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Genetic Variation of Foxtail Millet (Setaria italica (L.) P. Beauv.) Germplasm From Polewali Mandar West Sulawesi Based on Morphological Characters

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ABSTRACT

Foxtail millet (Setaria italica (L.) P. Beauv.) is a cereal crop that has long been domesticated worldwide and has been adopted as a model crop to provide a deeper understanding of plant biology. This plant has long been a mainstay of indigenous Indonesian people, especially in Polewali Mandar, West Sulawesi Province. This study aims to evaluate the genetic variation of foxtail millet germplasm from Polewali Mandar, West Sulawesi based on morphological characters. Three germplasm populations were collected from Polewali Mandar to assess genetic variation in millet germplasm. The observations of morphological traits were carried out based on the IBPGR of Setaria italica. Genetic variation in foxtail millet populations was measured using 28 morphological traits with nine qualitative and 19 quantitative characters. The research showed that seed color, stem diameter, plant height, panicle stalk length, and panicle stalk diameter had diverse variations. Cluster analysis of morphological characters using the UPGMA method shows a similarity coefficient ranging from 0.15-0.2. At a genetic similarity coefficient of 0.2, the three populations were divided into Cluster A and Cluster B. Cluster A consists of two populations (Minna' and Lasse'). In contrast, Cluster B consists of one population (Bulawang). The millet population SLB.C.3-SLB.A.1 has the highest genetic distance and is a potential candidate to be selected as a parent cross to produce higher het-erosis. Intensification of millet plant breeding programs is expected to increase the genetic basis for determining specific characteristics.

Keywords: *Foxtail millet; genetic variation; morphology; West Sulawesi*

INTRODUCTION

West Sulawesi Province has a strategic role in supporting Sulawesi's position at the national level as a production and processing center for biological plant, and agricultural products, especially millet plantations and fisheries. West Sulawesi Province, which directly faces the Makassar Strait, is one of the national and international shipping routes that provides added value to the economic development of this region.

Foxtail millet is one of the oldest cultivated crops and was potentially developed in West Sulawesi, Indonesia, mainly because of its expansive growth. This crop is extensively consumed as a staple food crop (Ramlah *et al.* 2023; Ramlah *et al.* 2020; Samtiya *et al.* 2023) and is produced in arid and semi-arid regions of China, India, Japan, South and North America, Australia, and Indonesia (Prasad, 2017). It is a multipurpose crop known for being genetically closely related to biofuel grasses, for its C4 photosynthesis, and for its excellent tolerance to abiotic stress (Prasad, 2017; Pramitha *et al.* 2023). Known as a drought tolerant crop (Bhinda *et al.* 2022), it responds to low nitrogen and phosphate by reducing and enhancing its root system (Nadeem *et al.* 2020).

In Indonesia, foxtail millet is commonly found in mountainous regions and cultivated in some areas, including West of Sulawesi, South of Sulawesi, Buru Island, East Nusa Tenggara, Bangka Belitung, Central of Java, Papua, Bengkulu, South of Sumatra, and West of Java. Mountain farmers value foxtail millet for its nutritional content and health promoting properties, ability to grow under low external input conditions, tolerance to extreme environmental stress, particularly drought, and patience with pests and diseases (Sintia *et al.* 2023). The farmers and local communities in West Sulawesi Indonesia use foxtail millet as an alternative food crop instead of rice in local culture (Ramlah *et al.* 2020); it is used to make pancakes, porridge, and puddings because of its low allergenic and highly digestible properties (Karpagapandi *et al.* 2023); It also known as food for bird even though millet is rich in fiber (Yulita *et al.* 2018). Currently, foxtail millet has received attention, mainly because it is rich in nutrients and highly beneficial to human health (Karamang *et al.* 2023). Millet is a cereal rich in mineral content, including iron, calcium, potassium, zinc, magnesium, vitamins, crude edible fiber, and gluten free with a low glycemic index (GI) (Singh *et al.* 2017). Millets are also rich in polyphenols, tannins, and phytosterols and are a good source of antioxidants. Millet consumption can provide a way to eliminate nutritional deficiencies (Dhaka *et al.* 2021).

The phenotypic characteristics of plants conform to their morphological characters (Claßen-Bockhoff, 2001), including qualitative and quantitative characters. A phenetic germplasm analysis must be conducted to determine the morphological variation (Adiansyah *et al.* 2023). The morphological study of plant taxa is essential to provide comprehensive information on their taxonomic status (Mahfut *et al.* 2021; Sofiyanti *et al.* 2022; Windiyani *et al.* 2022). It also gives an accurate way to determine the agronomical traits of plants (Purwanti & Prihanta, 2019), especially for plants breeding (George, 2007) such as Setaria taxa. Morphological characters are visible characteristics and can be used to construct genetic maps of organisms and determine genetic variability in organisms (Chahyadi *et al.* 2019). Oktavianingsih *et al.* (2019) state that information about morphological variability is needed to develop plant breeding populations as future alternative crops. Therefore, in crop improvement, the understanding of its biodiversity is an essential resource for plant breeding (Dias, 2015).

Information about diversity is essential for breeding and management strategies (du Plessis *et al.* 2019). It can support the sustainability of plant germplasm breeding activities by farmers and plant breeders (Liu *et al.* 2022). Both farmers and breeders take advantage of biodiversity by identifying traditional and better adapted plant varieties to heterogeneous various environments conditions (Brauner *et al.* 2021). According to Siswati *et al.* (2015), one of the factors that must be known in the assembly of varieties for plant genetic improvement is identifying the crossbreeding parents through characterization activities to obtain informative germplasm sources. However, studies on genetic variation using morphological markers are rarely available in foxtail millet germplasm in Indonesia. The variety and distribution of millet varies greatly. Therefore, it is necessary to explore and characterize which is helpful as a source of germplasm information. The collection of plants is felt to be very important, especially for plants that can be introduced for use as cultivated plants. Based on morphological characters, this study assessed the genetic variation of foxtail millet germplasm in Polewali Mandar, West Sulawesi Indonesia.

METHODS

This research was carried out on resident agricultural land. Plant material with three populations (each population consisting of 5 individuals) that was collected from Polewali Mandar, West Sulawesi, Indonesia (Table 1). The accession was compiled based on the phenotypic variation observed.

Table 1. Accession of foxtail millet (*Setaria italica* (L.) P. Beauv.) from Polewali Mandar West Sulawesi, Indonesia

No	Accession name	Population name
1	SLB.A.1	Minna’
2	SLB.A.2	
3	SLB.A.3	
4	SLB.A.4	
5	SLB.A.5	
6	SLB.B.1	Lasse’
7	SLB.B.2	
8	SLB.B.3	
9	SLB.B.4	
10	SLB.B.5	
11	SLB.C.1	Bulawang
12	SLB.C.2	
13	SLB.C.3	
14	SLB.C.4	
15	SLB.C.5	

Data Recorded

Morphological characteristics were observed directly in plants in the field and 4-5 months after planting. Data collection uses 28 morphological characters consisting of 9 qualitative characters and 19 quantitative characters (ratio) based on the International Board for Plant Genetic Resources (IBPGR) (1985), with some modifications and applying binary and multistate scoring, as shown in Table 2 and Table 3. Operational Taxonomy Units (OTUs) were compared to create the present and absent characters between accessions.

Data Analysis

Cluster analysis was carried out by the Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Ramlah *et al.* 2018), and the genetic distance was generated using MEGA 7.0 Software (Kumar *et al.* 2016).

RESULTS AND DISCUSSION

Based on the 28 characters observed on three populations of foxtail millet germplasm (Figure 1), characters varied the most, including seed color, plant height, stem diameter, panicle stalk length, panicle stalk diameter, and panicle length, which had the highest level of variability (Table 2; Table 3). The interaction between genotype and environment causes this significant difference in morphological characters. Polygenes will interact with various environmental factors so that they will influence the growth of a plant. According to Daryono *et* *al.* (2012), qualitative characters are expressions of dominant and recessive genes whose offspring are segregated so that a comparison of dominant phenotypic characters and recessive phenotypic characters emerges.

Figure 1. A. Hypocotyl; B. Leaf midrib; C. Stem bones; D. Leaves; E. Pigmentation;

Table 2. Morphological Variations of Millet (Qualitative Characters)

NO	Name of Germplasm	QUALITATIVE CHARACTER								
		1	2	3	4	5	6	7	8	9
		Hypocotyl color	Leaf midrib color	Stem bone color	Stem color	Leaf color	Leaf surface	Plant pigmentation	Panicle density	Seed color
1	SLB.A.1	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Yellowish cream
	Score	1	1	1	0	2	7	1	1	5
2	SLB.A.2	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Yellowish cream
	Score	1	1	1	0	2	7	1	1	5
3	SLB.A.3	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Yellowish cream
	Score	1	1	1	0	2	7	1	1	5
4	SLB.A.4	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Yellowish cream
	Score	1	1	1	0	2	7	1	1	5
5	SLB.A.5	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Yellowish cream
	Score	1	1	1	0	2	7	1	1	5
6	SLB.B.1	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Cream
	Score	1	1	1	0	2	7	1	1	5
7	SLB.B.2	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Cream
	Score	1	1	1	0	2	7	1	1	5
8	SLB.B.3	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Cream
	Score	1	1	1	0	2	7	1	1	5
9	SLB.B.4	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Cream
	Score	1	1	1	0	2	7	1	1	5
10	SLB.B.5	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Cream
	Score	1	1	1	0	2	7	1	1	5
11	SLB.C.1	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Brick red
	Score	1	1	1	0	2	7	1	1	1
12	SLB.C.2	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Brick red
	Score	1	1	1	0	2	7	1	1	1
13	SLB.C.3	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Brick red
	Score	1	1	1	0	2	7	1	1	1
14	SLB.C.4	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Brick red
	Score	1	1	1	0	2	7	1	1	1
15	SLB.C.5	Green	Green	Green	Dark green	Green	Rough	Green	Compact	Brick red
	Score	1	1	1	0	2	7	1	1	1

F. Panicles; G. Seeds

Table 3. Variation of Millet Morphology (Quantitative Characteristics)

NO	Name of Germplasm	QUANTITATIVE CHARACTER										
		1	2	3	4	5	6	7	8	9	10	11
		Plant height (cm)	Stem diameter (mm)	Leaf length (cm)	Leaf width (cm)	Leaf midrib length (mm)	Number of stem segments	Number of leaves	Number of tillers	Number of stem branches	Flowering age 50% (Days)	Harvest age (days)
1	SLB.A.1	139↑	5,23	54,1	3,3	4	10	11↑	1	1↓	50	86
	Score	2	2	3	2	1	3	3	3	3	2	1

2	SLB.A.2	139↑	5,07	53,3	3,4	5	10	11↑	1	2	47↓	86
	Score	2	2	3	2	5	3	3	3	3	1	1
3	SLB.A.3	127	5,32	54	3,5	6↑	10	11↑	1	3↑	50	86
	Score	2	2	3	2	5	3	3	3	5	2	1
4	SLB.A.4	116	5,19	54	3,6↑	4	10	11↑	1	3↑	50	86
	Score	1	2	3	2	1	3	3	3	5	2	1
5	SLB.A.5	142	5,51	52	2,6	4	10	11↑	1	2	52	86
	Score	3	3	3	2	1	3	3	3	3	2	1
6	SLB.B.1	76↓	4,1	54,2↑	3,2	4	9↓	9↓	1	1↓	56↑	91↑
	Score	0	2	3	2	1	3	1	3	3	2	2
7	SLB.B.2	83	2,98	49	3,4	3	11↑	11↑	2↑	1↓	54	91↑
	Score	0	1	2	2	1	5	3	3	3	2	2
8	SLB.B.3	121	2,9	51	3,5	4	9↓	9↓	1	2	54	91↑
	Score	2	1	3	2	1	3	1	3	3	2	2
9	SLB.B.4	140	4,34	45	3,2	3	10	10	2↑	3↑	51	91↑
	Score	3	2	2	2	1	3	3	3	5	2	2
10	SLB.B.5	100	3,83	50	3,2	3	10	10	1	1↓	53	91↑
	Score	1	2	2	2	1	3	3	3	3	2	2
11	SLB.C.1	82	5,45	38,5↓	3,3	2↓	9↓	10	2↑	1↓	54	83↓
	Score	0	2	2	2	1	3	3	3	3	2	1
12	SLB.C.2	100	2,24↓	43,3	2↓	3	9↓	9↓	1	2	53	83↓
	Score	1	1	2	1	1	3	1	3	3	2	1
13	SLB.C.3	91	4,63	46,4	2,5	2↓	10	10	1	3↑	49	83↓
	Score	0	2	2	1	1	3	3	3	5	1	1
14	SLB.C.4	106	7,73↑	49	2,6	2↓	9↓	10	2↑	2	52	83↓
	Score	1	3	2	2	1	3	3	3	3	2	1
15	SLB.C.5	87	3,2	44	2,7	3	10	10	0↓	1↓	53	83↓
	Score	0	1	2	2	1	3	3	1	3	2	1

*Next of Quantitative Character

NO	Name of Germplasm	QUANTITATIVE CHARACTER							
		12	13	14	15	16	17	18	19
		Panicle Stalk Length (cm)	Panicle stalk diameter (mm)	Panicle Length (cm)	Panicle Diameter (mm)	Panicle Branch	Length of Panicle Feathers (mm)	Heavy / Panicles (gr)	Weight 1000 Seeds (gr)
1	SLB.A.1	34,5↑	0,99	29,7↑	13,33	2↑	1,4	7,8	1,28
	Score	3	1	5	1	2	1	3	3
2	SLB.A.2	30	2,36	27,9	14,93	2↑	1,5	10,02↑	1,22↓
	Score	2	2	5	1	2	1	3	3
3	SLB.A.3	31,3	2,15	25,7	13,67	2↑	1,8	8,67	1,22↓
	Score	3	2	5	1	2	1	3	3
4	SLB.A.4	27,8	2,05	26,7	11,48	2↑	0,9	8,66	1,24
	Score	2	2	5	1	2	1	3	3
5	SLB.A.5	21,2	1,47	25	13,96	2↑	1,1	6,9	1,28
	Score	2	1	4	1	2	1	3	3
6	SLB.B.1	25,7	3,35	20,3	13,69	2↑	2	4,69	1,34
	Score	2	3	3	1	2	1	1	3
7	SLB.B.2	24,6	3,4	20,5	15,43	1	1,8	5,83	1,34
	Score	2	3	3	1	2	1	3	3
8	SLB.B.3	29,6	3,52	24,9	12,73	1	1,8	6,2	1,32
	Score	2	3	4	1	2	1	3	3
9	SLB.B.4	25,9	3,7↑	18,6	14,51	2↑	2,2↑	6,98	1,3
	Score	2	3	3	1	2	1	3	3
10	SLB.B.5	20,4	2,4	19,9	10,16↓	1	1,7	3,07↓	1,34
	Score	1	2	3	1	2	1	1	3

11	SLB.C.1	27,5	1,75	16	12,57	0↓	2	4,81	1,23
	Score	2	1	3	1	1	1	1	3
12	SLB.C.2	20	1,02	17,5	11,11	0↓	1,5	3,77	1,24
	Score	1	1	3	1	1	1	1	3
13	SLB.C.3	13,4↓	0,89↓	12,7↓	13,38	0↓	1,5	3,67	1,26
	Score	1	1	2	1	1	1	1	3
14	SLB.C.4	14,5	0,96	21,5	14,85	0↓	2	5,92	1,22
	Score	1	1	4	1	1	1	3	3
15	SLB.C.5	18,3	1,37	21,4	15,94↑	0↓	1↓	7,04	1,36↑
	Score	1	1	4	2	1	1	3	3

↑ : millet germplasm with the highest value ; ↓ : millet germplasm with the lowest value

The quantitative phenotype character can be measured clearly and has a continuous diversity; the phenotype forms a spectrum, and the population is large enough to form a normal distribution curve. In quantitative characters, the controlling genes are multiple genes (polygenes). This gene will interact with various environmental factors that affect the growth of a plant.

Qualitative characters can be seen directly and visually, and quantitative characters can be observed by looking at plant growth. Observation of qualitative characters is based on drawing or class guidelines and is realized in numerical scores. Although many genes control quantitative characters and the influence of genes on their environment, quantitative characters are still used as determinants of genetic variability in foxtail millet germplasm. Quantitative character data in measurements can be used in phenetic analysis if these characters distinguish characters between samples. Quantitative characters can be displayed as ratios. Using ratios in morphological characterization in the form of conversion is a solid basis for grouping samples. According to Rae (1998), applying statistical tests to quantitative morphological data empirically detects evolutionary changes, the raw material for phylogenetic reconstruction.

According to Poehlam and Sleper (1995), markers with morphological characters are visible characteristics and can be used in constructing genetic maps of organisms and determining genetic variability in organisms. It is essensial to select cross parents to get superior seed characters. The results of observations and measurements on three millet populations from 28 observed characters showed that the qualitative characters (Table 2) of hypocotyl color, leaf midrib color, plant pigmentation, stem color, panicle density, growth form, leaf color, leaf surface, and brightness of seed color were monomorphic. Meanwhile, seed color is polymorphic. On the quantitative characters, the length of the panicle hairs and the weight of 1000 grams of seeds are monomorphic. Meanwhile, the quantitative characters (Table 3) of plant height, stem diameter, leaf length, leaf width, leaf midrib length, number of stem segments, number of leaves, number of tillers, number of stem branches, flowering age, harvest age, panicle stalk length, panicle stalk diameter, panicle length, diameter panicles, panicle branches, weight/panicles are polymorphic. Breeders and the general public have used this variability to differentiate different populations of foxtail millet germplasm practically.

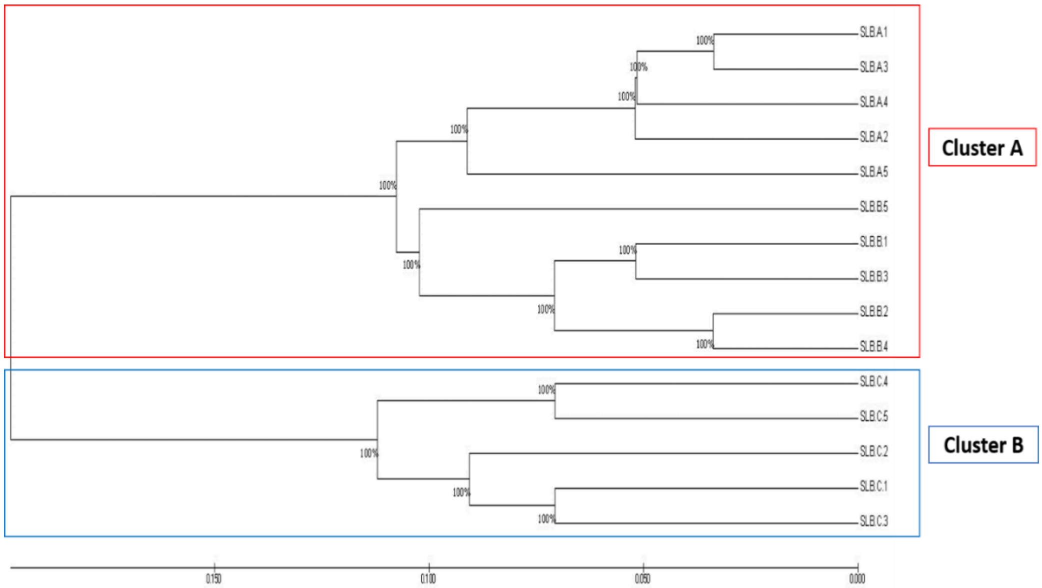

Figure 2. Dendogram of foxtail millet germplasm constructed using UPGMA method by MEGA Software 7.0

The cluster analysis results (Figure 2) show that the similarity coefficient ranges from 0.2-0.15. At a genetic similarity coefficient of 0.2, the three millet germplasm populations were divided into two large groups. Cluster A consists of 10 millet germplasm (Minna' and Lasse' Population), while Cluster B consists of 5 millet germplasm (Bulawang Population).

Table 4. Genetic distance of three populations of millet germplasm based on 28 morphological characters

	SLB.A.1	SLB.A.2	SLB.A.3	SLB.A.4	SLB.A.5	SLB.B.1	SLB.B.2	SLB.B.3	SLB.B.4	SLB.B.5	SLB.C.1	SLB.C.2	SLB.C.3	SLB.C.4	SLB.C.5
SLB.A.1															
SLB.A.2	0,141														
SLB.A.3	**0,067↓**	**0,067↓**													
SLB.A.4	0,103	0,103	0,103												
SLB.A.5	0,141	0,223	0,223	0,141											
SLB.B.1	0,181	0,223	0,223	0,181	0,268										
SLB.B.2	0,181	0,222	0,223	0,179	0,223	0,141									
SLB.B.3	0,223	0,268	0,268	0,222	0,181	0,103	0,103								
SLB.B.4	0,181	0,223	0,223	0,140	0,181	0,141	0,067	0,181							
SLB.B.5	0,223	0,267	0,223	0,141	0,315	0,181	0,181	0,315	0,141						
SLB.C.1	0,232	0,326	0,326	0,276	0,327	0,231	0,232	0,379	0,232	0,232					
SLB.C.2	0,434	0,628↑	0,557	0,432	0,494	0,378	0,378	0,434	0,432	0,278	0,181				
SLB.C.3	0,378	0,432	0,490	0,488	0,493	0,432	0,434	0,558	0,431	0,327	0,141	0,181			
SLB.C.4	0,327	0,494	0,434	0,323	0,232	0,494	0,329	0,380	0,328	0,278	0,181	0,181	0,223		
SLB.C.5	0,378	0,557	0,491	0,487	0,378	0,557	0,327	0,378	0,433	0,436	0,223	0,267	0,269	0,141	

Based on the genetic distance matrix values (Table 4). The lowest (closest) genetic distance is owned by SLB.A.3 (Minna') – SLB.A.2 (Lasse') with a genetic distance value of 0.067. Meanwhile, the highest (far) genetic distance is owned by SLB.C.2 (Bulawang) – SLB.A.2 (Minna'), with a genetic distance value of 0.628. The higher the genetic distance value between cultivars, the more closely the cultivars are related. On the other hand, the lower genetic distance value indicates that the cultivar is genetically similar and is still closely related. Each plant has a different morphological character, even in one species or the same habitat. Individual diversity can give rise to variation, and genes determine its unique nature. According to Handiwirawan (2006), the existence of several cultivars that have separated from their original group can be influenced by four factors, namely (1) Increased variation as a result of repeated mutations, (2) Increased variation due to gene flow from other populations (migration), (3) Variation that increases due to stochastic processes such as genetic drift, and (4) Variation that persists in populations due to the selection process.

Based on previous research, observations of millet samples carried out by Randall *et al.* (2016) showed that the characteristics of plant height, number of leaves, leaf area index, and harvest age of millet were the characteristics that contributed to the highest diversity, namely 46.82%. In research conducted by Brunda *et al.* (2015), the results of the morphological characterization of millet showed that flowering days showed the maximum contribution to genetic variation, followed by panicle weight and plant seed weight. The emergence of differences between plant varieties can cause physiological differences and different responsiveness to the environment.

CONCLUSSION

The genetic variation of the foxtail millet population from Polewali Mandar shows that the characteristics of the seed color, plant stem height diameter, the length of the panicle stalk, the diameter of the panicle stalk, and the size of the panicle were very diverse. Cluster analysis of morphological characters using the UPGMA method shows that the similarity coefficient ranges from 0.15-0.2. At the genetic similarity coefficient of 0.2, three populations were divided into Cluster A and Cluster B. Cluster A consisted of two populations, while Cluster B consisted of one person. The population of millets SLB.C.3-SLB.A.1 was the highest genetic distance and was an excellent candidate to be chosen as a crossing parent, resulting in higher heterosis. Information on the genetic variability of foxtail millet germplasm can be helpful for the selective breeding of specific characters and in enhancing the genetic base of millet breeding programs in the future.

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