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RESEARCH ARTICLE

Characterization of Parental Lines and Land Races of Pearl Millet [*Pennisetum glaucum* (L) R. Br.] by DUS Descriptors

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ABSTRACT

An experiment was conducted to characterize 31 pearl millet genotypes with 28 morphological traits using DUS descriptors at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, during *Kharif* 2019. The landrace Kizikuppam local was found to have a very long leaf length and a medium leaf width, indicating a high leaf area index, an important physiological trait that could be employed in future breeding programmes. The genotypes PT 6067, PT 6707, PT 6710, Cumbu 1, Uthangarai local and Shoolagiri local had compact panicle, an important ordinal trait for yield improvement. Small, yellow-brown color and elliptical shaped seeds were noted in Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3, and Pothu Cumbu, which could be used to promote the variation in the base population. The early flowering nature of these genotypes might incorporate drought resistance. Agglomerative hierarchical cluster (AHC) analysis showed PT 6582, Kizikuppam local, Uthangarai local, and Dhanashakti were placed under cluster VI with maximum cluster mean for single plant yield. Superior classification of these genotypes for plant growth habit, leaf sheath length, leaf blade length, leaf blade width, panicle exertion, panicle length, panicle girth, number of productive tillers, plant height, panicle density and thousand seed weight indicated the importance of these ordinal traits in yield improvement by using the genotypes of this cluster. The Shannon-Wiener index was high for the time of spike emergence, thousand seed weight, leaf blade length, panicle shape, panicle density and seed color.

Keywords : *Morphological Characterization; Diversity analysis; Cluster Analysis; Shannon-Wiener index (H); Pearl Millet*

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L) R. Br.] is a prominent warm-season cereal crop grown on 260 lakh ha in the arid and semi-arid tropical regions of Asia (>100 lakh ha) and Africa (150-160 lakh ha). India is the largest producer (> 90 lakh ha) of the crop (Rai *et al.*, 2009). Protogynous flowering nature evolved it into a highly cross-pollinated crop with an outcrossing rate of 85%, which fulfills one of the essential biological requirements for hybrid development and open-pollinated varieties. It can adopt to diverse agro-ecological conditions and is mainly grown on marginal lands (Singh *et al.*, 2016). Rajasthan is the largest producer of pearl millet in India with the production of 4.25 lakh tonnes, followed by Uttar Pradesh and Haryana (INDIASTAT, 2020).

Pearl millet has wide genetic diversity and adaptability. Hence, it is essential for genotype identification and classification using DUS morphological traits, which might help use specific genotypes in breeding programmes. These morphological traits help in differentiate the variety from the existing varieties and protect from infringement. To protect pearl millet varieties, one should prove the novelty among the DUS descriptor traits compared with existing varieties under a similar environment. Most of the qualitative traits are oligogenic and less influenced by the environment. Clear and distinct classification is possible with most of the qualitative traits. The trend of molecular characterization using molecular markers is also emerging and showed high polymorphism compared to morphological traits. Hence, there is a need for a more number of stable morphological traits to identify diversity in germplasm, differentiating the

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variety from the existing varieties and protecting from infringement. In this context, the present investigation was conducted on 31 pearl millet genotypes with the objective of characterization and diversity analysis with the help of morphological traits using frequency distribution and cluster analysis.

MATERIAL AND METHODS

A set of 31 pearl millet genotypes were used as experimental material, which included 17 restorers (R) lines (PT 6067, PT 6674, PT 6675, PT 6676, PT 6677, PT 6059, PT 6705, PT 6706, PT 6707, PT 6708, PT 6709, PT 6710, PT 6029, PT 6580, PT 6581, PT 6582 and PT 6583), three maintainer (B) lines (ICMB 98222, ICMB 06111 and ICMB 99222), ten land races (Cumbu 1, Nattu Cumbu, Cumbu 2, Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3, Kizikuppam local, Pothu Cumbu, Uthangarai local and Shoolagiri local) and an open-pollinated variety (Dhanashakti). They were grown in Randomized Complete Block Design (RCBD) at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, during *Kharif* 2019. Each entry occupied a 3 m row with a spacing of 45 cm × 15 cm. All agronomic practices were followed as per TNAU crop production guide. Observation on all 28 qualitative and quantitative traits wastaken and scored as per DUS (Distinct, Uniformity, and Stability) guidelines formulated for pearl millet by PPV& FRA, 2001. Frequency distribution and graphical representation of various traits were worked out in MS Excel 2016. Gower's coefficient of similarity (Gower, 1971) measure was calculated among the genotypes and grouped into different clusters by agglomerative hierarchical cluster (AHC) method in STAR (Statistical Tool for Agricultural Research) software version 2.0.1 using 28 DUS descriptors and single plant yield. Plant growth habit, time of spike emergence, leaf sheath length, leaf blade length, leaf blade width, number of nodes, panicle exertion, panicle length, panicle girth, number of productive tillers, plant height, panicle density, and thousand seed weight were considered as an ordinal variable. Anthocyanin coloration of first leaf sheath, leaf sheath pubescence, stigma pigmentation, anther color, node pubescence, node pigmentation, internode pigmentation, anthocyanin pigmentation of glume, panicle bristle, panicle bristle color, panicle bristle appearance, panicle shape, panicle tip sterility, seed color, and seed shape were considered as the nominal variable and single plant yield as a numerical variable. Shannon-Wiener index (*H*) (Shannon, 1948; Konopinski, 2020) of 28 DUS descriptors were computed in software PAST 4.0.

RESULTS AND DISCUSSION

Discrete morphological qualitative traits can be considered for the characterization of pearl millet genotypes. These traits can be observed with naked eyes. There is a need for more number of DUS traits for the classification and identification of individual genotypes.

Observation on 28 DUS traits was recorded and the frequency of genotypes was calculated in each class (Table 1). Most of the DUS traits showed at least two classes except for six traits like leaf sheath pubescence, stigma pigmentation, node pubescence, number of nodes, panicle bristle, and panicle bristle appearance, which showed no classification. This indicated the presence of diversity for observed morphological traits among the genotypes.

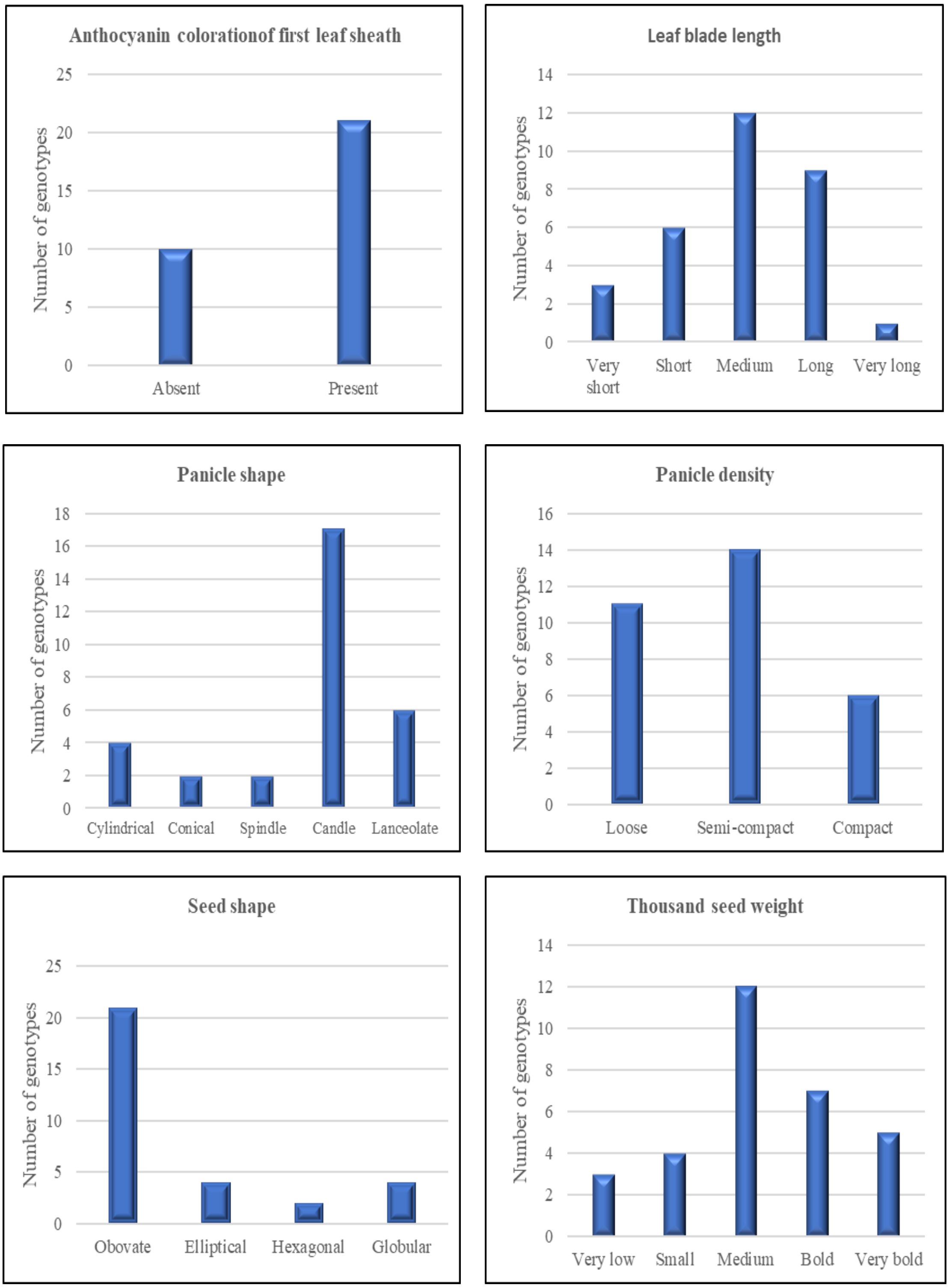
Around 67.74% of genotypes showed anthocyanin coloration of the first leaf sheath (Figure 1). Since anthocyanin pigment production was highly influenced by the environment, it was recommended to screen in controlled conditions. This trait is very important in the identification and purification of restorer and maintainer lines in a hybrid seed production programme (Kumar *et al.*, 2020). The phenotypic characters related to pigmentation have been commonly used as genetic markers in plants to study diversity connected with evolution, tolerance to abiotic stress, and disease resistance (Varalakshmi *et al.,* 2012). The time of spike emergence was very early in60.29% of genotypes and early to medium in 19.35% genotypes. Pearl millet is grown mostly in low rain fall areas rather than areas having high agronomic potential. Hence, the drought escape mechanism (early flowering) is an essential criterion for pearl millet (Vadez *et al.,* 2012). The landraces *viz.,* Kuttu Cumbu 1, KuttuCumbu 2, Kuttu Cumbu 3, Kizikuppam local and Pothu Cumbu land races were in the early category exhibiting an essential drought escaping mechanism.

Twenty-two of genotypes had long leaf sheath (58.06%) and medium leaf sheath length was observed in 12 genotypes, whereas short leaf sheath length was observed in one genotype. Leaf blade length was categorized into four groups, where maximum (51.61%) genotypes came under the medium group and the other two, four, and nine genotypes came under long, very short and short categories, respectively (Figure 1). The selection of plants with long leaf sheath and leaf blade plays a vital role in yield increment (Kalagare *et al.*, 2021, Vadez *et al.,* 2012), because of early suppression of weeds and availability of more leaf area index for photosynthesis. In tilled soil, water use efficiency was high mainly due to an increase in the leaf area index and dry matter production, which were also essential traits of forage pearl millet (Crookston *et al.*, 2020). The land race Kizikuppam local observed under very long leaf length category with medium leaf width can be utilized for improvement of leaf related traits in future breeding programmes.

Table 1. Frequency distribution of morphological traits of pearl millet parental lines and land races

Sl. No.	Traits	Category	Number	Frequency
1	Anthocyanin coloration of first leaf sheath (BLC)	Absent	10	32.26
		Present	21	67.74
2	Plant growth habit (PGH)	Erect	4	12.90
		Intermediate	27	87.10
		Spreading	0	0.00
3	Time of spike emergence (DTF)	Very early (<43 days)	6	19.35
		Early (43-46 days)	2	6.45
		Medium (47-50)	9	29.03
		Late (51-54 days)	10	32.26
		Very late (>54 days)	4	12.90
4	Leaf sheath pubescence (LSP)	Absent	31	100.00
		Present	0	0.00
5	Leaf sheath length (LSL)	Short (<11)	1	3.23
		Medium (11-15)	8	25.81
		Long (>15)	22	70.97
6	Leaf blade length (LBL)	Very short (<41)	3	9.68
		Short (41-50)	6	19.35
		Medium (51-60)	12	38.71
		Long (61-70)	9	29.03
		Very long (>70)	1	3.23
7	Leaf blade width (LBW)	Narrow (<3)	2	6.45
		Medium (3-4)	29	93.55
		Broad (>4)	0	0.00
8	Stigma pigmentation (SC)	Absent	31	100.00
		Present	0	0.00
9	Anther color (AC)	Yellow	13	41.94
		Brown	0	0.00
		Purple	18	58.06
10	Node pubescence (NP)	Absent	31	100.00
		Present	0	0.00
11	Number of nodes (NN)	Low (<11)	0	0.00
		Medium (11-15)	31	100.00
		High (>15)	0	0.00
12	Node pigmentation (NC)	Whitish	0	0.00
		Green	20	64.52
		Brown	3	9.68
		Red	0	0.00
		Purple	8	25.81
13	Internode pigmentation (INC)	Whitish	0	0.00
		Green	23	74.19
		Brown	0	0.00
		Red	0	0.00
		Purple	8	25.81
14	Panicle exertion (PE)	Incomplete	0	0.00
		Partial	5	16.13
		Complete	26	83.87
15	Panicle length (PL)	Very small (< 11)	0	0.00
		Small (11-20)	10	32.26
		Medium (21-30)	18	58.06
		Long (31-40)	3	9.68
		Very long (>40)	0	0.00
16	Anthocyanin pigmentation of glume (PGC)	Absent	29	93.55
		Present	2	6.45
17	Panicle bristle (PB)	Absent	0	0.00
		Present	31	100.00
18	Panicle bristle color (PBC)	Yellow	0	0.00
		Green	22	70.97
		Brown	0	0.00
		Purple	9	29.03
19	Panicle bristle appearance (PBA)	Non prominent	31	100.00
		Prominent	0	0.00
20	Panicle girth (PG)	Thin (<1.6)	0	0.00
		Medium (1.6-3.0)	21	67.74
		Thick (>3.0)	10	32.26
21	Number of productive tillers (NPT)	Monoculm (<2)	1	3.23
		Low (2-3 tillers)	23	74.19
		Medium (4-6 tillers)	7	22.58
22	Plant height (PH)	Very short (<101)	2	6.45
		Short (101-150)	9	29.03
		Medium (151-200)	20	64.52
		Tall (201-250)	0	0.00
		Very tall (>250)	0	0.00
23	Panicle shape (PS)	Cylindrical	4	12.90
		Conical	2	6.45
		Spindle	2	6.45
		Candle	17	54.84
		Lanceolate	6	19.35
		Dumb-bell	0	0.00
		Club	0	0.00
		Oblanceolate	0	0.00
		Globose	0	0.00
24	Panicle tip sterility (PTS)	Absent	10	32.26
		Present	21	67.74
25	Panicle density (PD)	Loose	11	35.48
		Semi-compact	14	45.16
		Compact	6	19.35
26	Seed color (SC)	Whitish	0	0.00
		Cream	5	16.13
		Yellow	0	0.00
		Grey	16	51.61
		Deep grey	0	0.00
		Grey brown	0	0.00
		Yellow brown	10	32.26
27	Seed shape (SS)	Obovate	21	67.74
		Elliptical	4	12.90
		Hexagonal	2	12.90
		Globular	4	9.68
28	Thousand seed weight (TSW)	Very low (<5)	3	12.90
		Small (5.0-7.5)	4	38.71
		Medium (7.6-10.0)	12	22.58
		Bold (10.1-12.5)	7	16.13
		Very bold (>12.5)	5	6.45

Figure 1. Graphical representation of frequency distribution of various morphological traits of pearl millet parental lines and land races



Anther color was observed in two categories. Fifty-eight percent of genotypes were purple colored and the remaining were yellow colored. This trait was important for the purification and identification of parental lines in the hybrid seed production programme. Medium panicle length between 21cm to 30cm was prominent in the population and others belonged to small (4) and long categories (1). A maximum number of genotypes showed medium panicle girth, while two genotypes had a thin panicle and 12 had a thick panicle. About 74.19% of genotypes came under the low number of productive tillers category and the rest had monoculed (one) and medium number of productive tillers (two). Reports of landraces with plant types having profuse tillering and small panicles were found to produce higher grain yield than the plant type having low tillering and large panicles under severe moisture stress (Van Oosterom *et al.,* 2006). The number of productive tillers was almost single for the genotype PT 6059.

Table 2. Estimates of descriptive statistics of different clusters based on single plant yield as numerical variable of 31 pearl millet genotypes

Cluster group	Single plant yield (g)				Genotypes
	Min	Max	Mean	Std Dev	
Cluster I	31.58	86.17	52.18	17.67	PT 6067, PT 6675, PT 6705, PT 6707, PT 6029, PT 6580, PT 6583, Cumbu 1, Nattu Cumbu, Cumbu 2 and Shoolagiri local
Cluster II	31.83	38.1	34.97	4.43	PT 6674 and PT 6709
Cluster III	25.3	71.94	44.92	16.02	PT 6676, PT 6677, PT 6710, PT 6581, ICMB 98222 and ICMB 99222
Cluster IV	19.2	19.2	19.2		PT 6059
Cluster V	48.56	74.49	61.52	18.34	PT 6706 and PT 6708
Cluster VI	55.14	79	69.02	10.21	PT 6582, Kizikuppam local, Uthangarai local and Dhanashakti
Cluster VII	43.45	43.45	43.45		ICMB 06111
Cluster VIII	26.37	35.85	30.63	4.04	Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3 and Pothu Cumbu

Plant height was categorized into four groups and larger number of genotypes (64.52%) fell under the medium group. Very short plant height was observed in PT 6059 and ICMB 06111. Candle-shaped panicle (54.84%) was prominent as compared to cylindrical, conical, spindle and lanceolate type (Figure 1). About 44.16% of genotypes came under semi-compact panicle density group and rest of the genotypes were loose (11) and compact (6) (Figure 1). The genotypes *viz*. PT 6067, PT 6707, PT 6710, Cumbu 1, Uthangarai local and Shoolagiri local were with compact panicle, which is one of the important ordinal traits for yield improvement.

Out of 31 genotypes, 16 genotypes showed grey color grains, whereas the grains of five and ten genotypes were of cream and yellow yellow-brown color. Grey grain colored hybrids exhibited a negative correlation for starch and fats, while white grain color hybrids resulted ina significant correlation for starch (Arya *et al.*, 2009). Most of the genotypes (67.74%) fell under obovate seed shape and the rest of them were elliptical (4), hexagonal (2), and globular shaped (4) (Figure 1). Observation of thousand seed weight showed that the maximum number of genotypes had bold seed (13). Very low, small, medium, and very bold seeds possessed two, three, twelve, and one genotype, respectively (Figure 1). Significant association of iron and zinc with thousand seed weight indicated that bold size seed would have more iron and zinc (Gupta *et al.*, 2009). PT 6706, PT 6029, PT 6581, Cumbu 2, and Dhanashakti came under the very bold seed group and can be utilized for the biofortification programme.

The four land races,Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3, and Pothu Cumbu were small-seeded, yellow-brown colored with elliptical shaped seeds. Most of the R lines were bold seeded with grey color. B lines were comparatively small-seeded compared to R lines and short plants. Comparison of seeds of these genotypes with R line (PT 6067), B line (ICMB 06111), and Dhanashakthi were depicted in Figure 2. Even though grain color did not influence the grain iron and zinc density (Govindaraj *et al.*, 2018), grain size was influencing the grain iron and zinc density (Kanatti *et al.*, 2019).

a). Land races (Seeds are Yellow brown colored, Elliptical in shape and Small sized)

Name: KuttuCumbu 1

Name: KuttuCumbu 2

Name: KuttuCumbu 3

Name: PothuCumbu

b). Restorer line

c). Maintainer line

d). Open pollinated variety

Name: PT 6067
Color: Grey
Shape: Obovate
Size: Bold

Name: ICMB 06111
Color: Yellow brown
Shape: Globular
Size: Small

Name: Dhanashakti
Color: Grey
Shape: Obovate
Size: Vey bold

Figure 2. Representation of diversity of pearl millet parental lines and land races in terms of seed color and seed shape

Cluster analysis using the agglomerative hierarchical cluster (AHC) approach revealed eight clusters depicted in a dendrogram (Figure 3). The cophenetic correlation coefficient measures of how faithfully a dendrogram preserves the pairwise distances between the original unmodeled data points. The cophenetic correlation coefficient of 0.649 for cluster analysis, was found to indicate the accuracy and stability of the hierarchical clustering results (Shah *et al.,* 2013, Azevedo *et al.*, 2012). Gower's similarity coefficient permits the simultaneous use of variables of different scales of measurement in the estimation of similarity. From the dendrogram, it was observed that the clusters were formed at around Gower's similarity coefficient of 0.3. A maximum number of genotypes (11) were under cluster I followed by cluster III, cluster VI, cluster VIII, cluster II, cluster V, cluster IV and cluster VII, and the seclusters had six, four, four, two, two, one and one genotypes respectively.

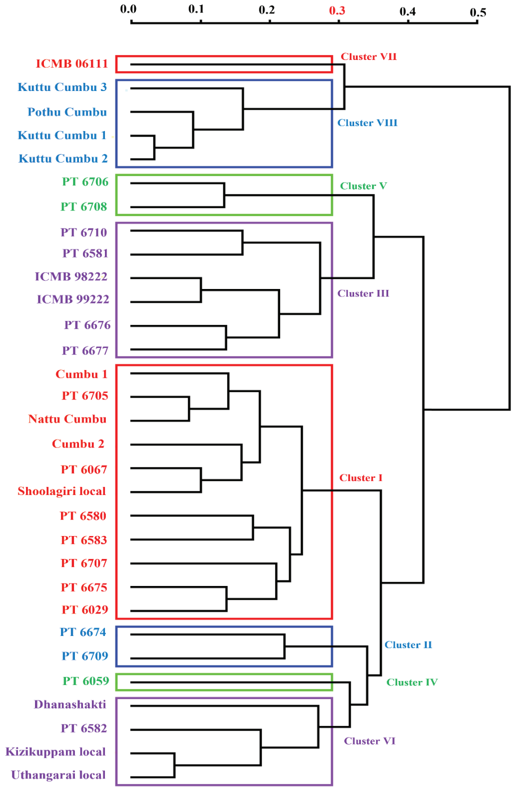


Figure 3. Dendrogram depicting the nine clusters among 31 pearl millet genotypes using UPGMA method of clustering

Descriptive statistics of single plant yield on different clusters were presented in Table 2. The highest cluster mean of single plant yield was observed for cluster VI (69.02), followed by cluster V and cluster I. The least cluster means of single plant yield 19.2 g was observed for cluster IV, followed by cluster VIII and cluster II. Cluster VI genotypes (PT 6582, Kizikuppam local, Uthangarai local, and Dhanashakti) showed higher-order classification for ordinal variables like plant growth habit, leaf sheath length, leaf blade length, leaf blade width, panicle exertion, panicle length, panicle girth, number of productive tillers, plant height, panicle density and thousand seed weight. They indicated the usefulness of the selection of these traits in the improvement of yield. The cluster V genotypes (PT 6706 and PT 6708) also showed similar kind of pattern for ordinal variables.

Table 3. Shannon-Wiener index (*H*) for 28 morphological traits of 31 pearl millet genotypes

Characters	Abbreviations	*H*
Anthocyanin coloration of first leaf sheath	BLC	0.629
Plant growth habit	PGH	0.385
Time of spike emergence	DTF	1.483
Leaf sheath pubescence	LSP	0.000
Leaf sheath length	LSL	0.704
Leaf blade length	LBL	1.381
Leaf blade width	LBW	0.239
Stigma pigmentation	SC	0.000
Anther color	AC	0.680
Node pubescence	NP	0.000
Number of nodes	NN	0.000
Node pigmentation	NC	0.858
Internode pigmentation	INC	0.571
Panicle exertion	PE	0.442
Panicle length	PL	0.907
Anthocyanin pigmentation of glume	PGC	0.239
Panicle bristle	PB	0.000
Panicle bristle color	PBC	0.602
Panicle bristle appearance	PBA	0.000
Panicle girth	PG	0.629
Number of productive tillers	NPT	0.668
Plant height	PH	0.819
Panicle shape	PS	1.265
Panicle tip sterility	PTS	0.629
Panicle density	PD	1.044
Seed color	SC	1.001
Seed shape	SS	0.969
Thousand seed weight	TSW	1.454

The small-seeded land races Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3, and Pothu Cumbu differed significantly from the other genotypes and were found in cluster VIII. These land races were of early flowering and had small panicles with small seeds with more tillers and also showed an early time of spike emergence, which is one of the droughts escaping mechanisms (Vadez *et al.,* 2012).

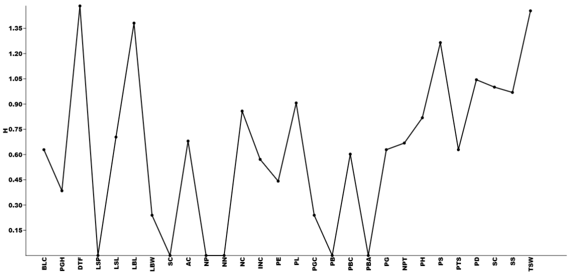


Figure 4. Graphical representation of Shannon-Wiener index (H) among 28 morphological traits

Shannon-Wiener index (*H*) is a measure that reflects the number of different species and how evenly the individuals are distributed among those species. The Shannon-Wiener index (H) of various traits ranging from zero to 1.483 was depicted in Table 3 and Figure 4. The highest Shannon-Wiener index (*H*) value of 1.483 was observed for the time of spike emergence, followed by thousand seed weight (1.454), leaf blade length (1.381) and spike shape (1.265), spike density (1.044) and seed color (1.001). The leastShannon-Wiener index (*H*) value of zero was observed for leaf sheath pubescence, stigma pigmentation, node pubescence, number of nodes, spike bristle and bristle appearance indicated that there existed no diversity among these traits in given experimental material. A higher value of indices indicates the presence of more diversity of genotypes for a particular trait (Upadhyaya *et al*., 2002). The result indicated that time of spike emergence, thousand seed weight, leaf blade length and spike shape, spike density, and seed color showed significant diversity in the traits, which can be exploited to improve of these traits.

CONCLUSION

From the above results, it was observed that there was significant diversity among the genotypes with respect to morphological traits. Anthocyanin coloration of first leaf sheath, anther color, node color and internodal colors were easy observable traits and were utilized in the rouging of off type in hybrid seed production programme. The land race Kizikuppam local observed under very long leaf length category with medium leaf width indicated high leaf area index, an important physiological trait which could be utilized for the future breeding programme. The number of productive tillers was almost single for the genotype PT 6059. The genotypes PT 6067, PT 6707, PT 6710, Cumbu 1, Uthangarai local, and Shoolagiri local with compact panicle is one of the important ordinal traits for yield improvement. PT 6706, PT 6029, PT 6581, Cumbu 2, and Dhanashakti came under the very bold seed group, with direct influence on the yield. Four land races (Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3 and Pothu Cumbu) showed distinct characteristics for small, yellow-browncolored and elliptical shaped seeds, which could be used to promote the variation of the base population and early flowering might be used to incorporate drought escape mechanism. Agglomerative hierarchical cluster (AHC) analysis resulted in eight clusters and showed Gower's similarity coefficient of 0.3. Cluster I showed a maximum of 11 genotypes. PT 6582, Kizikuppam local, Uthangarai local, and Dhanashakti showed maximum cluster means for single plant yield. They fell under superior category for the traits plant growth habit, leaf sheath length, leaf blade length, leaf blade width, panicle exertion, panicle length, panicle girth, number of productive tillers, plant height, panicle density and thousand seed weight. This indicated the importance of these ordinal traits in yield improvement utilizing the genotypes of this cluster. The small-seeded land races Kuttu Cumbu 1, Kuttu Cumbu 2, Kuttu Cumbu 3, and Pothu Cumbu differed significantly from the other genotypes and were found in cluster VIII. The Shannon-Weaver diversity index was found to be high for the time of spike emergence, thousand seed weight, leaf blade length and panicle shape, panicle density and seed color, indicating the existence of high variability in the material studied.

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Ethics statement

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

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

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