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Research Note

Genetic divergence studies in little millet (*Panicum sumatrense* Roth. ex. Roem. & Schult) using D^2 statistics and molecular markers

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Abstract
Little millet is well known for abiotic stress tolerance and high nutritional value. Focused research can bring this crop into mainstream cultivation with good economic return. In *kharif,* 2021, fifty little millet genotypes were assessed for genetic variation and diversity for 16 quantitative traits. Variability parameters revealed considerable variation among the genotypes for all the traits studied. Phenotypic parameters being higher than genotypic ones indicated only little environmental influence which was confirmed from high heritability and genetic advance. Most of the traits were found to be expressed additively. D^2 technique and analysis of 14 polymorphic microsatellite markers offered different clustering pattern indicating that in the present study morphological markers could not be considered true expressers of genotypic variation, but in both the clustering pattern, most IC genotypes were confined in one cluster indicating their relatedness. Moreover, total carbohydrate content was found to be the major contributor towards genetic divergence.

Keywords: Little millet, Variance parameters, Genetic Divergence, D^2 Statistics, Molecular markers

Little millet (*Panicum sumatrense* Roth. ex. Roem. & Schult syn. *Panicum millare* auct. Non Lam.) is an early maturing, self-pollinated, allotetraploid (2n = 4x =36) species of Poaceae. It grows well in arid and semi-arid climate, withstands marginal practices (Sapthagiri *et. al.,* 2020) and is commonly known as samai, samo, moraio, vari and kutki in India. The crop is rich in good cholesterol suitable for human consumption for growth and development. Its high fiber content along with high phosphorous, iron, protein, carbohydrate and fat (Patil *et al*., 2021) have made it an ideal replacement for major cereals (Reddy *et al*., 2017), and is especially good for people having low body mass. India is well known for its wide diversity in little millet and is the prime contributor of little millet germplasm collection maintained at the gene bank of International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad (Upadhyaya *et al.*, 2016). In India, total area of small millet cultivation is 21.98 million hectares with annual production and productivity of 42.95 million tonne and 1954 kg per hectare (https://agricoop.nic.in, 2022). Replacement of minor crops by modern high yielding cereals, pulses, vegetables and other cash crops offered immediate economic benefit to farmers causing traditional crops like little millet being pushed to further marginal conditions. It is on the verge of losing not only its diversity but even its existence (Devyani *et al.,* 2019). Gujarat, a state in west of India with ideal agro-climatic conditions for cultivation

of millets contributes 4.15% to total small millet production of the country (https://agricoop.nic.in, 2022) and has the potential in augmenting little millet production with proper research and management.

Success of any crop improvement programme largely depends on available genetic variability in natural population and extent to which desirable characters are heritable. Estimates of various genetic parameters like genotypic variance (σ^2g), genotypic coefficient of variation (GCV), phenotypic variance (σ^2p) and phenotypic coefficient of variation (PCV) offers an idea, respectively, on inherent variation and influence of environment in expression of traits. Heritability (H^2b) measures the proportion of phenotypic variance that is attributed to genetic variance, whereas high genetic advance for a trait indicates additive gene action for the expression of the trait. Hence, high H^2b and GA as % of mean provide better prediction of genetic gain to ensure effective selection for improvement (Sabiel *et al*., 2016; Anuradha *et al*., 2020; Matere *et al*., 2022). On the other hand, D^2 statistics (Mahalanobis, 1936) in agriculture assesses genetic divergence and identification of diverse genotypes for further exploitation through different breeding strategies.

Molecular markers being least influenced by environmental factors assess genotypic variation more efficiently than morphological traits. Among many such markers, Simple Sequence Repeat markers (SSR) are most desirable for being co-dominant, highly reproducible, frequent, multi-allelic, cost effective, better transferability, chromosome specific location, high allelic diversity and distribution throughout a genome (Manimekalai *et al*., 2018). However, reports on genetic diversity using molecular markers are rarely available in little millet mainly due to limited genomic information on the crop. Though very little attention and research were devoted to study little millet till date (Ali *et al*.,2017; Neelam *et al*., 2017), utility and transferability of SSR markers designed for other related crops (rice, maize, barnyard millet) to little millet are exploited successfully (Gautam *et al*., 2022).

Keeping the above facts under consideration, an experiment was conducted to study divergence in little millet using D^2 statistics and molecular markers.

A total of fifty genotypes of little millet was collected from Hill Millet Research Station, Navsari Agricultural University, Waghai, Gujarat (**Table 1**). The field experiment involving the above genotypes was conducted in randomized block design with three replications at the Experimental Farm of Department of Genetics & Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, during *kharif*, 2021 with a spacing of 30 × 10 cm. All the recommended package of practices was followed to raise a good crop.

Eight quantitative characters, *viz*., number of basal tillers per plant, days to 50% flowering, flag leaf blade length (cm), flag leaf blade width (cm), peduncle length (cm), panicle length (cm), plant height (cm) and 1000 grain weight (g) were recorded as per DUS guidelines (http://www.plantauthority.gov.in/). Five characters which were not mentioned in DUS guidelines, *viz*., number of productive tillers per plant, days to maturity, grain yield per plant (g), fodder yield per plant (g), and harvest index (%) were included after review of available literature by Suryanarayana and Sekhar (2018), Katara *et al*. (2019), Madhavilatha *et al*. (2020a) and Patel *et al*. (2023). Three biochemical parameters, *viz*., total carbohydrate (%), crude protein content (%) and total phenol (%) were assessed as per Anthrone method by Hedge and Hofreiter (1962), Standard Kjeldhal method by Association of Analytical Chemists, AOAC, (Washington, 1965) and Singleton *et al*. (1999), respectively.

Analysis of variance (ANOVA) was carried out following Panse and Sukhatme (1978), σ^2g and σ^2p were calculated as per the formulae suggested by Johnson *et al*. (1955). GCV and PCV were computed as reported by Burton (1952). Heritability in broad sense (H^2b) and GA as % of mean were calculated as per the formula by Hanson and Weber (1956) and Johnson *et al*. (1955), respectively. Traits were classified as having high, moderate or low genetic advance as per the method suggested by Johnson *et al*. (1955).

D^2statistics: The genetic divergence among the genotypes was computed by means of Mahalanobis' D^2 statistics followed by clustering the genotypes using Tocher's method (Rao, 1952). Intra and inter cluster distance, cluster mean and contribution of each trait to the total divergence were estimated (Singh and Chaudhary, 1985).

Genetic Diversity Using Molecular Markers: Genomic DNA from leaf samples of 50 genotypes of little millet were collected and isolated using modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1990). Quality assessment of DNA was carried out through agarose gel electrophoresis (1%) with 100 base pair (bp) DNA ladder (Fermentas, USA). Quantification of 1µl of isolated DNA of each genotype was carried out on NanoDrop-1000 (Software V.3.3.0).

A total of 32 Simple Sequence Repeat (SSR) primer pairs specific to little millet and allied species (Desai *et al*., 2021; Meniya *et al*., 2023) were used to screen the extracted DNA samples. PCR reaction consisted of 5µl of Emerald 2xPCR mastermix, 1µl of 100ng DNA, 10 picomole of both forward and reverse primer (1µl each); final reaction volume was adjusted to 10µl with 2 µl nuclease free water. Genomic DNA extracted from 50 genotypes were subjected to PCR amplification using SSR primers in a 200µl thin-walled PCR tube containing 10µl reaction mix in Applied Biosystem Thermocycler (Veriti 96 well). The amplified fragments were resolved using gel electrophoresis. Coefficients of similarity were calculated using Jaccard's similarity coefficient and cluster

Table 1. List of genotypes used in the present study

S. NO.	Genotype	S. NO.	Genotype	S. NO.	Genotype
1	IC-482815	18	IC-320419	35	IC-404902
2	IC-404842	19	IC-483263	36	IC-405052
3	IC-483193	20	IC-404903	37	IC-404844
4	IC-483142	21	IC-404956	38	IC-483220
5	IC-483221	22	IC-483180	39	IC-483082
6	IC-404846	23	IC-483286	40	DHLM-36-3
7	IC-483269	24	IC-483327	41	IC-309006
8	IC-482799	25	IC-328708	42	IC-483101
9	IC-589802	26	IC-404849	43	Waghai Vari -126
10	IC-483165	27	IC-482973	44	GPUL 11
11	IC-483434	28	IC-326747	45	GPUL 22
12	IC-483257	29	IC-483179	46	GPUL 31
13	IC-268169	30	IC-483154	47	TNPsu 227
14	IC-433197	31	IC-483292	48	TNPsu 231
15	IC-482826	32	IC-483113	49	IIMR-LM-4004
16	IC-482995	33	IC-482986	50	IIMR-LM-4006
17	IC-404910	34	IC-483155		

analysis was performed by agglomerative technique using UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SIMQUAL function and SAHN clustering function of NTSYS version 2.02 (Rohlf, 1998), respectively. Polymorphism information content (PIC) was estimated as per Botstein *et al*. (1980).

Correlation between Similarity Matrices (Mantel, 1967): The correlation between D^2 matrix and Jaccard's similarity matrix was analyzed through Mantel test using 'R' software (R-4.0.2) using "ape.5.0" package (Paradis and Schliep, 2019). Jaccard's similarity matrix was converted to dissimilarity through subtracting the values from 1.0.

ANOVA (**Table 2**) revealed high significant differences among the genotypes for all the characters indicating that the experimental materials were genetically diverse. Genotypic variances (σ^2g) and estimates of GCV were a little lower than phenotypic variances (σ^2p) and PCV, respectively, for all the traits conferring little environmental influence in the expression of the traits (Table 3). High heritability coupled with high GA as % of mean was observed for all the traits except total carbohydrate content, which established that those traits were under additive gene control and can be improved through selection in segregating populations. Similar findings in little millet were reported by Shinde *et al*. (2018), Devyani *et al.* (2019), Venkataratnam *et al.* (2019a), Anuradha *et al.* (2020) and Madhavilatha *et al.* (2020b), Behera *et al*. (2024).

D^2 Statistics :Based on the genotypic performances for different traits, D^2 technique divided the 50 genotypes under investigation into 10 clusters in which cluster I with 34 genotypes emerged as the largest (**Table 4**). Clustering pattern clearly revealed that most of the indigenous collections (IC) were closely related as they were grouped together in cluster I. However, the genotypes grouped in other clusters namely cluster II, III, IV, V, VI, VII, VIII & IX though were ICs, confirmed their distance from the genotypes in cluster I. Genotypes with unique genetic constitution than the rest of the experimental materials formed separate clusters individually (Cluster VI, VII, VIII, IX and X).

High intra cluster distance in cluster IV and cluster V with only three and two IC genotypes, respectively, further confirmed diverse genetic makeup of these genotypes (**Table 5**). As some germplasm shared same clusters with IC genotypes (**Table 4**) with few exceptions, it may be inferred that they share common ancestors or belong to the same gene pool. Higher inter cluster distance refers to wide divergence and *vice versa* (**Table 5**).

Contribution of individual characters towards total genetic divergence is given in **Table 6**. It was observed that total carbohydrate content contributed maximum (29.27%) towards total genetic divergence followed by total phenol (18.78%), peduncle length (18.04%), flag leaf blade length (9.06%) and panicle length (9.06%). These five characters together contributed to 84.21% of genetic divergence, whereas other 11 characters contributed only 15.79% cumulatively. The lowest contribution was made by number of basal tillers per plant, number of productive tillers per plant and days to maturity. On the contrary, Arunachalam *et al*. (2005) reported days to maturity to be the highest contributor for genetic divergence in little millet, whereas Selvi *et al*. (2015) reported grain yield,

Table 2. Analysis of variance for quantitative characters

S. No.	Df / Characters	Mean Sum of Squares		
		Replication	Genotype	Error
		2	49	98
1	Number of basal tillers per plant	0.7406	17.2235**	0.5259
2	Days to 50% flowering	1447.4767	421.5869**	9.9324
3	Flag leaf blade length (cm)	0.3134	54.4893**	0.1240
4	Flag leaf blade width (cm)	0.0015	0.1967**	0.0018
5	Peduncle length (cm)	0.1462	33.1656**	0.0500
6	Number of productive tillers per plant	1.1261	14.1563**	0.4644
7	Panicle length (cm)	0.1015	143.0040**	0.4732
8	Plant height (cm)	20.7954	967.1025**	10.0942
9	Days to maturity	9.2067	405.5659**	2.9822
10	1000 seed weight (g)	0.0221	0.4168**	0.0074
11	Garin yield per plant (g)	0.1174	27.4078**	0.7168
12	Fodder yield per plant (g)	25.0803	1034.2957**	8.3463
13	Harvest index %	0.0949	48.6750**	0.3972
14	Total carbohydrate (%)	0.0798	41.9026**	0.0550
15	Crude protein (%)	0.0326	5.1429**	0.0195
16	Total phenol (%)	0.0011	0.0011**	0.0011

Table 3. Estimates of different genetic parameters for 50 genotypes of little millet

S. No.	Characters	σ^2g	σ^2p	GCV (%)	PCV (%)	(H^2_b) %	G.A as % of mean
1	Number of basal tillers per plant	5.56	6.09	19.60	20.50	91.40	38.59
2	Days to 50% flowering	137.22	147.16	20.75	20.99	93.25	40.43
3	Flag leaf blade length	18.13	18.25	20.07	20.14	99.30	31.85
4	Flag leaf blade width	0.06	0.07	24.61	24.96	97.30	50.00
5	Peduncle length	11.04	11.09	25.12	25.18	99.50	51.63
6	Number of productive tillers per plant	4.56	5.03	21.39	22.46	90.80	41.98
7	Panicle length	47.51	47.98	21.52	21.62	99.00	44.10
8	Plant height	319.00	329.09	13.40	13.61	96.90	27.17
9	Days to maturity	134.19	137.17	13.40	13.54	97.80	27.29
10	1000 seed weight	0.14	0.15	16.97	17.42	94.80	34.04
11	Grain yield per plant	8.90	9.62	25.50	26.51	92.50	50.53
12	Fodder yield per plant	341.98	350.33	33.08	33.48	97.60	52.04
13	Harvest index	16.09	16.49	34.65	35.07	97.60	54.50
14	Total carbohydrate	13.95	14.00	5.79	5.80	99.60	11.90
15	Crude protein	1.71	1.73	12.61	12.68	98.90	25.83
16	Total phenol	0.0000296	0.0000298	38.90	39.02	99.40	79.90

σ^2g = Genotypic variance; σ^2p = Phenotypic variance ; GCV= Genotypic coefficients of variation; PCV= Phenotypic coefficients of variation; (H^2_b) % = Broad sense heritability % ; G.A as % of mean = Genetic advance as % of mean

Nirubana *et al*. (2017) and Patel *et al*. (2018) reported days to 50% flowering and Venkataratnam *et al*. (2019b) reported number of productive tillers to be the highest contributor.

Genetic Diversity using Molecular Markers*:* Assessment of genetic divergence was also carried out using 32 SSR markers, of which 14 markers were found to be 100% polymorphic (**Table 7**), indicating presence of variability

Table 4. Clustering of 50 genotypes of little millet based on D^2 Statistics

S. No.	Clusters	Number of genotypes	Name of genotypes
1	I	34	IC-482986, IC-483155, IC-482973, DHLM-36-3, IC-483165, IC-483220, IC-483082, IC-483257, IC-483434, IC-482826, IC-483154, IC-309006, IC-483221, IC-404846, IC-433197, IC-404910, IC-482995, GPUL 11, IC-483142, IC-482799, IC-482815, IC-328708, IC-404903, TNPsu 231, IC-405052, IC-268169, WV-126, IC-483180, IC-404849, IC-326747, IC-483286, IC-483269, IC-483113, IIMR-LM-4004
2	II	3	IC-483263, IC-404956, GPUL 22
3	III	3	TNPsu 227, IIMR-LM-4006, IC-404844
4	IV	3	IC-404842, IC-483327, IC-404902
5	V	2	IC-589802, IC-483179
6	VI	1	IC-483193
7	VII	1	IC-320419
8	VIII	1	IC-483292
9	IX	1	IC-483101
10	X	1	GPUL 31

Table 5. Average intra and inter cluster D values for 50 little millet genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**2211.29**									
II	3977.68	**1991.97**								
III	3285.88	3438.81	**1810.61**							
IV	3473.08	4970.48	6808.70	**2404.97**						
V	3101.98	4151.41	3376.16	3865.24	**2552.31**					
VI	3635.28	6798.89	4540.48	5425.77	3095.29	**0.00**				
VII	3696.17	6077.59	6846.95	3877.69	5329.88	5579.83	**0.00**			
VIII	3477.34	4190.71	3487.24	4852.83	3328.77	6515.11	7090.29	**0.00**		
IX	5759.55	10219.38	9765.45	4129.25	4596.74	3450.33	5394.98	10303.29	**0.00**	
X	3021.17	5158.16	3785.22	3827.72	3539.60	4822.49	3981.69	6201.84	4429.994	**0.00**

Table 6. Contribution of various traits towards total genetic divergence

S. No.	Characters	Contribution (%)	Cumulative (%)
1	Total carbohydrate	29.27	29.27
2	Total phenol	18.78	48.05
3	Peduncle length	18.04	66.09
4	Flag leaf blade length	9.06	75.15
5	Panicle length	9.06	84.21
6	Harvest index	7.76	91.97
7	Crude protein	5.14	97.11
8	Days to 50% flowering	1.14	98.25
9	Fodder yield per plant	0.65	98.9
10	Flag leaf blade width	0.49	99.39
11	1000 seed weight	0.24	99.63
12	Plant height	0.08	99.71
13	Grain yield per plant	0.08	99.79
14	Number of basal tillers per plant	0.07	99.86
15	Number of productive tillers per plant	0.07	99.93
16	Days to maturity	0.07	100.00

among the little millet genotypes under study. This was higher than that reported in little millet by Neelam *et al*. (2017) using RAPD markers (88.58%), and by Rajput *et al*. (2019) and Shankar *et al*. (2020) using ISSR markers (76.19% and 38.66%, respectively). PIC value which ranged from 0.400 (UGEP53) to 0.640 (DUPSSR) with an average of 0.496, was also higher than the reports (0.026 to 0.549) of Mayung *et al*. (2005) using EST-SSR markers.

The 14 responsive SSR primers generated 31 alleles with band size ranging from 52bp (UMC2252) to 558bp (LM_GE_7). Markers, *viz*., UMC1535, UMC2258 and DUPSSR were found to offer three polymorphic loci each and higher PIC value than the rest of the markers, which made those three markers useful for molecular study on little millet involving large and diverse population. Also, higher PIC value confirms wider divergence of the study material utilizable and their use in little millet improvement programmes.

Genetic relationship among Little Millet genotypes and cluster composition: Genetic similarity between genotypes was calculated based on Jaccard's similarity (J) coefficient. Genetic coefficient of similarity among the genotypes ranged from 0.19 to 0.94 and the average similarity coefficient was (0.57). Low genetic similarity as observed between IC-404842 and IC-483113

Table 7. Result of SSR marker analysis

S. No.	Primer	Primer name	F/R	Sequence (5′ - 3′)	T_m	Amplicon size (bp)	Number of alleles	PIC
1.	1L	LM_GE_1	F	TTTGGAACATGAAATAGCTTG	59.10	306-380	2	0.496
			R	GGAACAGCTTGTGATAGAGTG				
2.	2L	LM_GE_2	F	TGGATGAGATGTTGAAATACC	58.90	312-332	2	0.425
			R	ACCTGAAATTTTGGCTAAGTC				
3.	4L	LM_GE_4	F	CGAGAGAACAAAATCTGGATA	58.80	401-423	2	0.499
			R	GGAACAGCTTGTGATAGAGTG				
4.	5L	LM_GE_5	F	TATCCTCAAACAAAGCCAATA	58.80	406-433	2	0.496
			R	TTAACATGCTCCAATCAGTCT				
5.	7L	LM_GE_7	F	TTTTCCACGATGGAATATAGA	58.70	515-558	2	0.469
			R	GGAACAGCTTGTGATAGAGTG				
6.	5B	BM_GE_5	F	CTATAGCACGAAAAACCATTC	58.40	414-498	2	0.498
			R	AAAGAGAGAGCTTTGCATTCT				
7.	67F	UGEP53	F	TGCCACAACTGTCAACAAAAG	56.60	101-200	2	0.400
			R	CCTCGATGGCCATTATCAAG				
8.	20L	LM_Vg_5	F	GTATCTGTCTTGCTTTCCACA	59.50	484-741	2	0.546
			R	TAGAATAGAGGGAACGTGGTC				
9.	8M	UMC2252	F	CACTGCACTGCAAGGTACATACG	62.40	52-87	2	0.469
			R	GTCTTTGACCCCTTCCTCTTCTTG				
10.	9M	UMC1535	F	CAAGGCACCCACACACATACATA	61.50	61-295	3	0.401
			R	GGCAGAGAGATGAAAAAGAATGGA				
11.	41M	UMC2258	F	GAATAAGACCAGACAGCACCGAAC	61.50	136-352	3	0.612
			R	AAGATTGTATAAATGGCAGCCACG				
12.	47M	UMC2101	F	CCCGGCTAGAGCTATAAAGCAAGT	63.15	123-182	2	0.494
			R	CTAGCTAGTTTGGTGCGTGGTGAT				
13.	124M	UMC2226	F	TGCTGTGCAGTTCTTGCTTCTTAC	62.15	142-241	2	0.494
			R	AGCTTCACGCTCTTCTAGACCAAA				
14.	64M	DUPSSR	F	TCAGTGCTTTCATTGTAACGA	57.30	143-271	3	0.640
			R	ATAAACATCTTGCCAGCAAA				
				Total		-	31	-
				Average		-	2.21	0.496

L= Little millet B= Barnyard millet F= Finger millet M= Maize F: Forward; R: Reverse T_m: Melting temperature (°C) PIC = Polymorphism Information Content

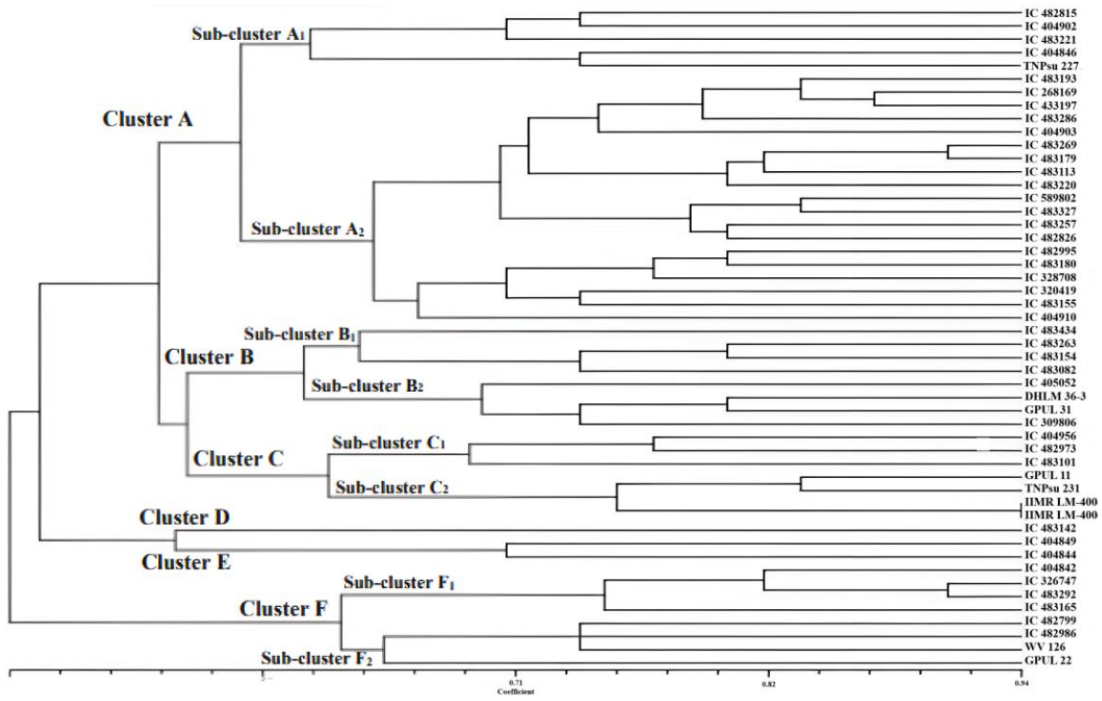

Fig.1. Dendrogram based on Jaccard's similarity coefficients

(**Fig. 1**) indicated wider diversity at genome level which can be exploited to develop variable segregating population suitable for genome mapping, whereas high genetic similarity as observed between IIMR-LM-4004 and IIMR-LM-4006 indicated their common gene pool.

A dendrogram was constructed based on the similarity coefficients revealing six main clusters, *viz*., Cluster A, Cluster B, Cluster C, Cluster D, Cluster E and Cluster F with 24, 8, 7, 1, 2, 8 genotypes, respectively (**Fig. 1**). A total of 24 IC genotypes were grouped in cluster A, indicating high genetic similarity, of which 17 genotypes were found to be in the same cluster (Cluster I) based on D^2 technique.

It was observed that in both the methods of clustering, 17 IC genotypes came together in same cluster, *i.e*., in cluster I (D^2 analysis) and in cluster A (molecular clustering), which suggested that genetic make-up of these 17 IC genotypes highly corresponds with their phenotypic expression and indicated their less interaction with environment. But cluster composition differed much for other clusters developed through the two methods indicating that clustering pattern based on SSR primers were not the true reflection of their morphological performances. This was confirmed through Mantel test (Mantel, 1967) where correlation between dissimilarity matrices was only r = 0.072 (p = 0.069). In many agricultural studies it was found that morphological diversity does not comply with molecular diversity much (Das *et al*., 2013). It could be overcome through inclusion of sufficient number morphological markers which contribute much to divergence among genotypes. Moreover, eight out of the 14 polymorphic SSR primers used in the study were not specific to little millet genome. Application of little millet genome specific EST-SSR markers, which could differentiate genotypes on their genomic expression rather than only on their genomic differences could come of much help in resolving the issue.

REFERENCES

Ali, A., Yu, M. C., Do, Y. H., Sukyeung, L., Jin, H. L., Sejong, O. and Myung, C. L. 2017. Development of EST-SSRs and assessment genetic diversity in little millet (*Panicum sumatrense*L.) germplasm. *Korean Journal of Plant Research*, **30** (3):287-297.

Anuradha, N., Kranti, P., Patro, T. S. S. K., Sandhya, Y. and Triveni, U. 2020. Association studies in little millet (*Panicum sumatrense* L.) for yield and other important traits. *International Journal of Current Microbiology and Applied Sciences,* Special issue, Pp 1465-1472.

Arunachalam, V., Rengalakshmi, R. and Kubera Raj, M. S. 2005. Ecological stability of genetic diversity among landraces of little millet (*Panicum sumatrense* L.) in south India. *Genetic Resources and Crop Evolution*, **52**:15-19. [Cross Ref]

Behera, R., Singamsetti, A., Rout, S., Nanda, S. and Sharma, S. 2024. Genetic variability and character associations for grain yield and other secondary traits in little millet *Panicum sumatrense* L. at

Eastern Ghats Zone of Odisha. *Electronic Journal of Plant Breeding*, **15** (1):239-245 [Cross Ref]

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, **32** (3):314-331.

Burton, G. W. 1952. Qualitative inheritance in grasses. In: Proceedings of the 6th International Grassland Congress, Pennsylvania State College, **3** (9):17-23.

Das, A., Pandey, S. and Dasgupta, T. 2013. Association of heterosis with combining ability and genetic divergence in sesame (*Sesamum indicum* L.). *International Journal of Scientific & Technology Research*, **2** (12):307-314.

Department of Agriculture, Corporation and Farmers Welfare 2022. Available from: https://agricoop.nic.in/

Desai, H., Hamid, R., Ghorbanzadeh, Z., Bhut, N., Padhiyar, S., Kheni, J. and Tomar R. S. 2023. Genic microsatellite marker characterization and development in little millet (*Panicum sumatrense*) using transcriptome sequencing. *Scientific Reports*, **11** (1):20620. [Cross Ref]

Devyani, K., Rajiv, K., Deepthi, R., Chaudhari, S. and Zapadiya, V. 2019. Genetic- morphological analysis in little millet (*Panicum sumatrense* L.) under different sown conditions. *International Journal of Current Microbiology and Applied Sciences*, **8** (5):177-189. [Cross Ref]

Doyle J. J. and Doyle J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**:13-15. [Cross Ref]

Gautam, A., Tantwai, K., Nema, S., Tripathi, N. and Tiwari, S. 2022. Cross transferability of ssr markers from finger millet, pearl millet and rice to Indian little millet and their genetic diversity analysis. *Biological Forum- An International Journal,* **14** (4): 1016-1023.

Hanson, W. D. and Weber C. R. 1956. Analysis of genetic variability from generations of plant progeny lines in soybean. *Crop Science*, **2** (1):63-67. [Cross Ref]

Hedge, J. E. and Hofreiter, B. T. 1962. Estimation of carbohydrate. Methods in carbohydrate chemistry. Academic Press, New York, Pp 17-22.

Johnson, H.W., Robinson, H. F. and Comstock, R. E. 1955. Genotypic and phenotypic correlation in soyabean and their implication in selection. *Agronomy Journal,* **47** (7):447-483. [Cross Ref]

Katara, D., Kumar, R., Rajan, D., Chaudhari, S. B. and Zapadiya, V. J. 2019. Genetic-morphological analysis in little millet (*Panicum sumatrance* L.) under different sown conditions. *International Journal of Current Microbiology and Applied Sciences*, **8** (5):177-189. [Cross Ref]

Madhavilatha, L., Rao, M. S., Priya, M. S. and Kumar, M. H. 2020a. Genetic divergence studies for yield and yield contributing traits in little millet (*Panicum sumatrense* L.). *Andhra Pradesh Journal of Agricultural Sciences*, **6** (4):202-206.

Madhavilatha, L., Rao, M. S., Priya, M. S. and Kumar, M. H. 2020b. Variability, character association and path analysis studies in little millet (*Panicum sumatrense* L.). *Andhra Pradesh Journal of Agricultural Sciences*, **6** (1):49-54.

Mahalanobis, C. R. 1936. On the generalization distance in statistics. In: *Proceeding of The National Institute of Sciences of India*, **11** (1):49-55.

Manimekalai, M., Dhasarathan, M., Karthikeyan, A., Murukarthick, J., Renganathan, V. G., Thangaraj, K., Vellaikumal, S., Vanniarajan, C. and Senthi, N. 2018. Genetic diversity in the barnyard millet (*Echinochola frumentacea*) germplasms revealed by morphological traits and simple sequence repeat markers. *Current Plant Biology*, **14**:71-78. [Cross Ref]

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach.

Matere, B. S., Bhavsar, V. V., Mate, P. A. and Girase, V. S. 2022. Genetic variability of yield and its components in little millet (*Panicum sumatrense* L.) local germplasm. *Journal of Agriculture Research and Technology*, **47**:258-263. [Cross Ref]

Mayung, C. L., Yu, M. C., Do, Y. H., Sukyeung, L. and Sejong, O. 2005. Assessment of genetic diversity in little millet (*Panicum sumatrense* L.) germplasm using EST-SSR. In: Proceedings of the Korean Society of Crop Science Conference*, The Korean Society of Crop Science*, **22**:230-232

Meniya, V. H., Padhiyar, S. M., Desai, H., Kheni, J. K. and Tomar, R. S. 2023. Development and validation of microsatellite markers for Barnyard Millet obtained by partial genome assembly. *Annals of Arid Zone*, **62** (1):83-89. [Cross Ref]

Neelam, T., Sharad, T. and Niraj, T. 2017. Genetic characterization of Indian little millet (*Panicum sumatrense* L.) genotypes using random amplified polymorphic DNA markers. *Agriculture and Natural Resources*, **52** (4):347-353. [Cross Ref]

Nirubana, V., Ganesamurthy, K., Ravikesavan, R. and Chitdeshwari, T. 2017. Genetic diversity studies in kodo millet (*Paspalum scorbiculatum* L.) germplasm accession based on biometrical and nutritional quality traits. *International Journal of Current Microbiology and Applied Science*, **6** (10):832-839. [Cross Ref]

Panse, V. G. and Sukhatme, P. V. 1978. Statistical methods for agricultural workers. Published by Indian Council for Agricultural Research. New Delhi, India.

Paradis, E. and Schliep, K. 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics, **35** (3):526-528. [Cross Ref]

Patel, S. N., Patil, H. E., Modi, H. M. and Singh, J. T. 2018. Genetic variability study in little millet (*Panicum miliare* L.) genotypes in relation to yield and quality traits. *International Journal of Current Microbiology and Applied Sciences*, **7** (6):2712-2725. [Cross Ref]

Patel, U. N., Patil, H. E. and Dela, G. J. 2023. Genetic diversity studies using Principal Component Analysis and D2 statistics in little millet (*Panicum sumatrense* L.) genotypes. *The Pharma Innovation Journal*, **12** (1):1106-1111.

Patil, H. E., Vavdiya, P. A., Vadodariya G. D. and Patel B. K. 2021. A dual purpose, high yielding little mille *Panicum sumatrense* variety 'GV-4' Ambika for cultivation in Gujarat. *Electronic Journal of Plant Breeding*, **12** (4):1321-1330. [Cross Ref]

Protection of Plant Varieties and Farmers' Right Authority Available from: https://www.plantauthority.gov.in/

Rajput, L. P., Tantwai, K., Puniya, S. K. and Koji, T. 2019. Assessment of genetic variability among the landraces of little millets *Panicum sumatrense* from different district of Madhya Pradesh. *International Journal of Current Microbiology and Applied Sciences*, **8** (4):2686-2693. [Cross Ref]

Rao, C. R. 1952. Advanced Statistical Methods in Biometrical Research, John Wiley and Sons, New York.

Reddy, M. C. V., Reddy, P. V. R. M., Munirathnam, P. and Gowada, J. 2017. Studies of Genetic variability in yield and yield attributing traits of finger millet (*Eleusine coracana* L.) Gaertn.). *Indian Journal of Agriculture Research*, **47** (6):459-552.

Rohlf, F. J. 1998. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0 user guide.

Sabiel, S. A., Noureldin, I., Baloch, S. K., Baloch, S. U. and Bashir, W. 2016. Genetic variability and estimates of heritability in sorghum (*Sorghum bicolor* L.) genotypes grown in a semiarid zone of Sudan. *Archives of Agronomy and Soil Science*, **62** (1):139-145. [Cross Ref]

Sapthagiri, S., Krishnan, R., Chinnamuthu, C. R., Chandra Sekaran, N., Chandrasekhar, C. N., and Geethalakshmi, V. 2020. Performance of little millet under rice fallow condition. *Madras Agricultural Journal*, **107** (1-3):43-47.

Selvi, V. M., Nirmalakumari, A. and Subramanian, A. 2015. Assessment of genetic diversity using morphometric traits in little millet (*Panicum sumatrense* L.). *Trends in Biosciences*, **8** (1):119-125.

Shankar, C. 2020. *Characterization of little millet genotypes using morphological traits and ISSR markers* (Master's thesis, Dr. Rajendra Prasad Central Agricultural University, Pusa).

Shinde, S., Karad, R. and Kakde, R. 2018. Correlation and path analysis studies in little millet (*Panicum sumatrense* L.). *Green Farming*, **9** (1):21-23.

Singh, R. K. and Chaudhary, B. D. 1985. Biometrical methods in quantitative genetics (3rd edition). Kalyani Publication, New Delhi.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: *Methods in enzymology,* **299**:152-178. [Cross Ref]

Suryanarayana, L. and Sekhar, D. 2018. Genetic divergence studies in little millet (*Panicum sumatrense* L.). *The Pharma Innovation Journal*, **7** (7):944-945.

Upadhyaya, H. D., Vetriventhan, M., Dwivedi, S. L., Pattanashetti, S. K. and Singh, S. K. 2016. "Proso, barnyard, little, and kodo millets," in genetic and genomic resources for grain cereals improvement. *Frontiers in Plant Science*, Pp 321-343. [Cross Ref]

Venkataratnam, T., Latha, L., Madhavi., Sekhar, M. and Nirmal, A. 2019a. Studies on genetic variability in little millet (*Panicum sumatrense* L.). *International Journal of Chemical Studies*, **7** (6):2948-2951. [Cross Ref]

Venkataratnam, T., Latha, L., Madhavi., Sekhar, M. and Nirmal, A. 2019b. Genetic divergence study in little millet (*Panicum sumatrense* L.). *Agricultural Science Digest*, **39** (3):210-214. [Cross Ref]

Washington, D. C. 1965. Association of analytical chemists, Official methods of analysis, 10th edition.