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Research Note

Multivariate analysis for assessing the genetic diversity and association patterns of yield attributing traits in little millet (*Panicum sumatrense*)

R Narasimhulu[1*], C. Vijaya Kumar Reddy[1], M. Jostna Kiranmayi[1], K. Hariprasanna[2], K. Prabhakar[1] and N. C. Venkateswarlu[1]

[1]Regional Agricultural Research Station, Nandyal, Acharya N.G. Ranga Agricultural University, Andhra Pradesh
[2]ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana 500 030
***E-Mail :** narsi46@gmail.com

Abstract
Little millet is an important small millet grown mostly in India. The availability of genetic variability is a critical requirement for crop improvement. Principal Component Analysis (PCA) and correlation study was undertaken in a set of 28 little millet genotypes to estimate genetic diversity, association pattern among seven quantitative traits and to select suitable genotypes for crop improvement. In cluster analysis, the genotypes were classified into three distinct clusters, each with one or two subgroups. Cluster I had the major genotypes with comparable ancestry, followed by Cluster II. The total variance was split into seven major principal components, with the top two PCs with eigenvalues greater than one accounting for 82.57% of the overall variation. PC1, which explained a larger part of the variance (58.05%), was strongly influenced by days to 50% flowering, days to maturity, number of productive tillers per plant, 1000-grain weight, and grain yield. PC2 was primarily influenced by plant height and fodder yield. PCA and association analysis revealed a significant positive association between grain yield and the number of productive tillers per plant, 1000-grain weight, plant height. These traits would be useful for direct selection for little millet crop improvement. In both cluster analysis and PCA, the genotypes DHLM 14-5, IIMR LM-8004, NDL LM1, TNPSu 242, VS 33, WV 168, DPLN 1 and VS 38 displayed diversity, implying that these lines may be used in crossing programme to generate further genetic variability to select suitable transgressive segregants.

Keywords: Little millet, genetic diversity, correlation, principal component analysis, biplot

Little millet (*Panicum sumatrense* L.) is an important small millet crop grown in India, particularly in rainfed areas with diverse soil types, and varying thermal and photoperiod conditions. It is an important staple crop in the hilly and tribal regions. The crop is distinguished by its rapid growth, short duration and grains high in nutrients such as dietary fibre, protein and minerals such as iron (Fe = 32.20 ppm) and zinc (Zn = 32.40 ppm) (Vetriventhan *et al.,* 2021). It performs remarkably well in adverse climatic situations and is invariably adapted to high temperature and moisture stress (Ajithkumar and Panneerselvam, 2014). It is also resistant to stored grain pests, allowing for extended storage life and better quality. Despite its importance in rainfed agriculture and nutritional value, the average yield of little millet remains low due to a lack of adoption of improved agronomic practises, poor soil fertility and non-availability of improved varieties (Manimozhi *et al.,* 2014).

To enhance little millet productivity, it is crucial to understand its genetic diversity and the association between different key yield-attributing traits. Multivariate and cluster analysis are the valuable tools for studying morphologically complex individuals and determining the degree of divergence between groups. Correlation analysis aids in the identification of association between different traits, whereas, Principal Component Analysis (PCA) reduces the multidimensional structure of the data and facilitates the interpretation of complex genotype interactions. Further, biplot analysis effectively visualises and interprets genotype diversity patterns by combining the results of correlation analysis and PCA. Diversity studies that integrate correlation analysis, PCA and biplot analysis can provide valuable insights into the relationships between different traits, identify key traits contributing to genetic variation, and visualise the distribution of genotypes in relation to these traits. Multivariate analysis was employed for genetic diversity and association study in little millet (Vaishali *et al.,* 2021; Patel *et al.,* 2023), finger millet (Suman *et al.,* 2019), and pearl millet (Sanjana Reddy *et al.,* 2021; Narasimhulu *et al.,* 2022, Khandelwal *et al.,* 2023). The current work used PCA and cluster analysis to estimate genetic diversity for various traits in a set of little millet genotypes. Associations between different traits were also investigated to identify appropriate traits for selection in order to improve grain yield.

The experimental material consisted of 24 little millet genotypes (BL 2021-2, BULM 18-174, BULM-18-111, BULM-18-21, DHLM 13-3, DHLM 14-5, DHLM-11-3, DLM 310, DPLN 1, GPUL 13, GPUL 14, IIMR LM-8004, IIMR LM-R21-1001, IIMR LM-R21-1002, IIMR LM-R21-1158, NDL LM 1, RLM 204, TNPSu 242, TNPSu 244, VS 3, VS 33, VS 38, WV 146 and WV 168) along with four checks (CLMV 1, BL 6, OLM 203 and DHLM 36-3) from the different centres of AICRP on Small millets. Genotypes were evaluated in three replications using a randomised complete block design (RCBD) at the Regional Agricultural Research Station, Nandyal, Andhra Pradesh, during *Kharif* season, 2022. Each genotype was sown in 10 rows of 3 m length, 22.5 cm apart, with 10 cm between hills. To ensure good crop growth, standard cultural and agronomic practises were used. Five competitive plants per genotype were selected randomly for recording observation on plant height (cm), number of productive tillers per plant, while observations on days to 50% flowering, days to maturity, fodder yield (kg/ha) and grain yield (kg/ha) were recorded on a plot basis. For 1000-grain weight, a random sample of 1000 grains were counted from the threshed seed and the weight was recorded in grams. The correlation analysis and PCA for the seven individual traits was computed using the opensource software package, grapesAgri1 (Gopinath *et al*., 2021). Clustering pattern of genotypes was worked out by using SAS-JMP software.

The data collected for grain yield and its contributing traits from 28 little millet genotypes were statistically analysed. The analysis of variance indicated significant variations among the genotypes for all quantitative traits tested, showing the presence of wide genetic variation in the experimental material. The cluster analysis identified three unique clusters, each with one or two subgroups (**Fig. 1**). This revealed that the genotypes had significant genetic diversity among them. Genotypes from the same cluster group are more closely linked than those from other cluster groups. Cluster I consisted of 12 genotypes (**Table 1**), which were further subdivided into two separate subgroups. Both subgroups exhibited early flowering and maturity traits, while varied in terms of plant height. Cluster II also comprised 12 genotypes, categorized into three subgroups. All genotypes in these subgroups exhibited medium duration for flowering and maturity. Notably, the genotype NDL LM1 in the third subgroup had a greater number of productive tillers and 1000-grain weight, both of which were related with a higher grain yield. Similarly, in the same subgroup, the entry IIMR LM-8004 demonstrated a higher 1000-grain weight. Cluster III consisted of four genotypes that exhibited longer duration for flowering and maturity. However, these genotypes exhibited a large number of productive tillers and a good grain yield. Particularly, the genotype TNPSu 242 showed higher grain yield. In any of the clusters, the genotypes with high values of grain yield and its attributing traits can be used for direct adoption or genotypes from different clusters with extreme divergence could be used for hybridization, followed by selections to obtain desirable transgressive segregants. Similar conclusions were drawn by Patel *et al.* (2023).

In general, the phenotypic correlation is impacted by the link between individual traits and the effect of environmental factors. If the two traits are connected, changing one will impact the other. Before initiating any effective selection programme for the traits in question, it

Table 1. Clustering of 28 little millet genotypes into different clusters

S. No.	Cluster No	Number of genotypes	Genotypes
1	I	12	BL 2021-2, IIMR LM-R21-1001, IIMR LM-R21-1002, WV 146, VS 38, OLM 203, BULM 18-174, WV 168, DPLN 1, BULM-18-21, IIMR LM-R21-1158, BULM-18-111
2	II	12	BL 6, GPUL 14, CLMV 1, TNPSu 244, VS 31, DHLM 13-3, DHLM-11-3, DHLM 14-5, DHLM 36-3, GPUL 13, IIMR LM-8004, NDL LM 1
3	III	4	DLM 310, VS 33, RLM 204, TNPSu 242

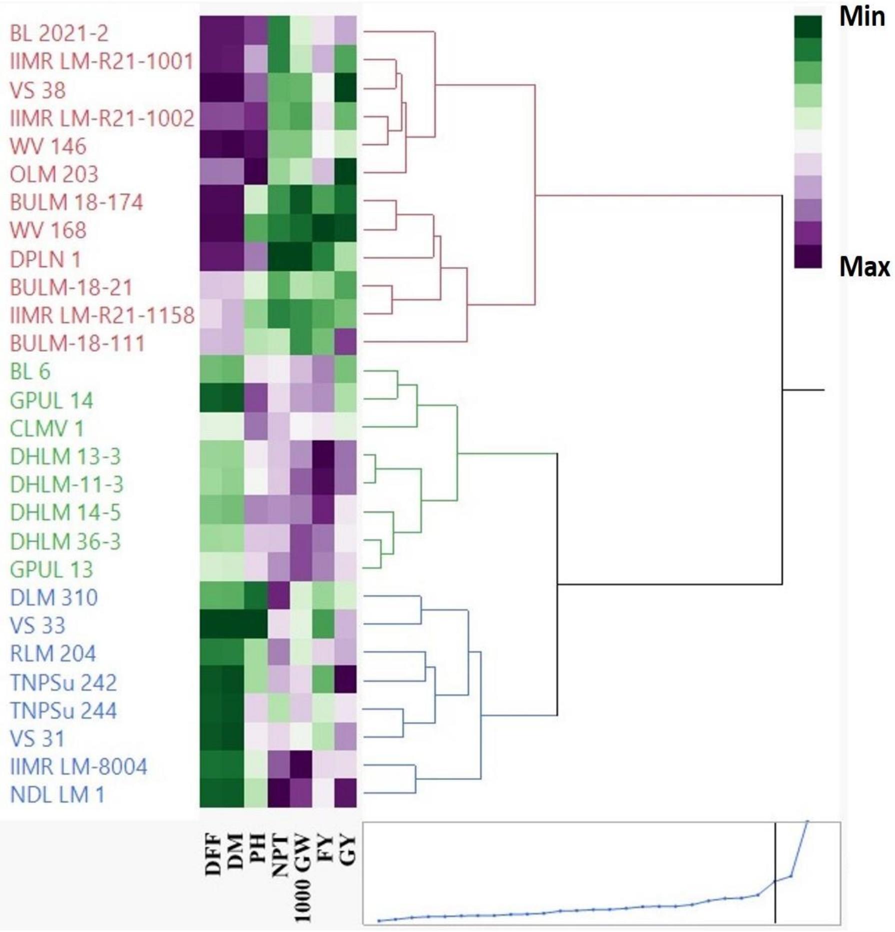


Fig. 1. Hierarchical cluster analysis and heat map visualisation of yield and yield attributing traits in little millet

DFF: Days to 50% flowering, DM: Days to maturity PH: Plant height, NPT: Number of productive tillers per plant, 1000 GW: 1000-Grain weight, FY: Fodder yield, GY: Grain yield

is critical to understand the relationship between distinct traits. The association between different yield-related traits in little millet has previously been studied, but the results varied significantly due to varietal differences and environmental conditions. One of the most notable findings in this investigation is the significant positive correlation between grain yield and the number of productive tillers (0.53) and 1000-grain weight (0.51). Grain yield, on the other hand, exhibited a significant negative connection with days to 50% flowering (-0.62), days to maturity (-0.61), and plant height (-0.28) (**Fig. 2**). Gopikrishnan *et al.* (2021) and Amaravel *et al.* (2023) reported similar results. This finding implies that genotypes with early maturity, short stature with a greater number of productive tillers and bold seed produced high grain yield. Such information is critical for little millet breeding programmes aimed at developing early-maturing cultivars with superior agronomic performance particularly those appropriate for lodging tolerance and mechanical harvest.

The inter-association analyses revealed a strong positive relationship between days to 50% flowering and days to maturity (0.99), as previously reported in little millet by Anuradha *et al.* (2017), Shinde *et al.* (2018) and Amaravel *et al.* (2023). Another important observation is the significant negative correlation between days to maturity and number of productive tillers (-0.77). Furthermore, the marginally positive relationship between plant height and days to 50 % flowering (0.36*) raises interesting concerns about the relationship between plant growth and flowering time. This study emphasises the importance of future research into the genetic and physiological factors that influence plant height and flowering period in little millet. The positive association between 1000-grain weight and number of productive tillers (0.68**) shows that genotypes with a large number of productive tillers were also have bigger seed size. Jyothsna *et al.* (2016) reported similar findings for the number of productive tillers per plant and 1000-grain weight. This association underscores the

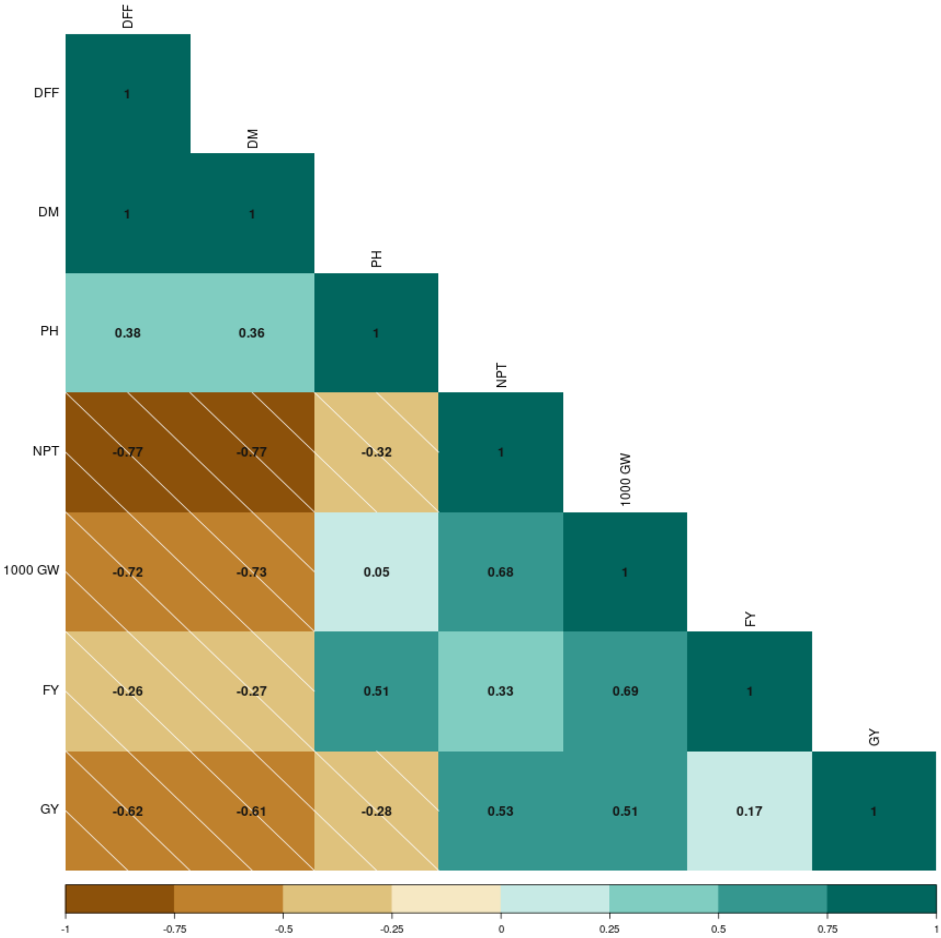
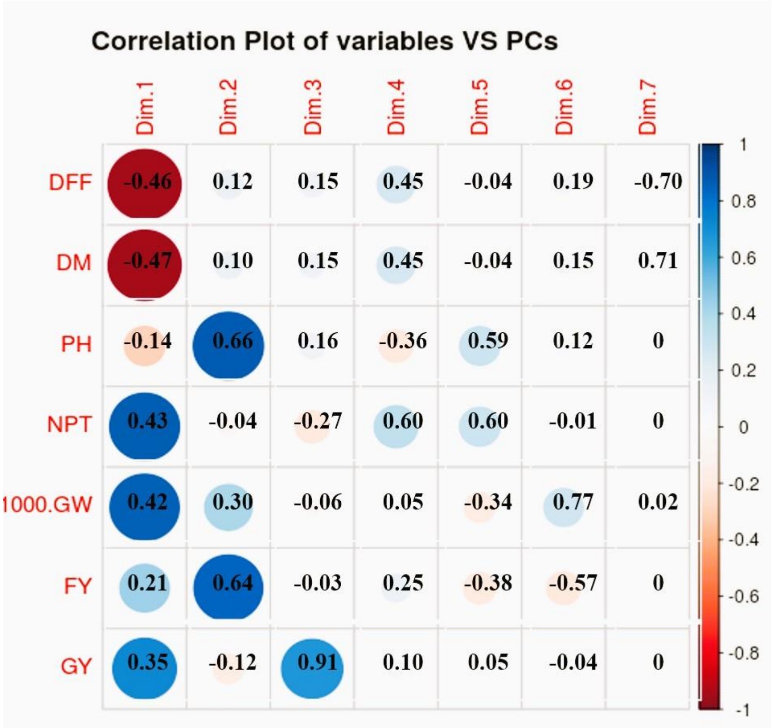


Fig. 2. Pearson correlation for yield and yield attributing traits in little millet

DFF: Days to 50% flowering, DM: Days to maturity PH: Plant height, NPT: Number of productive tillers per plant, 1000 GW: 1000-Grain weight, FY: Fodder yield, GY: Grain yield

significance of tiller production in achieving better grain filling and denser grains, both of which are desirable traits for enhanced yield and quality. Finally, a marginal positive correlation between fodder yield and grain yield implies that improving biomass may result in higher grain yields as well. This study underlines the possibility of selecting genotypes that have the dual benefits of increased fodder production without compromising grain yield. In conclusion, the majority of the genotypes were found to have short stature with a higher number of productive tillers in the early maturing type with bold seeded background, indicating that there is a better chance of establishing trait specific lines for future research and breeding efforts aimed at improving the agronomic performance and yield potential of little millet varieties.

The correlation between variables and principal components (PCs) provides valuable information about the relationships and contributions of each trait to the overall variation explained by the PCs (**Fig. 3**). According to the PCA results, the first two components had eigen values greater than one and offered around 82.56% of total accumulated variability for the seven distinct morphological variables evaluated in the 28 little millet genotypes. PC1, which accounted for a significant proportion of the total variance (58.05%), is strongly negatively correlated with traits such as days to 50% flowering and days to maturity, and positively correlated with number of productive tillers per plant, 1000-grain weight, fodder yield and grain yield. Similar findings were reported by Anuradha *et al*. (2017) and Shinde *et al*. (2018). This indicates that these traits have a major influence on the variation captured by PC1. The high negative correlation suggests that genotypes with shorter days to flowering and maturity, as well as higher test weight and yield, contribute to the positive direction of PC1. PC2 explains 24.52% of the variance and is positively correlated with plant height. It implies that plant height plays a dominant role in shaping the variation



	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7
DFF	-0.46	0.12	0.15	0.45	-0.04	0.19	-0.70
DM	-0.47	0.10	0.15	0.45	-0.04	0.15	0.71
PH	-0.14	0.66	0.16	-0.36	0.59	0.12	0
NPT	0.43	-0.04	-0.27	0.60	0.60	-0.01	0
1000.GW	0.42	0.30	-0.06	0.05	-0.34	0.77	0.02
FY	0.21	0.64	-0.03	0.25	-0.38	-0.57	0
GY	0.35	-0.12	0.91	0.10	0.05	-0.04	0

Fig. 3. Correlation plot of variable *vs* PCs in little millet genotypes

DFF: Days to 50% flowering, DM: Days to maturity PH: Plant height, NPT: Number of productive tillers per plant, 1000 GW: 1000-Grain weight, FY: Fodder yield, GY: Grain yield

explained by PC2. PC3, accounting for 7.63% of the variance, exhibits a positive correlation with grain yield. This indicates that genotypes with higher grain yields contribute more to the positive direction of PC3. Thus, the first three PCs explain 90% of variation in genotypes, while the other PCs explain the rest, according to Salini *et al.* (2010) in proso millet had reported that first five eigen vectors contributed 93.2% of total genetic variance, while similar study in finger millet reported 98.14% of total genetic variance explained by PCs (Suman *et al*., 2019). The correlations between the variables and PCs provide justification for the importance of each trait in contributing to the overall variation observed in the little millet dataset. These findings help us understand the underlying relationships and associations between the traits and the PCs, aiding in the interpretation of the genotypes.

Biplot analysis was used to thoroughly evaluate the multivariate relationships between the various traits in little millet genotypes by comparing the eigenvalues of PC1 and PC2 of PCA for all traits. In terms of the interrelationship between traits and genotypes, the first two PC axes (PC1, 58.05% and PC2, 24.52%) accounted for approximately 82.57% of total variability, revealing the complexity of the variation between the depicted components (**Table 2**). Vectors of traits (variables) with acute angles are positively correlated in the biplot, while those with obtuse or straight angles are negatively correlated (**Fig. 4**).

The distance between the raw (genotypes) is evaluated in terms of similarity. PC1's major traits were grain yield, number of productive tillers per plant, 1000-grain weight, and fodder yield, whereas, PC2 had days for 50% flowering, days to maturity and plant height as the major traits. The cosine of the angles between vectors revealed a strong positive link between grain yield, the number of productive tillers per plant and 1000-grain weight. These three traits were also positively associated with fodder yield. Days to 50% flowering and days to maturity, on the other hand, were negatively associated to grain yield and the number of productive tillers per plant. There was no link between 1000-grain weight and plant height. The biplot diagram enables the identification of high-performing genotypes for specific traits by assessing their proximity to the corresponding trait vectors within the same quadrant. Genotypes located in close proximity to the trait vectors within the respective quadrant are expected to demonstrate superior performance for those particular traits. Among the genotypes evaluated, NDL LM 1 had the most productive tillers with the higher grain yield, whereas, VS 38 had the maximum number

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Table 2. Principal components exhibiting eigenvalues, proportion of variation and total cumulative variation in 28 little millet genotypes

Principal components	Eigen value	Proportion of Variance (%)	Cumulative variation (%)
PC1	4.06	58.05	58.05
PC2	1.72	24.52	82.56
PC3	0.53	7.63	90.19
PC4	0.32	4.52	94.71
PC5	0.24	3.41	98.11
PC6	0.13	1.88	99.99
PC7	0.00	0.01	100.00

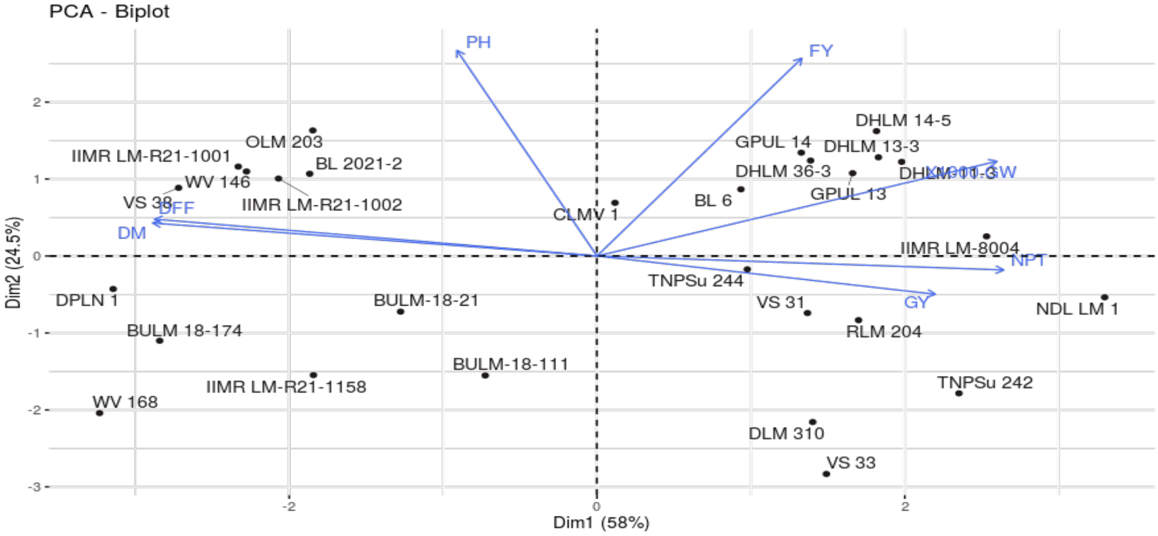

Fig. 4. Biplot association between yield and yield attributing traits of little millet genotypes on the first two principal components

DFF: Days to 50% flowering, DM: Days to maturity PH: Plant height, NPT: Number of productive tillers per plant, 1000 GW: 1000-Grain weight, FY: Fodder yield, GY: Grain yield

of days to 50% flowering coupled with a longer maturity period. The utilization of the biplot diagram in this study facilitated the identification of genotypes with desirable trait performances as previously reported by Selvi *et al*. (2015). The genotypes DHLM 14-5, IIMR LM-8004, NDL LM 1, TNPSu 242, VS 33, WV 168, DPLN 1, VS 38 and OLM 203 were assigned to distinct quadrants at the extreme ends of the plot. This suggests that these genotypes are significantly different from the others and differ in all attributes. Therefore, these genotypes could be considered as candidate parents in crop improvement programmes.

The current study on 28 distinct little millet genotypes revealed the presence of considerable genetic diversity and provided significant insights for future breeding efforts with the potential of developing possible early maturing high yielders with larger seed size and more productive tillers per plant with shorter plant stature. Selecting genotypes from this study and applying in hybridization followed by an intense selection approach for rapid development of dependent traits, such as yield attributes in little millet, can create more reliable cultivars with desirable traits.

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