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Research Article

Genetic diversity analysis of morpho-physiological and yield attributing traits in foxtail millet (*Setaria italica* L. Beauv.)

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Background: Selecting the right parents is essential in a breeding programme. It is necessary to access a large number of germplasm accessions for genetic diversity in order to select a diverse set of accessions as the parents for a hybridization program that aims to produce superior hybrids or better segregants in subsequent generations. Therefore, understanding the degree of diversity and assembling the germplasm is crucial. Thus, the aim of the current study was to quantify diversity and explore the impact of several traits that promote increased diversity within the population.

Methods: The current study was laid out in a Randomised Complete Block Design with two replications to quantify the genetic diversity among the 50 germplasm accessions of foxtail millet at the Regional Agricultural Research Station, Nandyal, during *Rabi*, 2023-24. Upon Mahalanobis D^2 analysis of 16 morpho-physiological and yield-attributes.

Results: Six clusters were formed from 50 accessions studied. Cluster I was the largest comprising 22 accessions, followed by clusters II and III, which was 16 and 9 accessions respectively. The remaining three clusters, IV, V, and VI, were solitary. Clusters V (SiA 3159) and VI (SiA 4389) had the greatest inter-cluster distance of 35.11%. The traits that contributed the most to genetic divergence were photosynthetic rate (39.59%), 1000-grain weight (17.47%), relative water content (16.41%) and fodder yield per plant (10.45%). This study suggests that selecting for these traits might be beneficial for improving foxtail millet. Based on their overall appearance and per se performance from a diverse cluster, the accessions SiA 3290, SiA 4345, SiA 4391, SiA 4281, SiA 4389, SiA 4396, and SiA 3159 were found desirable.

Conclusion: These selected foxtail millet accessions can be employed in the hybridization programmes and interbreed to produce better transgressive segregants, offering a high potential for yield in subsequent generations.

Keywords: *foxtail millet, germplasm, genetic diversity, clusters, grain yield*

Introduction

Small millets include a group of small-seeded grain crops of the grass family. This comprises foxtail millet, little millet, proso millet, barnyard millet, kodo millet and brown top millet (Dwivedi et al., 2012). Small millets were important crops used for food, feed, and fodder before modern crops took their place. Recent research has demonstrated that millets, which are resilient to several stresses, can reclaim their prominent place in agriculture, in the present scenario of rapid climate change, in the realm of food and nutritional security.

Foxtail millet is commercially cultivated in 23 countries, especially important in the arid and semi-arid regions of Asia, Africa, America, and Northern China. It belongs to the Poaceae family and is a short-duration, self-pollinating C_4 cereal crop (Lata et al., 2013). Foxtail millet is a staple food and feed in various regions of Asia and Africa and in North America, on the other hand, it is primarily cultivated for cover crops, silage and fodder (Goron & Raizada, 2015). According to studies by Zhang et al. (2007) and Lata et al. (2013), the grains of this crop are significantly more nutrient-dense than those of other major cereal crops (Vardhan et al., 2024). Additionally, they contain a high level of dietary fibre (6.7%) and 11-12% protein content (Amadou et al., 2011) and linoleic and oleic acid, enriched bran (Liang et al., 2010). In terms of food and nutritional security, foxtail millet has a promising future. The existence of diversity among the germplasm accessions determines much of the success of any plant breeding program (Allard, 1960). The choice of parents is of great significance in a breeding programme. It is well known that genetic variety is important for improving crops, especially foxtail millet (Ramesh et al., 2023). To select a diverse set of accessions for a hybridization programme and develop superior hybrids or to get better segregants in advanced generations, it is essential to access a large number of germplasm accessions for genetic diversity. As such, it is crucial to understand the magnitude of diversity, assessment and assemblage of germplasm. Therefore, diversity analyses, such the D^2 analysis, are used to quantify genotype diversity and examine the impact of traits that increase more diversity in the given population (Mahalanobis, 1936).

Materials and Methods

Site of experimentation

During *Rabi*, 2023-2024, the study was conducted at the Regional Agricultural Research Station (RARS), Nandyal (15°29' N latitude and 78°29' E longitude with an altitude of 211.76 meters above mean sea level).

Treatments and experimental design

The study was designed using a Randomised Complete Block Design with two replications with a spacing of 22.5 cm x 10 cm. Fifty foxtail millet germplasm accessions including three check varieties (SiA 3156, SiA 3223 and SiA 3159) were sown at a 3m length of each accession.

Cultural practices

A good crop was raised by practicing standard agronomic techniques, by applying a recommended dose of fertilizer (20 kg ha^{-1} N and 20 kg ha^{-1} P_2O_5 as basal dose and 20 kg ha^{-1} of N as top dressing). Weeding and other intercultural operations were taken up properly as per the need.

Data collection and analysis

Observations were recorded from five randomly selected plants in each accession such as plant height (cm), number of productive tillers per plant, flag leaf length (cm), flag leaf width (cm), panicle length (cm), relative water conductance (%), abortive grain rate, fodder yield per plant (g), harvest index (%), and grain yield per plant (g), while observations on days to 50% flowering and days to maturity were recorded on a plot basis. For 1000-grain weight, a random sample of 1000 grains were counted from the threshed seed and the weight was recorded in grams. Using a 'CI-340 handheld photosynthesis system', photosynthetic rate, transpiration rate and stomatal conductance were recorded. The data was subjected to assessment of genetic divergence by utilising D^2 analysis developed by P C Mahalanobis (1936) and categorize the accessions into distinct clusters by Tocher's method (Rao, 1952).

Results

Genetic divergence of 50 foxtail millet germplasm accessions including three checks varieties was quantitatively analysed by using the Mahalanobis D^2 analysis. The findings indicated that the variance due to different accessions was significant for various traits under study, which prompted further investigation based on D^2 values. The accessions were then grouped into six distinct non-overlapping clusters using the Tochers method (Rao, 1952), as shown in Figure 1 and Table 1. These findings revealed a sizable amount of genetic diversity. Cluster I was the largest comprising of 22 accessions followed by cluster II (16 accessions), cluster III (9 accessions) and cluster IV, V and VI were solitary with one accession each, suggesting significant heterogeneity among the accessions. The intra and inter-cluster D^2 values were provided for six clusters in Table 2. Higher inter-cluster distances than intra-cluster distances (Figure 2) suggest that there is more genetic diversity between the clusters than within them. The intra-cluster distances varied from 0 to

14.35. The largest intra-cluster distance (14.35) in cluster III represented considerable genetic variation among the germplasm accessions within the same cluster. On the contrary, clusters IV, V and VI showed no inter-cluster distance (0), because they contained a single genotype each. The average value of the inter-cluster distances varied from 16.19 (between clusters I and IV) to 35.11 (between clusters IV and V).

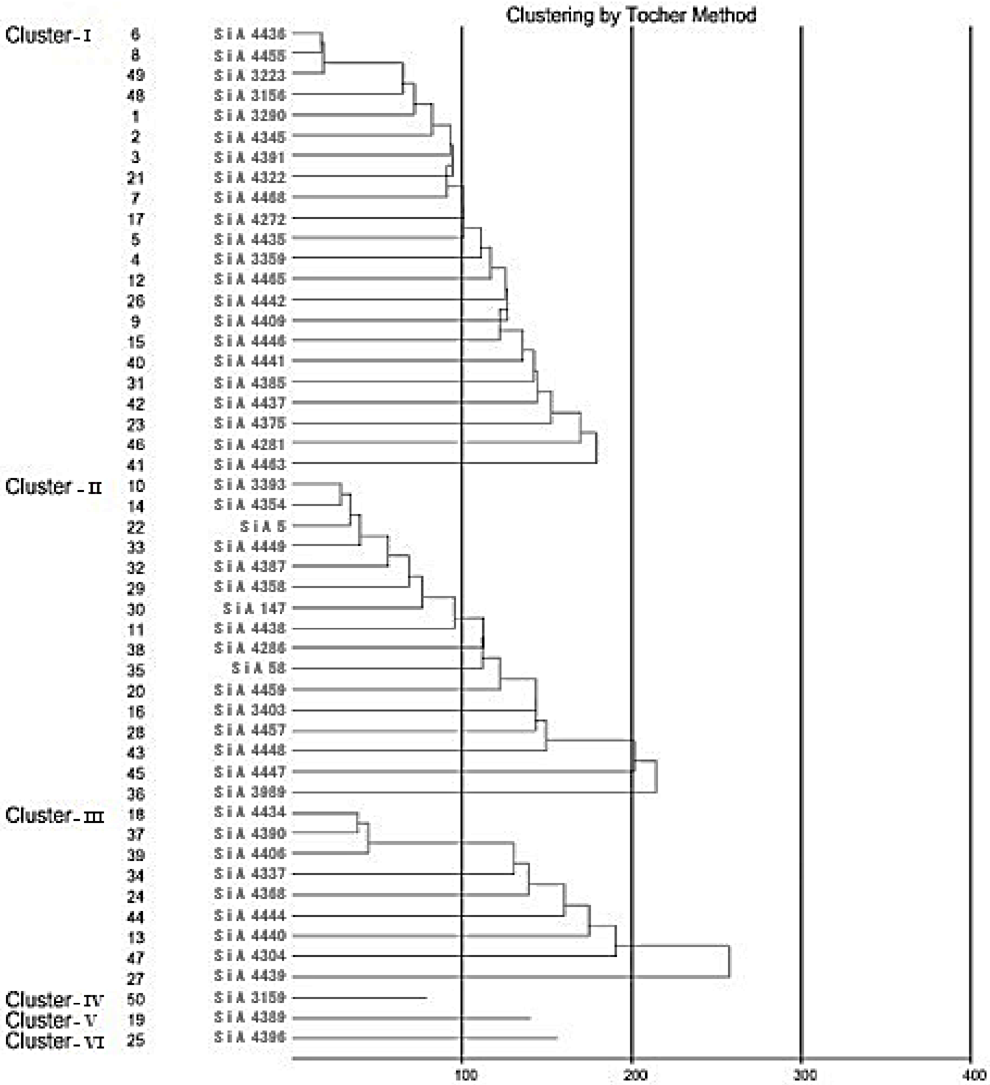

Figure 1. Dendrogram showing the relationship among 50 foxtail millet accessions in six clusters based on Mahalanobis D^2 values

Table 1. Clustering pattern of 50 foxtail millet accessions by Tocher's method

Cluster Number	Number of accessions	Accessions
I	22	SiA 4436; SiA 4455; SiA 3223; SiA 3156; SiA 3290; SiA 4345; SiA 4391; SiA 4322; SiA 4468; SiA 4272; SiA 4435; SiA 3359; SiA 4465; SiA 4442; SiA 4409; SiA 4446; SiA 4441; SiA 4385; SiA 4437; SiA 4375; SiA 4281 and SiA 4463
II	16	SiA 3393; SiA 4354; SiA 5; SiA 4449; SiA 4387; SiA 4358; SiA 147; SiA 4438; SiA 4286; SiA 58; SiA 4459; SiA 3403; SiA 4457; SiA 4448; SiA 4447 and SiA 3989
III	9	SiA 4434; SiA 4390; SiA 4406; SiA 4337; SiA 4368; SiA 4444; SiA 4440; SiA 4304 and SiA 4439
IV	1	SiA 3159
V	1	SiA 4389
VI	1	SiA 4396

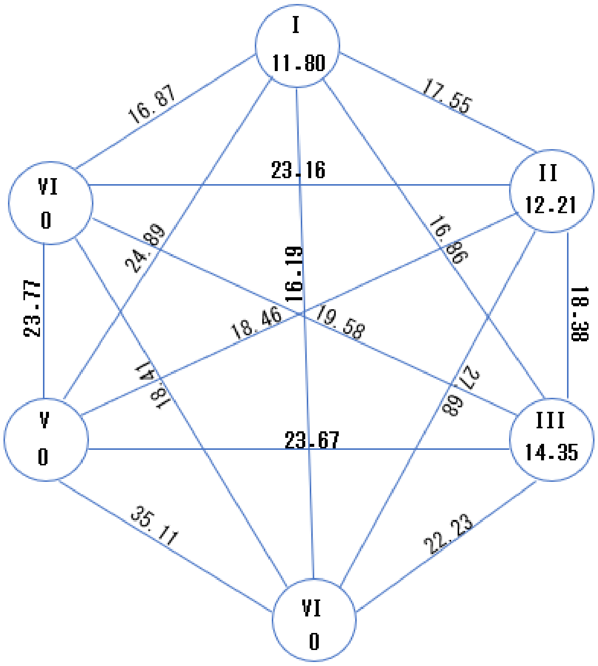

Figure 2. Intra-cluster and inter-cluster distances among the six clusters of 50 foxtail millet germplasm accessions

Table 2. Intra and inter-cluster D^2 values among six clusters with 50 foxtail millet germplasm accessions

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	11.80					
Cluster II	17.55	12.21				
Cluster III	16.86	18.38	14.35			
Cluster IV	16.19	27.68	22.23	0.00		
Cluster V	24.89	18.46	23.67	35.11	0.00	
Cluster VI	16.87	23.16	19.58	18.41	23.77	0.00

Table 3. Cluster mean values of 50 foxtail millet germplasm accessions based on Tocher's method for 16 morpho-physiological and yield-attributing traits

S. No	Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
1	Days to 50% flowering	55.55	55.44	54.46	50.00	53.00	56.50
2	Days to maturity	89.16	89.16	86.44	84.00	82.50	90.50
3	Plant height (cm)	142.00	142.99	132.99	143.20	128.20	155.70
4	Number of productive tillers per plant	2.27	2.08	2.49	2.10	1.80	2.15
5	Flag leaf length at flowering (cm)	32.93	30.16	28.58	32.50	39.70	35.90
6	Flag leaf width at flowering (cm)	2.09	2.03	1.87	2.29	2.18	2.15
7	Panicle length (cm)	19.02	17.41	16.38	21.05	21.50	21.10
8	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	34.19	22.95	31.17	45.97	16.62	37.80
9	Transpiration rate (mmol H_2Om^{-2} s^{-1})	5.17	3.56	4.92	6.24	5.02	5.95
10	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	0.24	0.16	0.23	0.29	0.22	0.32
11	Relative water content (%)	87.11	83.39	78.99	90.56	72.11	73.86
12	Abortive grain rate	0.15	0.18	0.18	0.11	0.16	0.12
13	1000-grain weight (g)	2.79	2.77	2.24	2.95	2.90	2.95
14	Fodder yield per plant (g)	14.51	13.70	12.42	13.82	12.01	13.59
15	Harvest index (%)	36.05	33.12	33.89	42.06	38.17	36.10
16	Grain yield per plant (g)	9.15	7.63	7.10	11.04	8.34	8.30

The average cluster means for the 16 traits in each cluster are provided in Table 3. A wide spectrum of variation was observed in clusters for the studied traits implying that the clusters formed were unique. The cluster VI recorded maximum means for the traits *viz.*, days to 50% flowering (56.50); days to maturity (90.50); plant height (155.70) and stomatal conductance (0.32). cluster IV recorded desired highest means for most of the traits *viz.*, flag leaf width at flowering (2.29), 1000-grain weight (2.95), harvest index (42.06), photosynthetic rate (45.97), transpiration rate (6.24), relative water content (90.56) and grain yield per plant (11.04) and desired lowest mean value for days to 50% flowering (50.00). Cluster V reported the highest means for flag leaf length at flowering (39.70) and panicle length (21.50), which also had the lowest mean for days to maturity mean (82.50). Clusters I and III showed the highest means for fodder yield per plant (14.51) and no. of productive tillers (2.49), respectively. The highest mean for 1000-grain weight (2.95) was reported by both cluster IV and cluster VI, similarly, the maximum mean for abortive grain rate (0.18) was found in clusters II and III.

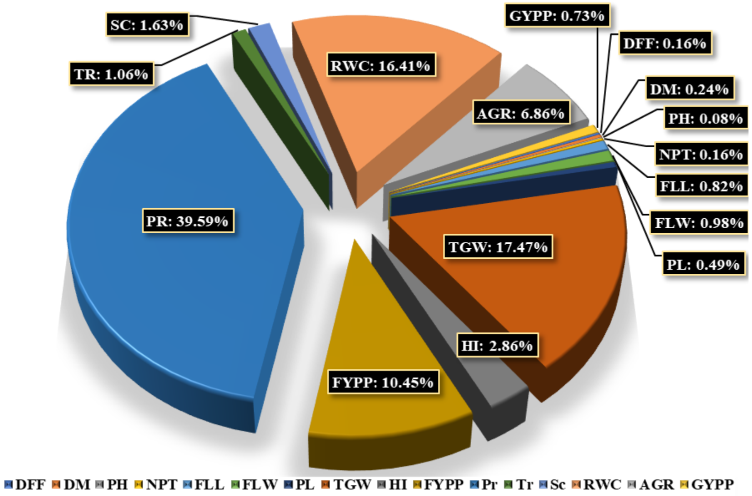

Figure 3. Relative contributions of each trait towards genetic diversity of the 50 foxtail millet germplasm accessions

Table 4 Relative contribution of 16 morpho-physiological and yield-attributing traits towards divergence among 50 foxtail millet accessions

S. No	**Source**	**Times the trait got 1st rank**	***Per cent* contribution**
1	Days to 50% flowering	2	0.16%
2	Days to maturity	3	0.24%
3	Plant height (cm)	1	0.08%
4	Number of productive tillers per plant	2	0.16%
5	Flag leaf length at flowering (cm)	10	0.82%
6	Flag leaf width at flowering (cm)	12	0.98%
7	Panicle length (cm)	6	0.49%
8	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	485	39.59%
9	Transpiration rate (mmol H_2Om^{-2} s^{-1})	13	1.06%
10	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	20	1.63%
11	Relative water content (%)	201	16.41%
12	Abortive grain rate	84	6.86%
13	1000-grain weight (g)	214	17.47%
14	Fodder yield per plant (g)	128	10.45%
15	Harvest index (%)	35	2.86%
16	Grain yield per plant (g)	9	0.73%

The number of times each of the 16 traits appeared at the top and its corresponding percentage contribution to the total divergence was determined and depicted in Table 4 and the relative contribution of the traits towards diversity was illustrated through a pie diagram in Figure 3. Among the traits studied, photosynthetic rate contributed maximum (39.59%) towards genetic divergence by ranking first 485 times followed by the 1000-grain weight (17.47% by ranking first for 214 times), relative water content (16.41% by ranking first 201 times), fodder yield per plant (10.45% by ranking first for 128 times), abortive grain rate (6.86% being ranked first for 84 times), harvest index (2.86% by getting first rank for 35 times), stomatal conductance (1.63% by ranking 20 times as first) and transpiration rate (1.06% by ranking first for 13 times).

The traits flag leaf width at flowering contributed 0.98% (being ranked first 12 times), flag leaf length at flowering 0.82% (by ranking first 10 times), grain yield per plant 0.73% (by ranking nine times first), panicle length 0.49% (by ranking first for six times), days to maturity 0.24% (by ranking three times first), 50% flowering and no. of productive tillers per plant 0.16% (by ranking first for two times) and plant height 0.08% (by ranking one time) contributed very less towards total divergence inferring more homogeneity for the traits in the genetic material evaluated.

Discussion

Six clusters were formed from fifty germplasm accessions studied, cluster I was the largest comprising 22 accessions, clusters IV, V and VI were solitary. Ayesha et al. (2019), Amarnath et al. (2019) reported 8 clusters for 50 foxtail millet accessions, Venkatesh et al. (2020) clustered the accessions into 9 clusters, Karvar et al. (2022) reported 5 clusters and Karvar et al. (2023) into 6 clusters. The emergence of discrete isolated clusters could be attributed to either geographic obstacles impeding gene flow or strong natural and human selection favouring an array of adaptive gene complexes as the origin of genetic diversity. Low levels of diversity have been observed in the germplasm accessions within the same cluster, thus choosing parents within the cluster may not be beneficial. These results align with the findings reported by Kumar et al. 2010. These findings are in consistence with the reports of Kumar et al., 2010. The clusters IV vs. V, II vs. IV, I vs. V, V vs. VI, III vs. V, II vs. VI and III vs. IV exhibited divergence in descending order of their inter-cluster D^2 values. Thus, genotypes from these clusters namely SiA 4436, SiA 4281, SiA 3290, SiA 4345, SiA 4391, SiA 3223, and SiA 3156 from cluster I; SiA 3159 from cluster IV; SiA 4389 from cluster V and SiA 4396 from cluster-IV were proposed to be included in the crossing programme to produce more desirable and superior recombinants in later generations. Based on the cluster mean values, germplasm accessions with the desired values for a given trait can be selected and used in a hybridization program to improve that trait. No cluster was found to have at least one germplasm accession with all desired traits, ruling out the possibility of choosing a single accession for immediate usage. Therefore, in order to incorporate all the desirable traits, hybridization among the selected germplasm accessions from divergent clusters is required.

The traits *viz.*, flag leaf width at flowering, flag leaf length at flowering, grain yield per plant, panicle length, days to maturity, days to 50% flowering, and number of productive tillers per plant had contributed little to the overall divergence, suggesting a lack of diversity for these traits in the germplasm accessions. This could be because of the directional selection by the breeders during the development of these accessions. Inter-crossing of genotypes from these clusters could be suggested to generate a large range of variability and then effective selection for these traits.

Conclusion

Mahalanobis D^2 analysis revealed a significant genetic divergence between the genotypes. The traits such as photosynthetic rate contributed the maximum to genetic divergence, followed by 1000-grain weight, relative water content and fodder yield per plant, while traits like days to 50% flowering, no. of productive tillers per plant and plant height contributed the least, indicating limited diversity for these traits. Inter-crossing genotypes from diverse clusters are recommended to enhance variability and subsequent improvement in these traits. Out of six clusters, cluster I was the largest with 22 accessions followed by clusters II and III with 16 and 9 accessions, respectively, while clusters IV, V, and VI were monotypic, indicating substantial heterogeneity. Inter-cluster distances were generally greater than intra-cluster distances, suggesting broader genetic diversity between clusters. Cluster IV exhibited the highest means for traits like days to flowering and plant height, while cluster IV excelled in traits like grain yield and photosynthetic rate. The analysis highlighted that no single cluster contained all desirable traits, necessitating hybridization between diverse clusters to achieve comprehensive trait improvement. The highest inter-cluster distance was observed between cluster IV (SiA 3159) and V (SiA 4389), making them potential parents for hybridization.

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Author contributions

B. Harsha Vardhan, M. Shanthi Priya and R. Narasimhulu: Conceived and designed the study.
B. Harsha Vardhan and R. Narasimhulu: Planned, performed experiments and wrote the manuscript. R. Narasimhulu, M. Shanthi Priya, A. Chandra Sekhar, D. Venkatesh Babu revised the manuscript.

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Conflict of interest

The author declares no conflict of interest. The manuscript has not been submitted for publication in any other journal.

Ethics approval

Not applicable

AI tool usage declaration

Author(s) hereby declare that no AI, related tools and text-to-image generators have been used during the writing or editing of the manuscript.

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