GMR
The Original

GENETIC VARIABILITY, TRAIT ASSOCIATION AND MULTI-TRAIT SELECTION FOR GREEN FODDER YIELD IN PEARL MILLET GERMPLASM

Aswathy N B[1], Asish K Binodh[2*], R Pushpam[3], N Manikanda Bhoopathi[4], S Vellaikumar[5]

[1]Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641003, India.
[2]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641003, India.
[3]Department of Forage Crops, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641003, India.
[4,5]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641003, India.

***Corresponding author**: Asish K Binodh; Email: asish@tnau.ac.in

ABSTRACT

Fodder pearl millet (*Pennisetum glaucum* (L.) R. Br.), an important forage crop, and identifying germplasm that combines high biomass production with suitable maturity is important for crop improvement. In this study, 114 germplasm accessions were evaluated for 11 phenological, morphological and fodder-related traits during summer 2026 at Tamil Nadu Agricultural University, Coimbatore, using an augmented block design with three repeated checks. Block-adjusted observations were used to characterize trait variation and associations, followed by path analysis, principal component analysis (PCA), hierarchical clustering and Smith–Hazel selection-index analysis. Considerable variation was observed among accessions. Leaf weight (LWT), stem weight (STWT) and green fodder yield (GFY) showed high estimates of variability and expected genetic advance in the present analysis. GFY was strongly associated with STWT (r = 0.979) and LWT (r = 0.847), while LWT and STWT were also positively associated (r = 0.722). Path analysis produced positive coefficients for STWT (0.742) and LWT (0.317), although strong collinearity and the component total relationship require cautious interpretation. PCA highlighted biomass-related variation as a major dimension. Ward's hierarchical clustering grouped the accessions into four clusters containing 17, 12, 23 and 62 accessions, respectively, providing a broad characterization of phenotypic similarities. A Smith–Hazel index combining STWT, LWT and days to 50% flowering (DFF) identified preliminary candidates with different combinations of biomass and maturity. The predicted responses at 10% selection intensity were +165.248 g plant−1 for STWT, +62.790 g plant−1 for LWT and −2.530 days for DFF, with a predicted correlated response of +224.964 g plant−1 for GFY. Thirumanar local, PT4843, PT4120, IP20374, IP20334 and PT4685 were shortlisted for further evaluation.Because the test accessions were unreplicated and the genetic-parameter estimates require verification, these findings should be considered preliminary and validated in replicated, multi-environment trials.

KEYWORDS: fodder pearl millet, green fodder yield, trait association, principal component analysis, Smith–Hazel selection index

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important fodder crop because of its biomass potential and adaptation to warm, semi-arid production environments. Increasing demand for livestock feed has strengthened the need for fodder cultivars that combine high forage productivity with suitable maturity and adaptation. Germplasm collections provide a source of variation for identifying potential parents and developing improved populations. Green fodder yield is a complex productivity trait associated with several morphological and biomass components. Estimates of variation, heritability and expected genetic advance can support preliminary assessment of selection potential, while correlation analysis describes the strength and direction of trait associations. Path analysis partitions correlations into direct and indirect statistical components, but its interpretation requires particular care when predictors are strongly correlated or are components of the response trait. Recent forage pearl millet studies have documented variation for flowering, plant architecture, leaf traits, biomass and fodder yield, while also showing that the relative importance of individual traits can vary among populations and environments (Annamalai et al., 2020; Sharma et al., 2026; Supriya et al., 2024).

Multivariate analyses provide complementary descriptions of phenotypic variation. Principal component analysis (PCA) identifies major dimensions of standardized trait variation, whereas hierarchical clustering summarizes similarities among accessions. Multi-trait selection is relevant to fodder breeding because high biomass must be considered alongside maturity and other agronomic objectives (Khandelwal et al., 2024; Parray et al., 2025). This study integrated adjusted trait

characterization, association analysis, PCA, hierarchical clustering and a Smith–Hazel selection index to identify preliminary pearl millet accessions with potential for high biomass production and relatively early flowering. The findings are intended as an initial screening framework rather than definitive evidence of breeding value.

MATERIALS AND METHODS

Plant material and experimental site

A set of 114 pearl millet (*Pennisetum glaucum* (L.) R. Br.) germplasm accessions representing diverse genetic backgrounds were evaluated during summer 2026 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The experiment comprised 114 unreplicated test accessions arranged in three blocks, with 38 test accessions and three repeated checks (CO 8, BAIF Bajra and Giant Bajra) per block. Each entry was grown in rows of 4 m, with 60 cm row spacing and 15 cm plant spacing. Five plants were selected randomly from each genotype for biomass assessment. days to 50% flowering was recorded as the number of days from sowing to approximately 50% flowering, and each genotype was harvested at its respective 50% flowering stage, with harvesting commencing from 26 March 2026. Green fodder yield was measured as fresh above-ground biomass immediately after harvest. The harvested biomass was then separated into leaf and stem fractions, which were weighed separately to obtain leaf weight and stem weight, respectively. leaf stem ratio was calculated as leaf weight /stem weight.

Experimental design and block adjustment

The experiment comprised 114 unreplicated pearl millet germplasm accessions and three repeated checks (CO 8, Baif Bajra and Giant Bajra) evaluated in an augmented block design with three blocks. Each block contained 38 test accessions and three checks. Trait data were analyzed using the augmented RCBD package in R (Aravind et al., 2023). Adjusted treatment means were obtained from a block-plus-treatment model. The reported ANOVA had 2 degrees of freedom for blocks and 116 degrees of freedom for treatments; the treatment component was partitioned into checks, test entries and the test-versus-check contrast. The residual error had 4 degrees of freedom. Because the test accessions were unreplicated and the residual degrees of freedom were limited, variance parameters and derived genetic statistics should be interpreted as preliminary, design-dependent estimates.

Traits recorded

Days to 50% flowering (DFF) was recorded as the number of days from sowing until approximately 50% of the plants in an entry had flowered. Plant height (PH), stem girth (SG), number of leaves (No L), number of tillers (No T), leaf length (LL) and leaf breadth (LB) were recorded on representative plants. At harvest, fresh plant material was weighed for green fodder yield (GFY), and the leaf and stem fractions were subsequently separated and weighed to obtain leaf weight (leaf weight) and stem weight (stem weight), respectively. Leaf weight (Lwt) and stem weight (Swt) were expressed as g plant^{-1}, and the leaf-to-stem ratio (LSR) was calculated as leaf weight /stem weight. Green fodder yield was recorded as fresh green biomass in g plant^{-1}

Genetic variability parameters

Genetic variability parameters were summarized using the augmented-design analysis. GCV and PCV were calculated from the corresponding genotypic and phenotypic variance estimates, broad-sense heritability was calculated as $H^2 = (\sigma^2g/\sigma^2p) \times 100$, and expected genetic advance was calculated using a selection intensity of $k = 1.755$ for a 10% selection proportion. GAM was calculated as (GA/trait mean) × 100. Since the test entries were unreplicated and the residual error had limited degrees of freedom, these estimates are treated as preliminary indicators of variation under the present screening conditions.

Genotypic association and path analysis

Correlations among the 11 block-adjusted entry values were calculated to describe trait associations. Path coefficient analysis followed Dewey and Lu (1959), with GFY as the response variable and the other traits as explanatory variables. Multicollinearity was assessed using variance inflation factors (VIFs) and the condition number. High VIF and condition number values were interpreted as evidence that individual direct and indirect coefficients may be unstable. Because leaf and stem weights are components of harvested biomass, the associations with GFY also reflect a component total relationship; path coefficients are therefore interpreted as descriptive statistical partitions, not independent causal effects.

Principal component and hierarchical cluster analyses

PCA was conducted as per Jolliffe & Cadima (2016) on standardized block adjusted observations. Eigenvalues, percentage variance explained and trait loadings were calculated. The first two components were used to visualize genotype trait relationships. PCA was interpreted as a complementary multivariate representation of the trait structure rather than as an independent statistical validation of the correlation analysis.

Hierarchical cluster analysis was performed to assess phenotypic similarities among the 114 pearl millet accessions based on 11 agro-morphological traits. Before clustering, the trait values were standardized to a common scale using z-score transformation to avoid differences in measurement units influencing the analysis. Euclidean distance was used to estimate

dissimilarity among accessions, and Ward's minimum-variance method (Ward, 1963) was applied to construct the hierarchical dendrogram. Cluster-wise mean values were calculated to characterize the groups for the evaluated traits. The average silhouette coefficient was used to assess cluster cohesion and separation, with lower values indicating greater overlap among clusters. The resulting clusters were interpreted as groups of phenotypically similar accessions.

Smith Hazel selection index

A Smith–Hazel selection index was constructed by combining stem weight, leaf weight, and days to 50% flowering, following Smith (1936) and Hazel (1943). Index coefficients were derived from the phenotypic and genotypic variance–covariance matrices and the assigned economic weights. Stem weight and leaf weight were assigned positive selection directions and days to 50% flowering a negative direction. Green fodder yield was excluded from the index because of its strong overlap with the biomass components and was evaluated as a correlated response. The index coefficients were obtained from b = P−1Ga, where P is the phenotypic variance covariance matrix, G is the genotypic variance–covariance matrix used in the analysis, and a is the vector of economic weights. The diagonal elements of the genotypic variance–covariance matrix (G) were obtained from the genotypic variance estimates derived from the augmented-design ANOVA, where $\sigma_g^2 = MS_{\text{Treatment:Test}} - MS_E$. Genotypic covariances between traits were then derived from the corresponding genotypic correlation coefficients as $\sigma_{g,ij} = r_{g,ij}\sqrt{\sigma_{g,i}^2\sigma_{g,j}^2}$. The resulting matrix was used as the genotypic covariance matrix in the Smith–Hazel index. All three index traits were standardized before index construction. Equal economic weights were used because empirically derived economic weights were not available and the objective was exploring multi-trait ranking. A 10% selection intensity (k = 1.755) was adopted to identify a manageable subset of preliminary pre-breeding candidates for subsequent replicated testing. Direct responses in the indexed traits and the correlated response in green fodder yield were estimated from the covariance structure.

Results and Discussion

Phenotypic variation and adjusted genetic parameters

The evaluation of 114 pearl millet germplasm accessions showed substantial entry variation for all 11 fodder-related traits. The magnitude of phenotypic variation was relatively high for leaf weight, stem weight, green fodder yield, leaf:stem ratio and number of tillers, while comparatively lower variation was recorded for stem girth, days to 50% flowering, leaf length and plant height (Table 2). The presence of significant variation among germplasm accessions provides opportunities for identifying superior material for fodder improvement, as reported in earlier evaluations of pearl millet germplasm (Khairwal et al., 2007; Annamalai et al., 2020). Days to 50% flowering ranged from 36.81 to 71.44 days and plant height from 138.20 to 192.92 cm. Number of leaves ranged from 27.15 to 84.90, number of tillers from 3.19 to 10.57, leaf length from 50.40 to 76.52 cm and LB from 2.63 to 6.07 cm. The largest ranges occurred for biomass traits: leaf weight varied from 54.45 to 407.15 g plant−1, stem weight from 113.89 to 768.62 g plant−1 and green fodder yield from 177.45 to 1090.94 g plant−1. leaf stem ratio ranged from 0.245 to 1.085. These ranges demonstrate substantial phenotypic contrast and provide scope for preliminary identification of contrasting parental material (Table 1). Annamalai et al. (2020) similarly reported substantial variation among 50 ICRISAT pearl millet accessions, with green fodder yield ranging from 130 to 970 g plant−1 and considerable variation for plant height, tiller number, leaf traits, stem girth and leaf: stem ratio.

Genotypic and Phenotypic Coefficients of Variation

Table 2 showed that the magnitude of variability differed among the evaluated traits. The highest GCV and PCV estimates were recorded for leaf weight (40.63 and 42.13%), stem weight (38.60 and 41.38%) and green fodder yield (35.34 and 38.24%). High variability was also observed for leaf:stem ratio (33.62 and 33.63%) and number of tillers (26.26 and 27.09%). Number of leaves exhibited high variability, with GCV and PCV values of 21.39 and 23.20%, respectively, whereas leaf breadth showed moderate variability (13.98 and 15.25%). Comparatively low GCV and PCV values were recorded for days to 50% flowering (8.28 and 9.09%), plant height (6.37 and 6.79%), stem girth (6.58 and 6.86%) and leaf length (7.65 and 8.06%).

The relatively high GCV and PCV estimates for green fodder yield and its biomass components indicate substantial variation among the evaluated accessions for these traits. This pattern is consistent with previous pearl millet studies. Bika and Shekhawat (2015) reported high GCV and PCV for green fodder yield, number of tillers and leaf:stem ratio, while Thomas et al. (2018) reported considerable variability for green fodder yield and associated fodder traits. Annamalai et al. (2020) also observed high GCV and PCV for green fodder yield and several fodder-related traits in ICRISAT pearl millet accessions. Similar variation for biomass-related traits has been reported in pearl millet fodder materials (Rani et al., 2022; Sharma et al., 2026). Differences among studies may reflect variation in germplasm, environments, population size and experimental design.

Heritability and Genetic Advance

Broad-sense heritability estimates reported in Table 2 ranged from 82.97% for days to 50% flowering to 99.94% for leaf:stem ratio. Other comparatively high estimates included number of tillers (93.97%), leaf weight (93.01%), stem girth

(92.003%), leaf length (90.01%), plant height (88.01%), stem weight (87.01%), number of leaves (85.01%), green fodder yield (85.41%) and leaf breadth (84.04%). Genetic advance as a percentage of mean (GAM), estimated at 10% selection intensity (k = 1.755), exceeded 20% for leaf weight (68.77%), stem weight (63.19%), leaf:stem ratio (58.99%), green fodder yield (57.32%), number of tillers (44.68%), number of leaves (34.61%) and leaf breadth (22.49%). Moderate GAM estimates were recorded for days to 50% flowering (13.24%), leaf length (12.74%), stem girth (11.08%) and plant height (10.49%). The combination of high reported heritability and high GAM for several biomass-related traits suggests that these traits may be useful targets for preliminary selection. Nevertheless, because the entries were evaluated in an augmented, unreplicated design in one season and at one location, these estimates should not be treated as definitive predictions of realized genetic gain.

High heritability together with high genetic advance as a percentage of mean is often considered useful when assessing the potential response to selection (Johnson et al., 1955). In this study, the reported combination of high heritability and high GAM for leaf weight, stem weight, leaf:stem ratio and green fodder yield highlights these traits as potential priorities for further evaluation. However, broad-sense heritability includes both additive and non-additive genetic effects and does not, by itself, establish additive gene action. In addition, the present estimates come from a single location and season. Genotype × environment interaction may affect the expression of forage and biomass traits; therefore, replicated multi-environment trials are required to confirm the stability of these estimates and the usefulness of the accessions for selection, as emphasized in studies of forage improvement (Daduwal et al., 2026; Puttamadanayaka et al., 2026; Parray et al., 2025).

Associations among traits

Associations among the 11 traits were examined using the block-adjusted entry values (Table 3). Stem weight showed the strongest association with green fodder yield (r = 0.979) followed by leaf weight (r = 0.847) and leaf weight and stem weight were also strongly associated (r = 0.722). These relationships suggest a strong correlation between variation in total green biomass and stem and leaf biomass accumulations. These results support the importance of biomass related traits in selection for fodder yield reported in forage pearl millet (Sharma et al., 2024). Number of leaves and number of tillers were strongly positively associated (r = 0.651), suggesting coordinated variation in canopy development and tillering; however, neither trait showed a meaningful association with green fodder yield (r = 0.005 and 0.029, respectively). Thus, greater leaf or tiller number alone did not necessarily translate into greater total biomass in this material. Leaf stem ratio was positively associated with leaf weight (r = 0.307) and negatively associated with stem weight (r = −0.361), reflecting the ratio nature of this trait. Its association with green fodder yield was weak (r = −0.191). days to 50% flowering, plant height, number of leaves, number of tillers, leaf length and leaf breadth also showed weak relationships with green fodder yield (r = 0.128, 0.083, 0.005, 0.029, 0.008 and 0.051, respectively). Thus, absolute leaf and stem biomass were more closely associated with green fodder yield than individual architectural counts or the leaf: stem ratio in the present material. Because leaf weight and stem weight are components of harvested biomass, the strength of their associations with green fodder yield should be interpreted partly as component total relationships rather than as evidence of independent biological causation.

GFY showed strong positive associations with STWT (r = 0.979) and LWT (r = 0.847), while LWT and STWT were also positively associated (r = 0.722). Number of leaves and number of tillers were positively associated (r = 0.651), although neither showed a meaningful association with GFY in this dataset. LSR was positively associated with LWT and negatively associated with STWT, consistent with its ratio definition. Because GFY was measured from the same harvested material that was separated into leaf and stem fractions, the strong associations with LWT and STWT partly reflect a component–total relationship. These results identify biomass components as useful descriptors of variation in this dataset but do not establish independent causal effects or guarantee the same relationships in other environments.

Path coefficient analysis

Path coefficient analysis was used to separate the correlations between GFY and the explanatory traits into direct and indirect effects (Table 4; Fig. 1). Stem weight (STWT) showed the highest positive direct coefficient (0.742), followed by leaf weight (LWT; 0.317). The indirect effect of LWT through STWT was 0.536, while the corresponding effect of STWT through LWT was 0.229. Days to 50% flowering had a small positive direct coefficient (0.016), whereas plant height (PH), number of leaves and leaf length (LL) showed small negative direct effects. Stem girth, number of tillers (NOT) and leaf breadth (LB) had negligible direct effects, while leaf ratio exhibited a small negative coefficient (−0.021).

These results highlight the close association of stem and leaf biomass with GFY. However, the coefficients should be interpreted cautiously because strong correlations between biomass components can affect the stability of individual path estimates. Sharma et al. (2024) identified leaf breadth and dry fodder yield as important direct contributors to green forage yield under North Gujarat conditions. Similarly, Prakash et al. (2026) reported associations of GFY with stem girth, plant height, dry matter yield and root-related traits across seasons. Differences between these findings and the present results suggest that the relative contribution of individual traits may vary with the germplasm and growing environment. In the present study, the path analysis therefore supports the importance of biomass-related traits for preliminary selection but does not establish their independent causal effects on GFY.

In the present study, multicollinearity diagnostics indicated substantial overlap between the biomass predictors, with variance inflation factors of approximately 10.27 for STWT and 9.98 for LWT, and a condition number of approximately 44.87. The direct path coefficients should therefore be interpreted cautiously, as collinearity can make their individual magnitudes unstable. Furthermore, because leaf and stem weights are components of total harvested green biomass, their strong associations with GFY partly reflect a component–total relationship. Collectively, the correlation and path analyses identify STWT and LWT as important biomass-associated traits for preliminary selection; however, their independent causal contributions cannot be established from these coefficients alone.

Principal component analysis

Principal component analysis (PCA) was performed to identify the major sources of variation among the evaluated pearl millet germplasm accessions and to examine the contribution of phenological, morphological and fodder-related traits to overall variability. The principal components, their eigenvalues and percentage of variance explained are presented in Table 5. The first six principal components accounted for 86.04% of the total variation, indicating that a substantial proportion of the variability could be represented by a reduced set of components. PC1, PC2 and PC3 explained 25.78%, 15.46% and 14.57% of the total variation, respectively, while the first two components together accounted for 41.24%. PCA provides a dimensionality-reduction framework in which correlated variables are transformed into orthogonal components that successively capture variation in the dataset (Jolliffe and Cadima, 2016).

PC1 was primarily associated with green fodder yield (GFY; −0.977), stem weight (STWT; −0.951) and leaf weight (LWT; −0.855), indicating that biomass-related traits constituted the major dimension of variation. Their alignment in the biplot agreed with the strong correlations of GFY with STWT (r = 0.979) and LWT (r = 0.847). The current analysis's emphasis on biomass-related characteristics is in line with earlier research on forage pearl millet. While Supriya et al. (2024) reported significant multivariate variation among forage pearl millet genotypes, with leaf-related traits contributing to the principal components, Dilbagh et al. (2024) identified biological yield and other yield-associated traits as significant contributors to multivariate variation. All these results point to the significance of plant morphology and biomass accumulation in characterizing variation within forage pearl millet germplasm. PC2, which was defined by the number of tillers (NOT; 0.898) and leaves (NOL; 0.850), accounted for 15.46% of the variation. PC2 was defined by the number of tillers (NOT; 0.898) and number of leaves (NOL; 0.850), which were consistent with their positive correlation (r = 0.651) and accounted for 15.46% of the variation. However, rather than proving that they are useful indirect selection criteria for higher fodder output, their weak correlations with GFY suggest that they explain variance among accessions. PC3 was associated mainly with leaf breadth (0.759), leaf length (0.646) and stem girth (0.571), representing additional variation in leaf and stem morphology. The contribution of these traits suggests that morphological differences among accessions were not restricted to biomass accumulation and tillering. The importance of morphological traits in differentiating pearl millet genotypes is also evident in previous multivariate assessments of the crop (Dilbagh et al., 2024; Supriya et al., 2024).

The distribution of accessions across the biplot (Fig. 2) indicates appreciable phenotypic dispersion, particularly along PC1, reflecting differences in the expression of biomass-related traits. Accessions positioned toward the left-hand side of the plot are associated with the negative PC1 direction, along which the GFY, STWT and LWT vectors point. Such accessions may therefore exhibit comparatively higher values for these traits, subject to their positions relative to the trait vectors. The negative signs of the PC1 loadings do not indicate negative biological relationships because the signs of principal components are arbitrary. Similarly, the distribution of accessions along PC2 reflects variation associated with tiller and leaf number. However, the first two components together explained only 41.24% of the total variation, indicating that a substantial proportion of the multivariate information is represented by subsequent components. Consequently, the biplot provides a partial representation of the relationships among accessions and traits and should not be interpreted as a complete description of their variation.

Cluster analysis of pearl millet accessions

Hierarchical cluster analysis was performed to characterize phenotypic variation among the pearl millet germplasm accessions based on their measured traits. Ward's method was used to group accessions according to their multivariate similarity, and the silhouette coefficient was employed to assess cluster cohesion and separation (Ward, 1963; Rousseeuw, 1987). This analysis complemented principal component analysis by providing a hierarchical classification of accessions and facilitating the identification of groups with contrasting biomass and fodder-related characteristics.

Ward's hierarchical clustering grouped the 114 accessions into four clusters based on 11 standardized agro-morphological traits (Table 6; Fig. 3). Cluster I comprised of 17 accessions, Cluster II 12, Cluster III 23 and Cluster IV 62. The average silhouette coefficient was low (0.126), indicating that the four-cluster solution provides a broad summary of phenotypic similarity rather than sharply separated groups. Cluster I had the highest cluster means for GFY, LWT and STWT; however, cluster membership alone does not establish the superiority of an individual accession. Candidate selection should be guided by individual trait values and the selection index, followed by replicated validation.

The clusters differed mainly in biomass-related traits. Cluster I had the highest mean green fodder yield (GFY; 738.92 g plant^{-1}), leaf weight (LWT; 196.97 g plant^{-1}), and stem weight (STWT; 533.52 g plant^{-1}). Cluster II showed intermediate values for these traits, with a mean GFY of 412.93 g plant^{-1}. Cluster III was characterized by higher mean numbers of

leaves (60.38) and tillers (7.99), but its mean GFY was lower (399.46 g plant^{-1}) than that of Cluster II. Cluster IV, the largest group, had the lowest mean GFY (369.77 g plant^{-1}) and relatively low biomass values. These results suggest that differences in fodder yield among the clusters were more closely associated with biomass accumulation than with leaf and tiller numbers alone. The distribution of individual accessions among the four clusters is presented in Table 7.

Cluster I had the highest mean GFY (738.92 g plant^{-1}), LWT (196.97 g plant^{-1}) and STWT (533.52 g plant^{-1}). Cluster II had a mean GFY of 412.93 g plant^{-1}, while Cluster III showed higher mean numbers of leaves and tillers but a lower mean GFY of 399.46 g plant^{-1}. Cluster IV, the largest cluster, had the lowest mean GFY (369.77 g plant^{-1}). These cluster means summarize patterns in the data, but the low silhouette coefficient and the overlap among biomass-related traits mean that the groups should not be interpreted as distinct genetic populations. Because GFY, LWT and STWT are closely related, their joint inclusion may also have increased the influence of biomass variation on the clustering.

Smith Hazel selection index and predicted response

The Smith–Hazel index was employed to identify promising pearl millet accessions by simultaneously considering stem weight, leaf weight and days to 50% flowering. The index integrates information on phenotypic and genotypic variance–covariance structures, enabling the combined evaluation of multiple traits with different selection objectives (Smith, 1936; Hazel, 1943). Stem weight and leaf weight were given positive selection directions, while days to 50% flowering was given a negative direction to promote biomass buildup and faster maturation. Green fodder yield was excluded from the index and considered as a correlated response because of its strong association with the biomass components.

The index coefficients and expected responses are reported in Table 8. The standardized index coefficients were 0.821 for stem weight, 0.961 for leaf weight and −0.858 for days to 50% flowering. The genotypic variance accounted for 87.0%, 93.0% and 83.0% of the phenotypic variance in stem weight, leaf weight and days to 50% flowering, respectively. At 10% selection intensity, the current analysis reports responses of +165.248 g plant−1 for stem weight, +62.790 g plant−1 for leaf weight and −2.530 days for days to 50% flowering, with a correlated response of +224.964 g plant−1 for green fodder yield. These are theoretical predictions, not observed gains, and require validation in replicated trials.

Identification of promising pre-breeding accessions

The Smith–Hazel index identified pearl millet accessions with different combinations of biomass productivity and flowering time (Table 9). Thirumanar local recorded the highest index score (6.801), with a stem weight of 672.70 g plant−1, a leaf weight of 407.15 g plant−1 and a green fodder yield of 1090.94 g plant−1. Its high index score reflects its substantial leaf and stem biomass, although it flowered relatively late (56.51 days). PT4843 combined a high stem weight (687.97 g plant−1) with earlier flowering (42.65 days) and a green fodder yield of 924.43 g plant−1. PT4120, IP20374 and PT4685 flowered earlier (39.17, 36.87 and 36.81 days, respectively), with green fodder yields of 825.45, 764.78 and 656.08 g plant−1. IP20334 recorded the highest stem weight among the top-ranked accessions (768.62 g plant−1) and a green fodder yield of 997.53 g plant−1. IP23479 showed a different biomass pattern, with a relatively high leaf weight (287.94 g plant−1) but a lower stem weight (265.14 g plant−1) and green fodder yield (554.37 g plant−1). It may be worth evaluating where leaf biomass is a specific objective, although forage quality traits would need to be measured before drawing conclusions about its nutritional value. The usefulness of multi-trait selection in pearl millet has been demonstrated by Kalagare et al. (2021), who evaluated selection indices for identifying promising genotypes, and Khandelwal et al. (2024), who combined agronomic performance and stability to identify suitable cultivars across locations. Prakash et al. (2026) also highlighted the value of integrating morphological and molecular diversity to identify potential parents for high-biomass fodder pearl millet. In the present study, the Smith Hazel index considered biomass components together with flowering time rather than ranking accessions solely by GFY. However, because the test entries were evaluated in an augmented, unreplicated design, these accessions should be treated as preliminary candidates. Their performance and ranking need confirmation through replicated, multi-environment trials before further breeding use.

CONCLUSION

The evaluation of 114 pearl millet germplasm accessions revealed substantial variation in flowering time, plant morphology and fodder-related traits. Biomass-related traits, particularly LWT, STWT and GFY, showed promising variation in the present screening, and the strong associations of LWT and STWT with GFY highlighted their value for describing biomass production. However, because these traits are components of harvested biomass and are strongly collinear, the path coefficients should be interpreted cautiously. PCA identified biomass-related variation as a major dimension of the dataset. Ward's hierarchical clustering grouped the accessions into four clusters of unequal sizes, providing a broad characterization of phenotypic similarities; the low silhouette coefficient indicated that the groups were not sharply separated. The Smith–Hazel index identified preliminary candidates with different combinations of biomass and flowering time. Thirumanar local, PT4843, PT4120, IP20374, IP20334 and PT4685 warrant further evaluation based on their individual trait values and index rankings. Because the test accessions were unreplicated and the genetic-parameter and index calculations require verification, the predicted gains and rankings should be considered preliminary. The selected accessions should be tested in replicated, multi-environment trials before breeding advancement.

The Smith–Hazel selection index helped identify accessions with favourable combinations of biomass traits and flowering time. The predicted responses indicated increases in stem weight, leaf weight and green fodder yield, along with a

reduction in days to 50% flowering. Thirumanar local, PT4843, PT4120, IP20374, IP20334 and PT4685 were identified as promising candidates for further pre-breeding evaluation, as they showed different combinations of biomass production and maturity. Although Cluster I included several accessions of interest, cluster membership alone was not sufficient to establish their superiority; individual trait performance and selection index values should guide further selection. Overall, the findings highlight the importance of considering both leaf and stem biomass while accounting for flowering time, rather than selecting solely for high biomass. Since the accessions were evaluated using an augmented, unreplicated design, the predicted genetic gains and accession rankings should be treated as preliminary. Replicated trials across multiple environments are needed to confirm their performance and determine their value for fodder pearl millet improvement

Table 1. Adjusted means, range and analysis of variance for eleven traits

Trait	Adjusted mean	Range	F	P	CV (%)
DFF	50.19	36.81–71.44	5.84	0.047	3.75
PH	166.57	138.20–192.92	8.32	0.025	2.35
SG	4.68	3.87–5.78	15.00	0.008	1.94
NOL	48.50	27.15–84.90	6.27	0.042	8.99
NOT	6.41	3.19–10.57	15.97	0.007	6.63
LL	62.87	50.40–76.52	9.80	0.018	2.55
LB	3.96	2.63–6.07	5.71	0.049	6.10
LWT	116.65	54.45–407.15	13.69	0.010	11.15
SWT	310.77	113.89–768.62	7.82	0.028	14.92
LSR	0.395	0.245–1.085	1303.86	<0.001	0.92
GFY	437.37	177.45–1090.94	6.88	0.035	14.61

Table 2. Adjusted estimates of genetic variability and expected response parameters.

Trait	GCV (%)	PCV (%)	ECV (%)	Heritability (h^2, %)	GA	GAM (%)
Days to 50% flowering	8.28	9.09	3.75	82.97	6.64	13.24
Plant height	6.37	6.79	2.35	88.01	17.47	10.49
Stem girth	6.58	6.86	1.94	92.003	0.52	11.08
Number of leaves	21.39	23.20	8.99	85.01	16.79	34.61
Number of tillers	26.26	27.09	6.63	93.97	2.86	44.68
Leaf length	7.65	8.06	2.55	90.01	8.01	12.74
Leaf breadth	13.98	15.25	6.10	84.04	0.89	22.49
Leaf weight	40.63	42.13	11.15	93.01	80.22	68.77
Stem weight	38.60	41.38	14.92	87.01	196.38	63.19
Leaf:stem ratio	33.62	33.63	0.92	99.94	0.23	58.99
Green fodder yield	35.34	38.24	14.61	85.41	250.69	57.32

Table 3. Correlations among block-adjusted entry values for fodder-related traits.

Trait	DFF	PH	SG	No L	No T	LL	LB	L Wt	S Wt	LSR	GFY
DFF	1.000	0.105	0.212*	−0.019	−0.109	−0.016	0.108	0.132	0.096	−0.041	0.128
PH	0.105	1.000	0.237**	−0.110	−0.074	−0.026	−0.104	0.020	0.112	−0.154	0.083
SG	0.212*	0.237**	1.000	−0.058	0.048	0.208*	0.266**	−0.018	−0.008	−0.097	−0.014
No L	−0.019	−0.110	−0.058	1.000	0.651***	−0.038	0.177	0.023	0.010	−0.024	0.005
No T	−0.109	−0.074	0.048	0.651***	1.000	−0.116	0.047	−0.034	0.058	−0.140	0.029
LL	−0.016	−0.026	0.208*	−0.038	−0.116	1.000	0.331***	0.083	−0.005	0.091	0.008
LB	0.108	−0.104	0.266**	0.177	0.047	0.331***	1.000	0.138	0.016	0.141	0.051
L Wt	0.132	0.020	−0.018	0.023	−0.034	0.083	0.138	1.000	0.722***	0.307***	0.847***
S Wt	0.096	0.112	−0.008	0.010	0.058	−0.005	0.016	0.722***	1.000	−0.361***	0.979***
LSR	−0.041	−0.154	−0.097	−0.024	−0.140	0.091	0.141	0.307***	−0.361***	1.000	−0.191
GFY	0.128	0.083	−0.014	0.005	0.029	0.008	0.051	0.847***	0.979***	−0.191	1.000

Note: * indicates significance at P < 0.05, ** indicates significance at P < 0.01, and *** indicates significance at P < 0.001.

Table 4. Direct path coefficients for green fodder yield.

Trait	Direct effect	Key interpretation
SWT	0.742	Largest positive direct coefficient; strongly correlated with green fodder yield
LWT	0.317	Positive direct coefficient; strong association with green fodder yield
DFF	0.016	Small positive direct coefficient
PH	−0.012	Small negative direct coefficient
NOL	−0.014	Small negative direct coefficient
LL	−0.014	Small negative direct coefficient
SG	0.004	Negligible direct coefficient
NOT	0.002	Negligible direct coefficient
LB	0.003	Negligible direct coefficient
LSR	−0.021	Small negative direct coefficient

Table 5. Principal component eigenvalues and variance explained.

Component	Eigenvalue	Variance explained (%)	Cumulative (%)
PC1	2.836	25.78	25.78
PC2	1.701	15.46	41.24
PC3	1.603	14.57	55.82
PC4	1.446	13.14	68.96
PC5	0.991	9.01	77.97
PC6	0.888	8.07	86.04

Table 6. Cluster-wise mean values of agro-morphological traits among 114 pearl millet accessions based on Ward's hierarchical clustering.

Cluster	Number of accessions	DFF	PH	SG	NOL	NOT	LL	LB	LWT	STWT	LSR	GFY
I	17	50.77	170.37	4.61	50.70	6.56	62.71	4.10	196.97	533.52	0.40	738.92
II	12	45.39	158.03	4.29	51.45	7.77	55.43	3.13	106.07	294.49	0.36	412.93
III	23	50.59	165.01	4.92	60.38	7.99	63.83	4.36	109.60	280.19	0.40	399.46
IV	62	50.70	167.20	4.65	42.80	5.55	63.73	3.95	99.20	260.53	0.40	369.77

Table 7. Composition of the four clusters and distribution of pearl millet accessions based on Ward's hierarchical clustering

Cluster	Number of accessions	Accessions
I	17	PT 6227, PT 6237, Thirumanar local, GP 15074, GP 15189, GP 15953, GP 15988, TNFB 9902, GP 16060, IP 20334, IP 20338, IP 22268, IP 23479, PT 4685, PT 4120, PT 4843, IP 20374
II	12	PT 6244, PT 6246, Kinathu kadavil local, Thiruchengode local, Thiruvennamalali local, Karamadi local, Kalipalayam local, Sulkania, RBB -1, PT 5360, IP 3110, PT 3836

III	23	PT 6247, PT 7053, Gobi local, APFB-9, GFB -1, GP 15072, IP 20378, PT 5365, IP 5951, IP 6105, IP 8327, IP 9527, IP 11907, IP 15527, IP 18308, IP 22269, IP 11949, IP 13295, PT 5652, PT 5682, PT 5701, PT 5474, GP15445
IV	62	PT 6226, PT 6234, PT 6235, PT 6241, PT 6243, Vellakavil local, Salem local, PT 6231, Sathy local, Trichy local, Coimbatore local, Thidivanam local, Vellaimalali local, Bavani sagar local, PCB -166, Moti bajra, GP 15076, GP 15495, GP 15958, GP 15974, GP 16016, TNFB 9901, GP 16021, GP 16026, GP 16257, GP 16271, GP 18215, GP 18219, IP 3629, IP20273, IP 20333, IP 20346, IP 20347, IP 20349, IP 20379, IP 20840, PT 5142, PT 5151, PT 5164, PT 5165, PT 5217, PT 5222, PT 5259, PT 5143, IP 3616, IP 11838, IP 15251, IP 20475, IP 20538, IP 20540, IP 20563, IP 11919, IP 11920, IP 14911, PT 4806, IP 11950, IP 12431, ICMR 1002, PT 5581, PT 5588, PT 4061, GP15415

Table 8. Smith–Hazel selection-index coefficients and predicted responses at 10% selection intensity.

Trait	Index coefficient (standardized)	Expected response	Direction
stem weight	0.821	+165.248 g plant−1	Increase
leaf weight	0.961	+62.790 g plant−1	Increase
days to 50% flowering	−0.858	−2.530 days	Decrease
green fodder yield	Excluded	+224.964 g plant−1	Correlated response

Table 9. Top 15 accessions ranked by the Smith–Hazel selection index.

Entry	Index score	Stem weight (g plant−1)	Leaf weight (g plant−1)	Days to 50% flowering (days)	Green fodder yield (g plant−1)
Thirumanar local	6.801	672.70	407.15	56.51	1090.94
PT4843	6.016	687.97	228.53	42.65	924.43
PT4120	5.459	647.07	179.91	39.17	825.45
IP20374	5.214	589.26	164.12	36.87	764.78
IP20334	4.883	768.62	220.35	50.56	997.53
PT4685	4.497	503.31	154.91	36.81	656.08
IP23479	3.406	265.14	287.94	48.34	554.37
GP15953	3.214	712.80	197.47	55.16	917.32
PT6237	2.145	477.93	177.52	50.80	668.18
PT5365	2.142	448.23	151.74	47.13	609.49
IP11907	1.953	258.13	104.97	36.82	377.09
PT5581	1.879	300.13	120.73	40.27	423.00
IP22269	1.786	314.18	199.64	49.45	517.56
TNFB9902	1.703	547.48	184.89	56.27	740.62
GP16060	1.662	453.20	136.78	48.29	607.65

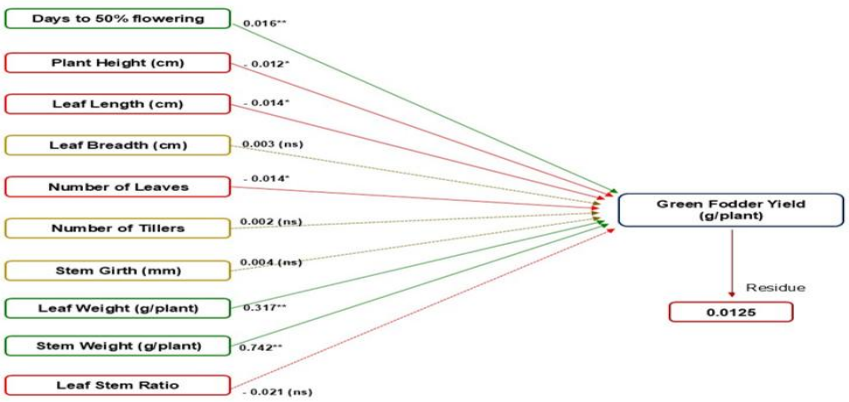

Fig. 1. Path coefficients for phenological, morphological and biomass-related traits in relation to green fodder yield.

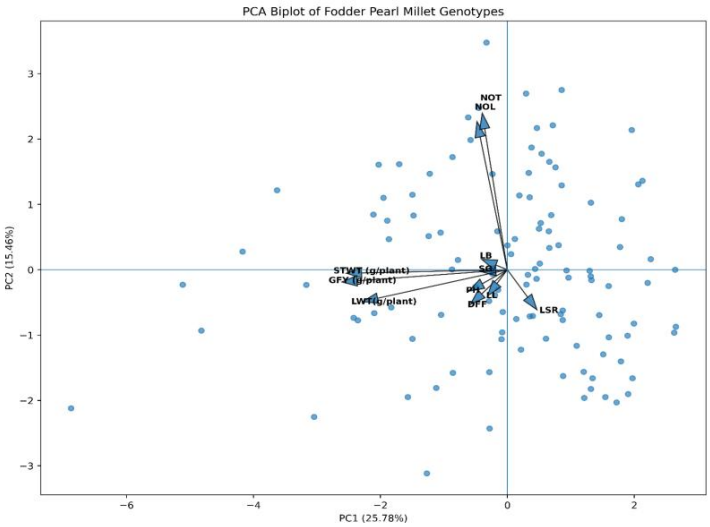

Fig. 2. Principal component biplot showing the distribution of pearl millet germplasm accessions

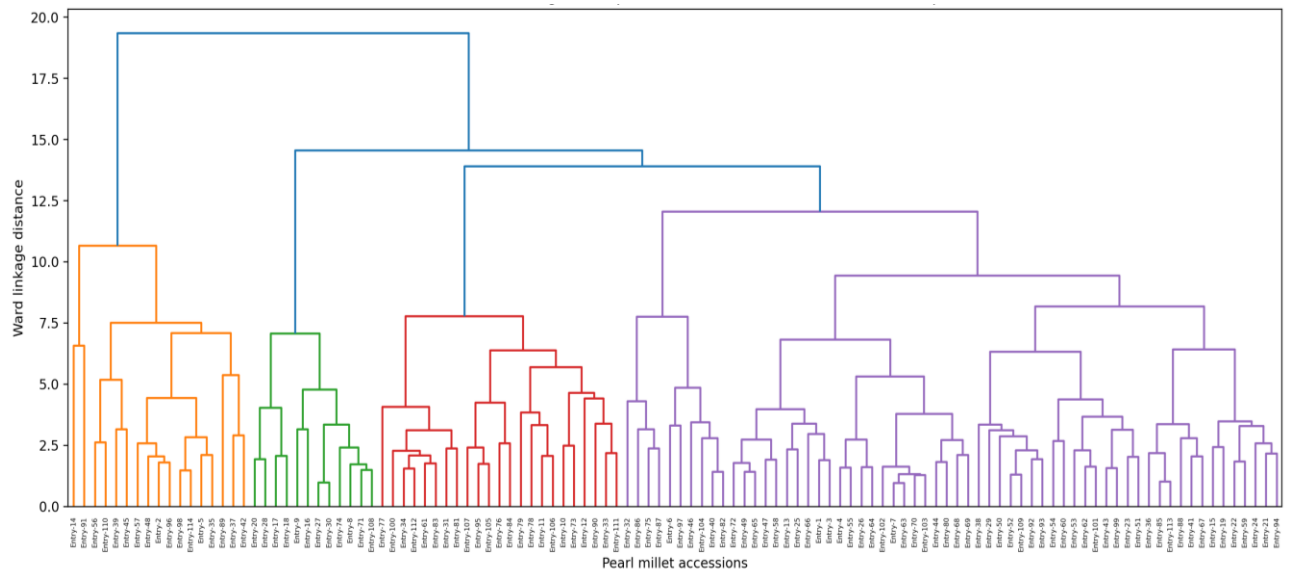

Fig. 3. Ward's hierarchical clustering of the 114 pearl millet accessions based on standardized agro-morphological traits

REFERENCES

1. Annamalai, R., Aananthi, N., Arumugam Pillai, M., & Leninraja, D. (2020). Assessment of variability and character association in pearl millet [Pennisetum glaucum (L.) R. Br.]. International Journal of Current Microbiology and Applied Sciences, 9(6), 3247–3259. https://doi.org/10.20546/ijcmas.2020.906.388
2. Aravind, J., Mukesh Sankar, S., Pandhari Wankhede, D., & Kaur, V. (2023). augmentedRCBD: Analysis of augmented randomised complete block designs. Zenodo. https://doi.org/10.5281/zenodo.8267052
3. Bika, N. K., & Shekhawat, S. S. (2015). Genetic variability study in pearl millet (Pennisetum glaucum (L.) R. Br.) for green fodder yield and related traits. Electronic Journal of Plant Breeding, 6(2), 600–602.
4. Daduwal, H. S., Bhardwaj, R., Sohu, R. S., Lamba, J. S., Singh, M., & Srivastava, R. K. (2026). Genetic variability and genotype-by-environment interaction for forage quality and biomass traits in pearl millet. Cereal Research Communications, 54, 1609–1622. https://doi.org/10.1007/s42976-026-00794-0
5. Dewey, D. R., & Lu, K. H. (1959). A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal, 51(9), 515–518. https://doi.org/10.2134/agronj1959.00021962005100090002x
6. Dilbagh, Sehrawat, K. D., Pahuja, S. K., Panchta, R., & Arora, S. (2024). Evaluation of variations in pearl millet (Pennisetum glaucum) genotypes for yield and its attributing traits under semi-arid conditions by principal component analysis. Forage Research, 50(1), 9–14. https://forageresearch.in/wp-content/uploads/2024/10/3.-9-14-DILBAGH.pd
7. Hazel, L. N. (1943). The genetic basis for constructing selection indexes. Genetics, 28(6), 476–490. https://doi.org/10.1093/genetics/28.6.476
8. Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans. Agronomy Journal, 47, 314–318.
9. Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 374(2065), Article 20150202. https://doi.org/10.1098/rsta.2015.0202
10. Kalagare, V. S., Iyanar, K., Chitdeshwari, T., & Chandrasekhar, C. N. (2021). Strategy of multiple selection indices for discrimination of potential genotypes and associated traits for yield improvement in pearl millet [Pennisetum glaucum (L.) R. Br.]. Electronic Journal of Plant Breeding, 12(3), 895–906. https://doi.org/10.37992/2021.1203.124
11. Khairwal, I. S., Yadav, S. K., Rai, K. N., Upadhyaya, H. D., Kachhawa, D., Nirwan, B., Bhattacharjee, R., Rajpurohit, B. S., Dangaria, C. J., & Srikant. (2007). Evaluation and identification of promising pearl millet germplasm for grain and fodder traits. Journal of SAT Agricultural Research, 5(1).
12. Khandelwal, V., Patel, R., Choudhary, K. B., Pawar, S. B., Patel, M. S., Iyanar, K., Mungra, K. D., Kumar, S., & Satyavathi, C. T. (2024). Stability analysis and identification of superior hybrids in pearl millet (Pennisetum glaucum (L.) R. Br.) using the multi trait stability index. Plants, 13(8), Article 1101. https://doi.org/10.3390/plants13081101
13. Parray, S. A., Ramalingam, A. P., Tugoo, M. Z., Serba, D. D., Vara Prasad, P. V., & Perumal, R. (2025). Harnessing genetic diversity for drought tolerance and forage yield improvement in pearl millet germplasms through a prebreeding framework. Scientific Reports, 15(1), Article 40033. https://doi.org/10.1038/s41598-025-23884-1
14. Prakash, U., Yadav, V. K., Mehta, B. K., Dwivedi, K. K., Keerthana, D., Choudhary, R., Shweta, & Kaushal, P. (2026). Integrating morphological and molecular diversity to develop high-biomass fodder pearl millet composites. Frontiers in Plant Science, 17, Article 1767075. https://doi.org/10.3389/fpls.2026.1767075
15. Puttamadanayaka, S., Sannagoudar, M. S., Siddaiah, C. N., Kumar, V., Mehta, B. K., Kumar, A., Dwivedi, K. K., Ponnaiah, G., & Gupta, S. K. (2026). Multi-environment evaluation and stability analysis for the selection of elite pearl millet genotypes with better fodder yield and quality component traits. Plants, 15(7), Article 1034. https://doi.org/10.3390/plants15071034
16. Rani, R., Khandelwal, V., Ramesh, Junjhdia, S., Singh, A., & Kumar, V. (2022). Genetic variability and association analysis for dry fodder yield and its component traits in pearl millet inbred. Forage Research, 48(1), 57–61.
17. Rousseeuw, P. J. (1987). Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics, 20, 53–65. https://doi.org/10.1016/0377-0427(87)90125-7
18. Sharma, R., Viradiya, Y. A., Donga, A. R., Parsaniya, T. A., Beniwal, J., & Jivan, P. P. (2026). Genetic variability in distinct genotypes of forage pearl millet (Pennisetum glaucum (L.) R. Br.) for forage yield and forage yield attributing traits. International Journal of Economic Plants, 11(4), 515–520.
19. Sharma, R., Viradiya, Y. A., Jivan, P. P., & Donga, A. R. (2024). Delineation of selection criterion using correlation and path analysis in forage pearl millet (Pennisetum glaucum (L.) R. Br.) in North Gujarat condition. Journal of Experimental Agriculture International, 46(10), 103–112. https://doi.org/10.9734/jeai/2024/v46i102929
20. Smith, H. F. (1936). A discriminant function for plant selection. Annals of Eugenics, 7, 240–250. https://doi.org/10.1111/j.1469-1809.1936.tb02143.x
21. Supriya, P., Singode, A., Reddy, D. M., Ronda, V., Nayak, D. S., & Reddi Sekhar, M. (2024). Multi-trait analysis and clustering of forage pearl millet genotypes in semi-arid conditions. Journal of Advances in Biology & Biotechnology, 27(11), 1602–1613. https://doi.org/10.9734/jabb/2024/v27i111747
22. Thomas, A. M., Babu, C., & Iyanar, K. (2018). Genetic variability and association studies in pearl millet for green fodder yield and quality traits. Electronic Journal of Plant Breeding, 9(3), 1263–1271. https://doi.org/10.5958/0975-928X.2018.00158.8

23. Ward, J. H., Jr. (1963). Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association, 58(301), 236–244. https://doi.org/10.1080/01621459.1963.10500845