

Genetic divergence studies for yield components and nutritional traits in foxtail millet (*Setaria Italica* (L.)

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Abstract

Analysis of variance revealed existence of significant differences among the genotypes studied and ample genetic variability for all the 16 quantitative and nutritional traits. All the 40 foxtail millet genotypes were grouped into six distinct non-overlapping clusters with maximum number of genotypes in cluster I (19) followed by cluster II (13), cluster V (five) and the remaining three clusters, III, IV and VI were mono-genotypic clusters with single genotype per cluster through Mahalanobis D² analysis. Maximum inter-cluster distance was observed between cluster II and III, II and IV, I and II, II and V. Maximum inter-cluster distance was found between clusters II (SiA 4349, SiA 4363, SiA 3156(C), SiA 4340, SiA 4334, SiA 4321, KOPF x 2126, SiA 4332, SiA 4325, KMF 2, SiA 4229, SiA 4323, SiA 4336) and III (SiA 4261) indicating high degree of genetic diversity in the genotypes. Cluster IV contained one genotype SiA 4316 with desirable traits and can be selected directly for immediate usage. Hybridization between the genotypes viz; SiA 4244, SiA 4251 and IIMR F x M15 with high means for majority of traits from divergent clusters can be made to incorporate all the desired traits and to get high frequency of heterotic hybrids. Inter crossing of genotypes from the cluster II (SiA 3156) and cluster III (SiA 4261) could be suggested to generate a wide range of variability followed by effective selection. Iron, Protein, Zinc content in grain and grain yield per plant were the major contributors towards divergence.

Keywords: Foxtail millet, Yield components, Nutritional traits, Genetic diversity

Introduction

Foxtail millet (*Setaria italica* (L.) referred as Italian millet is one of the oldest domesticated crops cultivated for food grain, tolerant to both biotic and abiotic stresses. The phenolics present in foxtail millet act as antioxidants by reducing the amount of free radical produced in the body. They are also known for their ability as reducing agents, metal chelators and reactive oxygen quenchers (Chandrasekara and Shahidi 2010). Nutritionally, foxtail millet grain contains Protein (10.56 g/100g) and is rich source of micronutrients including Iron (3.02 mg/100g), Zinc (2.46 mg/100g), Manganese (1.54 mg/100g), Calcium (23.94 mg/100g), and Magnesium (77.65 mg/100g) (Srilatha *et al.*, 2020). It has a moderate glycemic index (GI) of 59 and is a good source of β -carotene (Murugan and Nirmalakumari, 2006 and Coulibaly and Chen, 2011) ^[11]. A knowledge on the magnitude and nature of genetic variability present in a crop plays significant role in formulating a successful breeding programme. Mahalanobis D² statistics is an effective tool in quantifying the degree of genetic divergence at the genotypic level and provides measures of association between geographic distribution and genetic diversity based on generalized distances (Mahalanobis, 1936) ^[10]. Rao (1952) ^[14] suggested application of this technique for assessment of genetic diversity in plant breeding. Selecting the parents in crops for breeding programme is critical because, the success of such

programme depends upon the segregants of hybrid derivatives between the parents, particularly when the target is to improve the quantitative traits like yield. Most of the researchers have studied foxtail millet for yield and its components and indicated that clusters separated by largest statistical distance (D²) had maximum divergence and least distance had minimum divergence (Singh and Chaudhary, 1977; Brunda *et al.* 2015; Gangurdeet *et al.* 2016; Bheemesh *et al.* 2018; Venkatesh *et al.* 2020; Pawan Kumar *et al.* 2019; Anand *et al.* 2020; Gopi *et al.* 2021; Karvar *et al.* 2022 and Battula *et al.* 2025) ^[1, 4, 5, 7-9, 13, 16, 18]. A very little information is available on nutritional traits in contributing to genetic diversity in foxtail millet. Hence, the present investigation in foxtail millet was carried out to assess the genetic diversity among genotypes for grain yield, yield components and nutritional traits.

Material and Methods

The experiment was carried out during *Rabi*, 2024- 2025 at S.V. Agricultural college, Wet land Farm, Tirupati, ANGRAU, Andhra Pradesh, located at an altitude of 182.9 meters above the mean sea level, 13° N latitude and 79° N longitude. The experimental material comprised of 40 genotypes of foxtail millet along with two checks viz., SiA 3156 and SiA 3159 was laid out in Alpha Lattice design with two replications. All the genotypes were raised in two rows of 3m length with a spacing of 22.5 cm between the

rows and 10 cm between plants. All the recommended package of practices were followed in raising a healthy crop. Observations were recorded on five randomly selected competitive plants of each genotype and from each replication for yield component traits except for days to 50% flowering and days to maturity, for which data were recorded on per plot basis. Observations recorded on yield components include plant height (cm), number of productive tillers per plant, flag leaf length and width at flowering (cm), panicle length (cm), thousand grain weight (g), straw yield per plant (g) and grain yield per plant (g). SPAD Chlorophyll Meter Reading at 45 DAS was recorded using SCMR chlorophyll meter on the third leaf from top at three spots viz., top, middle and lower portion of the leaf top of the plant. Relative Water Content (%) calculated using the formula given by Barr and Weatherley (1962)^[2]. Harvest index (%) was worked out as the ratio of grain yield to biomass yield. Iron Content and Zinc Content (mg/100g) in grains were estimated as per procedure given by Tandon (1999)^[17]. The Nitrogen content (%) in the grounded flour sample was estimated by Micro Kjeldahl method and Protein content (%) was estimated as described by Sadasivam and Manickam (1996)^[15].

Statistical analysis

Mean data in respect of various traits were subjected to the Statistical analysis as per the procedure given by Panse and Sukhatme (1961). Mahalanobis (1936)^[10] D² statistic was adopted for assessing the genetic divergence among the test genotypes. The D² values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (s) values of any two uncorrelated genotypes (Rao, 1952)^[14]. All $n(n-1)/2$ D² values were clustered using Tocher's method as described by Rao (1952)^[14]. The intra and inter cluster distances were calculated adopting the formula given by Singh and Chaudhary (1977)^[16].

Wilk's 'Λ' criterion test: Wilk's 'Λ' (Statistical) criterion was used to test the significant differences between the groups based on the pooled effects for all the traits. The significance of 'Λ' (Statistical) value was tested by χ^2 at 624 degrees of freedom.

Results and Discussion

The mean data collected on 16 yield, yield components and nutritional traits for 40 foxtail millet genotypes were computed for genetic divergence analysis using Mahalanobis D² statistic. The groups or clusters were formed using Tocher's method in D² statistic. The values obtained, indicated the existence of considerable magnitude of variability among the genotypes studied that led to genetic diversity.

The 'Λ' statistic value was highly significant indicating that the genotypes differed considerably when all the traits were considered simultaneously. The significance of the genotypes clearly indicated the significant pooled effect of all the traits between different accessions. Hence, further analysis was made to estimate D² statistic.

Mahalanobis generalized distance D² values

The correlated unstandardized means [(X₁) - (X₂)] of the sixteen traits studied were transformed to standardized uncorrelated [(Y₁) - (Y₂)] set of variables by using pivotal condensation method. The statistical distance (Mahalanobis D² values) between a pair of genotypes was obtained as sum of squares of differences between pairs of corresponding uncorrelated values of any two germplasm accessions. These values were considered at a time and were used for final grouping of genotypes.

Clustering pattern

Forty genotypes, including two checks, using Tocher's method (Rao, 1952)^[14] were grouped into six distinct non-overlapping clusters indicating the presence of significant genetic diversity in the experimental material studied. Among all the six clusters, Cluster I was the largest comprising of a large number of genotypes (19), followed by cluster II (13), cluster V (5) and the remaining three clusters III, IV and VI were mono-genotypic (one) containing only one genotype each, indicating a high degree of heterogeneity among the genotypes (Table 1 and Fig.1). The formation of unique solitary clusters may be due to the fact that geographic barriers blocking gene flow or intensive natural and human selection for diverse and adaptable gene complexes must be cause for this genetic diversity. Ayesha *et al.* (2019) had reported eight clusters for 50 foxtail millet accessions, Venkatesh *et al.* (2020) clustered the 40 genotypes into nine clusters. Similarly, Anand *et al.* (2020), Karvar *et al.* (2022)^[9] reported five clusters and Battula *et al.* (2025) reported six clusters from 50 accessions.

Average intra and inter cluster distances

The average intra-cluster and inter-cluster D² values of six clusters were furnished in Table 2 and Fig. 2. The intra-cluster distances varied from 0 (clusters III, IV and VI to 291.02 (cluster V). Clusters III, IV and VI showed the lowest intra-cluster distance (0). On the contrary, the largest intra-cluster distance (291.02) in cluster V represented differences in the germplasm accessions within cluster. The inter-cluster distances were higher than the intra-cluster distances indicating the presence of wider genetic diversity between the clusters rather than within the clusters. Therefore, genotypes within the same cluster exhibit low levels of diversity and selection of parents within the cluster may not be considered rewarding.

The inter-cluster distance values ranged from 238.33 (cluster IV and cluster VI) to 999.39 (cluster II and III). Hence, the genotypes that lie between cluster II (SiA 3156) and cluster III (SiA 4261), with a maximum inter-cluster distance of 999.39 and a high degree of genetic diversity, could be considered potential parents. Superior recombinants can be obtained by involving these genotypes as parents in a hybridization program.

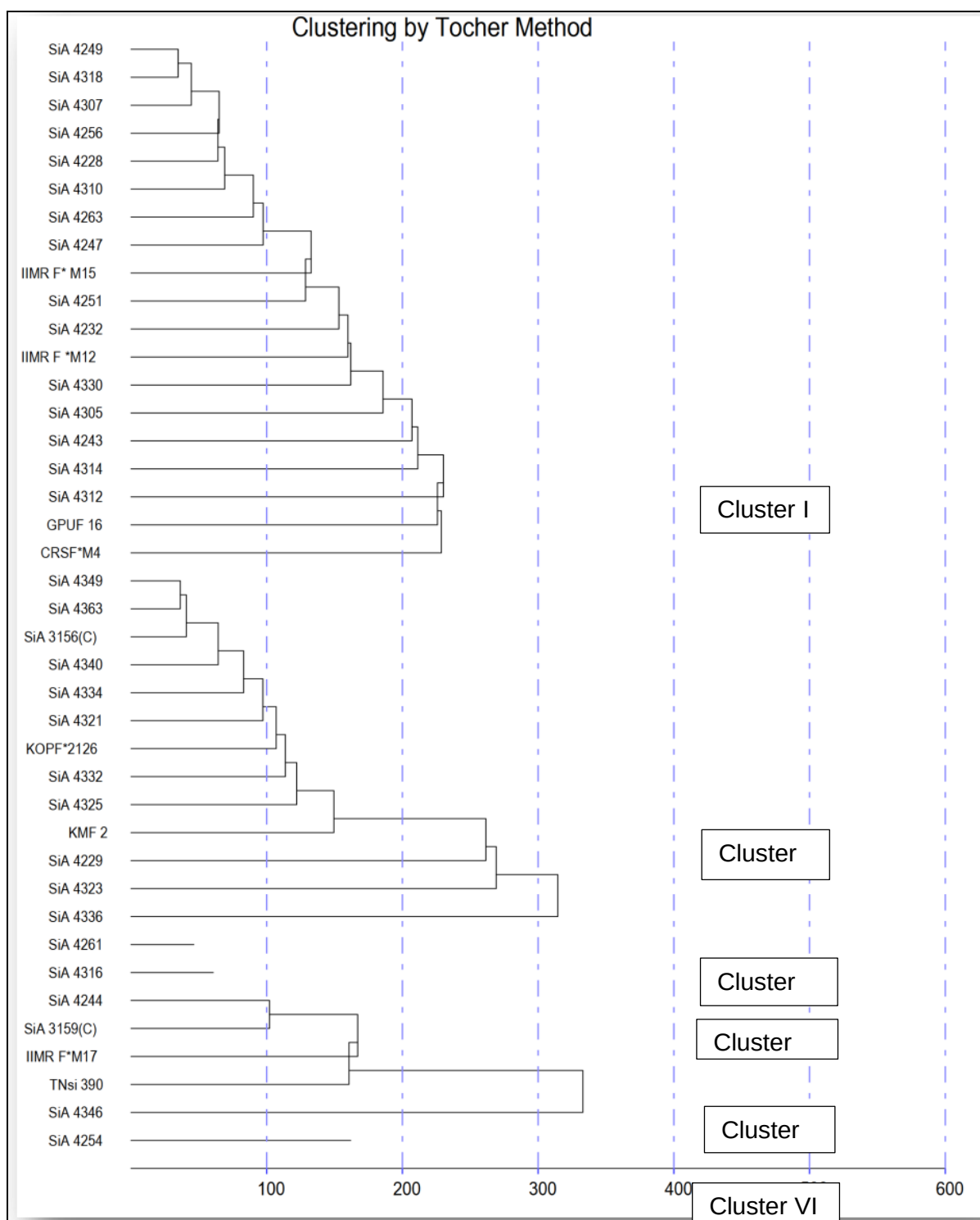


Fig 1: Clustering pattern of 40 foxtail millet genotypes by Tochers method

Table 1: Cluster composition of 40 foxtail millet genotypes based on Tochers method

Cluster Group	No. of Accessions	List of Accessions
I	19	SiA 4249, SiA 4318, SiA 4307, SiA 4256, SiA 4228, SiA 4310, SiA 4263, SiA 4247, IIMR F xM15, SiA 4251, SiA 4232, IIMR Fx M12, SiA 4330, SiA 4305, SiA 4243, SiA 4314, SiA 4312, GPUF 16, CRSFx M4
II	13	SiA 4349, SiA 4363, SiA 3156(C), SiA 4340, SiA 4334, SiA 4321, KOPF x 2126, SiA 4332, SiA 4325, KMF 2, SiA 4229, SiA 4323, SiA 4336
III	1	SiA 4261
IV	1	SiA 4316
V	5	SiA 4244, SiA 3159(C), IIMR F x M17, TNsi 390, SiA 4346
VI	1	SiA 4254

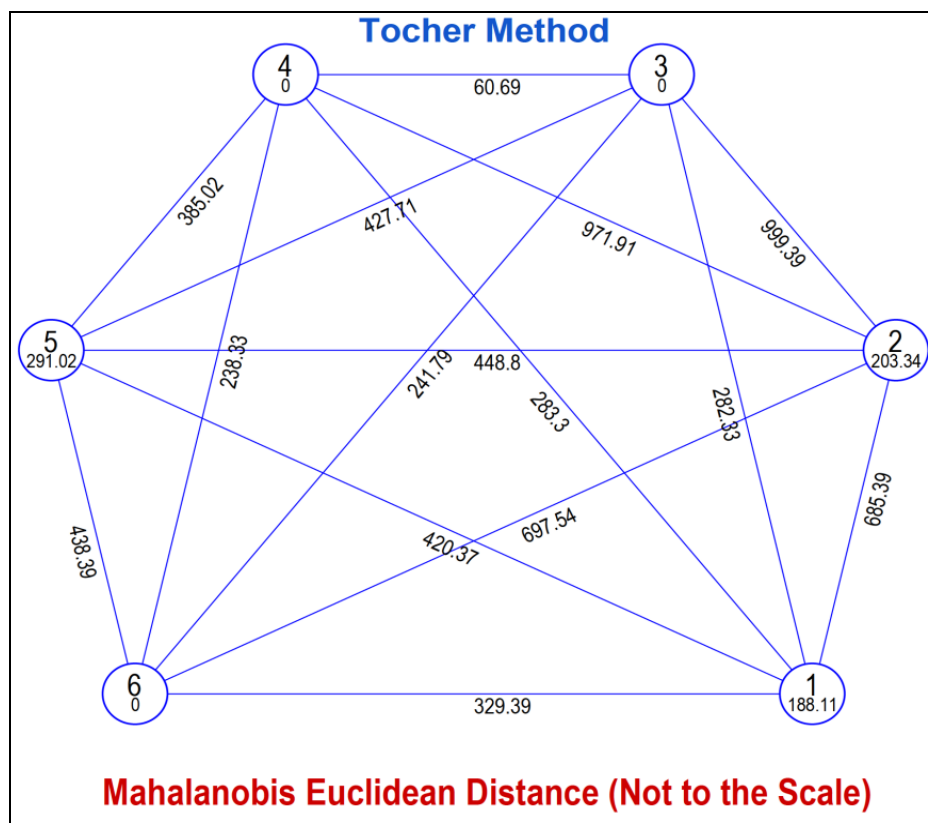


Fig 2: Cluster diagram showing average intra and inter cluster distances

Table 2: Intra and inter-cluster D^2 values among six clusters

Clusters	I	II	III	IV	V	VI
I	188.11					
II	685.39	203.34				
III	282.33	999.39	0.00			
IV	283.30	971.91	60.69	0.00		
V	420.37	448.80	427.71	385.02	291.02	
VI	329.39	697.54	241.79	238.33	438.39	0.00

Cluster means for various traits

Considerable variations between cluster means were observed for the majority of the traits under study. Cluster means of the days to 50% flowering was found to be low in cluster II (47.04) and high in cluster IV (54), whereas, the days to maturity cluster mean was noticed to be highest in cluster IV (91.50) and lowest in cluster II (86.88). The cluster means for plant height varied from 77.00 (cluster II) to 94.40 (cluster IV). Similarly, the maximum cluster mean for the number of productive tillers per plant was 2.70 in cluster IV, while, the lowest was 1.28 for cluster II. Highest cluster mean in cluster V (442.90) was found for SCMR and it was lowest in cluster VI (35.80). Relative water content had shown high cluster mean in cluster IV (88.30 %) and low in cluster II (79.83 %). Flag leaf length at flowering had the highest cluster mean of 31.05 cm in cluster V, whereas, cluster II had the lowest cluster mean of 26.36 cm. Flag leaf width at flowering recorded lowest cluster mean for cluster III (1.55 cm) and it was greatest for cluster IV (1.95 cm). Panicle length displayed highest cluster mean in cluster IV (16.10 cm) and lowest in cluster I (13.13 cm). Highest cluster mean (3.15 g) and lowest (2.80 g) were observed in cluster III, VI and cluster IV, respectively, for thousand grain weight. Straw yield per plant had maximum cluster mean in cluster IV (16.25 g) and minimum in cluster II (9.40 g). Harvest index had showed lowest cluster mean in

cluster III (32.75 %) and highest in cluster V (39.26 %). Highest cluster mean of 6.25 mg/100g was observed for cluster III and II and lowest of 2.31 mg/100g for the trait Iron content in grain. Zinc content registered high cluster mean in cluster I (4.48 mg /100g) and low in cluster VI (1.65 mg/100g). For the trait protein content, cluster III had a high cluster mean of 15.05 %, while, cluster II had a low cluster mean of 9.57 %. Cluster IV had the highest cluster mean for grain yield per plant (8.95 g), whereas Cluster II had the lowest cluster mean (5.91 g) (Table3)

A wide spectrum of variation was observed in clusters for the traits studied implying that the clusters formed were unique. The cluster IV recorded maximum cluster means for the traits viz. days to 50% flowering (54), days to maturity (91.50), plant height (94.40) and number of productive tillers per plant (2.70), relative water content (88.30%), flag leaf width (1.95 cm), panicle length (16.10 cm), thousand grain weight (3.15g), straw yield per plant (16.25g) and grain yield per plant (8.95 g). Cluster V recorded desired highest means for most of the traits viz., flag leaf length at flowering (31.05 cm), SCMR (42.90), harvest index (39.26%). Cluster II recorded desired lowest value for days to 50% flowering (47.04) and days to maturity (86.88). Cluster III reported highest means for Iron content in grain (6.25 mg/100g) and Protein content (15.05 %). Cluster I highest means for Zinc content in grains (4.48 mg/100g).

Table 3: Cluster means for yield, yield components and nutritional traits among foxtail millet genotypes.

CLUSTER	DFF	DM	PH	NPT	SCMR	RWC	FLL	FLW	PL	TGW	SYP	HI	IRON	ZINC	PC	GYP
I	49.95	88.34	82.20	1.54	39.76	86.38	28.16	1.64	13.13	2.81	11.43	35.82	5.58	4.48	9.62	6.37
II	47.04	86.88	77.00	1.28	40.93	79.83	26.36	1.56	11.50	2.83	9.40	38.38	2.31	3.65	9.57	5.91
III	50.50	88.00	81.15	1.95	40.00	84.30	27.65	1.55	12.85	2.80	13.30	32.75	6.25	3.95	15.05	6.50
IV	54.00	91.50	94.40	2.70	41.65	88.30	31.00	1.95	16.10	3.15	16.25	35.50	6.05	4.25	13.15	8.95
V	50.40	87.70	89.96	1.84	42.90	86.91	31.05	1.71	15.47	2.90	12.09	39.26	4.31	4.38	12.24	8.08
VI	51.00	91.00	89.45	1.55	35.80	82.90	29.40	1.65	15.90	3.15	15.00	30.80	4.90	1.65	11.80	6.70

DFF: Days to 50% flowering, DM: Days to maturity, PH: Plant height(cm), NPT: Number of productive tillers per plant, SCMR: SPAD Chlorophyll Meter Readings, RWC: Relative water content (%), FLL: Flag leaf length at flowering (cm), FLW: Flag leaf width at flowering (cm), PL: Panicle length (cm), TGW: Thousand grain weight (g), SYP: Straw yield per plant (g), HI:Harvest index (%), IRON: Iron content (mg/100g), ZINC: Zinc content (mg/100g), PC: Protein content (%), GYP: Grain yield per plant(g)

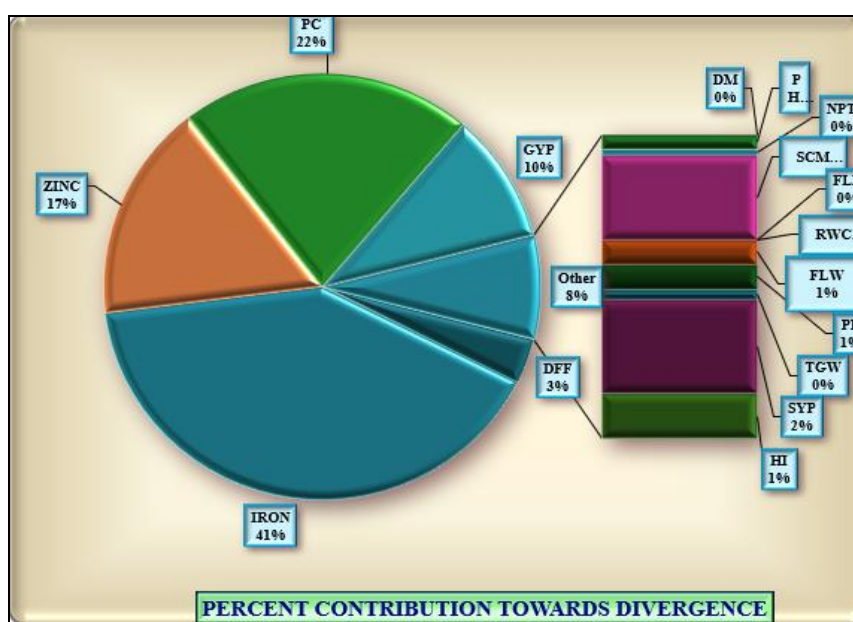
Relative contribution of traits towards diversity

Number of times each of the 16 traits appeared in first and its respective per cent contribution towards total divergence was determined and presented in Table. 4 and the per trait contribution towards diversity was illustrated through a pie diagram in Fig.3. Among all the traits studied, Iron contributed maximum (40.64%) towards genetic divergence

followed by the protein content (21.67%); zinc content (16.54%); grain yield per plant (9.87%)); days to 50% flowering (3.46%)); straw yield per plant (2.44%); SCM (2.18%) and harvest index (1.15%).

The traits flag leaf width at flowering and panicle length contributed 0.64%, plant height 0.38%, thousand grain weight 0.26%, number of productive tillers per plant 0.13% contributed very less towards total divergence inferring more homogeneity for these traits in the genetic material evaluated.

The traits viz., flag leaf length at flowering, days to maturity, relative water content had no contribution to the total divergence which is suggestive of lack of diversity for these traits in the genotypes which might be due to the operation of directional selection adopted by the breeders in the development of the genotypes. It is concluded that Iron, Protein and Zinc content in grain along with grain yield per plant were the major contributors towards divergence

**Fig 3:** Relative contribution of 16 traits to total genetic diversity in foxtail millet**Table 4:** Contribution of yield, yield components and nutritional traits towards total diversity in foxtail millet.

S. No	Trait(s)	Times ranked 1st	Contribution (%)
1	Days to 50% Flowering	27	3.46
2	Days to Maturity	0	0
3	Plant Height (cm)	3	0.38
4	Number of Productive Tillers per Plant	1	0.13
5	SCMR Reading	17	2.18
6	Relative Water Content (%)	0	0
7	Flag Leaf Length at Flowering (cm)	0	0
8	Flag Leaf Width at Flowering (cm)	5	0.64
9	Panicle Length (cm)	5	0.64
10	Thousand Grain Weight (g)	2	0.26
11	Straw Yield per Plant (g)	19	2.44
12	Harvest Index (%)	9	1.15

13	Iron Content (mg/100g)	317	40.64
14	Zinc Content (mg/100g)	129	16.54
15	Protein Content (%)	169	21.67
16	Grain Yield per Plant (g)	77	9.87

Conclusions

D² analysis resulted in the formation of six non-overlapping distinct clusters with maximum number of genotypes in cluster I (19) followed by cluster II (13), cluster V (five) and the remaining three clusters, III, IV and VI were mono-genotypic clusters with single genotype per cluster. Maximum inter-cluster distance was observed between cluster II and III, II and IV, II and VI, II and II in the decreasing order of magnitude indicating the presence of diversity in the material which can be utilized in hybridization programme. Intra-cluster distance was reported to be high in case of cluster V followed by II indicating that the accessions in the clusters V and II are more divergent than in cluster I. Cluster IV contained one genotype SiA 4316 with desirable traits and can be selected directly for immediate usage. Hybridization between the genotypes viz; SiA 4244, SiA 4251 and IIMR F x M15 with high means for majority of traits from divergent clusters can be made to incorporate all the desired traits and to get high frequency of heterotic hybrids. Inter crossing of genotypes from the cluster II (SiA 3156) and cluster III (SiA 4261) could be suggested to generate a wide range of variability followed by effective selection. Iron, Protein, Zinc content in grain and with grain yield per plant were found to be the major contributors towards divergence.

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