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Research Note

Genetic diversity for yield and morpho-physiological traits in foxtail millet (*Setaria italica* (L.) P. Beauv.)

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Abstract

Genetic diversity is a cornerstone of successful plant breeding programs, particularly for enhancing yield and stress tolerance in crops like foxtail millet. This study evaluated the genetic divergence among 40 foxtail millet genotypes based on 18 yield and morpho-physiological traits using Mahalanobis D² statistics. The analysis revealed significant genetic variability, with genotypes grouped into six distinct non-overlapping clusters using Tocher's method. Cluster I was the largest (14 genotypes), while Cluster II was monogenotypic, indicating high heterogeneity. Inter-cluster distances (506.52–3158.48) consistently exceeded intra-cluster distances (0–353.56), highlighting greater diversity between clusters. Cluster means varied significantly for traits such as days to 50% flowering (32.50–58.75 days), plant height (38.67–108.89 cm), and grain yield per plant (2.68–7.82 g). Cluster VI exhibited superior means for traits like days to maturity and panicle length, while Cluster IV excelled in grain yield and harvest index. Protein content (29.49%), transpiration rate (24.36%), and grain yield per plant (11.54%) were the primary contributors to genetic divergence. The results suggest that strategic hybridization between accessions from divergent clusters, particularly Clusters II and V, could generate superior recombinants with enhanced genetic potential for yield and stress tolerance in foxtail millet breeding programmes.

Key words: Clusters, Foxtail millet, Genetic diversity, Genotypes and Yield.

Foxtail millet (*Setaria italica* (L.) P. Beauv.), a self-pollinating, short-duration C_4 cereal in the Poaceae family, is the second most cultivated millet globally, with an estimated annual production of 5 million tonnes (Ayesha *et al.,* 2019). Predominantly grown in countries like China, India, and Japan, it plays a significant role in global agriculture, with China as the leading producer followed by India. In India, foxtail millet cultivation spans 0.43 million hectares, yielding 0.384 million tonnes annually with an average productivity of 893 kg/ha (Ram *et al.,* 2025). Key production areas include Andhra Pradesh, Tamil Nadu, Karnataka and Rajasthan, with Andhra Pradesh alone cultivating 8,350 hectares, producing 7,631 tonnes at an average yield of 914 kg/ha (Ram *et al.,* 2025). The grains of this crop are significantly more nutrient-dense than those of other major cereal crops (Vardhan *et al.,* 2024). Foxtail millet contains 11–12% protein (Amadou *et al.,* 2011), 6–8% dietary fiber, 31 mg/100g calcium, 2.8 mg/100g iron and low fat (4%), making it ideal for diabetic and gluten-free diets due to its low-to-medium glycemic index (Hariprasanna, 2016). Its small genome (515 Mb; 2n=2x=18) and short life cycle position it as a model crop for studying C_4 metabolism and drought resilience (Lata *et al.,* 2013). The crop's inherent drought tolerance is critical for rainfed semi-arid regions, where it ensures food security under water-deficient conditions. The existence of diversity among the germplasm 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 diversity is important for improving crops, especially foxtail millet (Ramesh *et al.,* 2023). Yield and productivity in foxtail millet are influenced by morpho-physiological traits such as the number of productive tillers, panicle length, primary branches, effective photosynthesis, leaf chlorophyll content, chlorophyll fluorescence, harvest index, and tolerance to drought, heat, pests and diseases. Trait-based breeding leveraging genetic diversity can enhance productivity and climate resilience. This study aims to assess genetic diversity among diverse foxtail millet genotypes using Mahalanobis D² statistics and hierarchical cluster analysis, focusing on 18 yield and morpho-physiological traits to identify superior lines and develop effective selection strategies for improving yield and adaptability in drought-prone environments.

The field experiment was carried out during *rabi,* 2024-25 at wet land farm, S. V. Agricultural College, Tirupati, Acharya N. G. Ranga Agricultural University, Andhra Pradesh situated in Southern Agro - climatic Zone of Andhra Pradesh, India located at an altitude of 182.9 m above from mean sea level, 13° N latitude and 79° E longitude. The experimental material utilized for the present study comprised of 36 foxtail millet genotypes with four standard check varieties obtained from various collections maintained at RARS, Nandyal. The experimental material was evaluated using Alpha lattice design and the total experimental area was divided into two replications, each consists of 4 blocks. In each block 10 genotypes were allocated and each genotype was planted in two rows each of 3 meters in length with spacing of 22.5 cm between rows and 10 cm within row. The observations were recorded on five randomly selected plants per plot for 18 morpho-physiological and yield-attributing traits such as days to 50% flowering, days to maturity, plant height (cm), number of productive tillers per plant, panicle length (cm), specific leaf area (cm² g^{-1}), net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}), relative water content (%), canopy temperature (°C), chlorophyll fluorescence, SPAD chlorophyll meter reading (SCMR), protein content (%), 1000-grain weight (g), straw yield per plant (g), harvest index (%) and grain yield per plant (g). The data recorded was subjected to analysis of variance as well as Mahalanobis D² statistics (Mahalanobis, 1928). The genotypes were grouped on the basis of minimum generalized distance using Tocher's method as described by Rao (Rao, 1952). The mean values for each character over two replications were subjected to the statistical analysis using INDOSTAT 9.3 and R software.

The analysis of variance revealed high significant differences among the genotypes under study for all 18 traits examined, suggesting considerable genetic variability among the evaluated genotypes and substantial scope for genetic improvement through selection (**Table 1**). Genetic divergence of 40 foxtail millet genotypes including four popular check 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 **Fig. 1. and Table 2**. These findings revealed a sizable amount of genetic diversity. Among six clusters, Cluster I was the largest, comprising 14 accessions, followed by cluster III (13 genotypes), cluster V (5 genotypes), cluster VI (4 genotypes), cluster IV (3 genotypes). The remaining cluster II was monogenotypic, containing single genotype indicating a high degree of heterogeneity among the genotypes**.** The intra and inter-cluster D^2 values were provided for six clusters in **Table 3**. The inter-cluster distances were constantly higher than the intra-cluster distances (**Fig. 2**), indicating greater genetic diversity between the clusters rather than within the clusters. Intra-cluster distances ranged from 0 to 353.56. Cluster II showed the lowest intra-cluster distance (0), as it was monogenotypic. In contrary, cluster V showed the largest intra-cluster distance (353.56), reflecting differences among genotypes within cluster. Therefore, genotypes within the same cluster possess low levels of diversity and selection of parents within the cluster may not be considered rewarding (Kumar *et al.,* 2010). The present results corroborate the findings documented by Battula *et al*. (2025) in foxtail millet.

Inter-cluster distances ranged from 506.52 (between cluster I and cluster III) to 3158.48 (between cluster II and cluster V). Maximum inter-cluster distance noticed between cluster II (SiA 3222) and cluster V (SiA 4322) suggesting, high degree of genetic diversity among the genotypes. Therefore, these genotypes might be considered as potential parents in a hybridization programme to generate superior recombinants with enhanced genetic potential.

A wide spectrum of variation was observed in clusters for the studied traits implying that the clusters formed were unique. The cluster VI recorded highest cluster means for important traits *viz.,* days to 50% flowering (58.75), days to maturity (92.75), plant height (108.89 cm), panicle length (19.01 cm), net photosynthetic rate (22.59 μmol CO_2 m^{-2} s^{-1}) and transpiration rate (3.78 mmol H_2O m^{-2} s^{-1}) (**Table 4**). Cluster V showed superior desired mean values for most of the traits *viz*., number of productive tillers per plant (3.34), stomatal conductance (0.38 mmol H_2O m^{-2} s^{-1}), relative water content (86.64%), SCMR (39.99) and 1000-grain weight (2.87 g). Cluster IV was notable for the highest means for chlorophyll fluorescence (0.69), straw yield per plant (11.33 g), harvest index (40.93%) and grain yield per plant (7.82 g), along with the lowest specific leaf area mean (150.52 cm² g^{-1}) and relative water content (75.24%). Cluster III exhibited

Table 1. ANOVA for yield and morpho-physiological traits among forty foxtail millet genotypes

S. No	Source of variation	Mean sum of squares			
		Replication (df = 1)	Genotype (df = 39)	Block (df = 6)	Error (df = 33)
1	Days to 50% flowering	1.012	48.146 **	1.273	3.086
2	Days to maturity	4.050	61.896**	1.558	2.018
3	Plant height	0.620	435.560**	0.15	34.042
4	Number of productive tillers per plant	0.002	0.534**	0.018	0.164
5	Panicle length	0.031	13.319**	0.933	2.716
6	Specific leaf area	15.720	906.810**	22.000	27.303
7	Net photosynthesis rate	0.050	12.316**	0.245	1.023
8	Transpiration rate	0.003	1.677**	0.003	0.018
9	Stomatal conductance	0.002	0.0202**	0.0001	0.0013
10	Canopy temperature	1.685	9.097*	0.340	4.819
11	Chlorophyll fluorescence	0.0012	0.0025**	0.0002	0.0007
12	Relative water content	10.860	49.346**	1.408	3.190
13	SPAD Chlorophyll Meter Reading (SCMR)	0.487	39.569**	2.237	8.824
14	Protein content	0.0065	4.8155**	0.0005	0.0514
15	1000 grain weight	0.00003	0.07595**	0.00020	0.02316
16	Straw yield per plant	0.038	9.485**	0.143	0.801
17	Harvest index	1.178	24.146**	1.058	9.075
18	Grain yield per plant	0.001	5.413**	0.049	0.716

Df = Degrees of freedom; ** Significance at 1

Table 2. Clustering analysis of forty foxtail millet genotypes using Tocher's method

Cluster Number	Number of genotypes	Genotypes
I	14	DHFt109-3, IIMRFxM14, KOPFx2107, SiA 4231, SiA 4255, SiA 4257, SiA 4262,SiA 4306, SiA 4309, SiA 4313, SiA 4315,SiA 4324, SiA 4348 and Suryanandi
II	1	SiA 3222
III	13	GPUF-18, IIMRFxM16, KMF-1, PPK-7, Prasad, SiA 4298, SiA 4311, SiA 4320, SiA 4326, SiA 4328, SiA 4331, SiA 4333 and SiA 4335
IV	3	SiA 4227, SiA 4228 and SiA 4252
V	5	SiA 4258, SiA 4279, SiA 4322, SiA 4338 and SiA 4350
VI	4	SiA 3156, SiA 3159, SiA 4276 and SiA 4317

Table 3. Average intra and inter-cluster cluster distances (D^2 values) for six clusters of forty foxtail millet genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**308.31**					
Cluster II	1,757.02	**0.00**				
Cluster III	506.52	1,740.92	**247.02**			
Cluster IV	664.52	2,068.08	722.58	**269.71**		
Cluster V	763.11	3,158.48	1,332.87	1,021.46	**353.56**	
Cluster VI	638.42	2,830.32	661.01	920.05	665.96	**204.03**

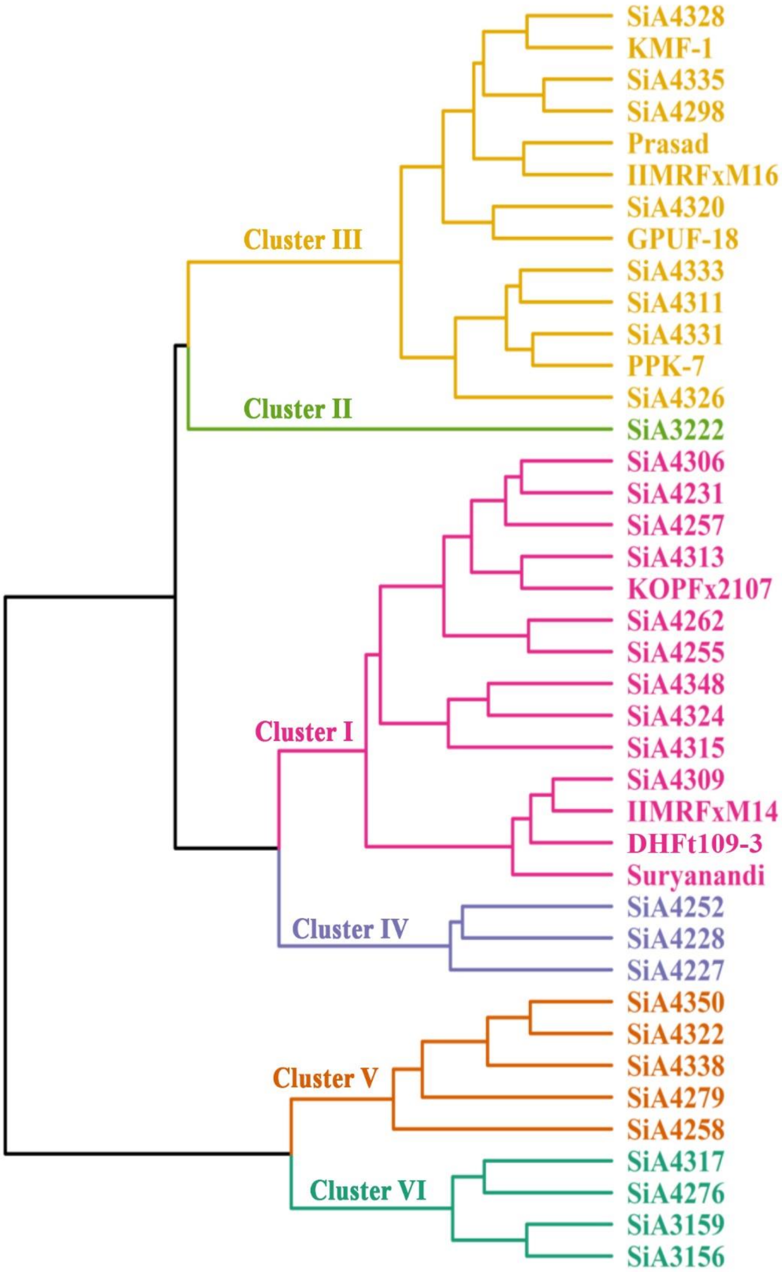

Fig.1. Dendrogram depicting relationship among forty foxtail millet genotypes based on Mahalanobis D^2 values

highest cluster mean for protein content (13.02%), while Cluster II recorded maximum cluster mean for canopy temperature (35.47°C) and lowest cluster means for majority of the traits *viz.,* days to 50% flowering (32.50), days to maturity (63.50), plant height (38.67 cm), number of productive tillers per plant (1.50), panicle length (6.97 cm), transpiration rate (1.59 mmol H_2O m^{-2} s^{-1}), stomatal conductance (0.15 mmol H_2O m^{-2} s^{-1}), chlorophyll fluorescence (0.56), SPAD chlorophyll meter reading (28.43), 1000 grain weight (2.58 g), straw yield per plant (4.03 g), grain yield per plant (2.68 g).

The results indicates that no single cluster owned the genotypes with all desired traits combinations, which ruled out the option of selecting directly a single genotype for immediate usage. Therefore, strategic hybridization between the selected genotypes from divergent and complementary clusters is essential to accumulate desired traits in the segregating population and selecting the desirable combinations in the later generation for the development of improved cultivars high yield and stress tolerance.

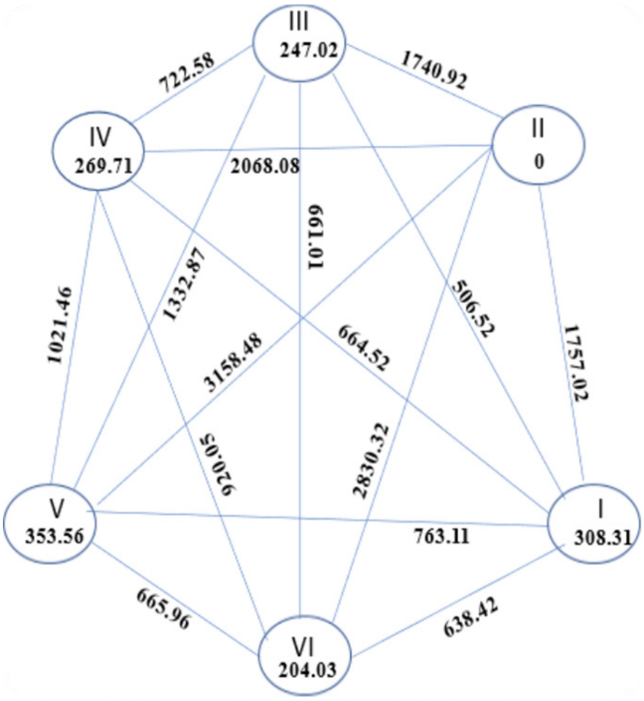

Fig.2. Mahalanobis Euclidean Distance (Not to the scale).

Table 4. Cluster mean values for eighteen yield and morpho-physiological traits among forty foxtail millet genotypes

S. No.	Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
1	Days to 50% flowering	57.46	32.50	58.15	55.50	57.40	58.75
2	Days to maturity	90.00	63.50	92.50	88.33	89.40	92.75
3	Plant height (cm)	95.36	38.67	95.60	98.10	107.58	108.89
4	Number of productive tillers per plant	2.79	1.50	3.06	2.90	3.34	3.30
5	Panicle length (cm)	16.52	6.97	15.76	15.86	18.21	19.01
6	Specific leaf area (cm^2 g^{-1})	187.22	162.16	165.35	150.52	168.03	152.96
7	Net photosynthesis rate (μm m^{-2} s^{-1})	19.85	20.30	20.48	21.45	21.23	22.59
8	Transpiration rate (mmol m-^{2} s-^{1})	2.46	1.59	2.15	1.65	3.67	3.78
9	Stomatal conductance (mmol m^{-2} s^{-1})	0.28	0.15	0.25	0.36	0.38	0.30
10	Canopy temperature (°C)	32.33	35.47	33.25	33.98	32.77	30.84
11	Chlorophyll fluorescence (%)	0.65	0.56	0.64	0.69	0.66	0.67
12	Relative water content (%)	83.56	81.50	80.53	75.24	86.64	84.54
13	SPAD Chlorophyll Meter Reading (SCMR)	36.55	28.43	38.17	37.99	39.99	37.97
14	Protein content (%)	11.16	12.56	13.02	10.12	9.02	11.87
15	1000 grain weight (g)	2.68	2.58	2.71	2.81	2.87	2.83
16	Straw yield per plant (g)	8.27	4.03	8.07	11.33	11.21	10.89
17	Harvest index (%)	36.75	39.98	36.81	40.93	38.35	38.86
18	Grain yield per plant (g)	4.83	2.68	4.74	7.82	7.05	6.98

Among the 18 traits studied, protein content contributed the most to genetic divergence (29.49%, ranking first 230 times) followed by transpiration rate (24.36%, ranking first 190 times), grain yield per plant (11.54%, ranking first 90 times) and specific leaf area (11.15%, ranking first 87 times) (**Table 5**). Other notable contributors included days to maturity (4.62%, ranking first 36 times), stomatal conductance (3.85%, ranking first 30 times), straw yield per plant (3.85%, ranking first 30 times), relative water content (2.95%, ranking first 23 times), harvest index (2.95%,

Table 5. Relative contribution for eighteen yield and morpho-physiological traits to genetic divergence among 40 foxtail millet genotypes

S. No.	Source	Rank	Contribution %
1.	Days to 50% flowering	1	0.13
2.	Days to maturity	36	4.62
3.	Plant height	22	2.82
4.	Number of productive tillers per plant	2	0.26
5.	Panicle length	0	0
6.	Specific leaf area	87	11.15
7.	Net photosynthesis rate	14	1.79
8.	Transpiration rate	190	24.36
9.	Stomatal conductance	30	3.85
10.	Canopy temperature	1	0.13
11.	Chlorophyll fluorescence	0	0
12.	Relative water content	23	2.95
13.	SPAD Chlorophyll Meter Reading (SCMR)	0	0
14.	Protein content	230	29.49
15.	1000 grain weight	1	0.13
16.	Straw yield per plant	30	3.85
17.	Harvest index	23	2.95
18.	Grain yield per plant	90	11.54

ranking first 23 times), plant height (2.82%, ranking first 22 times) and net photosynthetic rate (1.79%, ranking first 14 times). In contrast, traits such as number of productive tillers (0.26%, ranking first 2 times), days to 50% flowering (0.13%, ranking first once), canopy temperature (0.13%, ranking first once) and 1000-grain weight (0.13%, ranking first once) made minimal contributions to total divergence, suggesting greater homogeneity among the evaluated genotypes. This limited diversity likely results from directional selection pressures by breeders, favouring uniform traits like flowering time or tiller number during genotype development. Inter crossing accessions from divergent clusters could generate broader variability for these traits, followed by targeted selection to enhance diversity.

Overall, protein content, transpiration rate, grain yield per plant and straw yield per plant were the primary drivers of genetic divergence. Prioritizing these traits, alongside the performance of promising genotypes, is recommended for the genetic improvement of foxtail millet to enhance yield and drought resilience.

Mahalanobis's D^2 analysis, using Tocher's method, revealed significant genetic diversity among 40 foxtail millet genotypes, grouping them into six distinct clusters. Cluster I was the largest, comprising; 14 genotypes, followed by Cluster III with 13 genotypes. Cluster V, VI and IV contained 5, 4 and 3 genotypes, respectively. Notably, Cluster II was monogenotypic, consisting solely of 1 genotype SiA 3222, indicating substantial heterogeneity. Clusters II (SiA 3222) and V (SiA 4258, SiA 4279, SiA 4322, SiA 4338 and SiA 4350) exhibited the greatest inter-cluster distance, suggesting their potential as parents in hybridization programmes to generate superior transgressive segregants for high yield and adaptability. The absence of a single cluster with all desired traits underscores the need for strategic hybridization between divergent clusters to accumulate favourable traits in segregating populations. Among the eighteen traits, protein content contributed most to genetic divergence followed by transpiration rate, grain yield per plant and specific leaf area. By prioritizing these key traits such as protein content, transpiration rate, grain yield and specific leaf area and intercrossing genotypes from Clusters II and V, breeders can enhance genetic variability and develop improved foxtail millet cultivars with higher yield and drought resilience, contributing to food security in semi-arid regions.

REFERENCES

Allard, R.W. 1960. Principles of Plant Breeding. *John Willey and Sons*, New York. 485.

Amadou, I., Amza, T., Shi, Y.H. and Le, G.W. 2011. Chemical analysis and antioxidant properties of foxtail millet bran extracts. Songklanakarin *Journal of Science and Technology,* **33**(4): 509-515.

Ayesha, M. and Babu, D.R. 2019. Genetic divergence studies in foxtail millet [*Setaria italica* (L.) Beauv.]. *Electronic Journal of Plant Breeding,* **10**(1): 18-29. [Cross Ref]

Battula, H.V., Ragimekula, N., Mallapuram, S.P., Akila, C.S. and Dasari, V.B. 2025. Genetic diversity analysis of morpho-physiological and yield attributing traits in foxtail millet (*Setaria italica* L. Beauv.). *Journal of Innovative Agriculture,* **12**(1): 1-8. [Cross Ref]

Hariprasanna, K. 2016. Foxtail millet: Nutritional importance and cultivation aspects. *Indian Farming,* **73**(1): 47-49.

Kumar, D.B.M., Kantti, A. and Mallikarjunaiah, H. 2010. Genetic divergence in chilli accessions. *Electronic Journal of Plant Breeding,* **1**(5): 1363-1366.

Lata, C., Gupta, S. and Prasad, M. 2013. Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. *Critical Reviews in Biotechnology,* **33**(3): 328-343. [Cross Ref]

Mahalanobis, P.C. 1928. A statistical study at Chinese head measurement. *Journal of Asiatic Society of Bengal,* **25**: 301-307.

Ram, U., Naik, B., Ragimekula, N., Babu, D. and Sekhar, M. 2025. Character association and path coefficient analysis of yield and morpho-physiological traits in foxtail millet (*Setaria italica* (L.) P. Beauv.). *Journal of Advances in Biology & Biotechnology,* **28**: 710-721. [Cross Ref]

Ram, U.S., Naik, B.S.K., Narasimhulu, R. and Sekhar, D.V.B.M.R. 2025. Studies on genetic variability and genetic parameters for grain yield and its components in foxtail millet (*Setaria italica* (L.) P. Beauv.). *Andhra Pradesh Journal of Agricultural Sciences,* **11**(2): 118-125.

Ramesh, P., Juturu, V.N., Yugandhar, P., Pedersen, S., Hemasundar, A., Yolcu, S., Reddy, P.C.O., Reddy, C.M.C.V., Chari, P.V., Mohan, R. and Sekhar, A.C. 2023. Molecular genetics and phenotypic assessment of foxtail millet (*Setaria italica* (L.) P. Beauv.) landraces revealed remarkable variability of morpho-physiological, yield, and yield-related traits. *Frontiers in Genetics,* **14**: 1052575. [Cross Ref]

Rao, C.R. 1952. *Advanced statistical methods in biometrical research*. John Wiley and Sons, New York. 357-363.

Vardhan, B.H., Narasimhulu, R., Priya, M.S. and Sekhar, A.C. 2024. Genetic variability studies for morpho-physiological and yield-attributing traits in foxtail millet (*Setaria italica* L. Beauv.). *International Journal of Environment and Climate Change,* **14**(8): 76-84. [Cross Ref]