Identification of Superior Forage Pearl Millet Genotypes through Multi-Trait Analysis and Cluster Grouping in Diverse Temporal Semi-Arid Environments

Abstract

Pearl millet (*Pennisetum glaucum* L. R. Br) is an important crop in arid and semi-arid regions, providing essential fodder for livestock. The present investigation evaluated genetic variability and trait associations in 103 forage pearl millet genotypes (97 genotypes and 6 checks) across three environments during 2022-23. Sixteen morphological traits were assessed using a randomized complete block design with two replications. Analysis of variance revealed significant differences among genotypes, environments, and genotype × environment interactions for all studied traits. Correlation analysis revealed several traits that were positively associated with total green fodder yield, including leaf length, leaf width, number of leaves, plant height at first cut, number of tillers at first cut, green fodder yield at first cut, and green fodder yield at second cut. Principal component analysis indicated that four principal components accounted for 72% of the total variability. The first principal component (29%) was primarily influenced by the number of leaves and leaf width, while the second component (21%) was dominated by green and dry fodder yield first cut. Hierarchical cluster analysis grouped the genotypes into five distinct clusters, with Cluster IV showing superior performance for key forage traits. Cluster III contained the maximum number of genotypes (34), while Cluster I had the minimum (8). This comprehensive analysis identified promising genotypes with superior forage attributes and important trait associations for future breeding programs aimed at developing improved forage pearl millet varieties and hybrids to meet the rising demand for fodder in arid and semi-arid regions of the world.

Key words: Forage improvement, Trait associations, Principal component analysis, Cluster analysis, Biomass yield

1.Introduction

Pearl millet (*Pennisetum glaucum* L. R. Br.), a diploid (2n = 14), is a highly cross-pollinated crop with a protogynous flowering habit and belongs to the Poaceae family. It is an important staple crop in the arid and semi-arid tropical regions of Asia and Africa, ranking as

the fifth most significant cereal. In India, pearl millet covered 7.57 million hectares, producing 11.43 million tons with an average yield of 1510 kg/ha in 2022-23. However, production is estimated to decrease to 9.53 million tons in 2023-24 (Ministry of Agriculture & Farmers Welfare, Government of India).The availability of genetically diverse resources developed specifically for forage pearl millet has supported progress in this field (Yadav *et al.*, 2021). Several high-yielding varieties and hybrids of forage pearl millet have shown promising results in trials across India. Efforts have been directed toward developing stable, high-yielding genotypes and hybrids to meet the rising demand for fodder (Harinarayana*et al.*, 2005).

The complexity of forage yield as a trait necessitates a comprehensive analytical approach to understand its various components and their interrelationships, ultimately supporting informed breeding decisions for yield improvement(Javed *et al.,* 2016). To effectively enhance forage yield, breeders must understand the intricate network of relationships between various morphological and physiological traits. Analysis of variance (ANOVA) serves as a fundamental statistical tool in this context, enabling researchers to assess the significance of genetic variability among genotypes and determine the extent of environmental influence on trait expression. This analysis provides crucial insights into the heritable components of variation, which is essential for developing effective breeding strategies.

Correlation analysis plays an important role in understanding the complex associations between different agronomic traits and their direct or indirect effects on forage yield. As highlighted by(DaSilva *et al.,* 2017), correlation coefficients help identify traits that could serve as reliable selection criteria for yield improvement. However, the limitation of correlation analysis lies in its inability to account for the intricate multivariate relationships that exist within biological systems. Simple correlations may mask important underlying patterns when multiple traits interact simultaneously to influence yield (Yadav *et al.*, 2020). To address this limitation, Principal Component Analysis (PCA) emerges as a powerful multivariate statistical technique that can unravel complex patterns within large datasets. Following the methodological framework established by Sneath and Sokal (1973), PCA transforms correlated variables into uncorrelated principal components, with each component representing a linear combination of the original variables. The selection of significant principal components based on eigenvalues greater than one, as recommended by Kaiser (1958) and Jeffers (1967), ensures that only meaningful components are retained for

interpretation. This approach has proven particularly valuable in crop improvement programs, as demonstrated by recent studies in pearl millet (Sathya *et al*., 2014), where PCA effectively identified key trait associations and patterns of variation among genotypes.

Understanding genetic diversity is important for successful breeding program, as it directly influences the potential for genetic gain through selection and hybridization. Hierarchical cluster analysis provides a systematic approach to studying genetic relationships among genotypes, offering advantages over traditional Mahalanobis D^2 statistics in terms of visual interpretation and practical application (Bridges, 1966). Recent studies by Sharma *et al*. (2018) and Patil *et al*. (2020) have successfully employed hierarchical clustering to group pearl millet genotypes based on morphological and agronomic traits, facilitating the identification of promising parental combinations for breeding programs.The sequential clustering approach is particularly valuable as it helps identify genetically diverse parents from distinct clusters, potentially maximizing heterotic expression in hybrid combinations. This method has been effectively utilized in pearl millet breeding programs to develop superior hybrids with enhanced forage yield and quality traits (Govintharaj*et al.,* 2018). The identification of genetically diverse parents is crucial for achieving transgressive segregation, where progeny exhibit trait values exceeding those of either parent.

The present investigation employs this comprehensive statistical framework to evaluate genetic variability among pearl millet genotypes through ANOVA, determine trait associations using correlation analysis,identify key patterns of variation through PCA and assess genetic diversity using hierarchical cluster analysis.

2.Materials and methods

2.1 Experimental material and design

The experimental material consisted of diverse collection of 103 forage pearl millet genotypes, encompassing 97 breeding lines derived from indigenous landraces at the Indian Institute of Millets Research (IIMR), Hyderabadand sixcheck varieties.Field evaluations were conducted across three distinct temporal environments at IIMR spanning 2022-2023: the Kharif season of 2022 (E1), Summer season of 2023 (E2), and Kharif season of 2023 (E3). The experimental layout adopted a randomized complete block design (RCBD) featuring dual replication blocks, with the germplasm subdivided into four distinct trials containing 25 genotypes each.The experimental sites were situated within various research facilities of

IIMR in Hyderabad, positioned at coordinates 17°19'N latitude and 78°23'E longitude, with an elevation of 556 meters above sea level.

2.2 Data collection and statistical analysis

The phenotypic evaluation included sixteen distinct morphological characteristics, measured based on sample of five randomly identified plants per genotype within each environment. Sixteen morphological traits were recorded during the maturity and postharvest stages. The mean value of leaf length (LL, in cm), leaf width (LW, in cm), number of leaves (NL, in leaves in a plant), internodal length first cut (ILFC, in cm),stem thickness first cut (STFC, in mm), plant height first cut (PHFC, in cm), leaf stem ratio (LSR), number of tillers first cut (NTFC), number of new tillers (NNT), number of tillers regrowth (NTR), GFYF(green fodder yield first cut), DFYF(dry fodder yield first cut),GFYS (green fodder yield second cut), DFYS (dry fodder yield second cut),TGFY(total green fodder yield) and TDFY (total dry fodder yield) were made on 5 randomly chosen competitive plants of each genotype in each environment except for fodder yield which was calculated based on fodder harvested from plots during 2022-2023. Data processing and statistical computations were executed using R statistical software version 4.4.0 (2024-04-24 ucrt) within the RStudio. Genotype-by-environment interaction effects were investigated using the anova_joint function within the Metan package (Olivoto and Lúcio, 2020), which also provided tools for correlation analysis (corr_coef) to elucidate trait associations. Genetic diversity patterns were explored through hierarchical cluster analysis implemented via the stats package's hclust function. Multivariate data reduction was accomplished through principal component analysis using the MASS package, while the factoextra package enabled the generation of visualization tools, including eigenvalue plots and trait-genotype association biplots, to interpret complex patterns within the dataset.

3.Results and discussion

3.1 Analysis of variance for green fodder yield and related traits

The pooled analysis of variance for total green fodder yield and related traits across the three environments was presented in Table1. The results indicated that the mean sum of

squares across pooled environments for genotypes was significant, indicating variability among the genotypes. The mean sum of squares for environments was also found to be significant, showing differences among the environments used for evaluating the genotypes. The mean squares of genotype × environment interactions were observed to be highly significant for traits under study, indicating a significant amount of variation and a differential response of genotypes to different environments for various traits.The presence of variability among genotypes for green fodder yield and related traits in this study was in agreement with the findings of Harinarayan *et al.*,2005, Thomas *et al.*, 2019, Kapoor 2020, Shalini *et al.*,2020 and Parmar *et al.*, 2022.

Table1.Mean squares from pooled analysis of variance for forage yield and attributing traits of forage pearl millet genotypes in 3 seasons

Source of variation	Df	LL	LW	NL	ILFC	STFC	PHFC	LSR	NTFC
ENV	2	9781.3***	28.3***	70***	12.9***	12.7***	158937.8***	0.7***	424.3***
REP(ENV)	3	20.5	0.03	0.7	2.65.	3.4*	167.39	0	0.26
GEN	102	110***	0.3***	1.6***	8.1***	3.7***	747.52***	0.1***	2.11***
GEN: ENV	204	10992.4***	0.2***	0.6***	4.1***	1.5***	689.75***	0.02***	2.12***
Residual	306	7912.9	0.1	0.2	1.1	1	148.1	0	0.6
CV (%)		8.3	8.3	5.5	5.5	8.9	7.7	21.4	11.5
OV mean		61.5	3.4	7.4	19	11.3	157.2	0.5	6.8
Source of variation	**Df**	**NNT**	**NTR**	**GFYFC**	**DFYFC**	**GFYSC**	**DFYSC**	**TGFY**	**TDFY**
ENV	2	1090.4***	14.05***	7098.3***	1965***	21140.07***	4835.4***	38699.5***	10061.2***
REP(ENV)	3	0.6	0.12	134.68.	26.6	11.66	0.008	163.64.	26.12
GEN	102	1.3***	1.76***	434.3***	100.5***	103.17***	28.6***	396.2***	86.1***
GEN: ENV	204	2***	0.70***	194.2***	47.3***	124.68***	27.8***	149.8***	35.5***
Residual	306	0.6	0.4	52.6	15.7	13.8	3.1	68.9	20
CV (%)		13.8	19.1	20.9	24.2	20.2	19.8	15.6	17.7
OV mean		5.7	3.1	34.6	16.4	18.4	8.9	53.1	25.3

Where, (LL=Leaf length, LW=Leaf width, NL=Number of leaves, ILFC=Internodal length first cut, STFC=Stem thickness first cut, PHFC=Plant height first cut, LSR=Leaf stem ratio, NTFC=Number of tillers first cut, NNT=Number of new tillers, NTR=Number of tillers regrowth, GFYFC=Green fodder yield first cut, DFYFC=Dry fodder yield first cut, GFYSC=Green fodder yield first cut, DFYSC=Dry fodder yield second cut, TGFY=Total green fodder yield, TDFY=Total dry fodder yield

3.2 Correlation for fodder yield and related traits in pooled environments:

The Pearson correlation coefficient analysis revealed that traits that showed a positive and significant correlation with total green fodder yield across pooled environments included leaf length, leaf width, number of leaves, green fodder yield second cut, dry fodder yield second cut, plant height first cut, number of tillers first cut, number of new tillers, green fodder yield first cut, dry fodder yield first cut, and internodal length first cut (Figure 1).Forage yield is a complex trait influenced by numerous factors, and correlation analysis is crucial for understanding the traits contributing to green fodder yield(Aswini *et al.* (2023).

Plant height, number of tillers, and culm girth showed positive and significant correlations with green fodder yield in multiple studies (Bika and Shekhawat, 2015; Singh *et al*., 2018).Gopalan and Ramaswamy (1981) reported that the length of internodes was positively correlated with fodder yield in *Cenchrus ciliaris.* Similar positive associations have been reported in studies on pearl millet by Dhedi*et al*. (2016), Aswini *et al*. (2023) and Andhale*et al.* (2024). These findings emphasize the importance of selecting traits that contribute positively to enhancing forage yield in breeding programs.

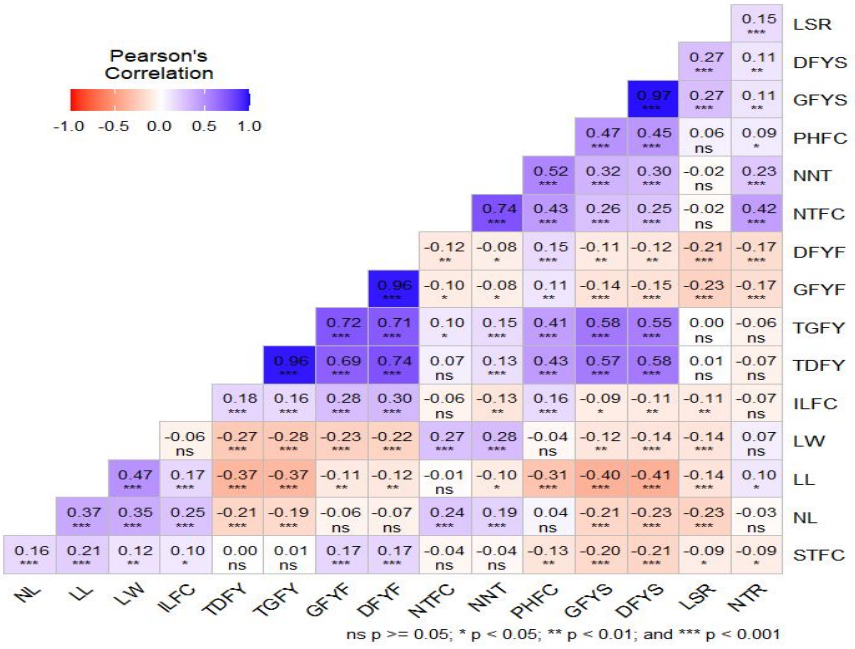

Figure 1. Genetic correlation for fodder yield and related traits amongforage pearl millet genotypesacross three environments

3.3 Principal component analysis

The results of the principal component analysis across pooled environments for total green fodder yield and related traits were presented in the Tables 2 and 3 and Figure 2. In our study four principal components had eigen values greater than one and they cumulatively explained 72% of the total variability. The first principal component explained 29% of the total variability. The second, third and fourth principal components explained 21%, 15% and 7% of total variability, respectively (Table 3.2).

Table 2. Principal component analysis summary for green fodder yield and related traits in forage pearl millet

Principal Component	Standard Deviation	Proportion of Variance	Cumulative Proportion	Eigen Value
PC1	2.14	0.29	0.29	4.57
PC2	1.84	0.21	0.50	3.37
PC3	1.57	0.15	0.65	2.48
PC4	1.03	0.07	0.72	1.05
PC5	1.00	0.06	0.78	0.99
PC6	0.96	0.06	0.84	0.92
PC7	0.86	0.05	0.88	0.73
PC8	0.75	0.04	0.92	0.57
PC9	0.67	0.03	0.95	0.44
PC10	0.58	0.02	0.97	0.33
PC11	0.54	0.02	0.98	0.29
PC12	0.43	0.01	1.00	0.19
PC13	0.21	0.00	1.00	0.04
PC14	0.13	0.00	1.00	0.02
PC15	0.00	0.00	1.00	0.00
PC16	0.00	0.00	1.00	0.00

Further principal component analysis was carried out using varimax rotation to check character association with respective principal components.The first principal component explains the highest variation in the dataset, with high positive loadings for traits such as the number of leaves and leaf width, indicating a strong contribution from these traits to the overall variation. In contrast, traits like total green fodder yield, total dry fodder yield, green fodder yield (second cut), and dry fodder yield (second cut) exhibit high negative loadings, suggesting a negative contribution to this component. The second principal component reflects traits like green fodder yield (first cut) and dry fodder yield (first cut), showing high positive loadings and contributing significantly, while traits such as plant height (first cut), number of tillers (first cut), and number of new tillers display high negative loadings, indicating their opposition to the variance explained by PC2. In the third principal component, the number of tillers (first cut) and number of leaves contribute strongly with high positive loadings, whereas traits like total green fodder yield and total dry fodder yield show negative loadings, suggesting an inverse relationship with this component's variance. The fourth principal component highlights internodal length (first cut) and green fodder yield (second cut) with high positive loadings, while traits like number of tillers (first cut) and number of new tillers have high negative loadings, reflecting their lower or inverse contribution to this component (Table 3).

Table 3. Factor loadings of principal components with eigen values greater than one for green fodder yield and related traits

Trait	PC1	PC2	PC3	PC4
Leaf length	0.3	0.06	0.23	0.22
Leaf width	0.2	-0.1	0.35	0.11
Number of leaves	0.15	0.03	0.43	0.35
Internodal length first cut	-0.03	0.24	0.18	0.53
Stem thickness first cut	0.06	0.23	0.19	0.04
Plant height first cut	-0.29	-0.2	0.26	0.07
Leaf stem ratio	-0.06	-0.2	-0.3	0.1
Number of tillers first cut	-0.1	-0.3	0.43	-0.23
Number of tillers regrowth	0.01	-0.3	0.1	-0.37
Number of new tillers	-0.13	-0.3	0.41	-0.23
Green fodder yield first cut	-0.22	0.43	0.14	-0.23
Dry fodder yield first cut	-0.23	0.43	0.14	-0.2
Green fodder yield second cut	-0.35	-0.3	-0.1	0.3
Dry fodder yield second cut	-0.35	-0.3	-0.1	0.29
Total green fodder yield	-0.43	0.15	0.06	0.03
Total dry fodder yield	-0.44	0.15	0.05	0.05

The PCA biplot illustrates the two main axes Dim1 (28.5%) and Dim2 (21.1%) represent the first two principal components. These two main axes explain a significant portion of the variability in the dataset, which captured from multiple original traits. The colored ellipses surrounding the data points represent confidence ellipses for each group (E_1, E_2 and E_3). The ellipses suggest that traits within each group share some similarity but are distinct from the other groups. The E_3 is more distinct from E_1 and E_2. The ellipses E_1 and E_2 were more overlapped and the observations within each group share some similarity but are distinct from the other groups (Figure 2). The arrows represent the contribution of the traits to the principal components. The longer the arrow, the stronger the correlation. Leaf width has a strong positive correlation with Dim 1 and plant height first cut is more strongly associated with Dim2. Leaf width is positively correlated with Dim1 and slightly with Dim2, meaning it contributes heavily to the variation captured by Dim1.The angle between two arrows indicates the correlation between the traits, small angle suggest a strong positive correlation, angle close to 90 degrees indicate little to no correlation and angle close to 180 degrees indicate a strong negative correlation. In E_1 the traits like total dry fodder yield and total green fodder yield were similar followed by green fodder yield first cut dry fodder yield first cut were similar. In E_2 green fodder yield second cut and dry fodder yield second cut were more similar. In $E_3$the number of leaves and leaf length were similar. Small angles between plant height first cut and leaf stem ratio suggest a strong positive correlation, meaning that

when plant height first cut is high, leaf stem ratio is also likely to be high.Conversely, arrows for plant height first cut and number of new tillers are close to 180 degrees apart, it indicates a negative correlation higher values of plant height first cut are associated with lower values of number of new tillers. Total green fodder yield and total dry fodder yield have longer arrows which explain a larger portion of the variance in Dim1 and Dim2, meaning they are key factors in differentiating the observations in your data.

Principal component analysis was used to identify the most influential traits contributing to overall variation in the data. By reducing correlated variables into independent components, the analysis revealed that four principal components explained 72% of the total variability. Traits such as leaf width and number of leaves contributed strongly to the first component, while fodder yields were key to the second component. This approach helped clarify trait relationships and guide breeding decisions based on the most impactful variables.

The findings of this study explain strong reliability with the studies on forage pearl millet, by Gupta (2022); Khandelwal *et al*. (2023). The identification of key traits contributing to yield and quality, such as plant height, dry fodder yield, and productive tillers, reflects patterns observed in previous studies of pearl millet.

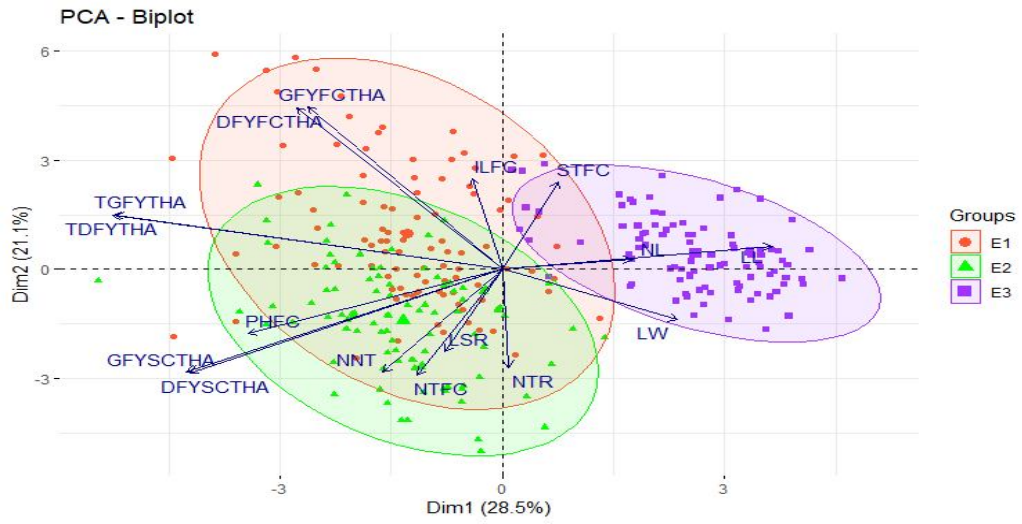

Figure 2: Principal component analysis (PCA) biplot showing trait distribution for forage pearl millet genotypes across three environments

The hierarchical clustering of forage pearl millet genotypes for forage yield and its attributes across three seasons revealed five distinct clusters. Cluster I consisted of 8 genotypes, indicating a relatively smaller group (Figure 3).

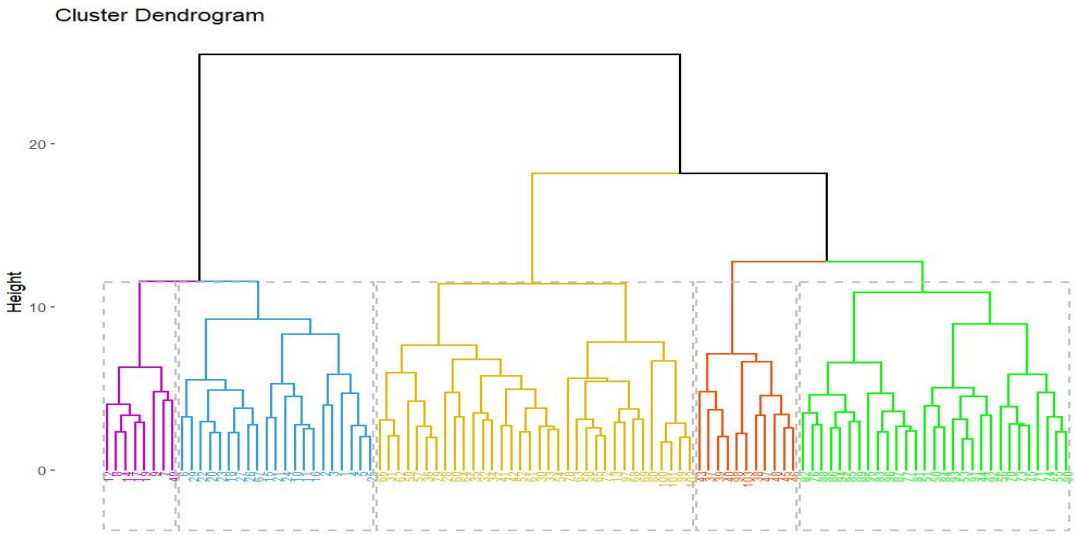

Figure 3. Hierarchical clustering plot for pooled data of 97 genotypes and 6 checks for green fodder yield and related traits in forage pearl millet pooled seasonal data

Moderately sized cluster was observed in Cluster II with 21 genotypes. Cluster III was the largest, with 34 genotypes, indicating a significant portion of the diversity within the genotypes. Cluster IV had 11 genotypes, while Cluster V was quite extensive, containing 29 genotypes. These clusters highlight the variability and grouping of genotypes based on their performance across multiple seasons (Figure 3 and Table 4)

Table 4.Pooled cluster composition of forage pearl millet genotypes for forage yield and its attributes in three seasons

Cluster	Number of Genotypes	Name of the Genotypes
I	8	IIMR FB12, IIMR FB8, IIMR FB14, IIMR FB17, IIMR FB19, IIMR FB9, IIMR FB7, IIMR FB49
II	21	IIMR FB6, IIMR FB29, IIMR FB22, IIMR FB20, IIMR FB23, IIMR FB18, IIMR FB 27, IIMR FB26, IIMR FB67, IIMR FB15, IIMR FB21, IIMR FB24, IIMR FB10, IIMR FB11, IIMR FB16, IIMR FB2, IIMR FB3, IIMR FB1, IIMR FB4, IIMR FB5, IIMR FB25
III	34	IIMR FB66, IIMR FB31, IIMR FB62, IIMR FB59, IIMR FB57, IIMR FB36, IIMR FB82, IIMR FB28, IIMR FB60, IIMR FB64, IIMR FB35, IIMR FB32, IIMR FB34, IIMR FB41, IIMR FB42, IIMR FB52, IIMR FB61, IIMR FB30, IIMR FB33, IIMR FB54, IIMR FB81, IIMR FB63, IIMR FB58, IIMR FB65, IIMR FB73, IIMR FB13, IIMR FB102, IIMR FB69, IIMR FB70, IIMR FB83, BULK 1, BULK 4, BULK 2, TSFB15-8
IV	11	IIMR FB43, IIMR FB37, IIMR FB39, IIMR FB40, Wonder Leaf, ForagenRaftaar, IIMR FB38, IIMR FB47, IIMR FB48, IIMR FB45, IIMR FB46

V	29	IIMR FB86, IIMR FB79, IIMR FB93, IIMR FB91, IIMR FB99, IIMR FB90, IIMR FB94, IIMR FB100, IIMR FB87, IIMR FB101, IIMR FB92, IIMR FB80, IIMR FB85, IIMR FB51, IIMR FB50, IIMR FB88, IIMR FB98, IIMR FB53, IIMR FB96, IIMR FB44, IIMR FB97, IIMR FB56, IIMR FB71, IIMR FB74, IIMR FB77, IIMR FB72, IIMR FB75, IIMR FB55, IIMR FB95

Table 5. Pooled cluster mean estimates of forage yield and related traits in forage pearl millet genotypes in three seasons

Trait	Cluster	Hierarchical Clustering				
		CI	CII	CIII	CIV	CV
Leaf length		0.46	-0.28	-0.29	1.31	-0.42
Leaf width		0.39	0.18	-0.31	0.45	-0.13
Number of leaves		-1.09	-0.92	0.31	0.82	0.37
Internodal length first cut		-0.76	-1.24	0.46	1.01	-0.03
Stem thickness first cut		-0.80	-0.16	-0.25	0.98	0.54
Plant height first cut		-0.22	-0.94	0.24	-0.96	0.51
Leaf stem ratio		0.73	-0.04	0.00	0.10	-0.56
Number of tillers first cut		-0.02	0.40	-0.28	0.80	-0.07
Number of tillers regrowth		1.16	0.26	-0.18	-0.10	-0.66
Number of new tillers		-0.47	0.65	-0.27	-0.19	0.55
Green fodder yield first cut		-1.14	-0.62	-0.28	0.68	1.07
Dry fodder yield first cut		-1.14	-0.59	-0.30	0.57	1.13
Green fodder yield second cut		0.55	1.71	-0.41	0.17	-0.45
Dry fodder yield second cut		0.58	1.94	-0.38	0.05	-0.52
Total dry fodder yield		-0.90	0.48	-0.55	0.65	0.92
Total green fodder yield		-0.91	0.22	-0.51	0.80	0.89

The hierarchical clustering analysis of forage pearl millet genotypes for pooled environments across different traits showed that Cluster IV had the highest positive mean for leaf length at 1.31, while negative values was observed Cluster II and Cluster III. For total green fodder yield, Cluster IV had a mean of 0.80, while Cluster I displayed a negative mean of -0.91. (Table 5). Particularly Cluster IV exhibited the highest positive means for important attributes such as leaf length, leaf width, number of leaves, internodal length and stem thickness, green fodder and dry fodder yield in both cuts. This shows that the genotypes within Cluster IV possess superior growth characteristics, contributing positively to forage yield. Conversely, Clusters II and III displayed several negative mean values across traits, indicating a need for further investigation to enhance their performance. The cluster sizes also indicate the genetic diversity among the genotypes, Cluster III had the largest number of genotypes, which provides broader genetic base for selection and improvement. These findings show the importance of cluster analysis in identifying and characterizing genotypes with desirable traits, paving the way for targeted breeding strategies aimed at improving forage quality and yield in diverse environments.

The results of this study align well with the findings of Chaudhary *et al.*(2015), Ramya*et al.* (2017), Kumar *et al.* (2020), Jain and Diwan (2021), Tomar *et al.* (2021), Gupta *et al.*(2022) and Kalagare *et al.* (2022), which also identified important traits contributing to yield within the same clusters. This highlights the stability of cluster analysis in uncovering key traits associated with yield in pearl millet and suggests that these traits could serve as valuable targets in breeding programs aimed at enhancing yield potential and in recovering high performing segregants through hybridization programs.

4.Conclusion

The present study identified leaf length, leaf width, number of leaves, plant height first cut, and number of tillers first cut as traits positively associated with total green fodder yield. These relationships were validated through principal component analysis, where four principal components explained 72% of total variation, with leaf characteristics and fodder yield components contributing significantly to the first two components (29% and 21% respectively). Hierarchical cluster analysis grouped genotypes into five clusters, with Cluster IV emerging as superior, showing high positive means for leaf length (1.31), leaf width (0.45), and total green fodder yield (0.80). The consistency of these trait associations across diverse analytical methods and clustering patterns provides robust selection criteria and identifies high-performing genotypes for forage traits. This comprehensive insight will guide future varietal and heterosis breeding programs in developing improved forage pearl millet varieties and hybrids, specifically adapted to thrive in arid and semi-arid regions.

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