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Characterization of Niger breeding lines (*Guizotia abyssinica* (L. f) Cass) on the basis of morphology

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Abstract
A field experiment was conducted with 42 including two check Niger breeding lines to study the morphological characterization using self-assumed description and nominal variables of morphological characters, which were used as an input for Nbclust hierarchical cluster analysis in which clustering was done using Ward's minimum variance method and Euclidean's method of genetic distance was derived. In this study, Niger breeding lines were grouped into five clusters based on analysis of divergence at the genetic distance. They revealed a considerable amount of genetic diversity. The largest cluster among all is cluster IV. Cluster IV has 20 lines, cluster V has 16, Cluster III, II & I has contained 2 lines each. Whereas, morphological characterization observation was recorded on 13 traits *viz*., leaf color, leaf shape, leaf serration of margin, leaf angle of branching, stem hairiness, stem color, color of ray florets, color of disc florets, plant branching habit, pollen color, seed color, seed shape and seed texture, etc.

Keywords: Hierarchical cluster, leaf angle of branching, stem hairiness

Introduction
Guizotia abyssinica (L. f) Cass mainly known as Niger, which belongs to family compositae, diploid species (2n=30) is an annual dicot crop and has epigeal germination. Niger originated from high lands of Ethiopia, is the only cultivated member of the taxon, Guizotia. It is introduced as a minor oilseed crop mainly used for the human consumption as well as for industrial uses. The oil content of Niger varies from 30-50 percent of the seed weight. Niger oil has four major fatty acid which are main unsaturated fatty acid *viz*., linoleic acid (75-80%) and oleic acid (5-8%) and two major saturated fatty acid *viz*., palmitic acid and stearic acid (7-8%). Indian Niger oil is being reported to be higher in oleic acid (25%) and has 10-30% protein content. Niger is completely out-crossing species with self-incompatibility mechanism (Chavan, 1961; Mohanty, 1964; Shrivastava and Sujatha, 1993) [3, 6, 15] and entomophilous particularly via bees. (Ramachandran and Menon, (1979) [9]. Variability exists for morphological characters (Pradhan *et al*. 1995) [7]
The description of Niger breeding lines on the basis of morphological characters is easily observable and characterization helps identify suitable lines while assist breeders in selecting diverse parents for breeding and adopt effective breeding methodologies which may aid in the genetic improvement of crops (Shilpashree *et al*., 2021) [13]. Keeping the above points in view, the present investigation was carried out to characterize 42 Niger breeding lines based on morphological characterization.

Method and Materials
The present studies on "Characterization of Niger (*Guizotia abyssinica* (L. f.) cass) breeding lines on the basis of morphology was conducted at the JNKVV, Zonal Agricultural Research Station, All India Co-ordinated Research Project on Niger, Chandangaon, Chhindwara (MP) India. The experiment was conducted using RBD design with three replications for two *kharif* seasons of 2021 and 2022. The materials for the present study comprised of 42 niger breeding lines including two national checks JNS-28 and JNS-9. The soil of the experimented site is sandy soil and the crop was sown in 4 rows with 1.5m row length. Intra row spacing 30 cm and inter plant distance 10 cm.
The observations were recorded on 13morphologicaltraits *viz*., leaf color, leaf shape, leaf serration of margin, leaf angle of branching, stem hairiness, stem color, color of ray florets, color of disc florets, plant branching habit, pollen color, seed color, seed shape and seed texture.

Table 1: List of material used in Trial

S. No.	Accession	S. No.	accession	S. No.	Accession	S. No.	Accession
1	JCN-1	11	JCN-11	21	JCN-21	31	JCN-31
2	JCN-2	12	JCN-12	22	JCN-22	32	JCN-32
3	JCN-3	13	JCN-13	23	JCN-23	33	JCN-33
4	JCN-4	14	JCN-14	24	JCN-24	34	JCN-34
5	JCN-5	15	JCN-15	25	JCN-25	35	JCN-35
6	JCN-6	16	JCN-16	26	JCN-26	36	JCN-36
7	JCN-7	17	JCN-17	27	JCN-27	37	JCN-37
8	JCN-8	18	JCN-18	28	JCN-28	38	JCN-38
9	JCN-9	19	JCN-19	29	JCN-29	39	JCN-39
10	JCN-10	20	JCN-20	30	JCN-30	40	JCN-40
41	JNS-28	42	JNS-9				

Result and Discussion

Morphological traits of the niger breeding lines were studied using self-assumed description. Results revealed that a significant amount of variation was recorded on almost all the characters recorded (Table 2).

The leaf color is one of the important characters for characterization. Based on the variation in the leaf color, Niger breeding lines were categorized in three group *viz*., light green, green and dark green. Five lines had light green, twenty-nine had green and eight had dark green in leaf color. Similar results were reported by Rani *et al.* (2010) [11].

Based on the leaf shape, lines were categorized in three group *viz.*, narrow, medium and broad. Sixteen lines had narrow, twenty-one had medium and five lines had broad leaf shape.

On the basis of leaf serration of margin, lines were categorized into three group *viz*., entire, serrate and dentate. Four lines had entire and twenty had serrate and eighteen lines had dentate-type leaf serration of margin. This type of result was also observed by Gobeyehu *et al.* (2021) [4] and Kumar *et al*. (2021) [5] for the same trait.

Based on leaf angle of branching, breeding lines were also categorized in three groups *viz*., erect, horizontal and hanging. Twenty-eight lines had erected, ten had horizontal and only four lines had hanging leaf angle of branching. This type of result was also observed by Kumar *et al* (2021) [5] and Saraswat and Bisen (2022) [12].

Based on stem color, Niger lines were categorized in three groups *viz*., green, purple green and purple, thirty-four had purple-green in color and eight lines had purple stem color and none had green in color. On the basis of stem hairiness, accessions were categorized into four groups *viz*., glabrous, sparse, medium and dense. Thirty lines had sparse hairiness, five had medium and seven had dense stem hairiness but none of the line showed glabrous stem hairiness. Gebeyehu *et al*., 2021 [4]., Ranjih and Bisen, 2021 [10] and Kumar *et al*., 2021 [5] also reported this trait.

In the color of ray florets, lines were categorized into three groups *viz*., pale yellow, yellow and whitish yellow. Four lines had pale yellow ray floret, thirty-six had yellow ray florets and two lines had whitish yellow ray florets. Similar also observed by Kumar *et al*. (2021) [5]. On the basis of color of disc florets, lines were categorized in two groups *viz*., yellow and purple, where forty lines had yellow and two showed purple color of disc florets.

On the basis of pollen color**,** lines were categorized in two groups i.e yellow and pale yellow. Forty lines had yellow and only two showed pale yellow color of pollen.

On the basis of seed color, Niger breeding lines were classified in three groups i.e brown, dark brown and black. Out of forty-two lines, twenty-five lines were brown, six were dark brown and eleven lines were black in color.

On the basis of seed texture, lines were categorized into smooth and rough and all lines had smooth seed texture. In case of seed shape, lines were classified in three groups *viz*., elongated, ovate and elongated ovate. Out of forty-two lines, two lines were having elongated seed shape and forty lines had elongated ovate shape and none of the line had ovate seed shape.

In case of plant branching habit, lines were categorized in two groups *viz*., erect and drooping, where twenty-nine lines were having erect and thirteen lines had drooping plant branching.

Table 2: Frequency distribution and percentage score of morphological traits.

S. No	Traits	Class	Score	No. of accessions	Frequency (%)
1	Leaf shape	Narrow	1	16	38.0
		Medium	2	21	50.0
		Broad	3	5	11.9
2.	Leaf color	Light green	1	5	11.9
		Green	2	29	69.0
		Dark green	3	8	19.0
3.	Leaf serration of margin	Entire	1	4	9.5
		Serrate	2	20	47.6
		Dentate	3	18	42.8
4.	Leaf angle of branching	Erect	1	28	66.6
		Horizontal	2	10	23.8
		Hanging	3	4	9.5
5.	Stem color	Green	1	0	0.0
		Purple green	2	34	80.9
		Purple	3	8	19.0
6.	Stem hairiness	Glabrous	1	0	0.0
		Sparse	2	30	71.4

		Medium	3	5	11.9
		Dense	4	7	16.6
7.	Pollen color	Yellow	1	40	95.2
		Pale yellow	2	2	4.7
8.	Color of ray florets	Pale yellow	1	4	9.5
		Yellow	2	36	85.7
		Whitish yellow	3	2	4.7
9.	Color of disc floret	Yellow	1	40	95.2
		Purple	2	2	4.7
10.	Plant branching habit	Erect	1	29	69.0
		Drooping	2	13	30.9
11.	Seed color	Brown	1	25	59.5
		Dark brown	2	6	14.2
		Black	3	11	26.1
12.	Seed texture	Smooth	1	42	100.0
		Rough	2	0	0.0
13.	Seed Shape	Elongated	1	2	4.7
		Ovate	2	0	0.0
		Elongated ovate	3	40	95.2

Table 3: Distribution of niger breeding lines for different morphology characters

S. No	Traits	Class	Niger Breeding Lines
1	Leaf shape	Narrow	JCN-5, JCN-9, JCN-13, JCN-14, JCN-16, JCN-21, JCN-23, JCN-28, JCN-29, JCN-30, JCN-31, JCN-32, JCN-35, JCN-36, JCN-37 & JCN-40
		Medium	JCN-1, JCN-3, JCN-4, JCN-8, JCN-10, JCN-11, JCN-15, JCN-17, JCN-18, JCN-19, JCN-20, JCN-22, JCN-24, JCN-26, JCN-27, JCN-33, JCN-34, JCN-38, JCN-39, JNS-28 & JNS-9
		Broad	JCN-2, JCN-6, JCN-7, JCN-12 & JCN-25
2.	Leaf color	Light green	JCN-10, JCN-21, JCN-27, JCN-32 & JCN-36
		Green	JCN-1, JCN-3, JCN-7, JCN-8, JCN-11, JCN-13, JCN-14, JCN-15, JCN-16, JCN-18, JCN-19, JCN-20, JCN-22, JCN-23, JCN-24, JCN-25, JCN-26, JCN-28, JCN-29, JCN-30, JCN-31, JCN-33, JCN-34, JCN-35, JCN-37, JCN-39, JCN-40, JNS-28 & JNS-9
		Dark green	JCN-2, JCN-4, JCN-5, JCN-6, JCN-9, JCN-12, JCN-17 & JCN-38
3.	Leaf serration of margin	Entire	JCN-2 JCN-7, JCN-30 & JCN-37
		Serrate	JCN-1, JCN-3, JCN-8, JCN-10, JCN-13, JCN-15, JCN-16, JCN-21, JCN-23, JCN-25, JCN-29, JCN-31, JCN-32, JCN-34, JCN-36, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Dentate	JCN-4, JCN-5, JCN-6, JCN-9, JCN-11, JCN-12, JCN-14, JCN-17, JCN-18, JCN-19, JCN-20, JCN-22, JCN-24, JCN-26, JCN-27, JCN-28, JCN-33 & JCN-35
4.	Leaf angle of branching	Erect	JCN-1, JCN-2, JCN-3, JCN-4, JCN-5, JCN-6, JCN-7, JCN-8, JCN-12, JCN-14, JCN-15, JCN-17, JCN-19, JCN-20, JCN-24, JCN-25, JCN-26, JCN-27, JCN-29, JCN-30, JCN-35, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Horizontal	JCN-9, JCN-10, JCN-13, JCN-16, JCN-18, JCN-21, JCN-22, JCN-28, JCN-31 & JCN-33
		Hanging	JCN-11 JCN-23, JCN-32 & JCN-34
5.	Stem color	Green	Nil
		Purple green	JCN-1, JCN-2, JCN-3, JCN-4, JCN-7, JCN-9, JCN-10, JCN-11, JCN-12, JCN-13, JCN-14, JCN-15, JCN-16, JCN-17, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-25, JCN-26, JCN-28, JCN-30, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Purple	JCN-5, JCN-6, JCN-8, JCN-18, JCN-24, JCN-27, JCN-29 & JCN-31
6.	Stem Hairiness	Glabrous	Nil
		Sparse	JCN-1, JCN-4, JCN-5, JCN-6, JCN-7, JCN-8, JCN-9, JCN-10, JCN-11, JCN-13, JCN-14, JCN-15, JCN-16, JCN-18, JCN-20, JCN-21, JCN-22, JCN-23, JCN-25, JCN-26, JCN-27, JCN-29, JCN-31, JCN-34, JCN-35, JCN-36, JCN-37, JCN-40, JNS-28 & JNS-9
		Medium	JCN-3 JCN-17, JCN-33, JCN-38 & JCN-39
		Dense	JCN-2 JCN-12, JCN-19, JCN-24, JCN-28, JCN-30 & JCN-32
7.	Pollen color	Yellow	JCN-3, JCN-4, JCN-5, JCN-6, JCN-7, JCN-8, JCN-9, JCN-10, JCN-11, JCN-12, JCN-13, JCN-14, JCN-15, JCN-16, JCN-17,JCN-18, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-24, JCN-25, JCN-26, JCN-27, JCN-28, JCN-29, JCN-30,JCN-31, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Pale yellow	JCN-1 &JCN-2
8.	Color of ray florets	Pale yellow	JCN-5 JCN-7, JCN-10 & JCN-13
		Yellow	JCN-1, JCN-2, JCN-3, JCN-4, JCN-6, JCN-8, JCN-9, JCN-11, JCN-12, JCN-14, JCN-16, JCN-17,JCN-18, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-24, JCN-25, JCN-26, JCN-27, JCN-28, JCN-29, JCN-30,JCN-31, JCN-32, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Whitish yellow	JCN-15 & JCN-33
9.	Color of disc floret	Yellow	JCN-1, JCN-2, JCN-3, JCN-4, JCN-6, JCN-7, JCN-8, JCN-9, JCN-10, JCN-11, JCN-12, JCN-13, JCN-14, JCN-15, JCN-16, JCN-17, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-24, JCN-25, JCN-26, JCN-27, JCN-28, JCN-29, JCN-30,JCN-31, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-39,

			JCN-40, JNS-28 & JNS-9
		Purple	JCN-5 & JCN-18
10.	Plant branching habits	Erect	JCN-1, JCN-2, JCN-3, JCN-5, JCN-6, JCN-7, JCN-11, JCN-14, JCN-16, JCN-18, JCN-19, JCN-21, JCN-22, JCN-23, JCN-24, JCN-26, JCN-27, JCN-28, JCN-30, JCN-31, JCN-32, JCN-34, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Drooping	JCN-4, JCN-8, JCN-9, JCN-10, JCN-12, JCN-13, JCN-15, JCN-17, JCN-20, JCN-25, JCN-29, JCN-33 & JCN-35
11.	Seed color	Brown	JCN-1, JCN-2, JCN-5, JCN-6, JCN-8, JCN-9, JCN-11, JCN-14, JCN-15, JCN-17, JCN-19, JCN-20, JCN-21, JCN-26, JCN-28, JCN-29, JCN-30, JCN-31, JCN-33, JCN-34, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40.
		Dark brown	JCN-7, JCN-12, JCN-13, JCN-22, JCN-25 & JCN-35
		Black	JCN-3, JCN-4, JCN-10, JCN-16, JCN-18, JCN-23, JCN-24, JCN-27, JCN-32, JNS-28 & JNS-9
12.	Seed texture	Smooth	JCN-1, JCN-2, JCN-3, JCN-4, JCN-5, JCN-6, JCN-7, JCN-8, JCN-9, JCN-10, JCN-11, JCN-12, JCN-13, JCN-14, JCN-15, JCN-16, JCN-17, JCN-18, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-24, JCN-25, JCN-26, JCN-27, JCN-28, JCN-29, JCN-30,JCN-31, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-39, JCN-40, JNS-28 & JNS-9
		Rough	Nil
13.	Seed Shape	Elongated	JCN-15 & JCN-39
		Ovate	Nil
		Elongated ovate	JCN-1, JCN-2, JCN-3, JCN-4, JCN-5, JCN-6, JCN-7, JCN-8, JCN-9, JCN-10, JCN-11, JCN-12, JCN-13, JCN-14, JCN-16, JCN-17, JCN-18, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-24, JCN-25, JCN-26, JCN-27, JCN-28, JCN-29, JCN-30,JCN-31, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-40, JNS-28 & JNS-9

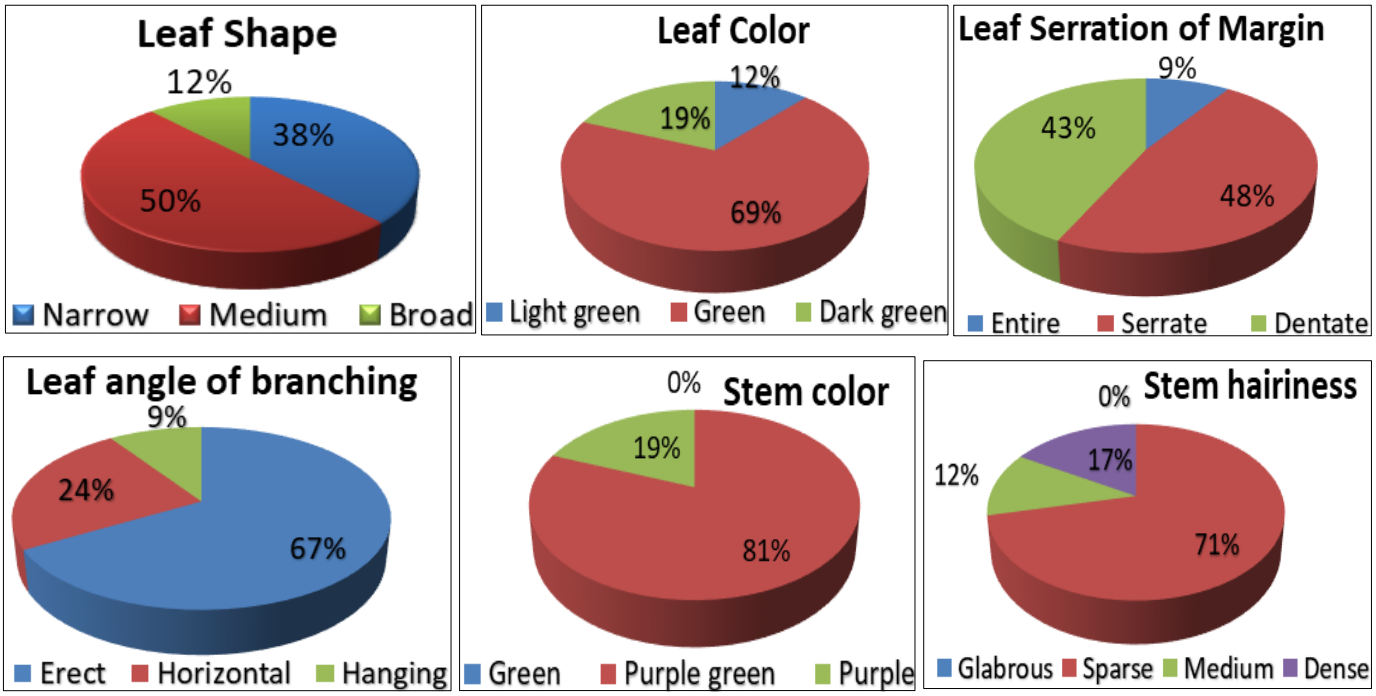
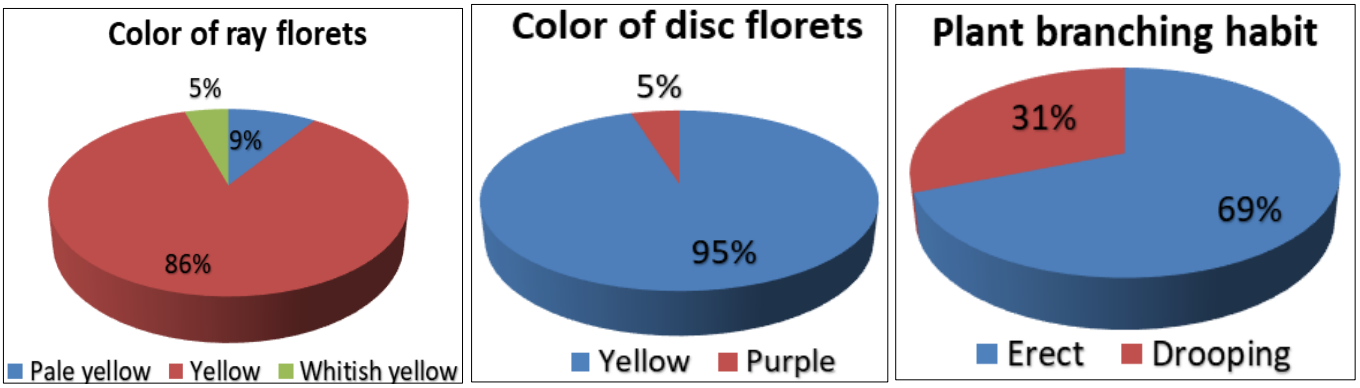


Fig 1: Graphical representation of morphological traits of Niger breeding lines through pie chart



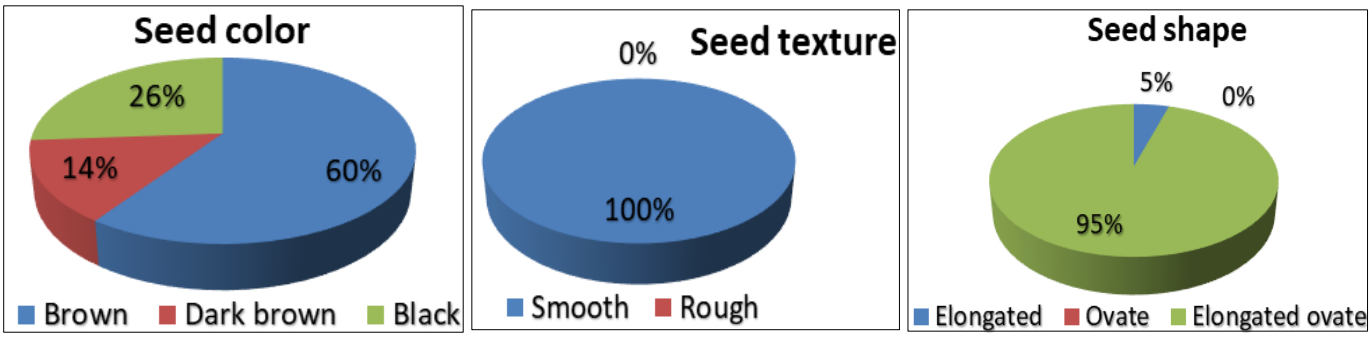


Fig 2: Graphical representation of morphological traits of Niger breeding lines through pie chart

Genetic diversity analysis using morphological traits: The study of genetic divergence using42 Niger breeding lines including two checks *viz*., JNS-28 & JNS-9 was determined by using nominal variables of morphological characters, which were used as an input for Nbclust hierarchical cluster analysis in which clustering was done using Ward's minimum variance method and Euclidean's method of genetic distance was derived. The dendrogram was further constructed based on clustering using the graph package. The dendrogram and phylogeny tree were constructed by using R Studio software.

Table 4: Distribution of Niger breeding lines into different clusters based on morphology traits.

Cluster Number	Number of lines	Name of lines
Cluster I	2	JCN-5 & JCN-18
Cluster II	2	JCN-1 & JCN-2
Cluster III	2	JCN-15 & JCN-39
Cluster IV	20	JCN-9, JCN-11, JCN-14, JCN-16, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-26, JCN-28, JCN-30, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-40
Cluster V	16	JCN-3, JCN-4, JCN-6, JCN-7, JCN-8, JCN-10, JCN-12, JCN-13, JCN-17, JCN-24, JCN-25, JCN-27, JCN-29, JCN-31, JNS-28 & JNS-9

Grouping of Niger breeding lines into different clusters: In this study, 42 Niger breeding lines were grouped into five clusters based on analysis of divergence atgenetic distance. (Table.4). The largest cluster among all is cluster IV. It had 20 lines, cluster V had 1 and, Clusters III, II & I contained 2 lines each.

The maximum genetic distance was found between cluster I & cluster IV followed by cluster I & cluster V. Therefore, good recombinants can be obtained on mating between cluster I (JCN-5 & JCN-18 & cluster IV JCN-9, JCN-11, JCN-14, JCN-16, JCN-19, JCN-20, JCN-21, JCN-22, JCN-23, JCN-26, JCN-28, JCN-30, JCN-32, JCN-33, JCN-34, JCN-35, JCN-36, JCN-37, JCN-38, JCN-40). The minimum genetic distance was found between cluster I, cluster II and III. As a result, on mating lines between those clusters, good recombinants may not be obtained. Birhanu (2019), Aboye (2021) [1] in Niger and Raza *et al*. (2019) [8] observed similar trends in diversity study of rapeseed and mustard germplasm using cluster analysis. Ahirwar *et al*., (2017) [1] grouped 114 Niger germplasm into 8 clusters in which 90 of germplasm were grouped in same cluster. Saraswat and Bisen (2022) [12] also observed similar result in Niger accessions.

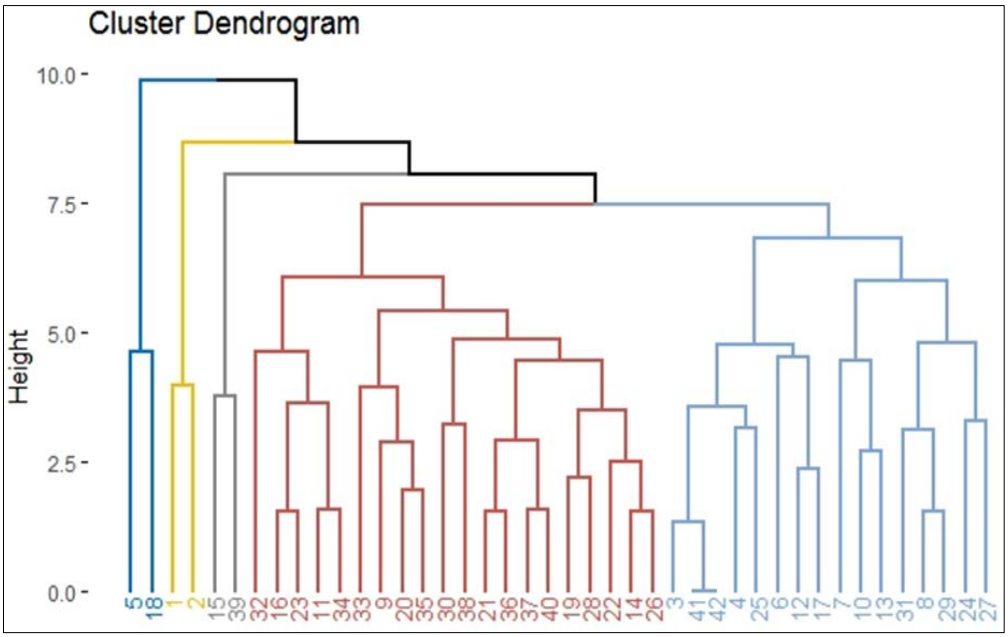


Fig 2: Hierarchical clustering for morphological traits

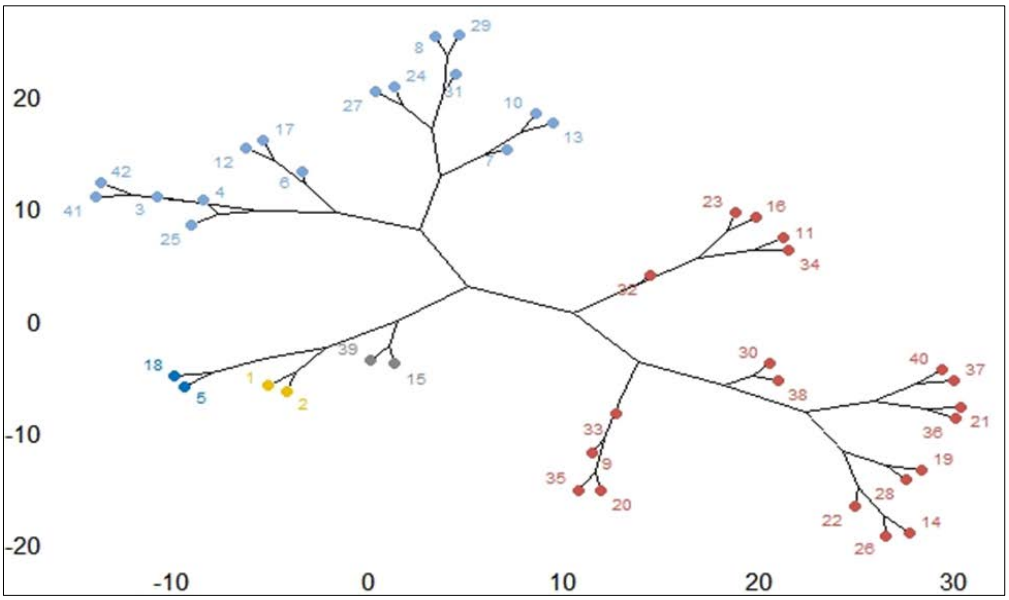


Fig 3: Phylogeny tree construction for morphological traits.

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