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Genetic Divergence in Foxtail Millet (*Setaria italica* L.) Germplasm Accessions for Quality and Yield Contributing Traits

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ABSTRACT

Background: Millets, particularly foxtail millet (*Setaria italica* L.), are gaining renewed attention for their nutritional, agronomic and ecological advantages. Despite its potential, genetic improvement of foxtail millet has been limited compared to major cereals. This study aims to evaluate the genetic divergence among 72 core germplasm accessions of foxtail millet and quantify carotenoid content in selected high-yielding lines to support future breedin g efforts. Although the genetic resources are large in this crop, it is kept unused and said to be underutilized crop. Thus, it is more important to expose the genetic parameters for the desirable traits in the germplasm accessions to improve its quality with yield. Hence, the evaluation of genetic resources had been taken for future research.

Methods: The present study aimed to assess the genetic diversity and carotenoid content among 72 foxtail millet accessions and two check varieties [CO (Te) 7 and ATL 1] sourced from the Ramiah Gene Bank. Field evaluations were conducted using a randomized complete block design (RCBD) at the Centre of Excellence in Millets, Tamil Nadu, during the 2023 *Rabi* season. Nine qualitative and eleven quantitative traits were recorded.

Result: Cluster analysis based on Mahalanobis D^2 statistics grouped the accessions into six clusters, indicating significant genetic diversity. Cluster III exhibited superior performance for key agronomic traits, while Cluster IV had the lowest mean for days to maturity, suggesting potential for breeding early-maturing, high-yielding varieties. The traits contributing most to genetic divergence included plant height (20.79%), panicle length (15.50%) and single plant stover yield (14.75%). Additionally, carotenoid content analysis in 25 elite accessions revealed a range from 0.18 to 1.68 mg/100 g, with accessions TGP/ISE–26 and TGP/ISE–183/1 showing the highest levels. The findings highlight promising genotypes for genetic improvement programs focused on yield and nutritional enhancement, particularly for biofortification with carotenoids.

Key words: Carotenoid, D^2 analysis, Foxtail millet, Genetic diversity.

INTRODUCTION

Millets, often referred to as “small-seeded grasses,” represent a significant C4 cereal crop within the Panicoideae subfamily of grasses (Purusotama and Chaturvedi, 2025). Millets are exceptionally nutritious food crops, boasting high levels of protein, dietary fiber, essential fatty acids and essential minerals such as potassium, zinc, magnesium, calcium and iron. They are also rich in vitamins, particularly those in the vitamin-B complex (Hegde *et al.,* 2005). Thanks to their remarkable nutritional attributes, millets are classified as “Nutri-cereals.” The Indian Council of Agricultural Research and the Indian Institute of Millets Research (ICAR-IIMR) have defined Nutri-cereals as grains with exceptionally high nutritional content, comparable to that of staple foods. Among these, foxtail millet (*Setaria italica*) holds a pivotal position as the second-largest crop, following pearl millet primarily cultivated for food consumption in Asia and as forage crops in Europe, North America, Australia and North Africa (Muthamilarasan and Manoj, 2015). Moreover, millets exhibit remarkable adaptability to climate, thriving efficiently even in conditions of low soil fertility, limited moisture and elevated temperatures (Lata *et al.,* 2013).

Foxtail millet, commonly referred to as Kangni, ranks as the second most extensively cultivated millet in India. It is typically grown in semi-arid regions and is well-suited to conditions with limited irrigation. Foxtail millets possess natural resistance to pests and offer a substantial nutritional profile, containing notable amounts of protein, dietary fiber, calcium, vitamins, iron and copper. It is non-acidic and non-glutinous, making it easily digestible, also it facilitates the

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gradual release of sugars in the body without disrupting metabolic processes (Gupta *et al.,* 2013). Moreover, it exhibits antimicrobial properties, possesses anti-tumorigenic potential and aids in detoxifying the body (Thakur and Tiwari, 2019). Once the core accessions are selected, the next logical step is to assess the level of genetic diversity within the core collection and identify sources of traits that are economically important, including resistance to biotic and abiotic stresses, yield and related characteristics, for further utilization in breeding programs.

Carotenoids are lipophilic pigments responsible for the vibrant yellow, red and orange hues seen in many flowers, fruits and vegetables. Beyond their role in plants, carotenoids are essential nutrients in human diets. They are primarily found in the forms of β-carotene, β-cryptoxanthin and α-carotene, which serve as the main dietary sources of vitamin A. Additionally, carotenoids such as lycopene, lutein and zeaxanthin function as potent antioxidants and scavengers of free radicals. These properties can contribute to a reduced risk of cancer and cardiovascular diseases.

MATERIALS AND METHODS

The genetic materials used in this study comprised 72 foxtail millet (*Setaria italica* L.) accessions from the core collection, including two check varieties, CO (Te) 7 and ATL 1. These germplasm accessions were sourced from the Ramiah Gene Bank, Plant Genetic Resources Division, Tamil Nadu Agricultural University, Coimbatore. Detailed information on the accessions is provided in Table 1. The selected germplasm represent the core collection of foxtail millet, which was developed to capture the diversity of the entire germplasm repository based on taxonomic descriptors and 12 qualitative traits.

Field evaluation was conducted during the *Rabi* season of 2023 at the Centre of Excellence in Millets, Athiyandal, located in Tiruvannamalai district, Tamil Nadu. The experiment was laid out using a randomized complete block design (RCBD) with three replications. Each accession was sown in a single row, 3 meters in length, on ridges spaced 30 cm apart, with a plant-to-plant spacing of 10 cm. A basal fertilizer dose of 20 kg N_2 and 50 kg P_2O_5 per hectare was applied, followed by a top dressing of 45 kg N_2 per hectare. Standard agronomic practices, including irrigation and hand weeding, were followed as required.

Data were recorded for nine qualitative traits namely plant growth habit, pigmentation at leaf sheath, leaf sheath pubescence, inflorescence shape, presence of inflorescence bristles, apical sterility, inflorescence compactness, presence of lobes on inflorescence and seed colour-on a plot basis. In addition, observations were made on eleven quantitative traits using five randomly selected competitive plants per accession across all replications. These traits included days to 50% flowering, days to maturity, plant height, number of basal tillers, flag leaf blade length, flag leaf blade width, panicle length, bristle length, thousand grain weight, single plant stover yield and single plant grain yield.

Table 1: List of foxtail millet germplasm accessions.

S. no.	Germplasm accession
1	TGP/ISE-15
2	TGP/ISE-23 SEL.
3	TGP/ISE-26
4	TGP/ISE-57A
5	TGP/ISE-128/1
6	TGP/ISE-183/1
7	TGP/ISE-213
8	TGP/ISE-213/1 sel.
9	TGP/ISE-254
10	TGP/ISE-365
11	TGP/ISE-440
12	TGP/ISE-454
13	TGP/ISE-472
14	TGP/ISE-509
15	TGP/ISE-518
16	TGP/ISE-520
17	TGP/ISE-708
18	TGP/ISE-740
19	TGP/ISE-1291
20	TGP/TNAU-4
21	TGP/TNAU-6A
22	TGP/TNAU-15
23	TGP/TNAU-29
24	TGP/TNAU-32
25	TGP/TNAU-59
26	TGP/TNAU-103/1 sel.
27	TGP/TNAU-108/2
28	TGP/TNAU-121
29	TGP/TNAU-141
30	TGP/TNAU-146
31	TGP/TNAU-155
32	TGP/TNAU-160
33	TGP/TNAU-179
34	TGP/GS-15/1
35	TGP/GS-20
36	TGP/GS-42
37	TGP/GS-43
38	TGP/GS-54
39	TGP/GS-59
40	TGP/GS-73
41	TGP/GS-108
42	TGP/GS-108/1 sel.
43	TGP/GS-146
44	TGP/GS-163
45	TGP/GS-230
46	TGP/GS-231
47	TGP/GS-247/1
48	TGP/GS-338
49	TGP/GS-467
50	TGP/GS-495

Table 1: Continue....

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51	TGP/GS-520
52	TGP/GS-537
53	TGP/GS-575
54	TGP/GS-622
55	TGP/GS-628
56	TGP/GS-641
57	TGP/GS-660
58	TGP/GS-669
59	TGP/GS-677
60	TGP/GS-699/1
61	TGP/GS-701
62	TGP/GS-711
63	TGP/GS-715
64	TGP/GS-716/1
65	TGP/GS-726
66	TGP/GS-734
67	TGP/GS-747/1
68	TGP/GS-753
69	TGP/GS-763
70	TGP/GS-764/1
Checks	CO - 7
	ATL - 1

Trait selection and recording were based on descriptors for *Setaria italica* and *Setaria pumila* as outlined by IBPGR (1985). Quantitative traits were subjected to D^2 (Mahalanobis, 1928) analysis to assess genetic divergence.

Carotenoids were extracted following a modified procedure based on the American Association of Cereal Chemists (AACC) method (Shen *et al.,* 2015). To summarize, 0.5 grams of millet powder were placed in a 50 mL centrifuge tube, which was wrapped in aluminum foil to prevent exposure to light. The sample was homogenized in 20 mL of water-saturated n-butanol for 30 seconds using a vortex mixer and then agitated for 3 hours at room temperature. After extraction, the mixture was centrifuged at 10,000 × g for 10 minutes at 4°C. The supernatant was carefully transferred to a new 50 mL centrifuge tube and diluted to a final volume of 25 mL with water-saturated butanol. The entire procedure was carried out under low light conditions to minimize light exposure. The absorbance of the extract was measured at 448 nm using an Eppendorf BioSpectrometer.

RESULTS AND DISCUSSION

Morphological diversity of qualitative traits

Qualitative traits, governed by oligogenic inheritance, do not exhibit continuous variation and are typically less influenced by environmental factors. In the present study, nine qualitative traits were evaluated to characterize seventy germplasm accessions along with two check varieties. Based on growth habit, genotypes were classified into three categories: Erect, prostrate and decumbent. The majority of accessions (92.85%) exhibited an erect growth habit, while 7% were decumbent. Both check varieties also displayed an erect growth habit. Leaf sheath pigmentation was assessed based on its presence or absence. Among the accessions, 34% exhibited purple pigmentation on the leaf sheath, while the remaining 66% lacked pigmentation.

Inflorescence shape was categorized into three types: Oblong, cylindrical and pyramidal. Oblong inflorescences were predominant, observed in 68% of the accessions, followed by cylindrical (22%) and pyramidal types (10%). Panicle compactness was used to classify panicles as lax, intermediate, or compact. Intermediate panicles were most common (42%), followed by compact (37%) and lax types (21%).

Grain color was also recorded, with 64% of the genotypes, including the checks, exhibiting yellow grains. The remaining 36% had white grains, except for one accession (TGP/GS-575), which displayed orange-colored grains. Leaf sheath pubescence was categorized based on its presence or absence. Approximately 46% of the accessions exhibited pubescence on the leaf sheath, while 54% lacked pubescence in this region.

Inflorescence bristles were classified based on their presence or absence on the panicle. Among the evaluated genotypes, 78% exhibited the presence of bristles, while 22% lacked them. Panicle tip sterility was similarly categorized into two classes: Presence or absence of apical sterility. Approximately 54% of the genotypes showed apical sterility, whereas 46% did not exhibit this trait. Inflorescence lobes were also classified based on presence or absence, with 76% of the genotypes possessing lobes and 24% lacking them.

Overall, the majority of the foxtail millet genotypes evaluated were characterized by an erect growth habit, absence of pigmentation on the leaf sheath, oblong and intermediate-type panicles, absence of leaf sheath pubescence, presence of inflorescence bristles, apical sterility, presence of lobes on the inflorescence and yellow grain color. These findings are consistent with the observations reported by Kavya (2017) in foxtail millet.

Cluster archetype

The Tocher's method was employed to cluster the 72 germplasm accessions including check varieties, resulting in the formation of six clusters, as given in Table 2. Cluster I was the largest with 56 genotypes along with one check ATL 1, followed by cluster III with 8 genotypes along with another check variety CO7. Cluster II comprised 4 genotypes and cluster IV had 2 genotypes. Clusters V and VI, each contained only one genotype. The distances within and between clusters were calculated and are presented in Table 3. Intra-cluster distances ranged from 0.00 to 69.70. Cluster III had the highest intra-cluster distance of 69.70, followed by cluster I with 62.80. Cluster IV had an intra-cluster distance of 53.18 and cluster II had 51.32. Due to being single clusters, clusters V and VI had intra-cluster distances of zero. Inter-cluster distances varied from 109.12 to 558.65. The largest inter-cluster distance was between cluster IV and cluster V (558.65), followed by cluster III and cluster V

(328.68), cluster I and V (301.91), cluster II and IV (285.53), cluster V and VI (283.84), cluster IV and VI (223.66), cluster III and IV (207.26), cluster III and VI (204.58), cluster I and IV (164.86), cluster II and VI (160.16), cluster II and III (141.51), cluster I and VI (139.10), cluster II and V (117.69), cluster I and III (115.49) and cluster I and II (109.12).

Cluster I had the highest number of accessions, followed by clusters III, II and IV. Clusters V and VI each represented solitary clusters. The presence of multiple clusters indicates substantial genetic diversity within the population. Selecting genotypes from these diverse clusters for hybridization can result in the development of superior progenies, as recommended by Subramanya and Ravikumar (2020) in finger millet. This approach allows for the utilization of genetic diversity in crop improvement and breeding programs. Cluster III exhibited the largest intra-cluster distance, indicating a diverse range of accessions within the same cluster. Clusters IV and V recorded maximum inter-cluster distances, highlighting the genetic diversity between these clusters.

Among the six clusters, cluster III showed higher mean values for yield-contributing traits such as days to 50% flowering, days to maturity, number of productive tillers, 1000 grain weight and single plant grain yield (Table 4). This suggests that cluster III contains more diverse accessions with favorable yield-related traits. Cluster V also exhibited higher mean values for plant height, flag leaf length, flag leaf width and panicle length. We might choose accessions from these clusters it will be used by foxtail millet crop improvement programme in future.

Table 2: Genetic divergence analysis of foxtail millet germplasm using mahalanobis D^2 statistics.

Cluster no.	No. of accessions	Constituents
I	56	TGP/ISE-15, TGP/ISE-23 SEL., TGP/ISE-26, TGP/ISE-57A, TGP/ISE-183/1, TGP/ISE-254, TGP/ISE-365, TGP/ISE-440, TGP/ISE-472, TGP/ISE-518, TGP/ISE-520, TGP/ISE-708, TGP/ISE-740, TGP/ISE-1291, TGP/TNAU-4, TGP/TNAU-15, TGP/TNAU-29, TGP/TNAU-32, TGP/TNAU-59, TGP/TNAU-103/1 sel., TGP/TNAU-108/2, TGP/TNAU-121, TGP/TNAU-141, TGP/TNAU-146, TGP/TNAU-155, TGP/GS-20, TGP/GS-42, TGP/GS-43, TGP/GS-59, TGP/GS-73, TGP/GS-108, TGP/GS-108/1 sel., TGP/GS-146, TGP/GS-163, TGP/GS-230, TGP/GS-231, TGP/GS-247/1, TGP/GS-338, TGP/GS-467, TGP/GS-495, TGP/GS-520, TGP/GS-622, TGP/GS-641, TGP/GS-660, TGP/GS-669, TGP/GS-677, TGP/GS-699/1, TGP/GS-701, TGP/GS-711, TGP/GS-715, TGP/GS-716/1, TGP/GS-726, TGP/GS-753, TGP/GS-763, TGP/GS-764/1, ATL-1
II	4	TGP/ISE-454, TGP/ISE-509, TGP/TNAU-160, TGP/TNAU-179.
III	8	TGP/ISE-213, TGP/TNAU-6A, TGP/GS-15/1, TGP/GS-537, TGP/GS-575, TGP/GS-628, TGP/GS-747/1, CO-7
IV	2	TGP/ISE - 213/1 sel., TGP/GS - 734
V	1	TGP/ISE - 128/1
VI	1	TGP/GS - 54

Table 3: Average intra and inter-cluster distance value for 11 traits.

Cluster number	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	62.80	109.12	115.49	164.86	301.91	139.10
Cluster 2		51.32	141.51	285.53	117.69	160.16
Cluster 3			69.70	207.26	328.68	204.58
Cluster 4				53.18	558.65	223.66
Cluster 5					0.00	283.84
Cluster 6						0.00

Table 4: Cluster mean value in germplasm accessions with checks for eleven quantitative traits.

Clusters	DFF	DM	PH	NPT	FLL	FLW	PL	BL	TGW	SSW	SGY
I	48.25	84.46	142.55	3.46	28.81	1.52	15.89	0.66	2.95	13.42	6.21
II	48.88	86.00	172.36	2.89	31.06	1.47	21.09	0.79	3.11	13.23	7.63
III	50.88	86.00	150.73	7.56	30.43	1.60	18.04	0.60	4.29	18.44	11.12
IV	47.00	82.25	119.99	2.08	25.96	1.49	15.95	0.49	2.63	32.78	5.37
V	47.50	82.50	199.37	4.13	37.14	1.82	28.27	0.43	2.50	12.97	5.42
VI	47.50	84.50	120.72	3.27	25.85	1.24	27.22	0.57	3.03	10.92	4.95

To develop high-yielding early-maturing varieties, it is to be considered for crossing genotypes from clusters III and IV. Cluster III, with its high mean for grain yield, can contribute to improved yield potential, while cluster IV had a low mean value for maturity can help in achieving early maturity in the progeny. Furthermore, traits like single plant grain yield, 1000 grain weight, panicle length and days to maturity have been identified as significant contributors to genetic divergence. Therefore, these traits should be given priority during the process of introgression and selection to enhance the successful breeding program in developing high-yielding early-maturing varieties.

Table 5: Contribution of individual traits towards genetic divergence.

Characters	Contribution (%)
Days to fifty per cent flowering	0.00
Days to maturity	3.17
Plant height	20.79
No. of productive tillers	12.43
Flag leaf length	6.52
Flag leaf width	1.81
Panicle length	15.5
Bristle length	10.16
Thousand grain weight	5.49
Single plant stover yield	14.75
Single plant grain yield	9.39

Table 6: Total carotenoid content of selected 25 foxtail millet germplasm accession.

Germplasm accession	Total carotenoid content (mg/100 g)
TGP/TNAU-4	0.31
TGP/ISE-15	0.92
TGP/ISE-23 SEL	0.90
TGP/ISE-26	1.68
TGP/TNAU-32	0.27
TGP/GS-54	0.95
TGP/ISE-57A	0.91
TGP/GS-108	0.10
TGP/ISE-183/1	1.25
TGP/ISE-213/1	0.94
TGP/GS-231	0.38
TGP/ISE-454	0.92
TGP/GS-467	0.97
TGP/ISE-472	0.90
TGP/ISE-509	0.91
TGP/ISE-520	0.90
TGP/GS-628	0.24
TGP/GS-699/1	0.63
TGP/GS-701	0.52
TGP/ISE-708	0.91
TGP/GS-726	0.95
TGP/GS-764/1	0.18
TGP/ISE-1291	0.15
CO 7	0.93
ATL 1	0.48

Relative contribution of each trait towards genetic divergence

Understanding the relative contribution of individual traits to genetic divergence is essential for guiding crop improvement strategies. A summary of the contributions of each trait to genetic divergence is presented in Table 5. Among the evaluated traits, plant height exhibited the highest contribution (20.79%), followed by panicle length (15.50%), single plant stover weight (14.75%) and number of productive tillers (12.43%). Bristle length (10.16%) and single plant grain yield (9.39%) also contributed substantially. Flag leaf length accounted for 6.52% of the divergence, while thousand grain weight contributed 5.49%. In contrast, days to maturity (3.17%) and flag leaf width (1.81%) had relatively minor contributions. Notably, days to 50% flowering showed no contribution to genetic divergence. Similar trends have been reported in kodo millet by Suthediya *et al.* (2021), particularly for panicle length and single plant yield and Shweta *et al.* (2022) in finger millet for days to 50% flowering.

Genotypes belonging to genetically distant clusters are ideal candidates for use as parents in inter-crossing programs aimed at maximizing heterosis, whereas genotypes within the same cluster may be more suitable for varietal development due to their genetic similarity. Clusters exhibiting high mean trait values can serve as valuable sources for the enhancement of specific agronomic traits. For the development of high-yielding, early-maturing varieties, hybridization between clusters III and IV is recommended, as cluster III demonstrated a high mean for grain yield, while cluster IV exhibited a lower mean for days to maturity. Key traits such as single plant grain yield, thousand grain weight, panicle length and days to maturity which showed substantial contributions to genetic divergence should be prioritized during introgression and selection processes.

Studies on carotenoid content

Total carotenoid content, representing the total yellow pigments, was quantified in twenty-five high-yielding accessions using the biochemical method described by Shen *et al.* (2015). Among the evaluated elite germplasm lines, the check variety CO7 demonstrated a superior yield performance, exceeding the population mean. Total carotenoids ranged from 0.18 mg/100 g to 1.68 mg/100 g (Table 6). It is observed that out of twenty-five accessions of foxtail millet, only 16 genotypes (64%) exhibited higher carotenoid content and remaining 36% of genotypes (9 genotypes) recorded relatively less amount of total carotenoids which was estimated less than 0.50 mg/100 g. The accessions TGP/ISE-26 (1.68 mg/100 g), TGP/ISE-183/1 (1.25 mg/100 g), TGP/GS-467 (0.97 mg/100 g), TGP/GS-699/1 (0.63 mg/100 g), TGP/GS-701 (0.52 mg/100 g) and the check CO7 (0.93 mg/100 g) recorded high amount of carotenoids present in the grains and TGP/GS-108 (0.10

mg/100 g), TGP/GS-628 (0.24 mg/100 g), TGP/GS-764/1 (0.18 mg/100 g) expressed relatively less amount of carotenoid content in the grains. The check ATL 1 revealed carotenoid content of 0.48 mg/100 g.

Carotenoid analysis determines the total yellow pigments present in the grains, including carotenes and xanthophylls, which are the primary pigments found in foxtail millet. These compounds serve as nutri-therapeutic agents, offering various health benefits. We conclude that there were 16 accessions exhibited high amount of total carotenoids predominantly in the accessions *viz.,* TGP/ISE-26, TGP/ISE -183/1 and TGP/GS-467 recorded more amount of carotenoids. The check CO7 reported significant carotenoid value with more single plant yield which resulted in the utilization of these accessions would be rewarded for the development of high carotenoid grains with more yield.

CONCLUSION

Significant genetic diversity was observed, suggesting potential for breeding high-yielding, early-maturing and carotenoid-rich foxtail millet varieties. Crosses between Cluster III (high yield) and Cluster IV (early maturity) are recommended. High-carotenoid accessions (*e.g.*, TGP/ISE-26) can be prioritized for biofortification programs.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

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