Maximum value **

Minimum value *

Table 5: Eigenvectors and eigenvalues of the first 12 principal components for 12 different traits of 30 linseed genotypes

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Days to 50% flowering	0.104	-0.026	-0.009	0.977	0.16	0.001	0.014	-0.048	-0.053	0.041	-0.027	0.032
Days to 75% maturity	-0.019	0.013	0.037	0.07	0.026	0.049	0.34	0.466	0.739	-0.317	-0.078	-0.073
Plant height (cm)	0.667	-0.134	-0.384	-0.12	0.218	-0.513	0.246	-0.015	-0.008	0.054	-0.011	-0.035
Technical height (cm)	0.453	-0.039	-0.394	-0.033	-0.044	0.731	-0.294	0.076	0.058	-0.053	0.03	0.024
Primary branches/ plant	0.023	-0.035	0.08	-0.044	0.006	0.289	0.477	-0.553	0.323	0.513	-0.077	-0.004
Secondary branches/ plant	0.015	-0.014	0.028	-0.018	-0.016	0.277	0.668	-0.001	-0.513	-0.458	0.027	0.036
Capsules/ plant	0.304	-0.724	0.595	-0.021	-0.123	0.04	-0.097	0.04	-0.004	-0.041	0.009	-0.006
1000-seed weight (g)	-0.007	0.015	0.035	0.007	-0.04	0.098	0.209	0.659	-0.238	0.635	0.19	-0.113
Seeds/ capsule	-0.001	0.001	0.005	0.018	0.014	-0.037	0.031	-0.098	0.149	-0.066	0.925	0.323
Aerial biomass (g)	0.486	0.673	0.551	-0.021	-0.038	0.009	-0.05	-0.013	-0.009	-0.029	0.012	-0.042
Harvest index (%)	-0.094	-0.037	0.164	-0.137	0.942	0.164	-0.083	0.018	-0.03	-0.016	0.051	-0.139
Seed yield/ plant (g)	0.017	0.019	0.054	-0.058	0.136	0.006	0.019	0.151	-0.006	0.093	-0.3	0.923
Eigenvalues	292.52	121.696	104.38	32.858	19.80	4.735	3.35	0.79	0.52	0.336	0.15	0.11
Percent of variance (%)	50.30	20.90	18.00	5.70	3.40	0.80	0.60	0.10	0.10	0.10	0	0
Cumulative percentage (%)	50.30	71.30	89.20	94.90	98.30	99.10	99.70	99.80	99.9	1.00	1.00	1.00

Table 6: Parameters of genetic variability for morphological traits of linseed

Genotypes	Mean	Min	Max	Heritability (%)	Genetic Advance (GA)	GA as % mean	GCV (%)	PCV (%)
Days to 50% flowering	130.57	114.00	144.67	92.56	11.59	8.88	4.48	4.66
Days to 75% maturity	183.44	181.33	186.00	42.96	1.30	0.71	0.53	0.80
Plant height (cm)	66.89	45.19	98.46	87.82	23.16	34.62	17.93	19.14
Technical height (cm)	38.95	22.29	62.05	81.69	16.00	41.08	22.06	24.41
Primary branches/ plant	9.57	5.47	12.93	62.13	2.38	24.84	15.30	19.41
Secondary branches/plant	7.91	4.07	10.33	68.46	2.38	30.09	17.65	21.34
Capsules/ plant	46.15	26.40	81.13	78.60	19.79	42.88	23.48	26.48
1000-seed weight (g)	6.56	3.76	8.07	77.06	1.64	25.07	13.86	15.79
Seeds/ capsule	7.96	7.20	9.20	57.66	0.61	7.72	4.93	6.50
Aerial biomass (g)	57.10	38.88	87.53	73.67	20.87	36.55	20.67	24.08
Harvest index (%)	21.69	13.35	35.99	77.49	8.47	39.04	21.53	24.46
Seed yield/ plant (g)	2.84	1.48	6.73	90.30	1.98	69.66	35.58	37.45

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