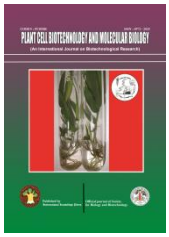

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Elucidating Diversity of Linseed (*Linum usitatissimum*) Germplasms by Applying DUS Guideline to Examine Morphological Features

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Flax, or *Linum usitatissimum*, is a multipurpose crop that may be produced in a variety of climates for use in industry, feed, fibre, and food. It is crucial to characterize and assess an array of genotypes to comprehend the potential of linseed in agriculture. The present investigation focused on agro-morphological characteristics to identify the best germplasm that may be utilized as future donors for linseed breeding. The goal of this study was to find the best germplasm to be used as future donors for linseed breeding by focusing on agro-morphological traits. In the current study, an expanded design was used to test 92 genotypes in total, both alien and native, throughout the course of two seasons, Rabi 2022-23 and Rabi 2023-24. The ninety-two genotypes were found to

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be organized into seven primary clusters by cluster analysis. Cluster I consist of twenty-four genotypes. Whereas cluster II and cluster III each encompass one genotype *i.e*., IC394118 and IC0599415 respectively. While cluster IV consists of seventeen genotypes, cluster V contain thirty-one genotypes. Cluster VI consist of seven genotypes. Whereas cluster VII which is the last cluster included eleven genotypes. A broad variation of genetic diversity was found using cluster analysis. The findings of this study open the door for focused breeding initiatives and environmentally friendly farming methods by offering insightful information about the genetic diversity and phenotypic variability of linseed.

Keywords: Cluster; characteristics; descriptor; DUS; morphological.

1. INTRODUCTION

Linseed (*Linum usitatissimum* L.) is an annual plant with several uses belonging to the Linaceae family and the genus Linum, which has over 300 species. Five wild species of *Linum usitatissimum*, besides the cultivated variety, have been identified in India. These are L. *perenne*, L. *strictum,* L. *mysorense,* L. *augustifolium* and L. *grandiflorum*. The Latin term *usitatissimum*, which means "most useful," is the source of the species name *Linum*. Linum itself comes from lin, which means "thread." Based on the variety of plant species, there are two main areas of origin for linseed: South West Asia, especially India [1,2] and the Mediterranean region of Europe [3]. Based on its application, flax may be broadly classified as fibre flax, oil flax, and oil-fibre dual-purpose flax [4]. These flax species are widely utilized in food production, paper manufacturing, textiles, and the remediation of heavy metal-contaminated soil, among other fields of use [5-7]. Worldwide linseed is a significant crop that is farmed on 3.22 million hectares, producing 3.06 million tons with 951 $kgha^{-1}$ productivity [8]. The top producer is Kazakhstan, followed by the Russian Federation and lastly Canada. With an area of 1.72 lakh ha, 9.9 lakh tons of production, and 574 $kgha^{-1}$ of productivity, India is the sixth largest producer of linseed in the world [8].

India's production is far lower than that of other countries, hence we need to find germplasm lines that can support high output [9]. The crop's limited genetic diversity makes it necessary to boost breeding programmes by introducing new germplasm, gathering regional ecotypes, and implementing interspecific hybridization. Therefore, it is important to examine and define the genotypes of linseed to identify the donor (s) for various attributes and to use these genotypes in various breeding programmes. For improved germplasm usage and conservation, crop species traits can be described using standard descriptors [10,11].

DUS characterization is now required to identify germplasm that can be a beneficial parent, carrying important features required for further development in a breeding programme. Determining whether a new variety is uniform in its features, stable and has consistent phenotypic qualities from generation to generation, and distinct from existing varieties are the three basic objectives of DUS testing. A variety can be categorized as distinct if any one or more of the conspicuous features that were identified throughout the assessment are different from those of any other variation whose presence is widely acknowledged. Furthermore, crop diversity management depends heavily on morphological description research. Therefore, it is essential to preserve the genotypes and investigate the breeding. With the aforesaid considerations in mind, present study was conducted to categorize 92 different linseed genotypes using linseed descriptors or the DUS guideline in accordance with UPOV [12].

2. MATERIALS AND METHODS

Ninety-two genotypes of linseed including exotic and indigenous lines acquired from All India Coordinated Research Project on linseed, Crop Research Farm Mauranipur, BUA & T, Banda U.P., India were used for experimentation. Experiment was conducted at Research Farm, Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior, M.P., India during two seasons *i.e*., *Rabi* 2022-23 and *Rabi* 2023-24 to generate qualitative data under managed field conditions. The experimental material was planted in augmented design keeping plant to plant distance 10 cm followed by 30cm row to row distance. To guarantee adequate germination, pre-sowing irrigations were implemented. The field trial plots were meticulously prepared before seeding, and Farm Yard Manure (FYM) was added. The recommended magnitude of manure and fertilizer were applied during the growth and development of crop. The experiment was routinely weeded

and irrigation was supplied as per requirement. In accordance, other essential cultural customs were carried out. Throughout the whole growing seasons, every accession was closely observed at different developmental stages, and any features that were off-type were eliminated. Observations were recorded as per DUS and UPOV [12] guidelines. Three random plants from each line were selected for recording data.

Data gathered from both individual plants and their parts as well as from groups of plants and their components were used to evaluate qualitative features, depending on the precise element used to characterize the accession. The DUS (Distinctness, Uniformity, and Stability) and International Union for the Protection of New Varieties of Plants [12] principles were followed to perform the outcomes.

3. RESULTS AND DISCUSSION

Following the UPOV [12] test standards for DUS (Distinctness, Uniformity, and Stability) evaluation of linseed, 12 specific traits/attributes as presented in Table 1, 2 and Fig.1 were used to determine the uniqueness of linseed types.

In respect to habitat sixty-three, or 68.4%, had a semi-erect growth habit, whereas twenty-two lines, or 23.91%, were classified as erect and seven or 7.60% were considered as bushy. Table 1 shows that thirty-eight lines (41.30%) of the total, have long plant height, forty-nine (53.26%) was classified as medium height while five lines (5.43%) was accepted as short. Only thirteen lines (14.13%) showed early flowering (less than 50 days), sixty-four lines (69.56%), displayed medium flowering (between 50 and 60 days), whereas fifteen lines (16.30%), exhibited late flowering (more than 60 days) out of ninety-two germplasm lines. Size of corolla was divided into three groups: large, medium, and small. Table 1 displays those thirty-seven lines (40.21%), had a large corolla, forty-two lines (45.65%), had a medium corolla, while thirteen lines (14.13%), were considered as a small corolla size. Three types of shape were recorded in present study. Seventy-two (78.56%) were disk shaped flower, twelve lines (13.04%) star shaped and only eight lines had funnel shaped flower.

Flower colour is divided into white and violet. Eighty-one lines (88.04%), were blue, while 11%, were demonstrated white. Flower aestivation was categorized into three classes including valvate, twisted, and semi-twisted. Of the ninety-two lines, sixty lines (65.21%) had semi-twisted aestivation, twenty-eight lines (30.43 %), were grouped in valvate whilst four lines (4.31%) fell under twisted estimation. Petal venation colour was divided into four classes: light violet, violet, blue, and white. Sixty-one lines (66.30%) were blue, eighteen lines (19.56%) violet, ten lines, (10.86%) white, whereas only three lines (3.26%) had light-violet petal veining. Fifty-three lines (57.60%), displayed blue filament colour, while forty-eight lines (41.30%) exhibited white filament colour.

Forty-five lines (48.91%) had a cream-colored anther; 28 lines (30.43%) blue, while 12 lines (13.04%) had a grey; and the finally 7 lines (7.60%) had a violet-coloured anthers. Flower sizes were large (> 20 mm), medium (15–20 mm), and small (< 15 mm). Of the ninety-two investigated germplasm lines, forty-two lines (45.65%) had medium-sized flowers, thirty-seven lines (40.21%), had large, while only thirteen lines (14.13%) had small flowers.

Another class of trait was seed characteristics. In present investigation. thirty-eight lines (41.30%), were grouped in medium size, thirty lines (32.60%,) into small sized capsules while twenty-four lines, (26.08%), were considered as bold capsules. Of the 92 lines, 37 (or 40.21%) fell into the semi-dehiscence category, while remaining 55 (or 59.78%) had a non-dehiscence character.

3.1 Cluster Analysis for Distinctiveness

Cluster analysis displayed that the ninety-two genotypes were grouped into seven major clusters. Each cluster contain a group of highly similar genotypes with analogous traits as shown in Fig. 2. Cluster I consist of twenty-four genotypes. Whereas cluster II and cluster III each encompass one genotype *i.e*., IC394118 and IC0599415 respectively. While cluster IV consists of seventeen genotypes, cluster V contain thirty-one genotypes. Cluster VI consist of seven genotypes. Whereas cluster VII which is the last cluster included eleven genotypes. A broad variation of genetic diversity was found using cluster analysis, which would be helpful for hybridization breeding programmes that aim to produce attractive transgressive segregants. Similar results were also reported by Mounika *et al*. [13] and Dhirhi *et al*. [14]. Wide variation of genetic diversity was also reported in many crops including linseed by Adugna *et al*. [15], Savita [16], Fulkar *et al*. [17], Fu *et al*. [18], Sinha [19] Mishra *et al*. [20], Sharma *et al*. [21], Asati *et al*. [22], Yadav *et al*. [23] and Rajpoot *et al*. [24].

Table 1. Characterization of 92 diverse linseed germplasm based on Distinctness (D), Uniformity (U) and Stability (S) as per DUS, UPOV [12]

Trait	Descriptor state (Assessment)	Stage of observation	Class or scale	Distribution by classes of descriptor (%)
Time of flowering **(**50% of plants with flower)	Number of days taken from the day of sowing to the day on which 50 % of the plants showed flowering was recorded. (VG)	50% Flowering stage	Early (<50)	13 (14.13%)
			Medium (50-60)	64(69.56%)
			Late (>60)	15 (16.30%)
Flower: Size of corolla (mm)	It is recorded in beginning of flowering. It is recorded in peak flowering. (MS)	50% Flowering stage	Small (<15mm)	13 (14.13%)
			Medium(15-20mm)	42 (45.65%)
			Large (>20mm)	37 (40.21%)
Flower**:** Shape	It must be recorded before noon. (VS)	50% Flowering stage	Disk	72 (78.26%)
			Funnel	8 (8.69%)
			Star	12 (13.04%)
			Tubular	0
Flower**:** Colour	It scored in fully opened flower by visual observation (VS)	50% Flowering stage	Violet	81 (88.04%)
			White	11 (11.95%)
Flower**:** Aestivation	It is recorded as arrangement of petals. (VS)	50% Flowering stage	Valvate	28 (30.43%)
			Semi-twisted	60 (65.21%)
			Twisted	4 (4.34%)
Flower**:** Venation colour	It is recorded in fully developed flower. (VS)	50% Flowering stage	Blue	61(66.30%)
			White	10(10.88%)
			Light-violet	3(3.26%)
			Violet	18(19.56%)
Stamen colour	It is recorded after flower opening (VS)	50% Flowering stage	Blue	53(57.60%)
			White	38(41.30%)
Anther**:** Colour	immediately after flower opening (VS)	50% Flowering stage	Grey	12(13.04%)
			Cream	45(48.91%)
			Blue	28(30.43%)
			violet	7 (7.60%)
Plant**:** Growth habit	Recorded considering both the angle of the basal branching and the crop canopy. (VG)	75 % Complete flowering stage	Semi-erect	63 (68.47%)
			Erect	22 (23.91%)
			Bushy	7(7.60%)
Plant**:** Height (cm)	The height of plant from the base, to the tip of the main stem was recorded in centimetres. (MS)	85% dough state	Tall	38(41.30%)
			Medium	49(53.26%)
			Dwarf	5 (5.43%)

Trait	Descriptor state (Assessment)	Stage of observation	Class or scale	Distribution by classes of descriptor (%)
Capsule: Size (mm)	It is recorded of fully developed capsule (MS)	85% dough state	Bold	24 (26.08%)
			Medium	38(41.30%)
			Small	30(32.60%)
Capsule**:** Dehiscence	It is recorded at the maturity time (VS)	95 % Physiological maturity	Semi-dehiscent	37 (40.21%)
			Non-dehiscent	55(59.78%)

**MG: Measurement by a single observation of a group of plants or parts of plants, MS: Measurement of a numbers of individual plants or parts of plants, VG: Visual assessment by a single observation of a group of plants or parts of plants, VS: Visual assessment by observation of individual plants or parts of plants*

Table 2. List of individual genotypes with their morphological descriptors

S.No.	Genotype	Time of flowering[a]	Flower shape[b]	Flower size[c]	Flower colour[d]	Flower aestivation[e]	Venation colour[f]	Stamen colour[g]	Anther colour[h]	Plant growth habit[i]	Plant height[j]	Capsule size[k]	Capsule dehiscent[l]
1	IC0096672	M	D	L	V	S-t	B	B	B	S-e	M	M	ND
2	IC0525976	M	D	L	V	S-t	B	W	B	S-e	M	M	ND
3	IC0498660	E	D	L	W	V	W	B	B	Bu	M	B	ND
4	IC0499165	M	S	L	V	S-t	B	W	C	Er	M	M	ND
5	IC0526058	M	D	L	V	S-t	B	W	V	S-e	M	S	SD
6	IC0448872	M	D	L	V	S-t	B	W	V	S-e	M	B	ND
7	IC0526063	M	D	L	V	S-t	LV	W	G	S-e	T	S	ND
8	IC0526118	M	F	M	V	S-t	LV	B	G	S-e	T	B	ND
9	IC0498486	M	D	L	V	S-t	B	B	G	Er	M	S	ND
10	IC0305141	M	D	M	V	S-t	B	W	B	S-e	T	B	ND
11	IC0525920	M	D	M	V	V	V	W	V	S-e	T	M	SD
12	IC0118855	M	D	L	V	S-t	B	B	B	Er	M	B	ND
13	IC0526166	L	D	L	V	S-t	B	W	V	Er	T	M	ND
14	IC0526087	L	F	L	V	V	B	B	G	S-e	T	B	ND
15	IC0498866	M	D	M	V	V	V	W	V	Er	M	M	SD
16	IC0356352	M	D	L	V	S-t	LV	W	G	S-e	M	S	ND
17	IC0385396	M	D	L	V	S-t	B	B	G	S-e	M	M	SD
18	IC0394130	M	F	M	V	T	B	B	G	S-e	M	S	SD
19	IC0424547	M	D	S	V	V	B	W	G	S-e	M	S	ND
20	IC0498538	M	D	M	W	V	W	W	C	Er	M	B	SD
21	IC0621689	M	D	L	V	S-t	B	W	G	Er	M	M	ND
22	IC0620658	M	D	L	V	V	B	W	G	Er	M	B	SD
23	IC0448921	L	D	L	V	S-t	B	B	G	S-e	T	M	ND
24	IC0199753	L	F	M	V	T	B	V	G	S-e	M	S	SD
25	IC0413173	M	D	S	V	S-t	V	B	G	S-e	M	S	ND

S.No.	Genotype	Time of flowering[a]	Flower shape[b]	Flower size[c]	Flower colour[d]	Flower aestivation[e]	Venation colour[f]	Stamen colour[g]	Anther colour[h]	Plant growth habit[i]	Plant height[j]	Capsule size[k]	Capsule dehiscent[l]
26	IC0053273	M	D	M	V	V	B	W	B	S-e	M	M	SD
27	IC0096540	M	D	M	W	T	W	W	C	Er	M	S	SD
28	IC0526118	M	D	L	V	V	V	W	G	S-e	M	S	ND
29	IC0054981	L	D	L	V	S-t	V	B	G	S-e	M	B	SD
30	IC0585316	E	D	L	V	V	B	B	G	S-e	M	M	SD
31	IC0342801	M	F	L	V	S-t	B	B	B	S-e	T	B	SD
32	IC0449113	M	S	S	V	V	B	B	C	S-e	T	S	SD
33	IC0499201	M	S	M	V	V	V	W	V	S-e	T	M	ND
34	IC0498517	M	D	M	V	S-t	B	B	C	S-e	T	S	ND
35	IC0498689	M	D	L	W	S-t	W	W	C	S-e	M	M	SD
36	IC0564592	M	D	L	V	S-t	B	B	G	S-e	M	S	ND
37	IC0498768	M	D	L	W	S-t	B	W	B	S-e	T	S	SD
38	IC0096638	M	S	M	V	V	B	B	G	S-e	T	S	ND
39	IC0498880	E	D	M	V	S-t	B	B	G	Er	T	M	ND
40	IC0498786	L	D	M	V	S-t	B	B	G	Bu	T	S	ND
41	IC0394118	L	D	L	V	S-t	B	B	G	S-e	M	B	SD
42	IC0498989	M	D	L	V	S-t	B	W	B	S-e	M	M	ND
43	IC0498843	E	S	M	V	V	V	W	G	S-e	M	M	SD
44	IC0526514	E	S	M	V	V	B	B	G	S-e	M	M	ND
45	IC0521450	M	D	L	V	S-t	V	B	G	Bu	T	S	ND
46	IC0521455	L	D	L	V	S-t	B	B	G	S-e	T	M	ND
47	IC0426926	M	D	L	V	S-t	V	W	B	S-e	T	M	ND
48	IC0498517	M	D	L	V	S-t	B	B	G	S-e	T	M	SD
49	IC0342805	M	F	M	V	T	B	W	B	S-e	M	B	SD
50	IC0342799	M	S	S	V	V	B	B	B	S-e	M	S	ND
51	IC0498482	M	D	M	W	S-t	W	W	C	Er	M	S	ND
52	IC0585324	E	D	M	V	S-t	B	B	G	S-e	M	B	SD
53	IC0967423	M	D	L	V	S-t	B	B	B	S-e	T	B	SD
54	EC0718843	M	F	S	V	V	B	B	G	Er	T	M	SD
55	IC0498489	L	D	L	V	S-t	B	B	G	Er	T	S	SD
56	IC0498561	E	D	M	V	S-t	B	B	G	S-e	T	S	ND
57	IC0499155	E	D	L	V	V	V	W	G	Er	T	B	ND
58	IC0499128	M	D	M	V	S-t	B	B	C	Er	M	S	ND
59	IC0572912	E	D	S	V	S-t	B	W	B	S-e	M	S	ND
60	IC0499013	M	S	M	V	V	W	W	C	S-e	M	M	SD
61	IC0599415	L	D	M	V	S-t	B	B	G	S-e	T	B	SD
62	IC0510935	E	D	M	V	S-t	B	B	G	Er	M	S	ND
63	IC0096678	M	D	M	V	V	V	B	G	S-e	T	S	ND
64	IC0998770	M	D	S	V	S-t	B	B	G	Er	T	M	SD

S.No.	Genotype	Time of flowering[a]	Flower shape[b]	Flower size[c]	Flower colour[d]	Flower aestivation[e]	Venation colour[f]	Stamen colour[g]	Anther colour[h]	Plant growth habit[i]	Plant height[j]	Capsule size[k]	Capsule dehiscent[l]
65	IC0296039	M	D	M	V	S-t	B	B	C	Er	T	S	ND
66	IC0498605	M	S	S	V	V	B	B	B	Er	M	M	SD
67	EC0041621	M	D	M	W	S-t	W	W	C	S-e	T	M	ND
68	IC0526162	M	D	M	V	S-t	B	B	B	Bu	T	M	ND
69	IC0564677	M	D	M	V	V	B	B	G	S-e	M	S	ND
70	IC0346107	E	S	S	V	V	B	B	B	S-e	T	S	SD
71	IC0523801	L	D	M	V	S-t	B	B	B	S-e	M	S	SD
72	IC0305053	E	D	S	V	S-t	B	B	G	S-e	T	S	ND
73	IC0498392	M	D	S	V	S-t	B	W	B	Er	T	B	SD
74	IC0498938	M	D	M	V	V	V	B	B	S-e	T	S	SD
75	IC0498795	M	D	S	V	V	B	B	G	S-e	M	M	ND
76	IC0385383	M	D	M	V	S-t	B	W	B	S-e	T	S	ND
77	IC0498427	M	D	M	V	S-t	B	W	G	S-e	M	M	ND
78	IC0259404	M	D	M	V	S-t	V	B	G	Bu	T	S	ND
79	IC0526105	L	S	M	V	V	V	B	B	Bu	D	B	SD
80	IC0498675	M	D	M	W	S-t	W	W	B	S-e	D	B	ND
81	IC0498724	M	F	L	V	S-t	V	B	G	S-e	D	B	ND
82	IC0498761	L	D	M	V	S-t	V	W	B	S-e	D	M	SD
83	IC0424878	L	D	L	V	V	V	W	V	S-e	M	B	SD
84	IC0499156	M	D	L	V	S-t	B	B	B	Bu	D	S	ND
85	IC0356165	M	D	M	V	S-t	B	W	G	S-e	M	M	ND
86	IC0118861	L	D	L	V	S-t	B	W	G	S-e	M	S	ND
87	LMS-2014-20	M	D	L	W	S-t	W	B	B	S-e	M	B	ND
88	LMS-2018-22	M	D	M	W	S-t	B	B	G	Er	M	S	ND
89	LMS-2015-42(AB)	M	D	M	V	S-t	B	B	G	S-e	M	B	ND
90	LMS-2015-81(AB)	M	D	M	V	S-t	B	B	B	S-e	M	B	ND
91	SLS-135 (D)	E	S	S	W	V	W	W	C	Er	T	S	SD
92	SLS-140 (D)	M	D	M	V	S-t	V	B	B	S-e	T	S	SD

E: early; M: medium; L: late; D: disk; T: tubular; S: star; F: funnel; S: small; L: large; M: medium; V: violet; W: white; T: twisted; S-t: semi-twisted; V: valvate; B: blue; W: white; V: violet; G: grey; S-e: semi-erect; Er: erect; Bu: bushy; SD: semi-dehiscent; ND: non-dehiscent

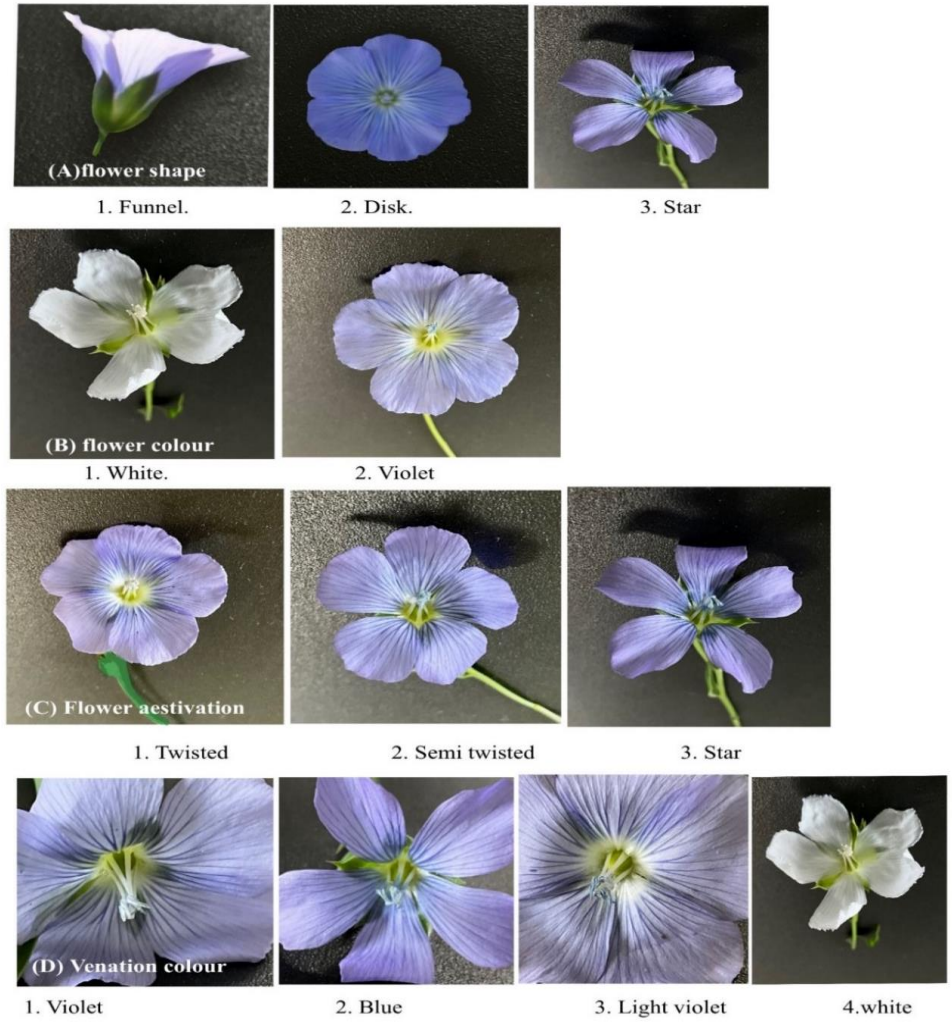

Fig. 1. Flower characteristics of linseed genotypes

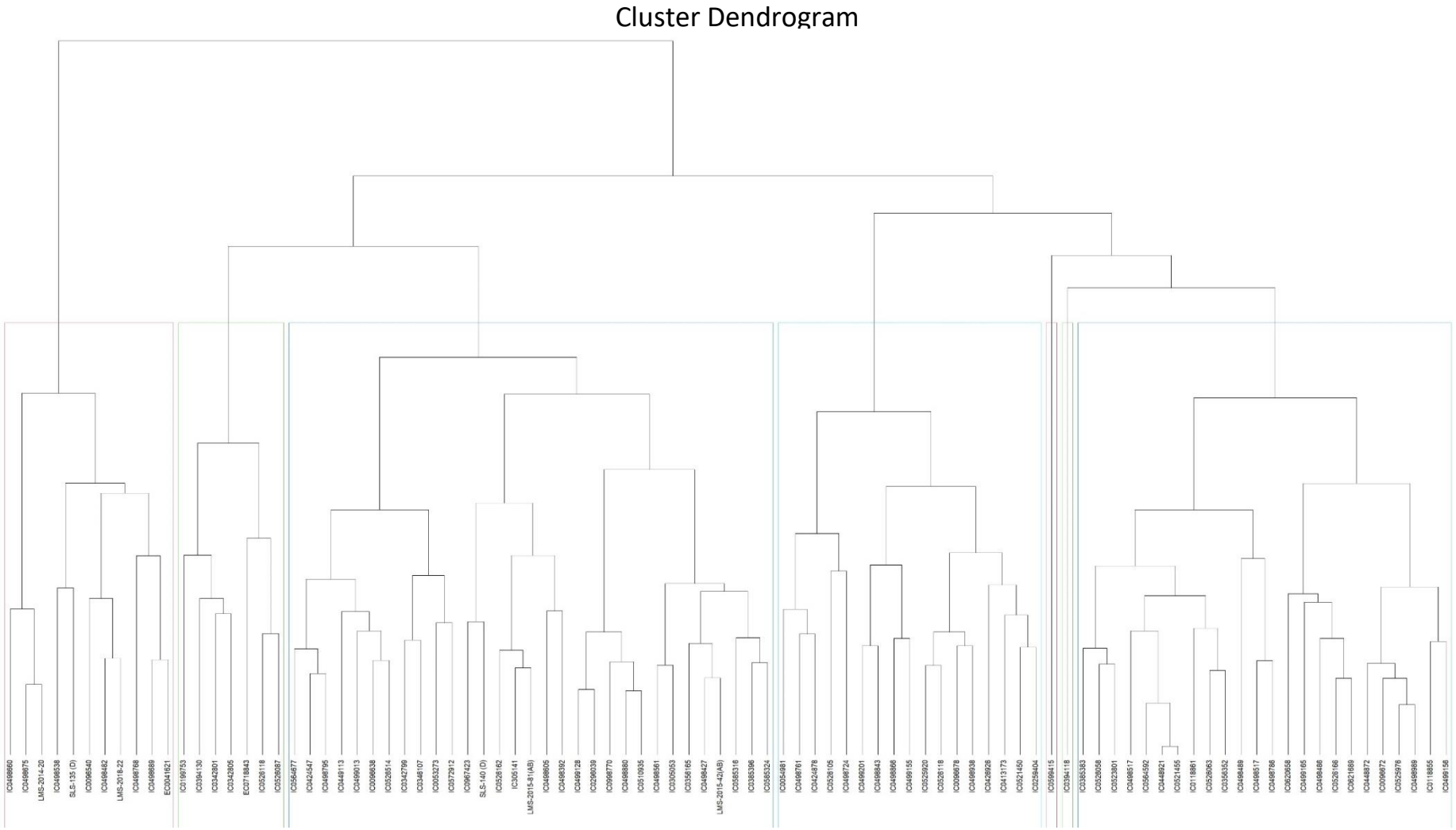

Fig. 2. Dendrogram for cluster analysis of linseed genotypes

4. CONCLUSION

The current study's findings suggest that a variety of genes interact to shape the formation of distinct personalities. The selection of identifiable, consistent, and stable features will be aided by measurements of morphological variation, and this will be extremely beneficial throughout the seed production and monitoring programme of linseed. In conclusion, the linseed germplasms have considerable morphological varieties that reflects variations in both altitude and area. The diverse range of descriptors used for classifying the linseed germplasm served as an effective means to divide them into different groups and provide substantial data to be worked upon further in breeding programmes. The significant morphological diversity found in the linseed germplasm reflects changes in both area and altitude. The linseed germplasm was effectively categorized into distinct groups using a wide range of descriptors, which also yielded valuable information that may be further refined in breeding initiatives. To help farmers and breeders distinguish true breeding identifications of a cultivar and produce variety that will be unique, novel, and useful from the already existing germplasm, morphological characterization aids in the creation and documentation of germplasm distinct features.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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