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Phylogenetic Relationships Based on Morphological Characterization of Niger (*Guizotia abyssinica* (L.f.) cass) Accessions

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ABSTRACT: Niger despite being nutritionally rich is an underutilized trivial oilseed crop and considered as one of the orphan crops. The present experiment aims to characterize a hundred and three accessions of niger. The experiment was carried out using un-replicated Augmented Block Design for a period of two seasons conducted during *Kharif*, 2020 and 2021 at Project Coordinating Unit, All India Coordinated Research Project on Sesame and Niger (ICAR), JNKVV Campus, Jabalpur, M.P. Twenty-two morphological traits distinguished the niger accessions. Most of the studied traits showed a reasonably large amount of variation. Maximum variation was recorded in leaf shape, leaf color, leaf angle of branching, leaf length, leaf width, the color of ray floret, number of ray florets, number of nodes, seed shape, and seed color. Among the different traits assessed, the number of bracts showed 100% frequency. Higher frequency for medium seed length (96.12%), yellow color of disc floret (92.23%), elongated ovate seed shape (91.26%), serrate leaf serration of margin (91.26%), smooth seed texture (87.38), erect plant branching habit (86.40%), yellow color of ray floret (85.43%) and yellow pollen color (84.46%) was observed. Genetic diversity analysis using morphological traits grouped 103 accessions into five clusters. The maximum genetic distance was found between cluster II and Cluster IV genotypes, followed by Cluster IV and Cluster V. Therefore, good recombinants can be obtained on mating between genotypes of clusters II and IV. Results revealed divergent characteristics of niger accessions and indicated the presence of diversity in the collection studied. Studying these traits at the genetic level will be very important to breeders since these traits may be further useful for genotype identification.

Keywords: Niger, morphology, phylogeny, accessions.

INTRODUCTION

Niger (*Guizotia abyssinica* (L.) Cass.) belonging to family Compositae is an oilseed crop grown in Ethiopia and India, accounting for about 50% of Ethiopian and 3% of Indian oilseed production (Getinet and Sharma 1996). Five of six species, including niger of the genus *Guizotia,* are native to the Ethiopian highlands. The species of the genus are diploid with 2n=30 chromosomes (Hiremath and Murthy 1992; Dagne, 1994). Niger is an annual dicot with epigeal germination, and seedlings have pale green to tan hypocotyls and cotyledons (Seegeler, 1983). It is usually grown on light, poor, coarse-textured soils (Chavan, 1961). It is a fully outbred species with a self-incompatibility mechanism (Chavan, 1961; Mohanty, 1964; Shrivastava and Shomwanshi 1974; Sujatha, 1993) and is entomophilous, particularly *via* bees (Ramachandran and Menon 1979).

The pale yellow niger seed oil has a nutty flavor and a pleasant odour (Getinet and Sharma 1996). The seed contains about 40% oil with a fatty acid composition of 75-80% linoleic acid, 7-8% palmitic and stearic acid, and 5-8% oleic acid (Getinet and Teklewold 1995). The Indian varieties contain 25% oleic acid and 55% linoleic acid (Nasirullah *et al.* 1982). The meal left after oil extraction is free of toxins but contains more crude fiber than most oilseed meals. Niger's oil, protein, and fiber content are affected by husk thickness, and thick-skinned seeds tend to contain less oil and protein and more fiber (Getinet and Sharma 1996).

Characterization is the description of plant germplasm. Morphological characters are easily observable, and characterization helps identify suitable genotypes, 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).

Phylogenetic relationships between various lines could be revealed using morphological characterization to limit recurring parents and benefit breeders in developing enhanced varieties with a broader genetic base (Thakur *et al*., 2022).

Considering the above point of view, the main objective of this experiment is to characterize the niger accessions based on their morphological traits.

MATERIAL AND METHODS

In the present research, a total of 103 accessions (Table 1), including three checks (JNS-9, JNS-30, and JNS-28), were evaluated which were collected from Project Coordinating Unit, All India Coordinated Research Project on Sesame and Niger (ICAR), JNKVV Campus, Jabalpur, M.P., India. Spacing between row to row and plant to plant was kept at 45.0 cm and 15.0 cm. The experiment was conducted using an un-replicated Augmented Block Design for two seasons during *Kharif*, 2020 and 2021. The observations were recorded on 22 traits *viz.* leaf serration, leaf shape, leaf color, leaf angle of branching, leaf length, leaf width, stem color, stem hairiness, pollen color, the color of ray floret, number of ray florets, the color of disc floret, number of bracts, number of nodes, internode length, plant branching habit, the diameter of capitula, lodging tendency, seed shape, seed length, seed color, and seed texture. Twenty-two morphological traits have been considered essential for describing hundred and three niger accessions.

RESULT AND DISCUSSION

A total of 22 morphological traits of the niger accessions were studied and results revealed that a significant amount of variation was recorded in almost all the traits studied. Grouping and the frequency distribution of accessions have been provided in Table 2 and its discussion in this section.

Various taxa of dicotyledonous plants have leaves possessing regular and familiar patterns of architectural organization for example; the dicot plant can have its margin as entire, lobed, or toothed (serrate and dentate) (Hickey, 1973). The niger accessions were grouped as entire, serrate, and dentate based on leaf serration of margin. A maximum number of accessions were observed as serrate (94) followed by dentate (05) and entire (04). Gebeyehu *et al*. (2021) and Kumar *et al*. (2021) also observed same trait in their experiments on niger.

The leaf is of prime importance to the rape, substitute as the plant's power generator and aerial environmental sensor (Bylesjo *et al*., 2008; Efroni *et al*., 2010). The number, area, shape, and size of leaves are important to plant science, allowing scientists to distinguish between different species and even to model climate change (Cope *et al*., 2012). In the case of leaf shape, maximum accessions were medium (58), followed by broad (28) and narrow (17). Kumar *et al*. (2021) reported similar findings.

Table 1: Experimental material.

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	BMD-181	27	BMD-207	53	AJSR-10	79	PCU-30
2	BMD-182	28	BMD-208	54	AJSR-11	80	PCU-31
3	BMD-183	29	BMD-209	55	AJSR-12	81	PCU-32
4	BMD-184	30	BMD-210	56	AJSR-13	82	PCU-33
5	BMD-185	31	BMD-211	57	AJSR-14	83	PCU-34
6	BMD-186	32	BMD-212	58	AJSR-15	84	PCU-35
7	BMD-187	33	BMD-213	59	AJSR-16	85	PCU-36
8	BMD-188	34	BMD-214	60	AJSR-17	86	PCU-37
9	BMD-189	35	BMD-215	61	AJSR-18	87	PCU-38
10	BMD-190	36	BMD-216	62	AJSR-19	88	PCU-39
11	BMD-191	37	BMD-217	63	AJSR-20	89	PCU-40
12	BMD-192	38	BMD-218	64	AJSR-21	90	PCU-41
13	BMD-193	39	BMD-219	65	AJSR-22	91	PCU-42
14	BMD-194	40	BMD-220	66	AJSR-23	92	PCU-43
15	BMD-195	41	BMD-221	67	AJSR-24	93	PCU-44
16	BMD-196	42	BMD-222	68	AJSR-25	94	PCU-45
17	BMD-197	43	BMD-223	69	AJSR-26	95	PCU-46
18	BMD-198	44	AJSR-1	70	AJSR-27	96	PCU-47
19	BMD-199	45	AJSR-2	71	AJSR-28	97	PCU-48
20	BMD-200	46	AJSR-3	72	PCU-23	98	PCU-49
21	BMD-201	47	AJSR-4	73	PCU-24	99	PCU-50
22	BMD-202	48	AJSR-5	74	PCU-25	100	PCU-51
23	BMD-203	49	AJSR-6	75	PCU-26	101	CHECK 1(JNS-9)
24	BMD-204	50	AJSR-7	76	PCU-27	102	CHECK 2 (JNS-30)
25	BMD-205	51	AJSR-8	77	PCU-28	103	CHECK 3 (JNS-28)
26	BMD-206	52	AJSR-9	78	PCU-29		

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

S. No.	Trait	Class	Score	No. of accessions	Frequency percentage
1	Leaf serration of margin	Entire	1	04	3.88
		Serrate	2	94	91.26
		Dentate	4	05	4.85
2	Leaf shape	Narrow	1	17	16.50
		Medium	2	58	56.31
		Broad	3	28	27.18
3	Leaf color	Very light green	1	00	0
		Light green	2	19	18.44
		Green	3	76	73.79
		Dark green	4	08	7.77
		Very dark green	5	00	0
4	Leaf angle of branching	Erect (acute)	1	52	50.4
		Erect	2	07	6.80
		Horizontal	3	39	37.87
		Hanging branches	4	05	4.85
5	Leaf length (cm)	Small	1	02	1.94
		Medium	2	81	78.64
		Long	3	20	19.41
6	Leaf width (cm)	Narrow	1	25	24.27
		Medium	2	77	74.75
		Broad	3	01	0.97
7	Stem color	Green	1	05	4.85
		Purplish green	2	19	18.44
		Purple	3	79	76.70
8	Stem hairiness	Glabrous	1	0	0
		Sparse	2	81	78.64
		Medium	3	17	16.50
		Dense	4	04	3.88
9	Pollen color	Yellow	1	87	84.46
		Pale yellow	2	16	15.53
10	Color of the ray floret	Pale yellow	1	10	9.70
		Yellow	2	88	85.43
		Whitish yellow	3	05	4.85
11	No. of ray florets	Up to six	1	04	3.88
		Seven to eight	2	79	76.70
		More than eight	3	20	19.42
12	Color of disc floret	Yellow	1	95	92.23
		purple	2	08	7.77
13	No. of bracts	Five	1	103	100
		More than five	2	0	0
14	No. of nodes	Up to ten	1	18	17.47
		Ten to fourteen	2	50	48.54
		More than fourteen	3	35	33.98
15	Internode length (cm)	Small	1	32	31.07
		Medium	2	66	64.07
		Long	3	05	4.85
16	Plant branching habit	Erect	1	89	86.40
		Drooping	2	14	13.59
17	Diameter of capitula	Small	1	71	68.93
		Medium	2	29	28.15
		Large	3	03	2.91
18	Lodging tendency	Absent	0	43	41.74
		Present	1	60	58.2
19	Seed shape	Elongated	1	06	5.82
		Ovate	2	03	2.91
		Elongated ovate	3	94	91.26
20	Seed length	Small	1	03	2.91
		Medium	2	99	96.12
		Large	3	01	0.97
21	Seed color	Brown	1	70	67.96
		Dark brown	2	23	22.33
		Black	3	10	9.70
22	Seed texture	Smooth	1	90	87.38
		Rough	2	13	12.62

The intensity of the green color is a clear indication of Chlorophyll pigment in leaves. The leaf chlorophyll content is often well correlated with plant metabolic activity, *e.g.*, photosynthetic capacity and RuBP carboxylase activity (Evans, 1983; Seeman *et al.*, 1987), plant stress (Eagles *et al*., 1983; Fanizza *et al*., 1991), and leaf N concentration as well. In the study for the trait of leaf color, maximum green leaf color (76) was observed, followed by light green (19) and dark green (08). None of the accessions had very light green and dark green leaf colors. Gebeyehu *et al*. (2021), Kumar *et al*. (2021), and Rani *et al*. (2010) also used this trait as one of the criteria for characterization of genotypes in niger.

Branch angle is a key morphological trait that shapes the canopy design and influences yield. Plants with effectively small branch angles exhibit compact canopy architectures, and these plants are, for that reason, more suitable for high-density planting (Wang *et al*., 2014), so a condensed canopy architecture maintains light capture under elevated densities by minimizing shade by adjacent plants (Sun *et al*., 2016). In the trait leaf angle of branching, maximum accessions were observed as having erect (acute) (52) followed by horizontal (39 accessions), erect (07 accessions), and 05 accessions were observed as having hanging branches. Kumar *et al*. (2021) reported similar findings.

Based on length of leaf, accessions were categorized into three classes i.e. small, medium and long. Most accessions were observed as medium (81) followed by long (20) and small (02). In leaf width trait, a maximum number of accessions (77) were observed as having medium width followed by narrow (25) and broad (01).

In the case of stem color, most accessions (79) were observed as purple colored, followed by purplish green (19), and 05 accessions had green stem color. Rani *et al*. (2010), Kumar *et al*. (2021), and Gebeyehu *et al*. (2021) observed same trait for categorizing niger genotypes. Hairiness and pubescence are reported to be a usual defense mechanism for biotic and abiotic factors, and they are rich in defense-related proteins also (Amme *et al.,* 2005). In the study for the trait of stem hairiness, maximum accessions were observed as sparse (81), followed by medium (17) and dense (04). None of the accessions were found to be glabrous (0). Gebeyehu *et al*. (2021), Ranjithkumar and Bisen (2021) and Kumar *et al*. (2021) also elucidated this trait.

Diverse flowers produce different carotenoids. The flowers become intensely dark as the amount of pigment elevates (Miller *et al*., 2011). In the case of pollen color, most of the accessions (87) were observed as a yellow color, and 16 accessions were pale yellow. In the study for the trait of the color of ray floret, was observed maximum yellow (88) followed by pale yellow (10) and whitish yellow (05) in niger accessions. Kumar *et al*. (2021) reported similar findings.

In the inflorescence of the family Asteraceae, the capitulum comprises the ray florets (outer florets) and the disc florets (inner florets) and can be regarded as the basic pollination unit (Leppick1977). In the case of no. of ray florets, most of the accessions (79) had seven-eight ray florets, followed by more than eight ray florets (20) and up to six ray florets (04). Kumar *et al*. (2021) reported similar results. In the study for the trait of the color of disc floret, maximum accessions were observed as yellow (95), and eight accessions had purple-colored disc floret.

In the case of the number of bracts, all the accessions under study (103) were observed as having five bracts. None of the accessions had more than five bracts (0). In the case of the number of nodes, most of the accessions (50) had ten to fourteen nodes followed by more than fourteen nodes (35) and up to ten nodes (18). In the study of internode length trait, the maximum number of accessions (66) were observed as having medium internode length followed by small (32) and long (05).

Plant growth habit is a significant agronomic trait since a dense ground cover affects the interception of light for photosynthetic buildup, the inhibition of weed development, and the decline of water evaporation from soil (Baum *et al*., 2003). In the case of plant branching habit, most accessions were erect (89), and fourteen accessions were drooping. In the trait of diameter of capitula, the maximum number of accessions (71) were small, followed by medium (29) and large (03). Gebeyehu *et al*. (2021) and Rani *et al*. (2010) elucidated this trait for characterization of niger germplasm. Lodging tendency was present in maximum accessions (60), followed by absent in 43 accessions. Rani *et al*. (2010) also explained this trait during their studies on the characterization of niger genotypes.

In the case of seed shape, most of the accessions were elongated ovate (94) followed by elongated (06) and ovate (03). Kumar *et al*. (2021) studied same trait and reported similar findings. In the case of seed length, maximum accessions were medium (99), followed by small (03) and large (01). All former researchers in sesame outlined seed coat color under digenic control with several puzzling segregants beyond possible explanation (Baydar and Turgut, 2000; Falusi, 2007). In recent times Zhang *et al*. (2013) using a high-density linkage map, analyzed the genetic segregation and quantitative trait loci (QTL) for sesame seed coat color and showed that two major genes with additive dominant- epistatic effects together with polygenes were answerable for controlling the seed coat color trait. In the trait of seed color, a maximum number of accessions (70) were brown, followed by dark brown (23) and black (10). Rani *et al*. (2010) and Kumar *et al*. (2021) also observed same trait in their experiments on niger. In the case of seed texture, most of the accessions were smooth (90), and 13 were rough.

Genetic diversity analysis using morphological traits

In the present investigation, the genetic divergence of 103 niger accessions including 03 checks *viz*., JNS-9, JNS-30 and JNS-28. 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 Phylogeny tree was further constructed based on clustering using the igraph package. The dendrogram and phylogeny tree were constructed by using R studio software ver. 4.1.2.

Grouping of genotypes into different clusters

In the present study, 103 niger accessions were grouped into 5 clusters based on analysis of divergence at the genetic distance of 10 (Table 3). The clustering of genotypes was grouped mainly by their morphological differences. Cluster V was the largest among all clusters comprising 61 genotypes. Cluster IV has 35 genotypes, Cluster II has five genotypes, and Cluster I and Cluster III have one genotype each *i.e.* monogenotypic.

Table 3: Distribution of genotypes into different clusters based on morphological traits.

Cluster no	No. of genotypes	Genotypes
Cluster I	01	BMD-189
Cluster II	05	PCU-32, PCU-30, PCU-47, BMD-202 and BMD-204
Cluster III	01	BMD-195
Cluster IV	35	BMD-197, BMD-198, BMD-222, PCU-31, JNS-9, JNS-28, BMD-187, BMD-186, AJSR-18, BMD—201, AJSR-27, BD-181, BMD-182, BMD-219, AJSR-11 AJSR-2, AJSR-5, BMD-193, BMD-194, AJSR-1, BMD-196, JNS-30, AJSR-15, AJSR-23, PCU-42, PCU-43, BMD-190, PCU-49, BMD-209, PCU-44, BD-199, PCU-45, PCU-25, PCU-24, PCU-38
Cluster V	61	BMD-218, PCU-51, PCU-26, PCU-29, BMD-216, BMD-210, AJSR-10, BMD-221, BMD-212, BMD-184, BMD-213, AJSR-9, AJSR-20, AJSR-16, BMD-183, BMD-220, AJSR-12, AJSR-19, BMD-217, AJSR-17, BMD-223, AJSR-13, AJSR-14, AJSR-3, BMD-203, AJSR-4, BMD-200, AJSR-6, AJSR-28, AJSR-7, AJSR-8, AJSR-22, AJSR-24, BD-185, BMD-192, BMD-191, AJSR-25, AJSR-26, BMD-188, BMD-211, AJSR-21, PCU-46, PCU-50, PCU-28, PCU-41, BMD-215, BMD-214, PCU-48, BMD-208, PCU-23, BMD-205, BMD-206, PCU-27, PCU-40, BMD-207, PCU-35, PCU-36, PCU-33, PCU-34, PCU-37, PCU-39

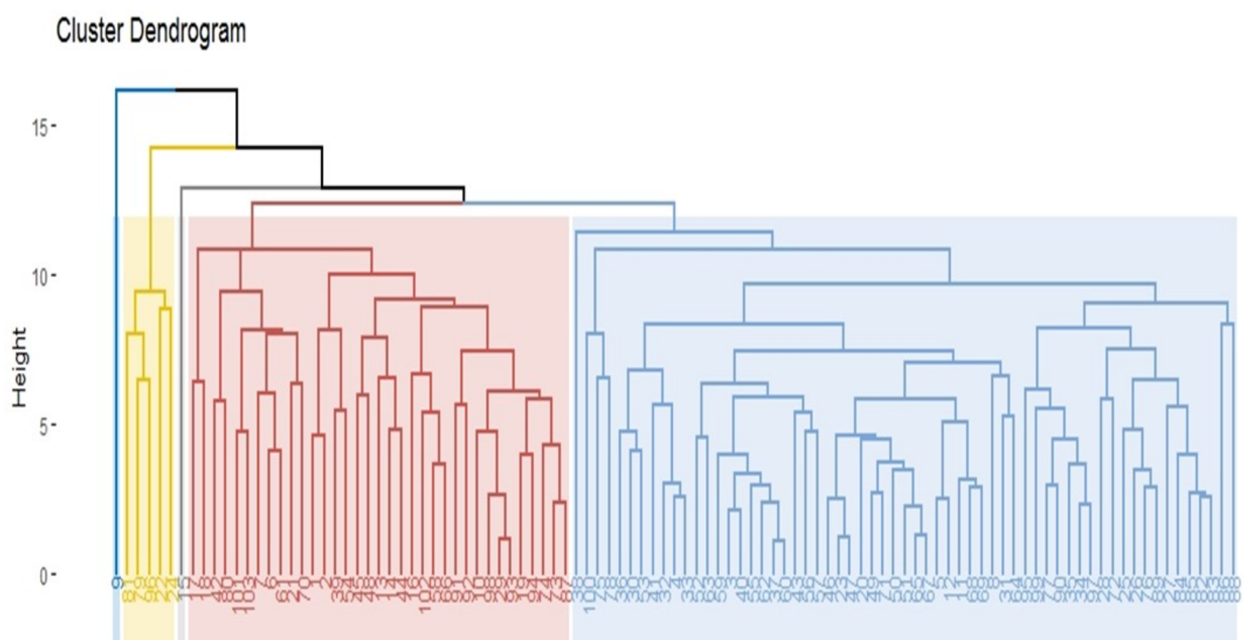

Fig. 1. Hierarchical clustering for morphological traits.

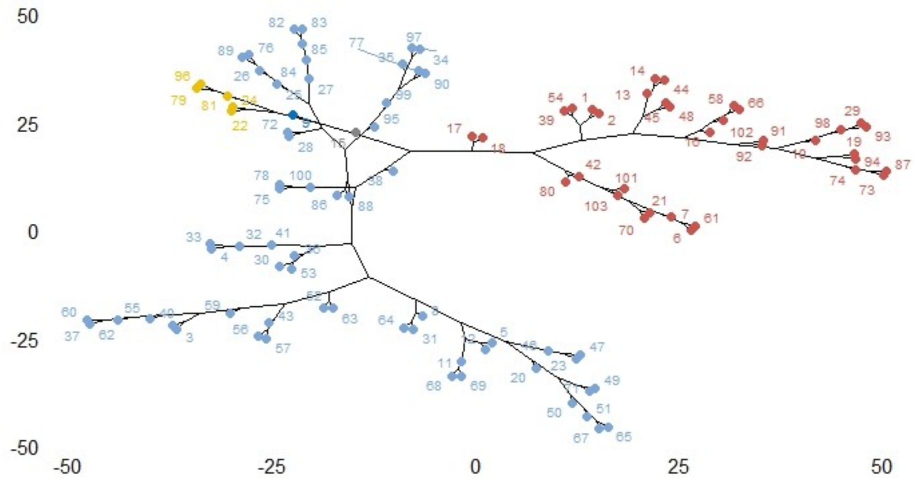

Fig. 2. Phylogeny tree construction for morphological traits.

The maximum genetic distance was found between the genotypes of cluster II and Cluster IV, followed by Cluster IV and Cluster V. So, good recombinants can be obtained on mating between clusters II ((PCU-32, PC-30, PCU-47, BMD-202, and BMD-204) and Cluster IV (BMD-197, BMD-219, AJSR-1, JNS-9, PCU-43, JNS-9, BMD-196, JNS-28, BMD-190, and AJSR-5). In contrast, the minimum genetic distance was found between cluster I and Cluster II genotypes, followed by Cluster I and Cluster III. As a result, on mating lines between those clusters, good recombinants may not be obtained.

CONCLUSION

A huge variation is present in 103 accessions for different morphological traits. In our experiment, maximum accessions acquired serrate leaf serration of margin, erect plant branching habit, erect leaf angle of branching and presence of lodging tendency, and sparse stem hairiness concerning a trait of color. Maximum accessions were green in leaf color, purple in stem color, yellow in pollen color, yellow in the color of ray floret, yellow in the color of disc floret, and brown in seed color. Most accessions were having medium leaf length and leaf width, exhibited seven to eight ray florets, five bracts, ten to fourteen nodes, and medium internode length. Concerning trait of shape, maximum accessions were medium in leaf shape, elongated ovate seed shape, small diameter of capitula, and medium seed length with smooth seed texture. Genetic diversity analysis using morphological characters was done, and hundred and three Niger accessions were grouped into five clusters. The maximum genetic distance was found between cluster II and Cluster IV genotypes, followed by Cluster IV and Cluster V. So, good recombinants can be obtained on mating between clusters II and IV. The present experiment indicates the presence of diversity in the collection. Studying these traits at the genetic level will be very important to breeders and researchers to identify and conserve favorable genes for crop improvement programmes.

FUTURE SCOPE

Assessment of heritability and other genetic parameters for the traits such as plant height and seed size can be performed to deduce the environmental influence on the traits and their further utilization in the crop improvement programmes.

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Conflict of Interest. None

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