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Elucidating the genetic diversity for morpho-physiological parameters in foxtail millet cultivars

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Abstract

Foxtail millet is an underutilized crop with eminent nutritional and climate resilience traits. In this study, a set of 31 genotypes collected from various research stations in India were subjected to a D² diversity approach for twelve quantitative traits. The 31 genotypes were grouped into five clusters with cluster I and III comprising of nine genotypes and the major contributor towards the genetic divergence was conferred by water use efficiency and transpiration rate. Among all, the cluster III, cluster II and cluster V were found to be comprised of genotypes with higher yield and photosynthetic efficiency. Moreover, the genotypes namely, Narasimharaya and ATL 1 were found to be desirable for higher yield and photosynthetic efficiency. Further, the highest inter cluster distance was found between cluster I and cluster II. Thus, the genotypes from these clusters viz., ATL 1, SR 16, GPUF-3, SiA 3159, Red Foxtail, Andhra Local, Kottatara thenai, GPUF-16, Kolli local, SiA 3085, RAU 2, SiA 326, Renadu, GPUF-16, Kolli local, HMT 100-1 and SiA 3156 could be identified as diverse parents for utilization in hybridization programs in future.

Keywords: Genetic diversity, foxtail millet, photosynthetic efficiency and water use efficiency

Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is one of the world's earliest cultivated crops which was domesticated over 8000 years ago in East Asia (Lu *et al.*, 2009) [8]. This is found to be an arid crop which is rich in high-quality protein, dietary fibre, and vital minerals like iron, calcium, magnesium, phosphorus, and potassium (Kumar *et al.*, 2019) [10]. Thus, foxtail millet is also known to be a hardy crop with a diploid chromosome number of $2n = 2x = 18$. This is a dual-purpose crop and its mode of pollination is self-pollination. Although it is self-pollinated, foxtail millet has been reported to have immense variability in its genetic resources which is crucial for effective selection and crop improvement (Suthamathi *et al.*, 2026) [15].

India's total foxtail millet production is estimated around 0.66 lakh tonnes (66,000 tonnes) annually, with 0.87 lakh hectares nationwide. Foxtail millet is a hardy crop known for its climate resilience from the earlier periods of history until now (Sun *et al.*, 2024). This is predominantly cultivated by the farmers in the arid zones. In India, Karnataka is the largest producer of foxtail millet followed by Andhra Pradesh and Telangana. Recently, foxtail millet has gained momentum owing to its nutritional values in the market. This is rich in protein, dietary fibre and essential minerals like iron and calcium (Beulah & Lavanya, 2024). Thus, day by day, the demand for foxtail millet grains and processed products is increasing and the efforts for breeding new varieties for higher grain and fodder yield needs to be focused to sustain food and nutritional security (Wang *et al.*, 2024) [14].

Genetic Variability for effective selection is an initial prerequisite that allows breeders to choose diverse parents to exploit heterosis. Foxtail millet is reported to have immense variability in its germplasm which needs to be further explored for developing elite cultivars in future (Xue *et al.*, 2025) [16]. Recently, 30 genotypes evaluated by Rao and Chaturvedi (2024) [12] across five environments in Nagaland for eleven morpho-physiological traits and single plant yield revealed, intra-cluster distances. In inter-cluster distances, clusters III and V were the highest in and the test weight was the major contributor for genetic divergence across environments.

Successively, another study conducted with 50 germplasm lines characterized for 16 morpho-physiological traits, grouped the genotypes into six clusters. Cluster I had the largest intra-cluster distance. Cluster IV and V were found to have the maximum inter-cluster distances. The largest contributor for genetic divergence was photosynthetic rate. The results found seven accessions viz., SiA 3290, SiA 4345, SiA 4391, SiA 4281, SiA 4389, SiA 4396, and SiA 3159 as desired genotypes for future breeding based on performance and diversity (Battula *et al.*, 2025) [1]. Recently, Ram *et al.* (2025) [11] evaluated 40 foxtail millet genotypes for 18 morpho-physiological traits. The genotypes were classified into six clusters. The maximum inter-cluster distance was observed between Clusters II and V and the highest intra-cluster distance was within Cluster I. The results exhibited the protein content as the highest contributor towards genetic divergence. Although, variability is being explored in foxtail millet, the genetic diversity of physiological and morphological traits in the germplasm facilitates the overall improvement in crop production and varietal development. Thus, in a view of this the current study was conducted to analyse the genetic variability and diversity among 31 genotypes by examining both morphological and physiological parameters.

Materials and Methods

The experiment was carried out with thirty-one foxtail millet cultivars collected from research stations in India at the Karunya Institute of Technology and Sciences in Coimbatore, during the season *Kharif* 2025 (Table 1). The genotypes were raised in a Randomized Block Design (RBD) with three replications under the spacing of 20×10 cm. From each genotype, under a replication five plants were chosen for recording of morphological and physiological traits. Twelve traits namely, flag leaf blade length (cm), flag leaf blade width (cm), 1000 seed weight (g), single plant yield (g), intercellular CO₂ Concentration (μmol mol⁻¹ (ppm), photosynthetic rate (μmol CO₂ m⁻² s⁻¹), cuvette temperature (°C), stomatal conductance (mol H₂O m⁻² s⁻¹), transpiration rate (mmol H₂O m⁻² s⁻¹), leaf temperature (°C), vapor pressure deficit (kPa), and water use efficiency (μmol CO₂/mmol H₂O (A/E) were observed. The recommended package of farming practices for raising the genotypes were followed (Indian Council of Agricultural Research, 2025) [4] and the collected data was subjected to a D² statistics developed by Mahalanobis (1936). For grouping of genotypes, hierarchical clustering by principal components was followed. The data analysis was carried out in Metan' in R software version 4.5.3.

Results and Discussion

The results of ANOVA for 12 quantitative traits revealed significant differences among 31 genotypes indicating the presence of genotypic variation for all the traits (Table 2). It was observed that, all traits exhibited highly significant differences among genotypes.

Mean Performance for yield and physiological traits

The overall mean performance for traits is presented in the Table 3. Among all for yield-related traits, the genotypes viz., Narasimharaya, Garuda and ATL 1 were identified as

highest performers for single plant yield and thousand grain weight. Similar results for single plant yield and its attributing traits were reported by Zhang *et al.* (2017) [18]. For physiological traits; among 31 genotypes; the genotypes viz., Narasimharaya, Andhra Local, DHFt 109-3 and K2 recorded a highest *per se* performance. Thus, genotypes with higher yield and photosynthetic efficiency could be forwarded in other breeding programs for developing high yielding cultivars in future.

Genetic Diversity for morphological and physiological traits

Among all 12 traits, the major contribution towards the genetic divergence was conferred by water use efficiency followed by that the transpiration rate and stomatal conductance were the major contributors for clustering of genotypes (Table 4). Similar results for genetic divergence were reported by (Zhao *et al.*, 2026) [19]. The 31 genotypes were subjected to D² analysis and were grouped into five clusters (Table 5). Among all, cluster I and cluster III were found to be the largest with nine genotypes respectively. Followed by that, cluster II and cluster V had eight genotypes each. Finally, the cluster IV had only 2 genotypes namely, DHF t 109-3 and ALT-2. Thus, all the genotypes were grouped based on the phenotypic variability present among them. None of the genotypes were found to be present in a solitary cluster (Figure 1).

Cluster Mean

Among all, cluster III recorded the highest mean for single plant yield and thousand grain weight (Table 6). This projects that the cluster comprised of the genotypes with higher performance for yield and thousand grain weight. Similar findings for yield related traits were reported by (Kandel *et al.*, 2020) [5]. For flag leaf blade length and flag leaf blade width, cluster IV and for photosynthetic traits cluster II and cluster V had genotypes conferring desirable photosynthetic efficiency. Cluster I recorded the highest mean for water use efficiency and cluster III had the highest mean for stomatal gas conductance. Thus, the genotypes in the respective above-mentioned clusters could utilized in breeding genotypes with higher photosynthetic and water use efficiency. Similar findings for photosynthetic traits were reported by Battula *et al.* (2025) [1].

Genetic Distance

Among five clusters, the highest inter cluster distance was found between cluster I and cluster II. Followed by that, it was in cluster III and cluster I (Table 7). Therefore, the genotypes from these clusters namely ATL 1, SR 16, GPUF-3, SiA 3159, Red Foxtail, Andhra Local, Kottatara thenai, GPUF-16, Kolli local, and from cluster I and SiA 3085, RAU 2, SiA 326, Renadu, GPUF-16, Kolli local, HMT 100-1 and SiA 3156 from cluster II, could be used as parents in hybridization programs to develop high yielding segregants. Further, among all; the cluster I had the highest intra cluster distance. This presented that genotypes grouped under this cluster were variable for the traits, other than the contributing characters Similar findings for inter and intra cluster distance were reported by (Ram *et al.*, 2025; Rao and Chaturvedi. 2024) [11, 12].

Table 1: List of Genotypes and their place of collection

S. No.	Genotype	Place of collection
1.	Lepakshi	(IIMR), Hyderabad.
2.	HMT 100-1	(IIMR), Hyderabad.
3.	SiA 3085	RARS, Nandyal, ANGRAU, Andhra Pradesh
4.	SiA 3156	RARS, Nandyal, ANGRAU, Andhra Pradesh
5.	RAU 2	(IIMR), Hyderabad.
6.	SiA 326	RARS, Nandyal, ANGRAU, Andhra Pradesh
7.	Suryanandi (SiA 3088)	RARS, Nandyal, ANGRAU, Andhra Pradesh
8.	DHF t 109-3	(IIMR), Hyderabad.
9.	HN 46	(IIMR), Hyderabad.
10.	ATL 1	TNAU, Coimbatore (Tamil Nadu).
11.	Garuda	RARS, Nandyal, ANGRAU, Andhra Pradesh
12.	Renadu	RARS, Nandyal, ANGRAU, Andhra Pradesh
13.	Narasimharaya	Andhra Pradesh/Karnataka
14.	krishnadevaraya	RARS, Nandyal, ANGRAU, Andhra Pradesh
15.	PS-4	(IIMR), Hyderabad.
16.	K2	(IIMR), Hyderabad.
17.	K3	(IIMR), Hyderabad.
18.	CO7	(IIMR), Hyderabad.
19.	SR16	(IIMR), Hyderabad.
20.	Srilakshmi	RARS, Nandyal, ANGRAU, Andhra Pradesh
21.	FxV- 652	(IIMR), Hyderabad.
22.	FxV-647	(IIMR), Hyderabad.
23.	CFxMV-1	(IIMR), Hyderabad.
24.	ATL 2	(IIMR), Hyderabad.
25.	GPUF-3	(IIMR), Hyderabad.
26.	GPUF-16	(IIMR), Hyderabad.
27.	SiA 3159(Mahanandi)	RARS, Nandyal, ANGRAU, Andhra Pradesh
28.	Red Foxtail	Telangana
29.	Andhra local	Andhra Pradesh
30.	Kolli local	Dhan Foundation, Tamil Nadu
31.	Kothatara thenai	Dhan Foundation, Tamil Nadu

Table 2: ANOVA for twelve traits

S. No.	Traits	Replication	Genotypes	Error
1	Flag leaf blade length (FLL)	2.7063	18.6409**	5.2363
2	Flag leaf blade width (FLW)	0.0015	0.1408**	0.0182
3	Intercellular CO ₂ concentration (Ci)	74.8892	7798.7197**	74.323
4	Photosynthetic rate (A)	0.0003	33.0881**	0.2725
5	Cuvette temperature (T _{cuv})	0.4891	0.6668	2.4699
6	Stomatal conductance (gs)	0.3815	1689.4903**	11.3362
7	Transpiration rate (E)	0.0004	0.3828**	0.0016
8	Leaf temperature (T _{leaf})	0.8031	0.7368	1.058
9	Vapor pressure deficit (VPD)	0.3019	6.3792**	0.2826
10	Water use efficiency (WUE)	0.1239	60.4465**	0.173
11	Thousand grain weight (TGW)	0.0114	2.3695**	0.0214
12	Single plant yield (SPY)	0.0106	30.6032**	0.1385

Table 3: Mean Performance for 31 genotypes of foxtail millet

Genotype	FLL	FLW	Ci	A	Tcuv	gs	E	Tleaf	VPD	WUE	TGW	SPY
1	28.18	1.48	286	6	28.5	95	1.92	28.3	13	4.85	3.5	6.73
2	29.18	1.58	161	7.5	28.3	54	0.86	28.8	15	8.53	3.8	6.86
3	30.74	1.78	179	15.5	28.4	123	1.66	28.2	13	8.29	2.8	5.91
4	26.76	1.38	203	5.7	28.4	58	1.02	29.1	15	7.09	4.2	8.1
5	25.18	1.28	125	11.5	28.3	71	1.07	28.8	15	10.25	2.1	5.11
6	29.08	1.58	156	8.9	28.2	66	0.97	28.5	14	9.53	1.9	4.9
7	28.18	1.48	145	8.2	28	59	0.87	28.6	14	10	3.7	6.38
8	31.48	1.88	70	6.5	28.1	38	0.59	28.9	15	12.72	3.5	7.16
9	26.98	1.38	116	13.2	28.1	83	1.14	28.7	13	11.46	3.6	6.75
10	24.76	1.28	127	8.4	28.2	52	0.79	28.6	14	10.8	4.5	11
11	23.48	1.18	201	5.1	28.3	46	1.32	28.3	13	8.01	4.1	14.74
12	22.68	1.08	111	14.1	28.3	83	1.36	28.7	15	10.04	2.2	4.16
13	23.98	1.18	179	10.6	28.1	76	1.2	28.1	14	8.42	4.8	16.29
14	23.08	1.08	255	5.7	28	68	0.91	28.1	14	6.1	4.1	10.98
15	25.38	1.28	177	9.2	28.3	63	0.95	28.7	15	8.4	3.8	8.8
16	24.28	1.28	219	9.1	28.3	104	1.42	28.1	13	7.01	4.4	11.83
17	24.88	1.28	249	7.9	28.1	102	1.38	28.9	14	6.23	3.7	8.56
18	25.78	1.38	220	8.2	28.3	116	1.35	28.2	11	4.23	4.1	9.26
19	25.28	1.38	98	11.89	28.09	53.62	0.61	28.59	10.12	18.09	2.6	6.61
20	26.18	1.48	174	14.75	26.06	81.54	1.32	29.91	14.45	9.13	4.5	12.66
21	30.08	1.78	163	11.6	28.2	79	1.04	28.6	12	10.88	3.6	5.33
22	29.48	1.68	136	13.7	28.2	81	1.08	28.6	12	11.32	2.8	4.55
23	28.58	1.58	155	8.67	29.39	63.56	0.88	27.16	13.08	9.98	3.5	7.33
24	28.08	1.48	146	2.4	28.1	14	0.22	28.6	14	11.21	2.8	6.72
25	25.18	1.38	167	11.9	28.1	79	0.94	28.9	12	12.82	4.1	11.76
26	25.18	1.38	80	11.3	28.2	90	1.1	28.6	11	12.3	1.9	4.25
27	29.78	1.78	205	14.4	28	49	0.65	28.9	12	18.03	4.7	12.59
28	24.38	1.28	97	12.6	28.3	56	0.6	28.8	10	19.33	2.1	5.15
29	26.87	1.5	175	9.2	28.4	35	0.43	29.3	12	24.79	3.5	9.29
30	29.77	1.77	146	8.4	27.99	63.2	0.99	28.29	14.17	8.92	1.9	5.05
31	23.48	1.18	127	14.8	28.6	59.6	0.82	29.6	14	16.2	3.8	8.48
Grand Mean	26.66	1.43	162.84	9.9	28.19	69.73	1.01	28.63	13.25	10.8	3.44	8.17
CV%	8.58	9.39	5.29	5.27	5.58	4.83	3.95	3.59	4.01	3.85	4.26	4.55

*FLL-flag leaf blade length (cm), FLW-flag leaf blade width (cm), TGW-1000 seed weight (g), SPY-single plant yield (g), Ci-intercellular CO₂ Concentration ($\mu\text{mol mol}^{-1}$ (ppm), A-photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), Tcuv-cuvette temperature ($^{\circ}\text{C}$), gs-stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), E-transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), Tleaf-leaf temperature ($^{\circ}\text{C}$), VPD-vapor pressure deficit (kPa), and WUE-water use efficiency ($\mu\text{mol CO}_2 / \text{mmol H}_2\text{O}$ (A/E)

Table 4: Percent contribution of twelve traits

Traits	Percent Contribution (%)
Flag leaf length (FLL)	1.46
Flag leaf width (FLW)	1.32
Intercellular CO ₂ concentration (CI)	2.64
Net photosynthetic rate (A)	2.21
Canopy temperature under UV (Tcuv)	0.66
Stomatal conductance (gs)	7.95
Transpiration rate (E)	4.52
Leaf temperature (Tleaf)	11.39
Vapor pressure deficit (VPD)	2.66
Water use efficiency (WUE)	20.46
Thousand grain weight (TGW)	16.36
Single plant yield (SPY)	28.37

Table 5: Distribution of Thirty-one germplasm accessions among different clusters

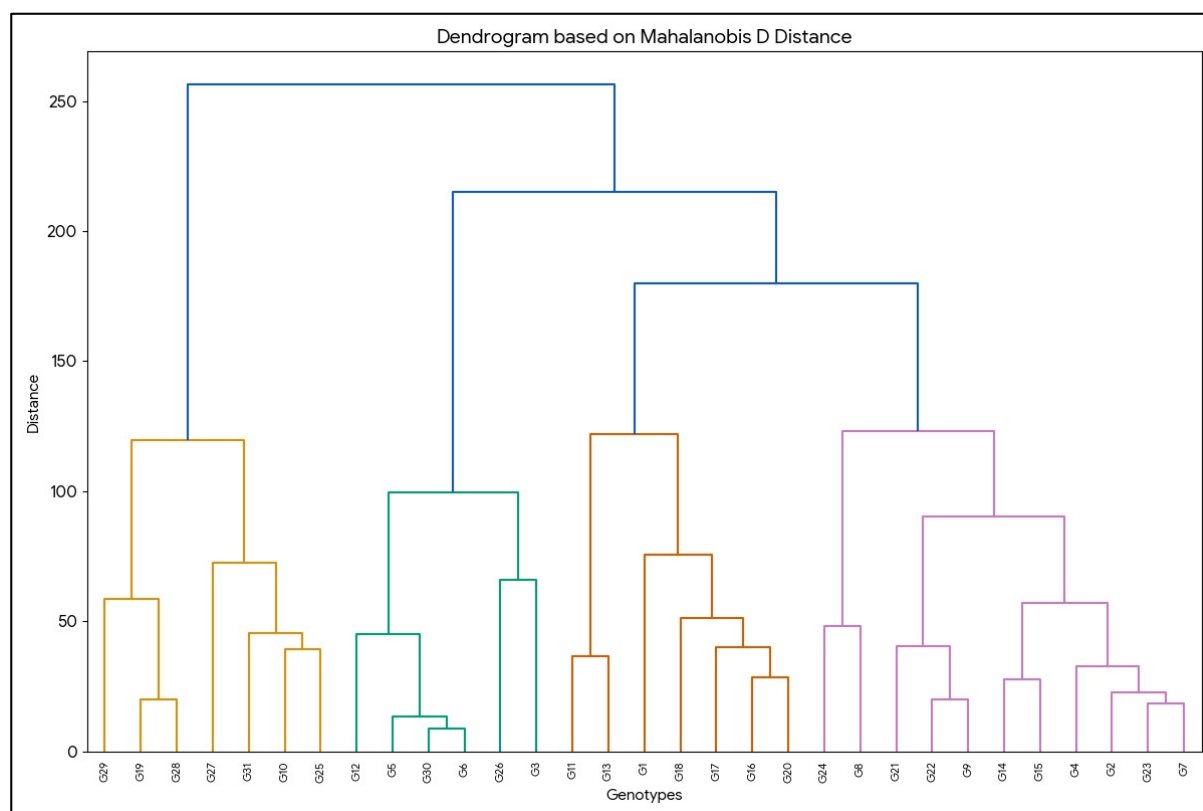
Cluster	Number of Genotypes	Genotype Names
I	9	ATL 1, SR 16, GPUF-3, SiA 3159, Red Foxtail, Andhra Local, Kottatara thenai, GPUF-16, Kolli local
II	8	SiA 3085, RAU 2, SiA 326, Renadu, GPUF-16, Kolli local, HMT 100-1, SiA 3156
III	9	Lepakshi, Garuda, Narasimharaya, K2, K3, Co7, Srilakshmi, ATL 1, SR 16
IV	2	DHF t 109-3, ALT-2
V	8	HMT 100-1, SiA 3156, SiA 3088, HN 46, Krishnadevaraya, PS-4, FxV-652, FxV-647, CFxMV-1

Table 6: Cluster mean values for Twelve traits

Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
FLL	25.68	27.1	25.25	29.78	27.52
FLW	1.4	1.48	1.32	1.68	1.47
Ci	142.29	132.83	218.29	108	167.89
A	11.88	11.62	8.81	4.45	9.27
Tcuv	28.24	28.23	27.95	28.1	28.32
gs	54.89	82.7	88.65	26	67.62
E	0.69	1.19	1.42	0.41	0.97
Tleaf	28.96	28.52	28.54	28.75	28.48
VPD	12.02	13.7	13.21	14.5	13.68
WUE	17.15	9.89	6.84	11.97	9.31
TGW	3.61	2.13	4.16	3.15	3.68
SPY	9.27	4.9	11.44	6.94	7.23

Table 7: Average Cluster distance (D^2) values among five clusters

D^2	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	67.11299	122.0087	117.0916	88.95427	87.19319
Cluster II	122.0087	57.47498	102.4631	94.93688	89.5584
Cluster III	117.0916	102.4631	67.02457	117.2302	76.04487
Cluster IV	88.95427	94.93688	117.2302	48.2552	78.08056
Cluster V	87.19319	89.5584	76.04487	78.08056	44.89179

**Fig 1:** Dendrogram of clustering of 31 genotypes

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