Madras Agric. J., 2023; https://doi.org/10.29321/MAJ.10.000723

RESEARCH ARTICLE

Genetic Diversity and Variability in Barnyard Millet [*Echinochloa frumentaceae* (L.)] Germplasm Based on Morphological Traits

Geethanjali S[1*], Dhandapani M[2], and Sivakumar V[3]

[1*]Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore – 641 003, India

[2]Tamil Nadu Rice Research Institute, Aduthurai – 612 101, India

[3]Department of Fruit Science, Tamil Nadu Agricultural University, Coimbatore - 641 003, India.

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Received: 15 February 2022

Revised: 26 February 2022

Revised: 01 March 2022

Accepted: 05 March 2022

ABSTRACT

Characterization of germplasm based on the phenotypic traits is essential for the identification of desirable genotypes in any crop improvement programme. In the present study, fifty three barnyard millet genotypes including checks were characterized for morphological traits. The genotypes exhibited considerable variation for the phenotypic traits studied. Single plant yield recorded the highest coefficient of variation of 35.47 per cent, followed by lower raceme length (32.22%), and peduncle length (30.52%). The least coefficient of variation (CV) of 6.18 per cent was observed for plant height. PCA analysis revealed that the first four principal components contributed to a maximum of 70.98 per cent of the total variation among genotypes. Cluster analysis based on quantitative traits categorized the 53 barnyard millet accessions into four distinctive clusters. Cluster I and cluster II included high-yielding genotypes, while cluster III and cluster IV consisted of low-yielding genotypes. The genotypes viz., GECH758, GECH746, GECH27, CO(KV)2, MA1, GECH10 and TNEf192 were found to be superior in terms of yield attributing traits viz., single plant yield and thousand-grain weight. The desirable genotypes can be utilized in hybridization programs for yield improvement in barnyard millet.

Keywords: *Barnyard millet; Genetic Diversity; PCA; Cluster analysis*

INTRODUCTION

Barnyard millet (*Echinochloa frumentacea* (Roxb.) Link) is cultivated in the humid tropical regions of India, Central African Republic, Tanzania, and Malawi (Wanous, 1990). India ranks first globally in terms of area and production of barnyard millet, with an average productivity of 1034 kg ha^{-1} (IIMR, 2018). It is a climate-resilient crop which supplies food and fodder with limited crop management practices even under unfavorable agro ecological climatic circumstances such as drought and flooding (Dwivedi *et al.,* 2012). Barnyard millet is primarily grown in two diverse agro-ecologies in India, such as in mid hills of Himalayan region and in the Deccan plateau region of Tamil Nadu (Sood *et al.,* 2015). In recent years, barnyard millet has received considerable attention mainly because of its high nutritive value and nutraceutical properties. It is an energy rich food and serves as a good source of carbohydrates (65%), protein (11%), fat (3.9%), and crude fiber (13.6%). The presence of notably high levels of micronutrients like Iron (Fe), Zinc (Zn), and anti-oxidative composites compared to other cereals and millets makes barnyard millet a nature's endowment for contemporary mankind (Saleh *et al.,* 2013; Renganathan *et al.,* 2017).

Genetic improvement for yield and yield attributes in any breeding program is highly dependent and influenced by the genetic variability in the gene pool. Although barnyard millet is a potential food and fodder crop, the genetic resources in this crop have not been extensively explored, and the crop remains under-utilized yet. Hence exploring the extent of genetic diversity and

*Corresponding author's e-mail: geethanjalitnau@yahoo.com

selecting superior genotypes with desirable attributes becomes a prerequisite for effectively exploiting genetic resources and developing improved cultivars in barnyard millet. Both morphological and molecular methods are employed in estimating genetic diversity in germplasm collections. Morphological characterization is the first step for assessing, describing, and classifying germplasm collections to increase their use in crop breeding (Wasala *et al.,* 2013). Although morphological evaluation is limited by factors such as ontogeny, a limited number of phenotypic traits, the effect of environment on trait expression, low heritability, time consuming and labour intensive process, it offers an unparalleled means of identification of phenotypic variation. Phenotypic descriptors have been efficiently used for analyzing diversity in cereal crops, including millets. Multivariate analysis such as D2 analysis, principal component analysis and cluster analysis help to efficiently categorize genotypes based on their level of diversity and help breeders in selecting diverse genotypes for hybridization programmes. Only limited studies have been attempted to explore the genetic diversity in this orphan crop (Mehta *et al.,* 2005; Gupta *et al.,* 2009; Sood *et al.,* 2015; Dhanalakshmi *et al.,* 2019; Vanniarajan and Chandirakala, 2020). Hence, the present study was undertaken to assess the extent of diversity and variability in a set of 53 barnyard millet accessions based on morphological characterization.

MATERIAL AND METHODS

Fifty-three national elite barnyard millet accessions, including checks obtained from the All India Coordinated Small Millet Improvement Project (AICSMIP) unit, Bengaluru, were evaluated in the present study. The trial was conducted during Kharif, 2014 at Millets Breeding Station (MBS), Tamil Nadu Agricultural University, Coimbatore, India, which is situated at about 11° N latitude and 77° E longitude at an altitude of 427 meters above MSL. The average annual rainfall is around 700 mm. The trial was laid out in an augmented block design (Federer and Raghavarao, 1975) with Co (KV)2 as check variety in each block. Each accession was grown in a row of three meters length with a spacing of 30 cm between rows and 10 cm between plants for morphological characterization and evaluation. The recommended agronomic packages of practices were followed during the experimental period. Ten morphological traits were recorded on five randomly selected plants of each accession as per the standard descriptors described for barnyard millet (IBPGR, 1983). The ten traits measured are as follows: plant height (PH) [measured in cm from ground level to the tip of the inflorescence at dough stage], number of basal tillers per plant (TPP) [measured as number of tillers at ground level or from the basal nodes], days to 50% flowering (DFF) [counted as number of days from sowing to the stage when the ears have emerged on 50% of the main tillers], flag leaf length (FLL) [measured in cm from ligule to flag leaf tip at flowering stage], flag leaf width (FLW) [measured in cm across the widest point of the flag leaf at flowering], peduncle length (PL) [measured in cm from top most node to the base of the inflorescence], inflorescence length (IL) [measured in cm from lowest raceme to the tip of the last raceme on the main tiller at dough stage], lower raceme length [measured in cm], 1000 seed weight (TW) [measured as weight of 1000 seeds (g) sampled randomly from the total harvest of an accession] and single plant yield (GPY) [measured in g as the mean grain yield per plant based on five random plants]. The data were subjected to the following statistical analysis. Basic descriptive statistics were obtained using the Excel software. Frequency distribution and principal component analysis (PCA) was performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL) 16.0 version. The complete linkage method and Euclidean distance were employed to group the 53 barnyard millet accessions based on the similarity matrix as implemented in Minitab software.

RESULTS AND DISCUSSION

The basic descriptive statistical measures viz., mean, minimum, maximum, variance, standard deviation (SD), coefficient of variation (CV), skewness and kurtosis coefficients for the morphological traits recorded are presented in Table 1. The traits showed a continuous variation and the frequency distribution of the ten traits is presented in Figure 1.

Plant height ranged from 101.50 to 154.0 cm. The number of basal tillers ranged from 2.5 to 5.25. The days to fifty percent flowering ranged from 40 to 67 days after sowing. GECH10 was the earliest to flower while ACM 331 recorded the maximum number of days to attain 50 per cent flowering. Among 53 genotypes, 33 genotypes attained 50% flowering within 50 days, 13 genotypes attained 50% flowering within 50-60 days

and seven genotypes attained 50% flowering after 60 DAS. The flag leaf length (16.33 cm) and width (1.43 cm) was recorded by ACM332. The mean peduncle length, inflorescence length and lower raceme length in the germplasm collection were 13.81 cm, 19.48 cm and 2.73 cm respectively. Thousand grain weight ranged from 2.26 to 3.93 g. The genotypes showed a wide variation for single plant yield ranging from 6.25 g to 26.75 g with a mean value of 13.45 g in the germplasm collection evaluated.

Among the ten traits, the largest variation was observed for single plant yield, with the coefficient of variation amounting to 35.47 per cent, followed by lower raceme length (32.22%), and peduncle length (30.52%). The least coefficient of variation (CV) of 6.18 per cent was observed for plant height. Sood *et al.,* (2015) also reported that grain weight and a number of basal tillers exhibited a high level of variation, with CV exceeding 30% in a global core collection of barnyard millet. Similarly, Mehta *et al*., (2005) and Gupta *et al*., (2009) reported high variability for grain yield and number of tillers.

Although the traits showed continuous variation, analysis of skewness and kurtosis, which are measures of symmetry and heaviness of distribution tails, indicated that flag leaf length, flag leaf width, and inflorescence length exhibited a fairly symmetrical normal distribution (skewness between -0.5 to 0.5). The vegetative parameters, such as plant height and a number of basal tillers, were negatively skewed, while floral and yield parameters, such as days to 50% flowering, peduncle length, lower raceme length, thousand-grain weight, and single plant yield showed a positively skewed distribution. Such a skewed distribution for morphological traits like number of basal tillers, flag leaf length, flag leaf width, peduncle length, single plant yield and 1000 seed weight has been reported in foxtail millet (Gowda *et al.,* 2002) and for traits such as plant height, number of productive tillers, days to 50% flowering, hundred grain weight and grain yield per plant in proso millet (Salini *et al.,* 2010). The skewed distribution indicates that these traits are governed by non-additive gene action and are influenced by the environment. Considering kurtosis, plant height, peduncle length, and lower raceme length showed a leptokurtic distribution, while all other traits exhibited a normal distribution. The high values of kurtosis recorded for peduncle length and lower raceme length could be attributed to the presence of outliers in the germplasm collection evaluated and can be influenced by the size of the germplasm.

Principal component analysis, a reductionist approach, helps assess the interrelationship among the various parameters and the independent contribution of each component to the total variance. PCA analysis revealed that the first four principal components contributed to a maximum of 70.98 per cent of the total variation among genotypes. These four principal components were retained based on the scree plot and threshold eigen value greater than 1 (Fig 2 and Table 2). The component plots for the ten morphological traits is presented in Figure 3. The first principal component accounted for 25.76 per cent of total variability. This was predominantly attributed by inflorescence length, lower raceme length, and thousand-grain weight in a positive direction, while days to flowering contributed in the negative direction.

The second component axis accounted for 23.21 per cent of total variability and exhibited positive loading for most traits except a number of tillers and peduncle length. However, high positive loadings were observed for flag leaf length, flag leaf width, plant height and single plant yield, which reflected the prominent role of flag leaf towards increasing the grain yield per plant. This is anticipated, since flag leaf functions as an important photosynthetic organ and serves as a primary source in increasing grain yield (Zhang *et al.,* 2015) and at least 50% of photosynthetic products for grain are provided by the flag leaf (Li *et al.,* 1998). Hence flag leaf length and width should be considered as important selection criteria for yield improvement. The loading of number of tillers in the negative direction indicated that tall accessions generally produced a lesser number of tillers. The tendency of tall accessions to be single stemmed or with very few tillers has also been observed by (Upadhaya *et al.,* 2009, Kim *et al.,* 2010, Geethanjali *et al.,* 2016) in foxtail millet.

The third component accounting for 11.85 per cent of total variability differentiated the accessions based on yield attributes, namely 1000 grain weight and grain yield per plant. The high positive loading of peduncle length in this axis, indicated that peduncle elongation rate has a positive impact on yield attributes in barnyard millet. Similar positive impact of peduncle elongation on grain yield has also been reported in foxtail millet (Geethanjali *et al.,* 2016).

The fourth principal component accounting for 10.16 percent of the total variation, differentiated the accessions based on floral parameters and thousand-grain weight with high positive loadings for peduncle length, inflorescence length, lower raceme length, and negative loading for 1000 grain weight. Similar to the present study, Prabhu *et al.,* (2020) also reported that loadings of peduncle length and thousand grain weight were in opposite directions based on the principal component analysis involving 40 barnyard millet accessions.

Similar to the present observations, PCA analysis based on morphometric traits in a global core collection of 95 barnyard millet accessions originating from several countries revealed that plant height, number of nodes, flag leaf length, flag leaf width, inflorescence length, raceme number, days to flowering, grain weight contributed most to diversity (Sood *et al.,* 2015).

The scatter plot drawn between PC1 and PC2 depicted the wide distribution of genotypes in the factor plane across different quarters (Figure 4). Most of the GECH genotypes and TNEf genotypes which are collections from Bangalore and Coimbatore grouped on the right hand side of the plane while ACM accessions which are collections from Madurai grouped on the left hand side of the plane. The outliers included two genotypes, namely ACM332 and GECH746.

Cluster analysis based on quantitative traits grouped the 53 barnyard millet accessions into four distinct clusters (Figure 5). Cluster I and cluster II included high yielding genotypes, while cluster III and cluster IV comprised of low yielding genotypes. Cluster I with 15 genotypes was characterized by high yielding genotypes which were late flowering in nature with increased flag leaf length and width. Similar observations on late flowering genotypes producing higher yields have also been reported in barnyard millet by Arunachalam and Vanniarajan (2012) and Vanniarajan and Chandirakala (2020). Cluster II comprised of 23 genotypes. This group included medium duration flowering genotypes (45-55days) with increased thousand grain weight, longer peduncle length, longer inflorescence length and longer lower raceme length. Cluster III consisted of 13 genotypes which included tall and early flowering genotypes. Although these genotypes recorded longer inflorescence length and longer lower raceme length, they were low yielding, indicating that these two traits may not be contributing as yield attributing traits. Cluster IV comprised two distinct genotypes ACM332 and GECH746 with low yield, early flowering and short statured nature, but had more number of tillers which were not productive. Based on cluster analysis involving morphological traits in barnyard millet germplasm, Renganathan *et al.,* (2017) also reported a similar finding that early maturing genotypes were found to be poor yielders and grouped separately.

Superior genotypes with desirable agronomic traits were mostly present in cluster I and II and can be selected for utilization in hybridization programmes for developing improved varieties in barnyard millet. Accessions obtained or developed from different locations grouping within same clusters indicated that geographical location was not the sole contributor for divergence. This finding is in correspondence with the earlier reports of Mehta *et al.,* (2005), Gupta *et al.,* (2009), Sood *et al.,* (2015), Vanniarajan and Chandirakala (2020). The similarity in the expression of phenotypic traits shared by genotypes collected from different eco-geographical locations may be a reason for this observation.

Table 1. Variation in quantitative traits of barnyard millet accessions

Variables	Mean	Min	Genotype	Max	Genotype	SD	CV (%)	Skewness	Kurtosis
PH(cm)	132.4±1.12	101.50	ACM332	154.0	TNef202	8.19	6.18	-0.609	3.13
TPP	3.62± 0.08	2.50	TNEf199	5.25	GECH746	0.55	15.29	0.713**	1.50**
DFF	49.66±1.03	40.00	GECH10	67.00	ACM331	7.53	15.8	0.875*	-0.462*
FLL(cm)	23.87±0.37	16.33	ACM332	29.50	GECH27	2.75	11.53	-0.282	0.340
FLW(cm)	2.45±0.05	1.43	ACM332	3.30	TNEf199	0.38	15.82	-0.390	0.239
PL(cm)	13.81±0.57	6.00	TNEf199	33.90	ACM296	4.21	30.52	2.014	8.809
IL(cm)	19.48±0.44	12.50	ACM332	28.30	TNEf202	16.65	16.65	0.467	0.860
LRL(cm)	2.73±0.12	1.50	ACM335	6.88	GECH1	0.88	32.22	2.352	8.922
TW(g)	2.77±0.05	2.26	ACM334	3.93	GECH351	0.34	12.38	1.006	1.351
GYP(g)	13.45±0.65	6.25	GECH18	26.75	GECH758	4.77	35.47	0.727**	0.294*

* PH- plant height (cm), TPP- number of tillers per plant, FLL- flag leaf length (cm), FLW- flag leaf width (cm), DFF- days to 50% flowering, PL- peduncle length (cm), IL- inflorescence length (cm), LRL- lower raceme length (cm), TW-thousand grain weight (g), and GPY- grain yield per plant (g).

Table 2. Eigen value and per cent of total variation and component matrix for the principal component axes

PC	PC 1	PC 2	PC 3	PC 4
Eigenvalue	2.57	2.32	1.18	1.01
% variance	25.76	23.21	11.85	10.16
Cumulative %	25.76	48.97	60.82	70.98
Component Matrix				
PC	PC 1	PC 2	PC 3	PC 4
PH(cm)	0.489	0.588	0.251	-0.176
TPP	0.287	-0.496	-0.295	-0.110
DFF	**-0.767**	0.250	0.009	0.083
FLL(cm)	-0.047	**0.728**	-0.302	-0.068
FLW(cm)	-0.297	**0.846**	-0.111	0.034
PL(cm)	0.141	-0.208	**0.627**	**0.580**
IL(cm)	**0.837**	0.333	0.054	0.201
LRL (cm)	**0.705**	0.312	-0.305	0.369
TW(g)	0.480	0.019	**0.401**	**-0.669**
GPY(g)	-0.356	0.411	**0.531**	0.002

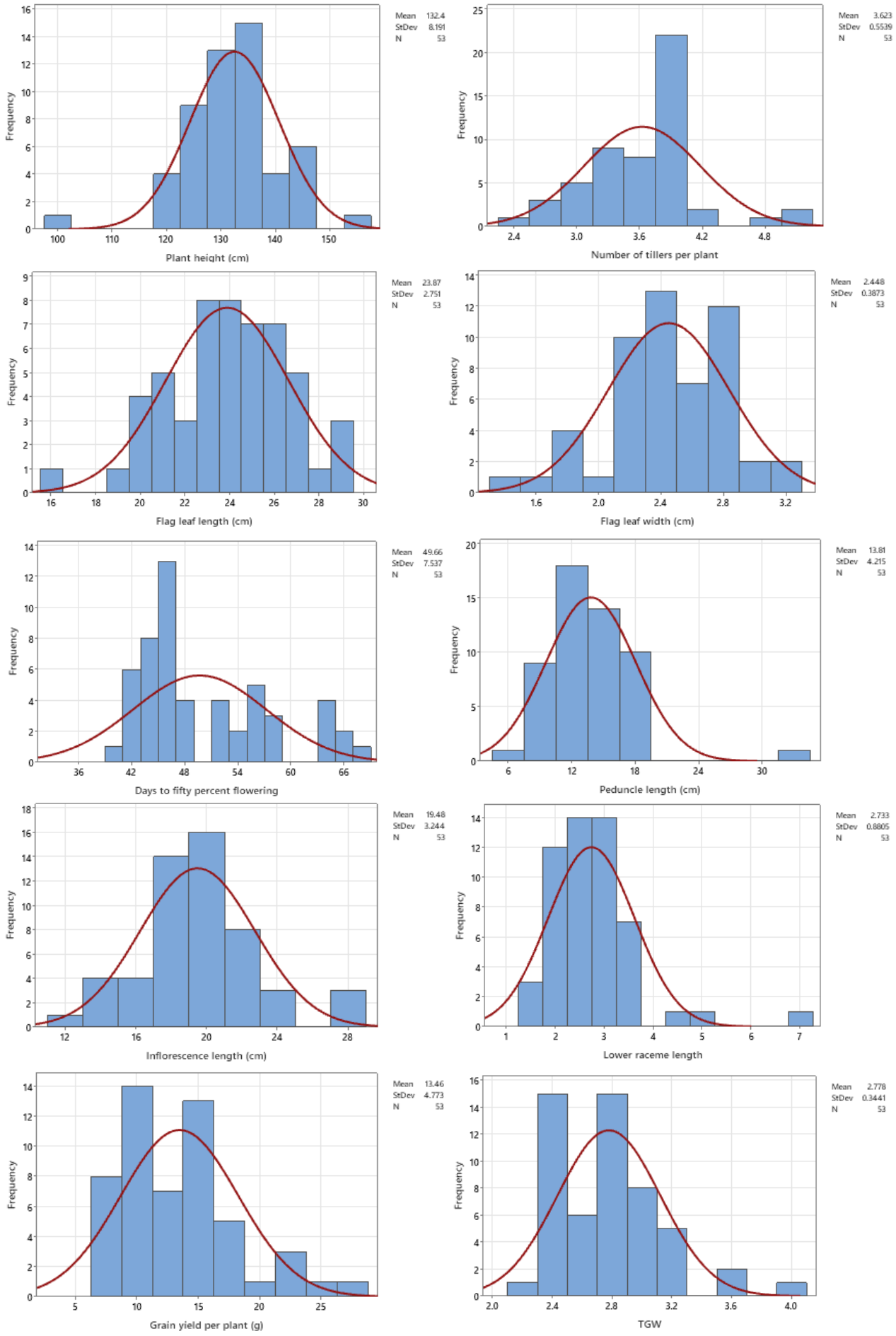

Figure 1. Frequency distribution of barnyard millet genotypes for ten morphological traits

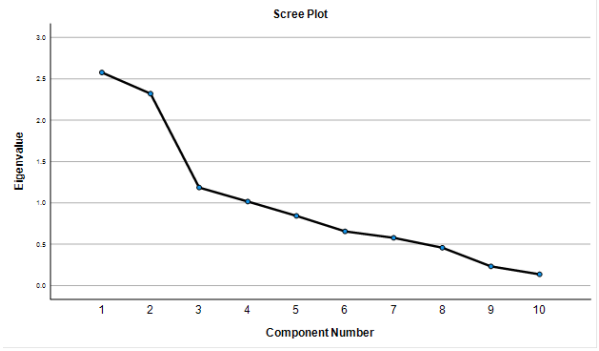

Figure 2. Scree plot showing the eigen value variation for quantitative traits in barnyard millet

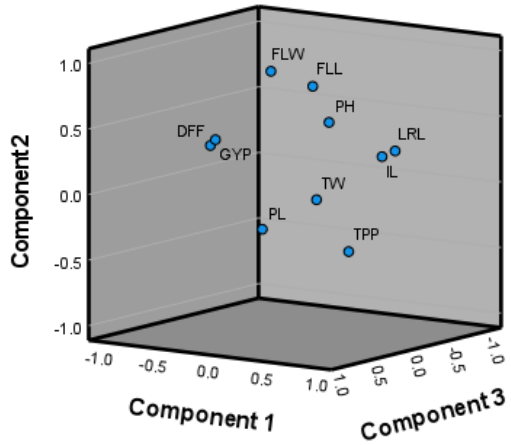

Figure 3. Component plots for the ten quantitative traits in barnyard millet germplasm

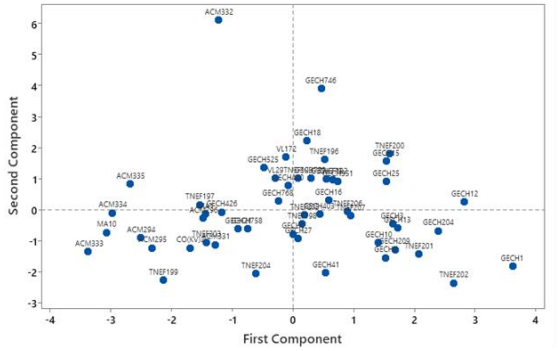

Figure 4. Distribution of barnyard millet genotypes across the two components

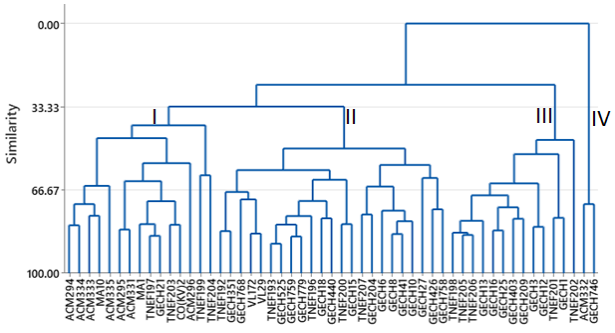

Figure 5. Dendrogram showing the genetic relationship among 53 barnyard millet accessions

Conclusion

The PCA analysis and cluster analysis in the present study revealed substantial divergence among the genotypes based on morphological traits. Each cluster with distinct phenotypic attributes contributed towards diversity and provides a base for the selection of desirable genotypes with specific traits for utilization in hybridization programmes. The present study has also helped in the identification of superior genotypes such as GECH758, GECH746, GECH27, CO(KV)2 and MA1 recording single plant yield greater than 20g. GECH10 and TNEf192 recorded thousand grain weight exceeding 3.5g. These genotypes serve as potential sources for utilization in breeding programmes for yield enhancement in barnyard millet.

Funding and Acknowledgment

The authors would like to thank All India Coordinated Small Millet Improvement Project (AICSMIP) unit, Bengaluru and Millets Breeding Station (MBS), Tamil Nadu Agricultural University, Coimbatore for the financial support and technical guidance.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

We assure that we have written and submitted only entirely original works.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflicts of interest in the publication of this content.

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; shivafruitscience@gmail.com.

Author contributions

Research grant- All India Coordinated Small Millet Improvement Project (AICSMIP) unit, Bengaluru and Millets Breeding Station (MBS), Tamil Nadu Agricultural University: Idea conceptualization - SG, MD: Experiments - SG, MD, VS: Guidance - SG, MD: Writing-original draft - SG, MD, VS: Writing- reviewing &editing - SG, MD, VS.

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