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Research Article

Assessment of genetic diversity in linseed germplasm using morphological traits

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Abstract

The genetic diversity among 61 linseed genotypes along with 4 checks was studied in the present investigation at the Experimental Farm of Punjab Agricultural University, Regional Research Station, Gurdaspur. Results revealed that the sufficient genetic variability was observed for all the characters studied based on various genetic variability parameters. High heritability coupled with high genetic advance for plant height, technical height, capsules per plant, test seed weight and seed yield per plant indicated additive type gene action and improvement can be made through simple selection. Principal component analysis (PCA) indicated that, out of total principal components, five PCs contributed 84.68 per cent to the total variance amongst the genotypes assessed for eight agronomic traits. PC1 contributed maximum towards the variability (30.91%) followed by PC2 (15.89%) and PC3 (14.95%). Cluster analysis grouped the accessions under four major clusters which indicated a fair association of genetic diversity. Sufficient variability was observed in the genotypes based on phenotypic and genotypic variance, principal component analysis and cluster analysis which can be utilized by researchers in future linseed breeding programmes.

Key words

Linseed, Diversity, Variability, PCA, cluster

INTRODUCTION

Flax (*Linum usitatissimum* subsp. *usitatissimum*) is a self-pollinated diploid ($2n=30$), annual plant species. *Linum* genus originated either in the Middle East or Indian regions and spread throughout Asia and Europe, and later into the New World (Soto-Cerda *et al.*, 2013). The domestication of flax was also observed on the Indian subcontinent near the Mediterranean Sea and this region is known to have high biological diversity of genus *Linum* (Fu, 2005 and Kaur, 2017). Divergent selection applied over thousands of years has resulted in flax and linseed types of *Linum* which are the same species but differ considerably in morphology, anatomy, physiology, and agronomic performance (Soto-Cerda *et al.*, 2013). *Linum bienne* Mill. (= *L. Angustifolium* Huds.) is perhaps the oldest flax wild form cultivated. Its cultivation for fibre and seeds is believed to have led to the development of *L. usitatissimum* L., the modern cultivated flax, one of the early domesticated plants (McDill *et al.*, 2009).

Flax has been used until the 1990s principally for the fabrication of cloths (linen) and papers, while flaxseed oil and its sub-products were used in animal feed formulation (Singh *et al.*, 2011). There is a small difference in using the terms flaxseed and linseed. Flaxseed is used to describe flax when consumed as food by humans while linseed is used to describe flax when it is used in the industry and feed purpose (Morris, 2008). In the last two decades, flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components. Flaxseeds have nutritional characteristics and are a rich source of ω -3 fatty acid: α -linolenic acid (ALA), short chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibres, Phyto estrogenic lignans, proteins and an array of antioxidants (Ivanova *et al.*, 2011; Singh *et al.*, 2011 and Alhassane and Xu, 2010). Keeping in view, the increasing demand

for linseed due to numerous health benefits, there is a consistent need to increase the genetic potential for seed yield. Limited competitiveness due to the narrow genetic base of released cultivars, cultivation under low input conditions (Sood *et al.*, 2007) and sensitivity to fungal diseases are the main constraints for its low productivity.

To develop such desirable genotypes, recombination breeding and trait manipulation offer the potential alternatives which however require suitable parents for the crossing programme. Genetic diversity is the key pillar of biodiversity and diversity within species, between species and of ecosystems. Sustainable utilization of crop diversity is exceptionally important at the present stage of global community development because of the direct linkage with national and global food security. Crop diversity is vital components of the agricultural production systems that ensure food security. The future success of world agriculture will depend on its ability to change and crops will have to be adapted to sustainable forms of agriculture while maintaining increased productivity to feed a growing world population. In the natural population too, severe reductions in population size, the so-called genetic bottleneck, leads to loss of genetic diversity and increased susceptibility to infectious pests and diseases that supervene increased chances of extinction of an individual crop in question. Genetic models indicate that there is a loss of five per cent of its heterozygosity per generation. This indicates that severe bottlenecks degrade heterozygosity and genetic diversity. Crop Diversity provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics by selecting suitable parents and to analyse the nature and magnitude of genetic variation present in the germplasm (Govindaraj *et al.*, 2015). Therefore, the present study on agro-morphological traits to identify the superior germplasm accessions that can contribute as potential donors for future exploitation in the selection and breeding of linseed.

MATERIAL AND METHODS

Sixty-one germplasm lines of linseed including four checks were evaluated for various quantitative traits in augmented block design at Punjab Agricultural University, Regional Research Station, Gurdaspur (Pb.) India during *rabi* 2015-2016. The soil of the experimental site was clay loam medium in organic carbon and phosphorus and low in potassium. The experimental field was divided into four blocks, the first three blocks having 16 entries and the fourth block having 13 entries. The checks (LC 2063, LC 54, T 397 and Binwa) were distributed randomly in each block. Each entry has 2 rows of 3 meter length spaced 30 cm apart with the plant to plant distance of 10 cm. The crop was raised following the practices as per the package of practices of Punjab Agricultural University, Ludhiana. A representative random sample of 5 individual plants from each plot was used for recording the observations on Plant height (PH) in cm, Technical height (TH) in cm, Seeds per capsules (SPC), Capsules per plant (CPP), 1000 seed

weight (TSW) in g. and Seed yield per plant (SYPP) in g. Data on Days to 50% flowering (DF) and Days to 75% maturity (DM) was recorded on a plot basis.

The data were subjected to analysis of variance using the augmented RCBD package (Aravind *et al.*, 2020) of R Software. The adjusted means values of all the quantitative traits were used for estimation of principal component analysis, cluster analysis and correlations with FactoMineR (Sebastien Le *et al.*, 2008), Cluster (Machler *et al.*, 2020) and ggplot2 (Wickham, 2016) packages. The Elbow method from the Cluster package was used to determine the optimum number of clusters.

RESULTS AND DISCUSSION

The basic descriptive statistics based on adjusted mean values of eight quantitative traits showed a wide range of phenotypic expression for agro-morphological traits such as DF (89.00 to 110.00), DM (144.00 to 155.00), PH (48.00 to 106.00 cm), TH (28.00 to 60.30 cm), SPC (5.40 to 10.00), CPP (26.00 to 142.00), TSW (4.57 to 8.43 g) and SYPP (1.00 to 3.88 g) (Table 1). The mean of DF 50% was 98.98, but it ranged from 89.00 for SJKO-13 to 110.00 for EC 1441-1. For DM 75% mean was 148.89, ranged from 144.00 days of EC 41684 to 155.00 for EC 1441-1. PH had a mean value of 75.96, which varied from 48.00 for 2680-RJK-22 to 106.00 for POLF-29-1. The mean of TH was 43.72, varied from 28.00 (2679-RJK-4) to 60.30 (EC 1441-1). The mean of SPC was 7.84, ranged from 5.40 (2663-PKY-11) to 10.00 (T 397). CCP had a mean value of 75.42, ranged from 26.00 (2663-PKY-11) to 142.00 (2674-PKY-22). The mean of TSW was 6.48 g, varied from 4.57 g (NEELAM 2) to 8.43 g (SJKO 14). The mean of SYPP was 2.12 g, ranged from 1.00 g (2663-PKY-11) to 3.88 g (UDNA-1-2-5). The wide range of variability was also observed in earlier studies by Adugna and Labushangne (2002) and Kaur *et al.* (2018) for DF, DM, SPC and TSW; Akbar *et al.* (2003) and Kaur *et al.* (2018) for PH, CPP and TSW and Sivaraj *et al.* (2012) for PH, SPP and TSW. The results of the present study are in agreement with Dikshit and Sivaraj (2015) and Kaur *et al.* (2018) who reported good variability for morphological traits.

The GCV and PCV were high for CPP and SYPP and medium for PH, TH, SPC and TSW, while the low for DF and DM. The heritability was found high (>68%) for all the traits. The estimates of GCV in relation to PCV showed a small difference indicates that observed variation is mainly due to genetic factors while a large difference indicates the role of the environment. The mean GA (%) was high (> 20) for CPP, SYPP, TSW, TH and PH and Medium for SPC and DF (10 to 20) and Low for DM (<10). The high heritability coupled with high genetic advance is predominantly controlled by the additive type of gene action. Accessions such as EC 1441-1, POLF 34-2 JRE-3, SJKO 14, AYOGI had more than 55 cm TH, accessions SJKO 14, 2662-PKY-10, NEELAM 4, LCK 9312 and 2664-PKY-12 had more than 8.20 g TSW and accessions

UDNA 1-2-5, EC 322661, BAU 610-A, LCK 9312 had high mean yield (>3.0 g/plant) can be subjected for fibre, bold seeded and high yield through simple selection based on phenotypic performance. The characters PH, TH, CPP, TSW and SYPP showed high heritability coupled with high genetic advance indicated additive type of gene action for these traits and can be exploited to produce better genotypes in linseed breeding as suggested by Kaur *et al.*

(2018) and Upadhyay *et al.* (2019). The parameter DF and SPC showed high heritability coupled with medium genetic advance and DM showed high heritability coupled with low GA which indicated the importance of dominance and epistatic effects in the inheritance of these traits and selection for these characters would be less effective, so hybridization will be needed for their improvement. The PCA or canonical root analysis is a multivariate

Table 1. Basic descriptive statistics for eight quantitative traits of 61 germplasm lines of linseed

Traits	Mean±SE	Range	Variance (P)	Variance (G)	GCV (%)	PCV (%)	Heritability (bs)	Genetic Advance as % of mean	Skewness	Kurtosis
DF	98.98±1.18	89.00-110	26.21	24.81	5.08	5.22	94.67	10.20	0.33	2.24
DM	148.89±1.19	144.00-155.00	8.95	7.62	1.86	2.02	85.10	3.54	0.47	2.25
PH	75.96±3.96	48.00-106.00	132.83	117.10	14.16	15.08	88.16	27.43	-0.12	3.06
TH	43.72±2.16	28.00-60.30	52.21	47.53	15.56	16.30	91.05	30.62	0.12	2.94
SPC	7.84±0.58	5.40-10.00	1.09	0.75	11.08	13.35	68.91	18.98	-0.39	2.78
CPP	75.42±8.09	26.00-142.00	841.75	776.28	39.20	40.82	92.22	77.67	0.83	2.99
TSW	6.48±0.29	4.57-8.43	1.38	1.29	17.16	17.74	93.54	34.23	-0.11	1.82
SYPP	2.12±0.12	1.00-3.88	0.38	0.37	28.27	28.81	96.30	57.24	0.91	4.07

statistical technique attempt to simplify and analyze the inter relationship among a large set of variables in term of a relatively a small set of variables or components without losing any essential information of original data set. The PCA reduce relatively a large series of data into a smaller number of components by looking for groups that have very strong inter-correlation in a set of variables and each component explained per cent variation to the total variability. The first principal component is the largest contributor to the total variation in the population followed by subsequent components. The criteria used by Clifford and Stephenson (1975) and corroborated by Guei *et al.* (2005), suggested that the first three principal components are often the most important in reflecting the variation patterns among accessions, and the characters associated with these are more useful in differentiating the accessions. Thus, it is useful for genetic improvement of important traits having larger contributions to the

variability rather than going for all the characters under study. The first five PCs having Eigenvalue of more than 0.85 contributed 84.68 %of the total multivariate variation present in linseed germplasm (**Fig 1**), 30.91% of which was accounted by PC1 followed by 15.89% in PC2 and 14.95% in PC3 (**Table 2**). The first three principal components together explained more than 50% of total variation through traits such as DF, DM, PH, TH, SPC, SYPP and CPP. Consequently, the most diverse accessions could be selected on the basis of these three PCs. Worku *et al.* (2015); Kaur *et al.* (2018) and Patial *et al.* (2019) reported similar results and thus confirmed the independence of some traits. The first two principal components biplot including loadings of the various characters along with the genotypes spread over is given in **Fig 2**. This figure indicates that the PCA showed a clear differentiation between most of the linseed genotypes from each others.

Table 2. Principal Component analysis (PCA)based on standard data for 8 quantitative traits of linseed

Eigen Value	2.48	1.27	1.20	0.98	0.85
Proportional Variation (%)	30.91	15.89	14.95	12.30	10.63
Cumulative Variation (%)	30.91	46.80	61.75	74.05	84.68
Traits	PC1	PC2	PC3	PC4	PC5
DF	0.89	0.24	-0.17	-0.22	0.08
DM	0.79	0.28	-0.18	-0.34	0.18
PH	0.64	-0.05	0.46	0.45	0.17
TH	0.58	-0.59	0.26	0.28	0.08
SPC	0.17	0.72	0.13	0.23	-0.07
CPP	-0.30	0.26	-0.43	0.55	0.54
TSW	-0.34	-0.04	0.44	-0.43	0.68
SYPP	-0.24	0.45	0.68	0.02	-0.16

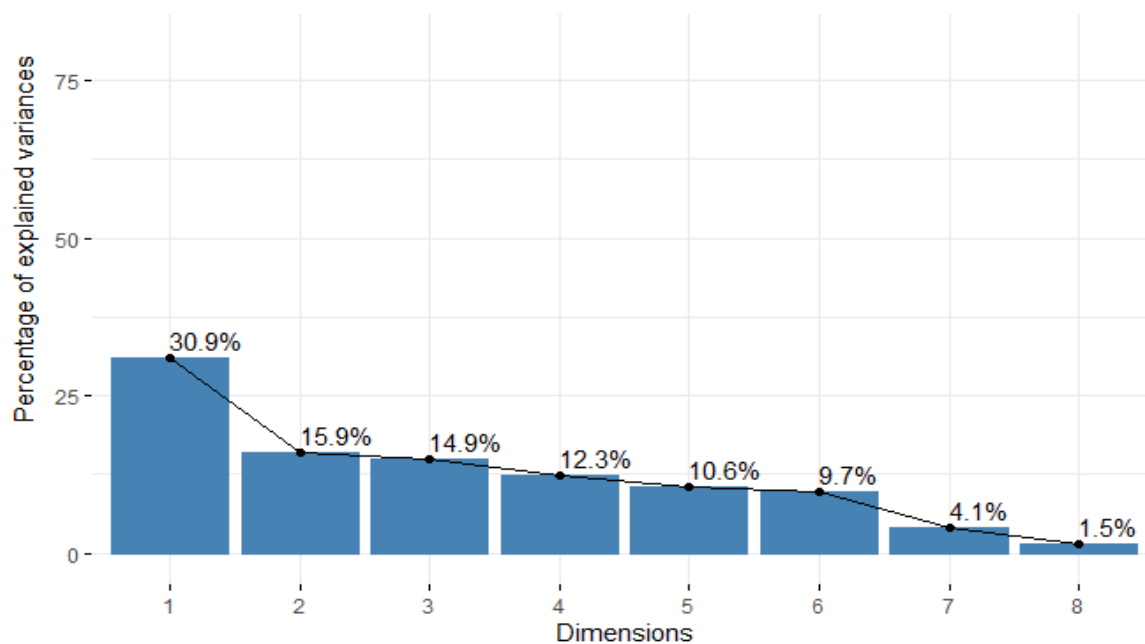


Fig. 1. Contribution of different principal components.

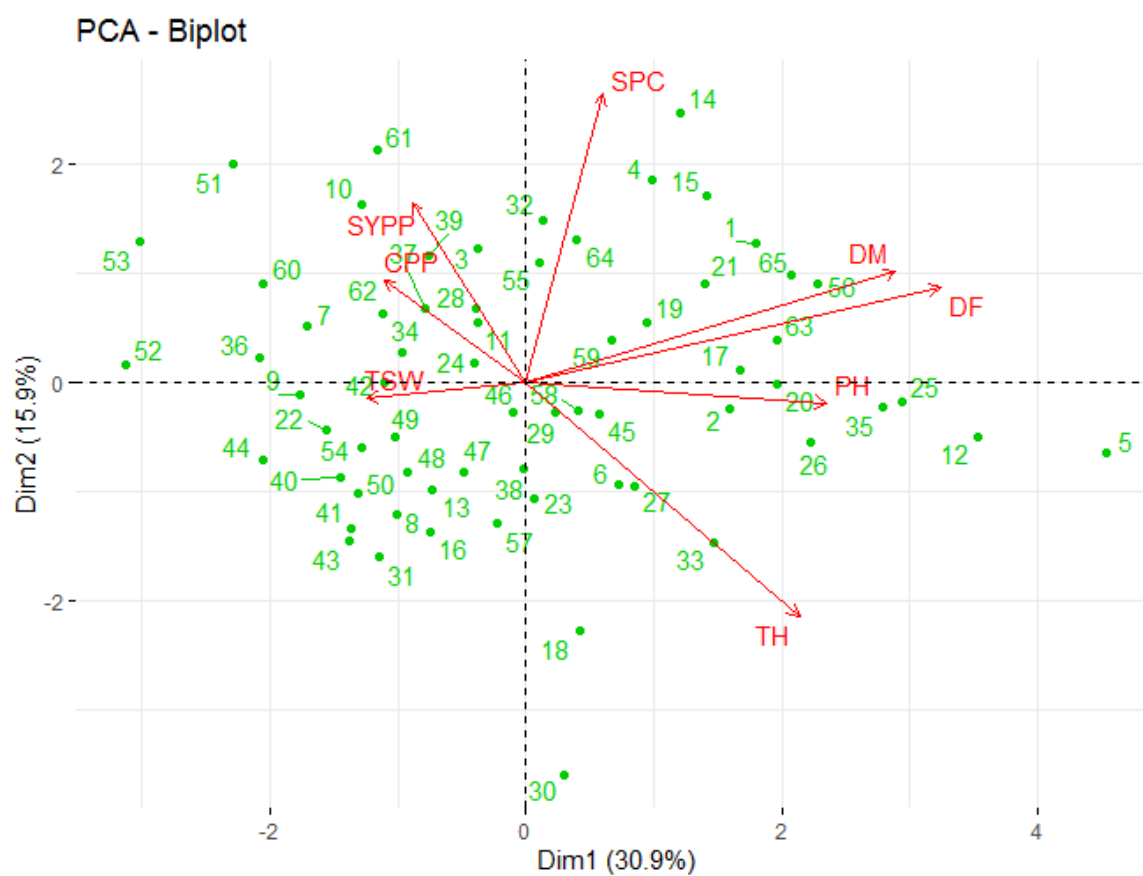


Fig. 2. Biplot of 65 genotypes of linseed on principal component axis 1 and 2.

The correlation matrix among the traits along with the correlation coefficient showed 28 associations, having 5 positive associations (**Fig 3**). DF showed significant association with DM, PH and TH. A significant positive association of DM with PH and PH showed a significant

positive association with TH. SYPP showed a positive association with TSW, SPC. Similar phenotypic correlations among yield attributing traits and other morphological traits have been reported earlier by Adugna and Labuschagne (2004) and Kaur *et al.* (2018).

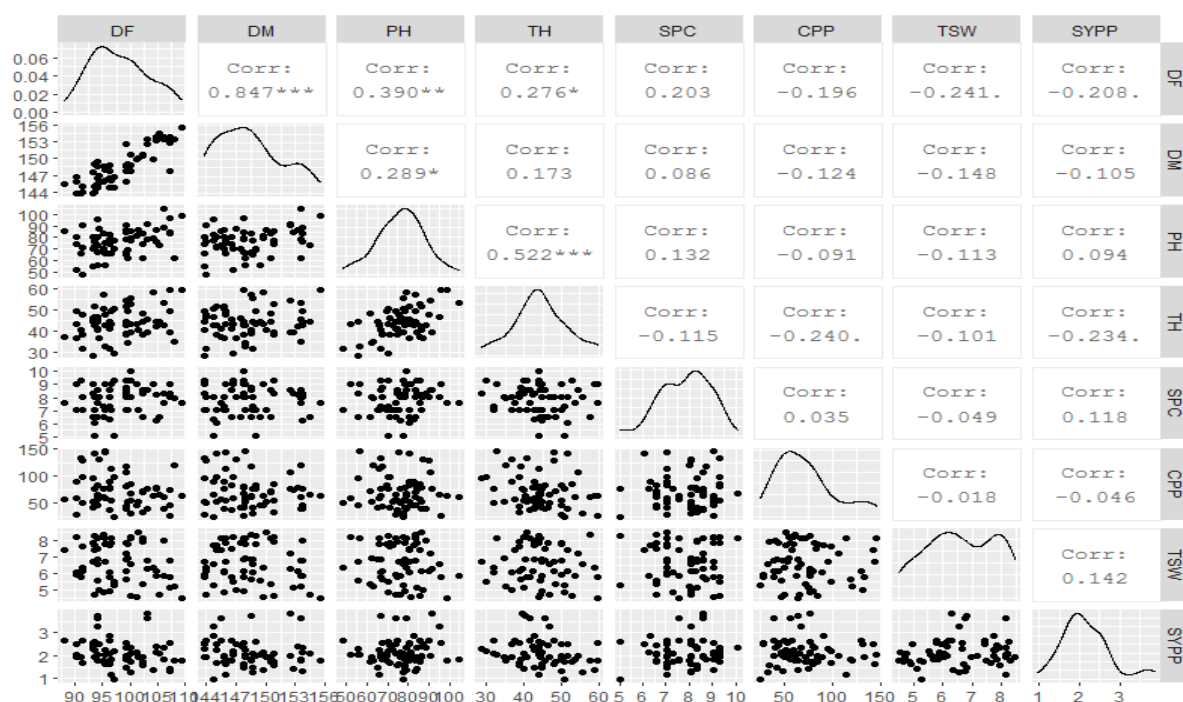


Fig. 3. The pairwise correlations of different quantitative traits of linseed germplasm lines. The upper panel show the numerical values of the correlation. The *, **, and *** star indicate correlation significance at 0.05, 0.01 and 0.001 level of probability, respectively. The lower panel show the scatter plots.

Cluster analysis groups a large number of accessions into a few numbers of homogeneous clusters which in turn facilitates the selection of diverse accessions. The number of accessions fallen under cluster C 3 was the highest (41) followed by cluster 1 (21), cluster 2 (2) and cluster 4 (1) as shown in **Table 3** and **Fig 4**. Different clustering patterns were also reported in linseed by some earlier workers (Kandil *et al.*, 2011; Pali and Mehta, 2017 and Patial *et al.*, 2019). The mean value of accessions grouped into each cluster showed that cluster 2 had accessions that were more in test weight, seed yield per plant and lesser days to flowering. Cluster 3 grouped accessions with more days to maturity and seeds per capsule. Cluster 1 grouped accessions with late in flowering and capsules per plant. Cluster 4 had accessions with more technical height and lesser days to maturity. The diversity present in the germplasm is also supported by an appreciable amount of variation among cluster means for different traits. Diversity among the linseed germplasm was also reported earlier by Nizar and Mulani (2015), Kumar *et al.* (2018), Kaur *et al.* (2018) and Patial *et al.* (2019). The selection of parents from cluster 4 for earliness should be

combined with higher seed yield from cluster 2 should be used in the breeding programme for the development of varieties having short duration coupled with high yielding ability.

The present study also helped in identifying trait specific superior accessions, which may be included in the linseed breeding program. The accessions EC 41684, A-4-3-101-3, 2679 RJK-4, A-58, G 1424 takes significantly lesser days to maturity (144 days) as compared to early check variety T 397 which takes 148.5 days to maturity under tested location climatic conditions. The accession EITA and UDNA 1-2-5 having significantly higher single plant yield (>3.0 g) than check varieties (LC 2063, LC 74, T 397 and Binwa) and having more plant height (> 93 cm) could be used for the development of dual-purpose linseed varieties. The accession POLF-29-1 having significantly more plant height and technical height than check varieties could be used for the development of fibre type linseed varieties. The accession EC 322661 having a high seed yield coupled with less plant height (68 cm) could be exploited for the development of dual-purpose linseed genotypes.

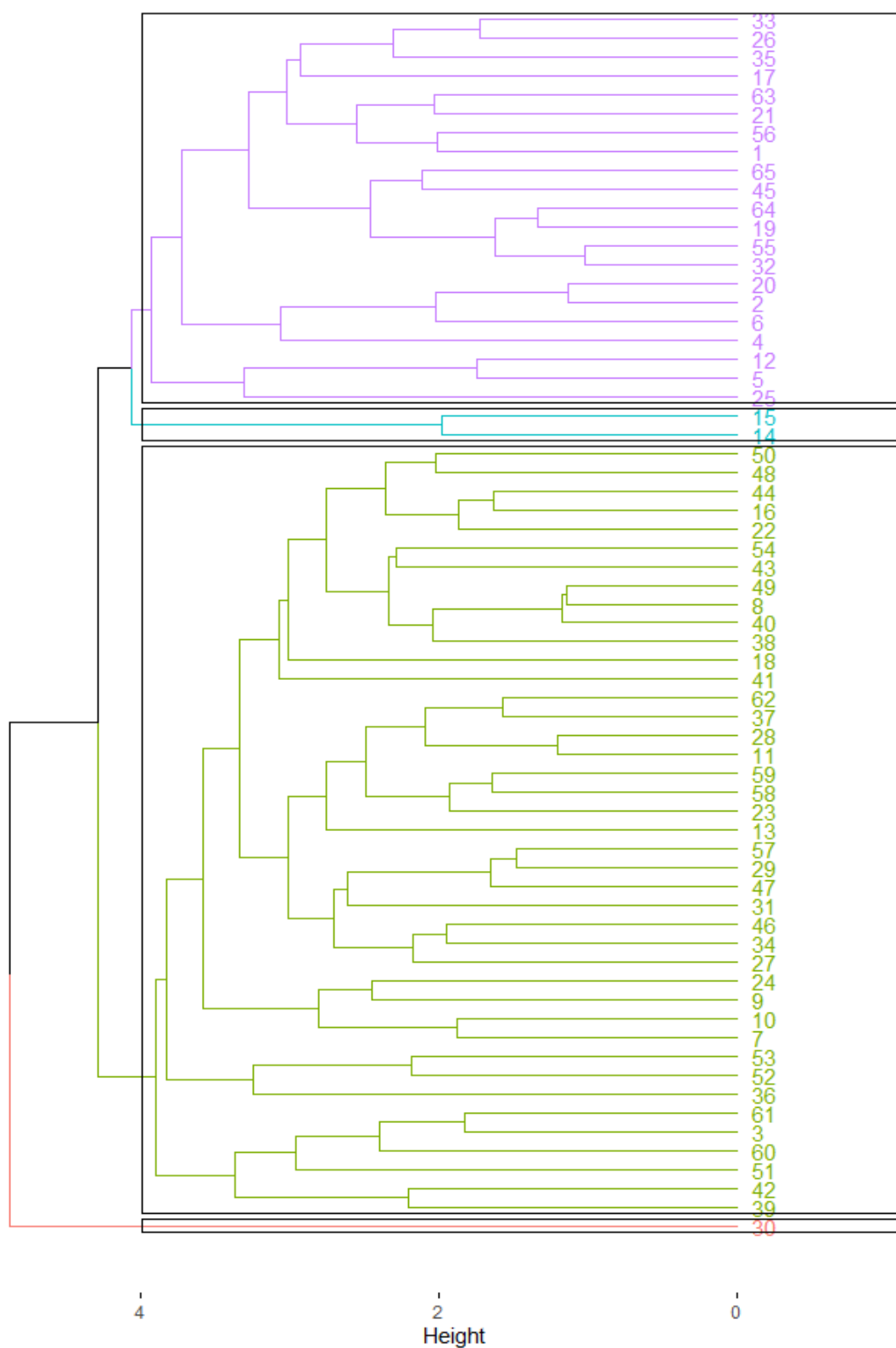


Fig. 4. Dendrogram depicting relationships among the linseed genotype for eight agronomical traits

Table 3. Means of quantitative variables in different clusters of linseed germplasm

Cluster variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4
DF	103.63	94.92	103.19	97.19
DM	151.46	146.52	153.00	144.75
PH	83.02	72.59	85.31	77.31
TH	47.59	42.57	42.56	50.81
SPC	8.23	7.66	8.56	5.06
CPP	66.06	75.44	57.63	24.38
TSW	6.36	6.80	6.52	5.30
SYPP	2.05	2.26	1.78	0.93
Number of accessions	21	2	41	1

The present study identified DF 50%, DM 75%, PH, TH, SCP, CPP, TSW and SYPP, traits are important traits for the variation in linseed germplasm. The correlation matrix suggested that early maturity, plant height and technical height should be given emphasis in breeding programs for further improvement of linseed varieties. Trait specific linseed accessions identified EC 1441-1, POLF 34-2 JRE-3, SJKO 14, AYOGL having more than 55 cm TH, accessions SJKO 14, 2662-PKY-10, NEELAM 4, LCK 9312 and 2664-PKY-12, UDNA 1-2-5, EC 322661, BAU 610-A, LCK 9312 had high heritability coupled with high GA for traits such as capsules per plant, Seed yield per plant, Test Seed Weight, Technical Height and Plant height. These accessions can be utilized in linseed breeding for the development of superior genotypes widely adapted under various agro-ecological zones.

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