Studying Genetic Diversity in Genotypes of Linseed (*Linum usitatissimum*L.) Using Agro-Morphological Traits for Rainfed Conditions

ABSTRACT

One of the oldest plants to have been grown for food, oil and fibre worldwide is Linseed, popularly known as flax. As an oilseed crop, linseed is unique in that it can produce technical-grade vegetable oil and high-quality fiber that are strong and durable. The trial was conducted in the winter cropping season (2022-23) at TCA Dholi, Department of Seed Science and Technology (Dr. Rajendra Prasad Central Agricultural University), Pusa, Samastipur in Bihar. Randomized Block Design (RBD) was employed during the study with statistical methods being used to analyze data. The D2 analysis was used to assess genetic divergence among 26 linseed genotypes. Significant genetic diversity was discovered with similar genotypes falling into fifteen clusters. The genetic differentiation of the genotypes was shown to be significant because the degree of variation within clusters was found to be less than that between them. The highest genetic difference among them was observed between IX and III cluster, indicating a high level of genetic divergence between these two clusters. Conversely, inter-cluster distance between IV & I was the smallest meaning close genetic association among their constituents. Capsules number per plant had the highest contribution towards genetic divergence followed by plant height; time to 50% flowering; 1000-seed weight; seed yield; days to maturity; and harvest index when all traits were considered. These discoveries demonstrate one thing: these traits should be considered when selecting superior genes for breeding programs aimed at improving quality and productivity in flax seeds production.

Keywords: Linseed, Genetic divergence, D2 analysis, Cluster analysis, Trait contribution.

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INTRODUCTION

Oils and fats are essential elements in our daily diets and serve as fundamental raw materials for a wide array of products used in everyday life. Oilseeds are also recognized for their therapeutic and curative properties. In terms of vegetable oil production, India ranks third globally,

following the USA and China, as a major contributor to the global oilseed industry. Oilseeds hold significant importance, second only to cereals, in terms of cultivated area, production, and economic value. At present, India comprises about 13% of the global oilseed area cultivated, produces 7%, and consumes 10% of all edible oils. More than 80% of India's vegetable oils and fats are sourced from seven primary annual oilseed crops - groundnut, rapeseed-mustard, soybean, Niger-seed, sesame, sunflower, and safflower - as well as two non-edible sources: castor and linseed. Linseed occupies approximately 26.1 million hectares with a yield of about 24.9 million tons, representing around 18% of the total cultivable land. In the 2020-21 report by the Ministry of Agriculture and Farmers welfare, Government of India, Directorate of Oilseed Development states that oil seeds are ranked second after cereals as far as agro-commodities are concerned accounting for nearly 3 percent of the gross national product (GNP) and around 10% of total agricultural value added.

Linseed is grown in both tropical and temperate regions globally, with major production in Argentina, the former USSR, India, the USA, Canada, Pakistan, Australia, and Nepal. In India, linseed cultivation covers approximately 437 thousand hectares, yielding around 173 thousand tonnes, making it the third-largest global producer. Australia and Canada exhibit the highest productivity, averaging about 7 quintals per hectare, whereas India's average yield is about 400 kilograms per hectare. According to Anonymous (2020), India cultivated linseed in an area of 182,000 hectares during the 2019–20 period and harvested 122,000 metric tons with an average yield of 671 kg/ha. Linseed oil, with a high iodine value exceeding 180, is ideal for applications in painting and varnishing due to its rapid drying properties. Consequently, a significant portion of the world’s linseed is utilized in industrial applications. Although linseed is primarily classified as a non-edible oil, approximately 20% of the linseed produced in India is used for food, particularly in states such as Uttar Pradesh, Madhya Pradesh, Bihar, and Chhattisgarh. In these states, linseed is cultivated over 26,900 hectares, producing 17,890 tonnes of seeds with a productivity of about 150 kg/ha (Anonymous, 2021). Linseed covers 9% of the oilseed area in India and contributes about 6.29% of the total oilseed production. It is predominantly grown on marginal and sub-marginal soils under rainfed conditions, often as a relay crop during the Rabi season (Agrawal *et al*., 2014).

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Linseed, with an oil content ranging from 33% to 45%, is integral to various agro-based sectors. Flax annual (*Linum usitatissimum subsp. usitatissimum*) is a self-fertilizing species as well as diploid (2n=30). The Linum genus is thought to have originated in the Middle East or Indian regions and then extended towards Asia, Europe, and America **(Soto-Cerda *et al.*, 2013)**.Flax domestication occurred around the Mediterranean basin, an area noted for its rich biodiversity within the *Linum*genus, extending to the Indian subcontinent **(Fu, 2005; Kaur, 2017)**. Selective breeding over time has led to various flax and linseed types within the Linum species, demonstrating diverse physiological characteristics, including morphology, anatomy, and agricultural performance **(Soto-Cerda *et al.*,2013)**. Early cultivated forms, such as *Linum bienne*were utilized for fiber and seeds, leading to the development of *Linum usitatissimum* L., one of the earliest domesticated plants **(McDill *et al.*,2009)**. Despite being primarily classified as a non-edible oil, linseed oil possesses nutritional value and is utilized in pharmaceuticals and animal nutrition **(Khan *et al.*,2013)**. Linseed's oil content ranges from 33% to 45% and contributes significantly to various sectors, including paints and varnishes, due to its high content of unsaturated fatty acids, particularly α-linolenic acid (ALA). The whole plant or processed linseed finds commercial applications.

Mahalanobis' D2 statistic multivariate analysis is a crucial tool for estimating genotypic divergence and evaluating the contribution of various traits to overall divergence. This technique helps organize germplasm collections into homogenous groups, facilitating the reduction of the collection size for evaluation. Divergent genotypes are selected to leverage heterosis and increase the frequency of desirable genes in segregants using D2 statistics **(Mahalanobis, 1936)**. Variations among genotypes may result from geographical isolation or genetic barriers to crossability. The D2 statistic measures differentiation at both intra-cluster and inter-cluster levels, aiding the selection of genetically diverse parents for hybridization programs. Three key considerations for using D2 statistics in parent selection are: (i) assessing the relative contribution of traits to overall genetic divergence, (ii) choosing clusters with maximum statistical distance, and (iii) selecting one or a few genotypes from these clusters. For example, **(Kumar *et al.*,2022)** utilized this method to predict diversity in green gram. Evaluating genetic divergence and similarity among genotypes is essential for selecting suitable progenitors for breeding programs that produce transgressive segregants. Despite efforts to develop improved

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mungbean cultivars through systematic breeding, linseed remains highly valuable for gene diversity studies. **(Joshi *et al.*,2022)** emphasized that this research aids in identifying variation among genotypes and forming diverse lines. Selecting diverse genotypes from broad genetic backgrounds is crucial for effective hybridization, increasing the likelihood of incorporating desirable genes and generating variability in subsequent generations **(Joshi *etal.*, 2022)**. Flaxseeds have been under investigation for many dietetic studies since they are reputed to contain omega 3 fatty acids (α-linolenic acid), PUFA, soluble and insoluble fibers, phytoestrogen lignans, proteins and antioxidants **(Ivanova *et al.*, 2011; Singh *et al.*, 2011; Alhassane and Xu, 2010)**. However, enhancing the genetic potential of flax is necessary to meet rising demands for its health benefits, addressing issues such as its low-input nature, susceptibility to fungal diseases, and limited genetic diversity in existing cultivars **(Sood *et al.*,2007; Guha Roy *et al.*,2012)**. For plant breeders, crop diversity provides a foundation for selecting suitable parents and evaluating genetic variation within germplasm **(Govindaraj *et al.*,2015)**. Thus, evaluating agro-morphological traits is essential for identifying superior germplasm accessions for future linseed breeding and selection programs.

MATERIALS AND METHODS

The investigation was done in TCA Dholi, Seed Science and Technology of Winter Crop Season 2022-23 at Dr. Rajendra Prasad Central Agricultural University Department Pusa, Samastipur Bihar. Geographically, it is located in an area that has an elevation of 55.21 meters above sea level, at coordinates which are roughly 25° 99' North latitude and 85° 59' East longitude. The experimental site featured a flat terrain with efficient drainage. The soil was alluvial, characterized by a uniform distribution of calcium carbonate, varying between 10% and 40% throughout the soil profile. This kind of soil is common in areas along the Burhi Gandak River, which is well-known for its calcareous characteristics. During the crop-growing season, the weather was favorable for the best possible plant development.

The study evaluated twenty-six linseed cultivars (see table 1) procured from the genetics and plant breeding department of Rajendra Prasad Central Agricultural University, Pusa, Samastipur-848125, Bihar. The experimental design was implemented using a Randomized Block Design (RBD) methodology, and data analysis was performed using the statistical techniques proposed by Fisher & Yates in 1963. To assess genetic divergence among the linseed genotypes, D2

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statistics, as described by Mahalanobis (1936), were employed, followed by clustering analysis based on the method outlined by Tocher (1952).

RESULTS AND DISCUSSION

Variance Analysis

Table 2 gives an overview of the findings from the variance analysis on the various attributes. Thus, this study measured their diversity by all genotypes. Some traits such as seed germination, plant height among others showed significant differences between genotypes. Other agronomic traits considered in the study were number of primary branches, secondary branches, capsules and seeds per capsule as well as seed yield **(Kumar *et al.*, 2019; Kasana*et al.*, 2018a)**. These results suggest considerable variability in the investigated material.

Genetic Diversity

Twenty-six linseed genotypes were classified into eleven different clusters using Tocher's method, according to Table 3. Among the twenty-six genotypes, the biggest cluster was cluster II incorporating eight of them while four of them were in cluster I. Additionally, D2 statistics over fifteen characters indicated significant genetic variation among these twenty-six genotypes. The D2-based metrics are distributed then into eleven clusters. Other than Clusters II and I with eight and four genotypes respectively, there were only three genotypes in Cluster III. With two genotypes each, Clusters IV, V, and VI had other members including single ones for Clusters VII, VIII, IX, X, and XI. The study findings indicate that across all types of flaxseed samples tested, there is vast genetic variability present in their genomes.

Table 4 shows all the eleven cluster pairs found in intra and inter-cluster distances (D2). What was seen is that, while genotypes from different places were clustered together, those with similar geographical locations were split into different clusters. Therefore, this picture means that genotype of members has little connection to their geographic origin. The largest distance was found between Cluster IX and III (D2 = 274.24) followed by Clusters VI and III (D2=233.79), or Clusters IV and III (D2=215.63). Conversely, the smallest between-cluster distances were observed in the pair of clusters IV and I (D2=35.08), II and I (D2=35.11), V and

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I(D2=36.64). The first biggest distance within a single layer is Cluster II with D2 of 23.17 while Cluster III has a slightly smaller value at D2 =22.24 for this parameter. Cluster I have the lowest values which is 21.79 for this value. But each cluster had only one genotype including Clusters VII,VIII,IX,X,XI which had least intracluster distances as shown in D2 = 0 for all of them. In total, therefore, it can be said that the intercluster distances are larger compared to intracluster ones which suggest high genetic diversity among the considered genotypes. As a result of this situation parents should be picked from diverse groups for breeding so that heterotic effects regarding yield attributes being discussed herein as well as quality traits associated with these features can be maximized in general terms.

The foregoing table (Table 5) presents primary determinants of genetic diversity. The number of capsules per plant leads with approximately 26.05% of the variation, followed by plant height at 25.73%, and days to flowering at 23.06%. Other differentiating factors included test weight (10.85%), harvest index (6.99%), seeds per capsule (6.04%), seed germination (5.17%), vigour index II (4.97%), number of secondary branches per plant (4.79%), seed yield (4.65), primary branches per plant (4.53%), days to maturity (4,20), vigour index I(2,54), days to flower in percentages(4:05). These results also demonstrate the significance of three traits: number of capsules, plant height, and days to flowering in determining genetic diversity levels within a population; therefore, these traits must be emphasized during selection process aimed at improving germplasm based on genetics potential maximization which is a step that should not be overlooked for its vitality.

In breeding programs, both genetic divergence and variability are crucial. This particular study made use of D2 analysis, a technique devised by Mahalanobis, to evaluate genetic divergence. The main objective of breeders is to evaluate genetic diversity in their germplasm collections, varieties, or advanced breeding materials that are important in incorporating them into the breeding programmes. This evaluation is very important due to two main reasons that genetically diverse parents may produce significant heterotic effects leading to an increase in vigor and performance of their progenies. Also, crossing genetically distant or unrelated parents within the same species brings about a wider range of variability in the offspring. In selecting appropriate parents for enhancing grain yield various traits exhibiting quantitative divergence need to be considered.

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The given context is appropriate for statistical methods like D2 analysis first suggested by Mahalanobis (1936) and the Tocher method discussed by Rao (1952). The distance between the clusters is an indication of their level of variation. If the distance is long, it means that there is a greater divergence between them whereas short distance implies less difference in terms of genetic variation among them. Those genotypes which are found within the same cluster exhibit a higher degree of similarity compared to those genotypes found in other clusters. This therefore means that genotypes grouped together within a cluster show lower divergence than those in different clusters.

The study revealed significant genetic diversity among the clusters, with the largest distance observed between Cluster IX and Cluster III. This substantial diversity offers opportunities for genetic improvement in linseed. Conversely, Clusters IV and I displayed the smallest inter-cluster distance, suggesting relatively minor genetic divergence between them. Similarly, Clusters II and I, as well as V and I, showed closer genetic relationships. A broad range of variation was evident across the multi-genotypic clusters for different traits. Genetic divergence was shown to be most significantly influenced by the number of capsules for each plant. contributing 26.05%. Other significant traits included plant height (25.73%), 1000-seed weight (10.85%), days to 50% flowering (23.06%), seed yield per plant (5.65%), harvest index (6.99%), and days to maturity (4.20%), which together were responsible for 98.87% of the genetic diversity found in the sample. These characteristics are vital for breeding activities that choose favorable genotypes for linseed. However, the genetic difference in plant's primary branches (1.54%), secondary branches (1.0%) and capsules number (0.95%), as well as vigor indices I and II (2.55% & 2.98%) were the least significant variations (below 3%). Across a wide range of genotypes, these attributes show relatively little variation, which suggests moderate to low heritability and increased homogeneity between genotypes. These results highlight how critical it is to assess genetic diversity in breeding efforts in order to make the most use of a variety of attributes to enhance cultivars of linseed.Similar findings about plant height, as well as the length of the roots and shoots of seedlings, have been recorded by **(Kasana*et al.*, 2018, Rizvi *et al.*, 2018, Kumar *et al.*, 2019, and Chaudhary *et al.*, 2016)**.

CONCLUSION

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A lot of genetic heterogeneity was found after twenty-six linseed genotypes were evaluated, and these genotypes were grouped into fifteen distinct groups. Within these clusters, highlight the genotype variety by the smaller genetic distances between than within the clusters. The greatest inter-cluster length was in Clusters IX and III meaning that these populations are highly genetically dissimilar. Genetic improvement applications of linseed breeding are possible because of this marked variation. A closer genetic link among genotypes in these clusters is illustrated by Clusters IV and I for which they had the least intercluster separation. The most important trait causing genetic differentiation among the individuals was the number of capsules per plant. The second most important traits were days to 50% flowering, height of the plant, seed yield, 1000-seed weight, days to maturity, and harvest index as important segregating traits for populations. These collectively constitute important genetic traits. These results reveal some importance of these characteristics in selecting superior genotypes for developing strains that will help boost both crop quality as well as yield levels.

Table 1. Genotypes Details

No.	Genotype	No.	Genotype	No.	Genotype
1	RLC197	10	OL-2013-14-2022-6	19	LCK2209
2	SLS143	11	SL-5	20	NL505
3	SHEKHAR(ZC)	12	LCK2220	21	NL408
4	LMS-2020-R-5	13	SLS144	22	RL18122
5	RLC198	14	BAU-2021-23	23	RCRLV-22-1
6	T397(NC)	15	OL-2013-14-2022-6	24	RL18116
7	NL506	16	DLV-115	25	BRLS 119-1
8	SLS145	17	NL407	26	SABOUR TISI2
9	LMS-2020-R-7	18	BRLS106-1		

Table 2. Shows the Analysis of Variance (ANOVA) for characters in linseed

Characters	Mean sum of squares

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	Replication ***df* = 2**	**Genotype** ***df* = 25**	**Error** ***df* = 50**
Seed germination (%)	2.62	39.01 **	3.81
Harvest index (%)	0.05	4.26 **	0.95
Test weight (g)	0.15	1.32 **	0.11
Seedling shoot length (cm)	0.24	1.10 **	0.17
Seedling root length (cm)	0.15	1.22 **	0.2
Vigour index I	118.99	3089.44 **	120.72
Vigour index II	23.25	3093.22 **	76.49
Days to 50% flowering	1.24	9.49 **	1.37
Days to maturity	0.66	11.70 **	1.94
Number of primary branches per plant	0.24	1.02 **	0.18
Number of secondary branches per plant	1.16	1.52 **	0.71
Plant height (cm)	4.51	141.88 **	3.73
Number of capsules per plant	36.82	65.29 **	5.68
Number of seeds per capsule	0.58	0.69 **	0.34
Seed yield (q/ha)	0.0074	0.017 **	0.008

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Table 3. Distribution of 26 genotypes of linseed to different clusters based on D2 statistics

Sr. No.	**Cluster**	**No. of genotypes**	**Name of genotypes**
1	I	4	DLV-115, LCK2209, BRLS106-1, BAU-2021-23

2	II	8	SL-5, LCK2220, T397(NC), RLC197, SLS145, LMS-2020-R-7, NL505, NL407
3	III	3	LMS-2020-R-5, OL-2013-14-2022-6, SABOUR TISI2
4	IV	2	SLS143, NL408
5	V	2	SLS144, OL-2013-14-2022-6
6	VI	2	NL506, RL18122
7	VII	1	SHEKHAR(ZC)
8	VIII	1	RLC198
9	IX	1	RCRLV-22-1
10	X	1	RL18116
11	XI	1	BRLS 119-1

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Table 4. Average Intra-cluster and Inter-cluster distances of twenty-six genotypes of linseed

Cluster	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**
I	**21.79**	35.11	114.90	35.08	36.64	48.06	166.93	53.72	93.07	58.56	47.10

II		**23.17**	74.64	75.81	53.75	135.94	150.51	73.24	155.01	78.62	54.51
III			**22.24**	215.63	170.72	233.79	140.64	169.58	274.24	139.27	71.07
IV				**18.64**	55.06	35.85	36.27	152.60	48.40	54.33	172.13
V					**20.05**	41.29	103.42	99.78	71.34	51.18	170.17
VI						**20.18**	159.49	60.09	180.28	77.28	172.24
VII							**0.00**	132.91	80.56	72.70	186.74
VIII								**0.00**	171.60	109.03	176.35
IX									**0.00**	141.03	133.24
X										**0.00**	164.54
XI											**0.00**

Table 5. Linseed Genotypes' Genetic Diversity in Terms of Various Characters' Contribution

Sr. No.	**Characters**	**Contribution to divergence (%)**
1	Seed germination	5.17
2	Harvest index	6.99
3	Test weight	10.85
4	Seedling shoot length	3.63
5	Seedling root length	4.81
6	Vigour index I	2.55
7	Vigour index II	2.98
8	Days to 50% flowering	23.06
9	Days to maturity	4.20
10	Number of primary branches per plant	1.54

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11	Number of secondary branches per plant	1.80
12	Plant height	25.73
13	Number of capsules per plant	26.05
14	Number of seeds per capsule	0.95
15	Seed yield	5.65

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