RESEARCH ARTICLE

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Understanding the Genetic Basis of Yield-related Traits in Little Millet (*Panicum sumatrense* Roth. ex. Roem. and Schultz.) Germplasm through Association and Diversity Analysis

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ABSTRACT

Background: Little millet is an important crop grown by tribal farmers in India. Genetic variability can be exploited to develop new varieties with higher yield. However, yield is complex and depends on multiple interconnected component characters. Diversity analyses, such as D² analysis, are used to evaluate the diversity among genotypes and determine the traits that contribute the most diversity in a given population. These analyses are crucial for achieving the goal of developing new varieties with increased yield. **Methods:** In this study, 323 little millet genotypes were evaluated using an Augmented RCBD, focusing on ten quantitative traits. The experiment was conducted during the rabi season of 2020-2021, and good agronomic practices were followed. D² cluster analysis and path analysis were used to analyze the data, with the "R" tool and the "biotools" and "agricolae" packages, respectively. **Result:** In this study, 323 little millet genotypes classified into thirteen distinct clusters based on Mahalanobis's D² statistics, reflecting differences in their phenotypic characteristics. The largest cluster (cluster I) included 243 genotypes, while the smallest clusters (Cluster IX, Cluster X, Cluster XI, Cluster XII and Cluster XIII) had only 1 genotype. The inter-cluster distance varied, with the largest value (577.7) between cluster V and XII. This analysis can be useful for identifying desirable genotypes and understanding the population's genetic diversity and structure.

Key words: Augmented RCBD, Mahalanobis's D2, Path analysis.

INTRODUCTION

Little millet (*Panicum sumatrense*), a climate-resilient crop belonging to the Poaceae family, is an important crop that originated in India and is primarily grown by tribal farmers as a rain-fed crop (Nirmalakumari *et al.*, 2010). Traditional tribal farmers have been growing and preserving small millets, which are underutilized crops that are nutrient-dense and have the potential to improve nutrition and address the current problem of micronutrient malnutrition. Little millets are rich in B vitamins and minerals such as potassium, phosphorus, iron, zinc and magnesium and outperform crops like wheat, rice and maize in terms of nutritional value. The crop is naturally grown in tropical regions and requires only a small amount of water throughout its lifecycle. It has a promising future in the realm of food and nutritional security (Selvi *et al.*, 2015 and Kundgol *et al.*, 2014).

In Tamil Nadu's hilly regions, such as the Javvadhu hills, the Kolli hills, and the Sitheri tribes, farmers grow a traditional landrace of little millet. However, rice, wheat and maize are the most commonly grown crops by farmers and many of them are unaware of nutritionally rich crops like little millet, which have high yield potential and greater nutritional value. Genetic variability exists in crops that can be exploited for yield and related traits *via* crop breeding. By doing so, a new variety can be developed that can meet the increasing demand of a population. It is necessary for breeders to select parents with genetically divergent characteristics for crossing and further selection in subsequent generations. For the development of self-pollinated crops such as millet in order

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to develop these varieties. Several measures utilizing quantitative characters were developed (Savankumar et al., 2018).

Yield is a complex trait that depends on multiple interconnected component characters and changing one component can affect the entire cause-and-effect system. While the correlation coefficient measures the association between two characters, it doesn't necessarily indicate a direct causal relationship or the contribution of one character's variation to the others. Therefore, although

correlation coefficients can help determine the components of yield, but it doesn't provide a complete understanding of the direct and indirect influences of each component character. Path coefficient analysis is a statistical tool developed by Wright (1921) that can provide a better index for selection by partitioning the association into direct and indirect effects through other independent variables. This investigation aims to estimate the direct and indirect effects of different characters on grain yield per plant using path coefficient analysis

In crop breeding program improvement of yield is the main objective for any crop. The diversity analyses such as D² analysis used to assess the diversity among genotypes and evaluate the influence of traits which contribute more diversity in the given population (Mahalanobis, 1936). Path analysis can provide valuable insights for improving the breeding and selection of little millets to enhance their yield, quality, and nutritional value. This method can be applied to understand how different factors influence the overall performance of the crop and can guide the breeding programs to select the best genotypes.

MATERIALS AND METHODS

In this study, a total of 323 little millet genotypes, which included four checks, were evaluated using an augmented randomized complete block design (ARCBD) (Federer et al. (1975). The little millet accessions were obtained from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) and the Ramiah gene bank, Department of Plant Genetics Resources at Tamil Nadu Agricultural University, Coimbatore. The experiment was conducted during the rabi season of 2020-2021 at the Centre of Excellence in Millets, Athiyandal. The little millet genotypes were sown with a spacing of 30 cm × 20 cm, and good agronomic practices were followed. The study focused on ten quantitative traits, including days to fifty per cent flowering, plant height (cm), flag leaf length (cm), flag leaf breadth, number of productive tiller/plant, panicle length (cm), number of nodes per plant, peduncle length (cm), dry fodder yield (g) and single plant grain yield (g). To analyze the data, D² cluster analysis and path analysis were performed using the "R" tool and the "biotools" and "agricolae" packages, respectively.

RESULTS AND DISCUSSION

Path analysis

The path analysis of grain yield indicated that several factors contributed significantly and positively to grain yield per plant at the phenotypic level (Table 1). These traits include days to 50% flowering, plant height, flag leaf breadth, number of productive tillers per plant, panicle length, peduncle length, number of node and dry fodder yield per plant. These traits had a direct effect on grain yield per plant and they exhibited a strong positive association with grain yield. Overall, these findings suggest that these traits are important for maximizing grain yield and it should be considered when selecting plants for breeding or cultivation. Similar results were also reported in little millet by Nandini et al. (2016). who found that the number of effective tillers per plant, panicle length, and plant height were positively associated with single plant grain yield. Nirmalakumari et al. (2010) also reported that days to 50% flowering, plant height, total number of effective tillers per plant and flag leaf breadth were important factors positively associated with single plant grain yield. Anuradha et al. (2017) and Venkataratnam et al. (2019) found that number of productive tiller and panicle length positively associated with single plant grain yield. In addition, Jyothsna et al. (2016) and Naidu et al. (2021) found that the number of effective tillers per plant, days to 50% flowering, flag leaf length, panicle length and dry fodder yield per plant were all positively associated with grain yield. Katara et al. (2020) conducted a path coefficient analysis in little millet, which indicated that dry fodder yield per plant and days to 50% flowering had significant and positive direct effects on the grain yield of individual plants. Sarak et al. (2023) found that number of productive tiller per plant positively associated with single plant grain yield. According to the study by Nagar et al. (2020), the traits that

Table 1: Phenotypic path coefficient analysis for yield and yield attributing traits in 323 little millet genotypes.

Traits	PH	FLL	FLB	NPT	PL	NN	PEL	DFY	DFF
PH	0.02	-0.07	0.07	-0.01	0.05	0.06	0.02	0.08	0.00
FLL	0.01	-0.12	0.09	0.00	0.03	0.01	0.01	0.07	-0.01
FLB	0.01	-0.07	0.14	-0.02	0.04	0.03	0.01	0.09	0.01
PT	0.00	0.00	-0.03	0.08	-0.02	-0.03	-0.01	0.07	-0.01
PL	0.01	-0.03	0.05	-0.01	0.13	0.02	0.01	0.11	0.01
NN	0.00	0.00	0.02	-0.01	0.01	0.26	0.01	0.11	0.02
PEL	0.01	-0.03	0.03	-0.01	0.04	0.04	0.05	0.02	-0.01
FW	0.00	-0.03	0.04	0.02	0.05	0.08	0.00	0.32	0.02
DFF	0.00	0.01	0.02	-0.01	0.01	0.05	0.00	0.07	0.10

DFF- Days to fifty per cent flowering, PH- Plant height (cm), FLL- Flag leaf length (cm), FLB- Flag leaf breadth (cm), DFW- Dry fodder yield (g)/plant, PEL- Peduncle length (cm), NN- Number of node/plant, PL- Panicle length (cm), NPT- Number of productive tiller/plant and SPY- Single plant grain yield (g).

had the highest positive direct effect on grain yield per plant in their experiments were dry fodder yield per plant, followed by days to 50% flowering, panicle length, peduncle length, and number of productive tillers per plant. The strong correlation between fodder yields with single plant grain yield in little millet suggests that selecting for these traits directly would lead to an increase in grain yield. This finding is similar to previous studies conducted by Anuradha et al. (2017) in little millet and Arya et al. (2017) in barnyard millet, which also found that these traits have a significant impact on grain yield. Sarak et al. (2023) revealed that single plant grain yield positively associated with dry fodder yield and panicle length, as identified in their study. Therefore, it may be beneficial to focus on direct selection for fodder yield and panicle length to improve grain yield in little millet. These consistent findings across different studies provide further evidence that these traits are crucial for achieving high grain yield in little millet, and they should be considered when selecting plants for cultivation or breeding.

D² analysis

The genetic divergence of three hundred twenty three little millet genotypes was quantitatively assessed by using the Mahalanobis D² statistic to measure their yield and contributing characters. The main factor contributing to genetic divergence in this study was the days to fifty per cent flowering from individual plants, followed by plant height, panicle exertion, plant height, dry fodder yield per plant and panicle length from individual plants (Fig 1). The

study conducted by Arunachalam et al. (2005), he observed that genetic divergence was mainly attributed to the traits of days to maturity, followed by plant height. Results showed that the variance due to genotypes was significant for all of the characters analyzed, which prompted further analysis using D2. Based on the D2 values and utilizing Tocher's method, the three hundred twenty three genotypes were grouped into thirteen different clusters. Within each cluster, the genotypes had a lower average D2 value than those belonging to different clusters. Details on the distribution of genotypes among the various clusters can be found in Table 2. Out of thirteen clusters, cluster I was the largest comprising of 243 genotypes followed by cluster II had forty four genotypes, cluster IV had eleven genotypes, cluster III had seven genotypes, cluster V had six genotypes, cluster VI had three genotypes, cluster VII and VIII had two genotypes and the remaining clusters IX, X, XI, XII and XIII consisted of one genotype each indicating high degree of heterogeneity among the genotypes.

The utilization of intra and inter cluster distances aided in identifying distinct parental candidates for crop enhancement initiatives. Table 3 and Fig 2. Outlines the intra and inter cluster distance values for the thirteen clusters. The average D² values for intra cluster distances varied from 0.00 to 88.50. The presence of a high degree of variability within the cluster is indicated by the high intra-cluster distance, suggesting potential for improvement through various selection methods. Cluster VIII showed the highest intra-cluster distance of 88.5, indicating the greatest

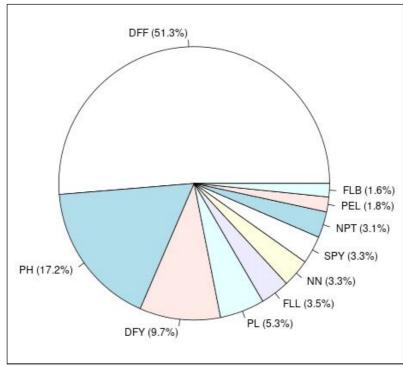


Fig 1: Relative contribution of quantitative characters towards genetic divergence in 323 little millet genotypes.

variability, followed by cluster VII (77.6), cluster V (73.7), cluster IV (67.0), cluster II (61.2), cluster III (51.9), cluster I (46.7) and cluster VI (44.9). This suggests that there is significant genetic variation among the genotypes in the same cluster. The results suggest that the genotypes within cluster VIII displayed a relatively higher level of diversity among themselves. This finding is consistent with previous reports by Madhavilatha *et al.* (2020), Venkataratnam *et al.* (2019), Sujata *et al.* (2021), Shanmugam *et al.* (2023), Suthediya *et al.* (2021) and Suryanarayana and Sekhar (2018), Negi *et al.* (2017) and Shinde *et al.* (2013) which also demonstrated substantial genetic diversity. Clusters IX, X, XI, XII and XIII had intra cluster distance values of zero because they contained only one genotype each. The largest

inter cluster D^2 value was observed between cluster V and XII (577.7), followed by between cluster VI and IX (553.5), cluster V and XII (467.2), cluster VIII and X (432.5), cluster VI and X (409.8), cluster VI and XIII (377.6), cluster VI and VII (372.4) and cluster IV and VI (371.8). This indicates that the genotypes from these clusters may be directly utilized in crop improvement programs to develop heterotic little millet varieties.

According to Kumar *et al.* (2010), Dinesh *et al.* (2010), Wolie *et al.* (2013), Sudeepthi *et al.* (2020), and Amegan *et al.* (2020) the highest level of heterosis is anticipated in crosses made between parents that belong to the most dissimilar clusters. The study found that the lowest D² value was observed between cluster XII and XIII (90.8), followed

Table 2: Clusters and its genotypes based mahalanobsis D² analysis

Clusters	Number of genotypes	Name of the genotypes
Cluster I	243	F0080062, ERP99, TNAU63, F00800174, MLT-3-K19, TNPSu223, TNPSu203, TNPSu224, TNPSu231, Paiyur-2, AYT-227, LMV-547, LMV-549, TNPSU230, LMV-523, LMV-536, LMV-548, LMV-541, LMV-528, LMV-544, LMV-578, MLT-2-K19, MLT-3-K19, MLT-4-K19, AYT-235, 541673, 405084, 371968, 418532, 326747, 589792, 268169, 483419, 483198, 404968, 404842, 483027, TK36, TNAU63, OLM217, OLM208, Paiyur-1, BIREGNDI, F00800099, ATL-1, 435032, 483263, 480173, 404985, 482923, 404845, 404353, 482995, 483088, 483257, 483082, 460083, 405082, 405050, 589793, 404910, 404966, 404960, 404908, 405043, 483425, 405083, 404959, 404932, 405048, 483023, 483563, 488043, 405026, 505114, 488204, 589798, 310568, 483270, 483114, 7102, 483292, 482987, 483132, 483264, 498110, 567454, 587687, 484241, 405042, 386409, 483081, 493420, 483430, 408102, 405052, 404907, 495785, 589799, 497971, 404952, 589784, 589905, 406043, 454964, 483188, 482185, 160, 206, 155, 210, 161, 163, 104, 102, 11, 7, 483139, 483085, 483149, 7058, 488134, 480986, 7051, 482826, 539787, 43439, 483240, 483247, 482973, 569476, 483107, 408650, 483108, 405069, 404956, 326802, 483195, 340135, 7006, 7106, 7107, IVT-165, IVT-157, IVT-159, IVT-111, IVT-212, IVT-154, IVT-166, IVT-214, IVT-216, IVT-105, IVT-101, IVT-114, IVT-112, 3001, 4079, 3009, 3120, 4117, 408, S-14, 4150, 4045, 4096, 4139, 3197, 3037, 3068, 3020,
		4194, 7013, 7055, 7066, 7112, 7015, 7007, 7012, 7108, 7113, 7016, 7062, 7004, 7110, 7002, 7054, 7101, 7057, 7111, 3011, F00800107, 4114, 4080, 2151, 202, 4058, 4056, 3072, 4007, 4092, 4019, 4006, 3073, 3186, 3196, 3076, 3163, S-1, 308, 420, 412, 407, 403, 307, 3141, 3140, 4109, 3084, 4198, 3200, 406, F00800177, 4163, 4140, 4167, 3191, 3187, 3202, 4043, 4171, 4002, 4190, 4182, 4083, 3122, 3040, 4122, 3182, 4004, 4003, 4143 and 4062.
Cluster II	44	MLT-05-K1, ELB-9, TNPSu74, 404933, 589888, 482909, 483165, 404018, GUAJARAT_VAR, 404382, 483192, 483201, 589786, 409328, 483003, 483051, 483269, 483494, IVT-16, IVT-211, IVT-155, IVT-213, IVT-207, IVT-108, 3027, 7061, 3088, 4082, 4072, 3178, 3089, 404, S-04, 3077, 3133, 4074, 3195, 3157, 4670, 3139, 3030, 3116, 4068 and 4001
Cluster III	7	482977, OLM-36, 7109, 4078, 4031, 4093 and 4138
Cluster IV	11	LMV-540, 306493, OLM203, 483155, IVT-116, 4149, 4113, 3013, 3190, 3108 and 4005
Cluster V	6	483101, IVT-162, IVT-151, IVT-103, 4124 and 4160
Cluster VI	3	404897, 493476 and 3012
Cluster VII	2	483197 and 4060
Cluster VIII	2	482891 and 4193
Cluster IX	1	TNPSu231
Cluster X	1	LMV-539
Cluster XI	1	LMV-543
Cluster XII	1	7008
Cluster XIII	1	4032

by cluster I and X (92.2), cluster IV and IX (93.1) and cluster II and VI (95.5). The results indicated that the inter-cluster distance was greater than the intra-cluster distance, suggesting a high level of genetic variation between the clusters. Genotypes within the same cluster exhibited minimal divergence, indicating that transgressive segregants cannot be expected from crosses between genotypes within the same cluster. To obtain desirable transgressive segregants, it is recommended to use parents from different clusters with significant divergence. Within any cluster, genotypes demonstrating high seed yield and favourable component traits can be utilized for hybridization or direct adoption, followed by selection. Similar findings were reported by Bedis et al. (2007) and Suthediya et al. (2021). As a result, genotypes from these clusters could be utilized as parents in a hybridization program.

Table 4 Shows the cluster means for ten quantitative traits, which reveals significant differences among the clusters for most of the traits. Clusters X and I exhibited early flowering genotypes at 49 and 51 days, respectively, while cluster VI had delayed flowering genotypes at 71 days. The height of plants in cluster XII was relatively tall at 108.3 cm, while plants in cluster III were short at 42.25 cm. Cluster IX had the least number of productive tillers per plant at 2, whereas cluster X had the most at 6.67. Panicle length was highest in cluster XII at 44.67 cm and lowest in cluster XIII at 16.23 cm. The maximum flag leaf length was recorded in cluster VIII at 35.83 cm, while cluster XI had the lowest at 14.0 cm. Flag leaf breadth was highest in cluster VIII at 1.03 cm and lowest in cluster X at 0.43 cm. Cluster IX had the highest number of nodes, whereas cluster XIII had the least at 3.67. Highest peduncle length was recorded about 14.00 cm in cluster XII and lowest peduncle length recorded about 7.65 cm in cluster III. Cluster VII had the highest dry fodder yield at 16.15 g, while cluster XIII had the lowest dry fodder yield at 1.50 g. Cluster XI showed a high single plant grain yield (g) at 9.85 g, while cluster XII had a low single plant grain yield at 0.68 g. This suggests that the genotypes within these clusters could be selectively utilized to improve specific traits in plant breeding programs [Devaliya et al. (2017), Patel et al. (2018) and Shanmugam et al. (2023)].

The genotype LMV-543 from cluster XI, which has a high yield, can be used in crop improvement programs. Another genotype, 7008 from cluster XII, exhibited early flowering and could serve as a valuable resource for future breeding programs aimed at producing early maturing varieties. Additionally, the dwarf genotype 7008 can be utilized in the development of non-lodging, dwarf little millet varieties. These findings demonstrate that there is sufficient genetic diversity among little millet accessions for further use in breeding programs. The genotypes identified as highly diverse in this study may be particularly valuable for exploitation in future breeding efforts focused on little millet.

CONCLUSION

Table 3: Intra-cluster (Bold) and Inter-cluster distance b	(Bold) and I	nter-cluster	distance betv	etween each clusters based on D^2 values.	clusters base	ed on D² va	lues.						
No of	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster1	Cluster1	Cluster
cluster	_		≡	<u>></u>	^	ΙΛ	III	ΛIII	×	×	X	IIX	IIIX
Cluster I	46.7	232.3	11002.5	121.0	331.5	368.8	176.2	329.2	182.8	92.2	187.5	144.5	104.6
Cluster II		61.2	187.9	209.2	135.5	95.5	283.8	182.8	345.6	332.4	282.2	326.6	191.6
Cluster III			51.9	222.7	232.6	213.0	197.9	338.2	328.6	104.4	191.2	356.5	233.4
Cluster IV				0.79	249.7	371.8	122.5	164.7	93.1	206.6	172.0	104.8	111.8
Cluster V					73.7	117.9	195.9	123.4	364.6	366.5	251.6	467.2	350.4
Cluster VI						44.9	372.4	263.0	553.5	409.8	366.9	577.7	377.6
Cluster VII							77.6	166.8	146.8	166.0	136.8	268.3	265.3
Cluster VIII								88.5	243.4	432.5	282.2	324.8	284.1
Cluster IX									0.0	266.4	103.6	154.4	156.4
Cluster X										0.0	163.8	281.2	266.5
Cluster XI											0.0	337.7	259.2
Cluster XII												0.0	8.06
Cluster XIII													0.0

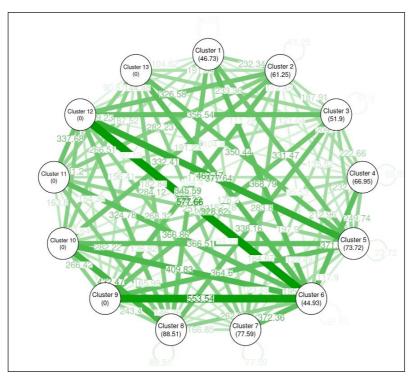


Fig 2: Relationship among thirteen clusters based on mahalanobsis D² analysis.

Table 4: Cluster mean for 10 quantitative characters among 323 little millet genotypes.

Cluster	SPY	PH	FLL	FLB	NPT	PL	NN	PEL	DFY	DFF
Cluster I	1.51	70.13	23.79	0.65	2.91	29.23	4.12	10.09	3.71	54.92
Cluster II	1.30	66.41	22.77	0.63	3.11	28.08	3.85	9.26	3.74	53.27
Cluster III	0.94	69.01	24.62	0.58	3.00	28.76	4.14	10.43	2.34	52.48
Cluster IV	1.46	66.06	22.34	0.62	3.09	28.83	3.82	9.21	3.72	53.52
Cluster V	2.19	79.49	23.03	0.69	2.72	30.28	4.78	11.18	3.94	56.56
Cluster VI	2.51	54.04	20.94	0.58	3.56	27.30	4.00	8.59	2.30	50.56
Cluster VII	1.43	73.40	23.85	0.75	1.17	35.50	4.33	8.73	2.25	55.50
Cluster VIII	1.78	70.67	24.83	0.78	3.00	32.92	4.83	9.33	12.50	56.33
Cluster IX	0.61	68.60	24.83	0.70	1.67	18.50	3.00	12.33	2.16	51.67
Cluster X	1.98	80.33	24.63	0.70	2.67	24.33	3.33	10.80	2.15	53.00
Cluster XI	2.89	75.17	21.83	0.67	1.67	35.83	4.33	8.47	4.27	56.67
Cluster XII	0.91	50.00	20.67	0.63	3.33	25.60	3.67	8.63	1.14	49.00
Cluster XIII	0.10	108.3	24.33	0.83	2.33	28.67	4.00	7.67	3.43	52.33

DFF- Days to fifty per cent flowering, PH- Plant height (cm), FLL- Flag leaf length (cm), FLB- Flag leaf breadth (cm), DFY- Dry fodder yield (g)/plant, PEL- Peduncle length (cm), NN- Number of node/plant, PL- Panicle length (cm), NPT- Number of productive tiller/plant and SPY- Single plant grain yield (g).

Path analysis revealed traits positively influencing grain yield in little millet: days to 50% flowering, plant height, flag leaf breadth, productive tillers per plant, panicle length, peduncle length, number of nodes and dry fodder yield. Previous studies supported their importance. D² analysis showed genetic divergence among genotypes, mainly driven by flowering time, plant height, panicle exertion, dry fodder yield, and panicle length. Thirteen distinct clusters with diverse genotypes were identified. Utilizing genotypes with greater inter-cluster distances could enhance crops. Cluster means analysis highlighted trait differences among clusters. LMV-

543 (cluster XI) and 7008 (cluster XII) were valuable for yield and early maturation. Consider these traits and genetic diversity in little millet breeding for improved yield and desired varieties.

Cinflict of interest: None.

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