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Identification of Superior Forage Pearl Millet (Pennisetum glaucum (L.) R.Br.) Inbred Lines

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ABSTRACT

⁹he experiment was conducted during *kharif*, (July–November, 2022) at ICAR – Indian Institute of Millet Research (IIMR), 🗘 Rajendranagar, Hyderabad, India. In this experiment, 81 forage pearl millet (*Pennisetum glaucum* (L.) R.Br.) lines was evaluated for its genetic diversity and variability. For all variables examined, an analysis of variance revealed a considerable variation between lines. Leaf-to-stem ratio, first-cut and second-cut of green fodder yield, as well as dry fodder yield, all had high PCV and GCV values. For Plant height, Leaf length, Number of leaves tiller⁻¹, Number of tillers plant⁻¹, Stem thickness, and Regeneration ability however, moderate PCV and GCV values have been identified. Plant height, Number of leaves tiller¹, Leaf-to-stem ratio, Green fodder yield in first cut, the Green fodder in second cut, and the yield of dry fodder all showed high heritability and high genetic advance. On the basis of Mahalanobis D² statistics for clustering, genetic diversity was examined. The Tocher method was used to grouping of genotype lines into 11 clusters. The most lines were found in cluster I (52), followed by cluster VI with 14 lines, cluster III with 6 lines, and cluster IX with 2 lines. Cluster VI has the greatest intra-cluster distance (86.59), followed by clusters I (54.11), III (52.99), and IX (52.30). Between cluster III and IX had observed greater inter-cluster distance of 594.54 followed by cluster III and X (471.67), cluster IX and XI (458.76) and cluster I and IX (410.82). Intercrossing of these clusters lines might be useful to develop better forage pearl millet hybrids.

KEYWORDS: Forage pearl millet, genetic diversity, genetic variability, R- package

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Pearl millet, [Pennisetum glacuum (L.) R.Br.] is an ethnobotanically important crop in the genus Pennisetum L. (Rich). This belongs to the Poaceae family. It is one of the few diploids (2n=14) in the genus that is well spread in the tropics and the subtropics. In the world's arid and semi-arid regions, it is among the most significant sources of food as well as fodder. Natural drought and heat tolerance, low hydrocyanic acid levels, and high protein, calcium, phosphorus, and other mineral content are all characteristics of forage pearl millet (Kaushal et al., 2024). It is a possible feed crop because its oxalic acid concentration is within the permissible range. In India, it is the fourth most important cereal after rice, wheat and sorghum. Pearl millet is not only a food crop, but also a forage crop. Pearl millet is grown on an area of 6.84 million ha, with an average production of 9.78 million tons and productivity of 1430 kg ha⁻¹ in 2021–22 (Anonymous, 2023).

Pearl millet grain serves as a staple diet for over 90 million individuals residing in the Sahelian region of Africa and northwestern India (Srivastava et al., 2020). Pearl millet is an ideal solution to current agricultural challenges due to its impressive ability to withstand harsh climates and its significantly lower consumption of energy, water, and production of greenhouse gases compared to other crops (Daduwal et al., 2024). Pearl millet is a C₄ type of grass that primarily grown in Africa and India. People eat its grain, and it's also used as food for animals. This crop is known for its ability to survive in dry, nutrient-poor soil (Crookston et al., 2020). In India, pearl millet breeding focuses on the development of dual-purpose varieties. It is primarily used for livestock maintenance and dairy farming. India hosts the world's largest livestock population (20%) and most people (16.8%) on 2.3% of the world's land area, and leads in cattle (16%) and buffalo (5.5%). Livestock contributes 32% of agricultural production and generates 22% of India's total GDP. However, there is a large gap between the supply and demand of feed. In recent years, feed shortage has remained a burning issue, so researchers need to make efforts to ensure regular feed supply for dairy cattle development and cattle welfare improvement (Meena et al., 2012, Meena et al. (2013)). Pearl millet is characterized by high leaves, high tillering. It produces a large amount of forage due to high regeneration ability, allowing for multi-cut harvests and grazing (Babiker et al., 2024). To meet this need, systematic breeding strategies need to be developed to meet the feed requirements. Currently, there are no publicly bred pearl millet forage hybrids in India. The major constraint is non-availability of forage specific lines with desirable forage traits and better combining ability to harness heterosis for forage yield or biomass. In the present study, a number of newly developed forage pearl millet lines

were evaluated to estimate diversity for each of the forage yield components, classify lines based on their performance, and identify superior genotypes with desirable traits for forage. Biometric estimates such as heritability, genetic advance, and genetic divergence using Mahalanobis distance (Mahalanobis, 1936, Rao, 1952) were calculated from data collected in the field. The basic purpose of this experiment to identify superior lines for higher yields in forage pearl millet. These basic estimates for breeding lines allow the breeder to select lines for A-line development, subgrouping for desirable traits, etc.

2. MATERIALS AND METHODS

The Indian Institute of Milliet Research (ICAR-IIMR), Hyderabad, developed 75 inbred lines (IIMR-AVS lines) that were used as the experimental material for this study in *kharif*, 2022 (July–November). A Randomised Block Design (RBD) with two replications was used to evaluate 75 lines for three trials with three leading checks and three advanced populations developed at ICAR-IIMR, 25 genotypes were used in each field trial at the Indian Institute of Millet Research's experimental farm in Rajendranagar, Hyderabad. The experiment was carried out of 2×0.45 m² area with two rows for per entry in a plot.

All recommended practices for growing a productive crop were followed. The designated stage was used to record observations to provide yield and its associated distinctive characteristics, such as Days to 50% flowering, Plant height (cm), Leaf length (cm), Leaf width (cm), Number of leaves tiller⁻¹, Number of tillers plant⁻¹, Internodal length (cm), Stem thickness (mm), Leaf-to-stem ratio, Green fodder yield in first cut, Green fodder yield in second cut, Dry fodder yield, and Regeneration ability.

Variability in the genetic material was estimated through Analysis of Variance using R package.

R code:gen.var(s4[3:14],s4\$Genotype,s4\$Replication) gene=gen.var(s4[3:14],s4\$Genotype,s4\$Replication) sink("sbdataanalysis") print(gene)

Further, using estimates from the ANOVA broad-sense heritability, genetic advance was calculated. Pooled mean values from three trials were used to calculate the Mahalanobis distance. This distance was further used to draw clusters using Tocher's method.

3. RESULTS AND DISCUSSION

Analysis of variance showed significant differences between lines for all yield and yield-related trait. All the characters evaluated in this study showed significant genetic differences. This is revealed that experiment material having ample variability.

3.1. Genetic variability

As expected, PCV was higher than GCV (Table 1). The assessed pearl millet genotypes had higher PCV and GCV for 13 traits viz. Days to 50% flowering (6.44, 5.81), Plant height (11.90, 11.64), Leaf length (10.69, 9.54), Leaf width (9.16, 7.82), Number of leaves tiller-1 (12.05, 11.41), Number of tillers plant-1 (12.95, 11.41), Internodal length (8.78, 8.39), Stem thickness (11.24, 9, 27), Leaf stem ratio (66.41, 64.95), Green fodder yield in first cut (28.30, 26.16), Green fodder yield in second cut (26.23, 23.90), Dry fodder yield (26.53, 24.03) and Regenerative ability (12.62, 10.73). The presence of high genetic variability is indicative and shows the potential effectiveness of selection.

High PCV and high GCV were recorded for the Leaf stem ratio, Green fodder yield in first cut, Green fodder yield in second cut, and Dry fodder yield. Similar results for leaf stem ratio were reported by Shanmuganathan et al. (2006), Satapute et al. (2014), Govintharaj et al. (2017), for green fodder yield by Shanmuganathan et al. (2006), Satapute et al. (2014) and Subbulakshmi et al. (2022), for dry fodder yield by Dhedhi et al. (2016), Rani et al. (2022), and Goswami et al. (2023).

Moderate PCV and GCV were obtained for Plant height (11.90, 11.64), Leaf length (10.69, 9.54), Number of leaves tiller⁻¹ (12.05, 11.41), Number of tillers plant⁻¹ (12.95, 11.41), the thickness of the stem (11.24, 9.27), and Regeneration ability (12.62, 10.73). Similar results

for plant height were observed by Shanmuganathan et al. (2006), Bhoite et al. (2008), Satapute et al. (2014), and Rani et al. (2022), for leaf length from Vidyadhar et al. (2007) and Parmar et al. (2022), for number of leaves tiller-1 from Vidyadhar et al. (2007) and Keerthana et al. (2022), for number of tillers plant⁻¹ by Satapute et al. (2014), Bind et al. (2015), and Goswami et al. (2023), for stem thickness by Shanmuganathan et al. (2006) and Rani et al. (2022) for low genotypic and moderate phenotypic coefficients of variation. Leaf width (9.16, 7.82), Internode length (8.78, 8.39), and Days to 50% flowering (6.44, 5.81) all had recorded low GCV. Similar findings were observed for Days to 50% flowering by Bind et al. (2015) and Subbulakshmi et al. (2022), as well as for leaf width by Satapute et al. (2014). Plant height (95.67, 23.46), Number of leaves tiller⁻¹ (89.65, 22.26), Leaf stem ratio (95.67, 130.88), Green fodder yield in first cut (85.41, 49.80), Green fodder yield in second cut (83.04, 44.87), and Dry fodder yield (82.08, 44.85), all exhibit a high heritability and high genetic advance. Similar findings have been published by Satapute et al. (2014), Bind

Day to 50% flowering (81.28, 10.79), Leaf length (79.79, 17.57), Leaf width (72.86, 13.75), Number of tillers plant⁻¹

et al. (2015), Shanmuganathan et al. (2006), Bind et al.

(2015), Satapute et al. (2014), Dhedhi. (2016), Thomas et al. (2018) and Subbulakshmi et al. (2022) for height of the

plant, number of leaves for every tiller, ratio of leaf stems,

and green fodder yield.

Tabl	e 1: Estimates of genetic a	dvancem	ent, herit	ability, ar	nd variability f	for yield and	associat	ed facto	ors in forage p	earl millet
S1. No.	Characters	Mean	Range		Phenotypic variance	Genotypic variance	PCV (%)	GCV (%)	% heritability	% Mean genetic
			Min	Max	variance	variance	(70)	(70)	(h ²)	advance
1.	Days to 50% flowering	56.28	51.00	69.00	13.15	10.69	6.44	5.81	81.28	10.79
2.	Plant height (cm)	153.29	104.80	195.30	333.07	318.63	11.90	11.64	95.67	23.46
3.	Leaf length (cm)	56.85	43.90	74.30	36.95	29.48	10.69	9.54	79.79	17.57
4.	Leaf width (cm)	3.00	2.30	3.75	0.07	0.05	9.16	7.82	72.86	13.75
5.	No. of leaves tiller-1	6.83	5.10	8.80	0.68	0.60	12.05	11.41	89.65	22.26
6.	No. of tillers plant ⁻¹	5.62	4.40	7.20	0.53	0.39	12.95	11.10	73.53	19.62
7.	Internodal length (cm)	19.21	14.70	22.40	2.84	2.60	8.78	8.39	91.44	16.54
8.	Stem thickness (mm)	11.15	8.70	14.2	1.58	1.07	11.24	9.27	67.70	15.72
9.	Leaf to stem ratio	0.72	0.30	3.26	0.23	0.22	66.41	64.95	95.67	130.88
10.	Green fodder yield (kg plot ⁻¹) in first cut	6.12	2.40	11.30	3.00	2.56	28.30	26.16	85.41	49.80
11.	Green fodder yield (kg plot ⁻¹) in second cut	4.88	2.55	10.35	1.64	1.36	26.23	23.90	83.04	44.87
12.	Dry fodder yield (kg plot ⁻¹)	2.39	1.44	5.34	0.40	0.33	26.53	24.03	82.08	44.85
13.	Regeneration ability	0.54	0.41	0.69	0.005	0.003	12.62	10.73	72.34	18.81

(73.53, 19.62), Internode length (91.44, 16.54), Stem thickness (67.70, 15.72), and Regeneration ability (72.34, 18.81) were all found to have high heritability and moderate genetic advance as a percentage of the mean. Selection can be advantageous for these qualities because it appears that it is controlled by both additive and non-additive gene action. Bind et al. (2015) confirmed comparable findings for Days to 50% flowering, for stem thickness El-Gaafarey et al. (2023) reported similar result and Vidyadhar et al. (2007) and Satapute et al. (2014) reported comparable findings for leaf length.

3.2. Genetic diversity

Calculation of distance and clustering is based on observed phenotypic diversity. It can be used as an initial step in selecting superior forage pearl lines that will produce better hybrids. This study's objective was to examine the genetic variation across pearl millet cultivars in terms of their morphological parameters. In this study, 81 pearl millet lines are evaluated for 13 quantitative traits and genetic diversity.

3.3. Cluster groups

Eleven distinct clusters were formed from the 81 genotypes of pearl millet (Table 2). Using Tocher's approach, the lines of forage pearl millet were divided into 11 clusters based on D² values. The largest group, cluster I, contained

52 genotype line. It was followed by cluster VI, which had 14 genotypes, cluster III, which had six genotype line, and cluster IX, which had two genotype line. The remaining genotypes, clusters II, IV, V, VII, VIII, X, and XI, were included only one line, to exhibit significant genotype heterogeneity.

3.4. Average inter-and intra-cluster distances

Cluster VI had the greatest intra-cluster distance (86.59), which was followed by cluster I (54.11), cluster III (52.99), and cluster IX (52.30), showing that there is some genetic dissimilarity present between the genotypes of these clusters. Clusters II, IV, V, VII, VIII, X, and XI were contain individual genotype line; hence the within-cluster, D² values for these clusters are zero. The largest average for the desired characteristics in these clusters, can be used as the basis for selection. It's probable that the degree of general combining ability, heterogeneity, and pedigree among distinct genotypes are the causes of this intra-cluster genetic diversity among the same cluster group.

The highest inter-cluster distance (594.54) was recorded between cluster III and IX followed by cluster III and X (471.67), cluster IX and XI (458.76) and cluster I and IX (410.82) (Table 3). This is indicating presence of high diversity, as these cluster showing high distance between

Table 2: Genotype clustering of forage pearl millet (Tocher's approach)									
Cluster no.	No. of genotypes	Name of genotypes							
Cluster I	52	IIMR AVS4, IIMR AVS5, IIMR AVS6, IIMR AVS11, IIMR AVS13, IIMR AVS24, IIMR AVS25, IIMR AVS26, IIMR AVS28, IIMR AVS29, IIMR AVS30, IIMR AVS32, IIMR AVS33, IIMR AVS34, IIMR AVS35, IIMR AVS36, IIMR AVS37, IIMR AVS38, IIMR AVS39, IIMR AVS40, IIMR AVS41, IIMR AVS42, IIMR AVS43, IIMR AVS48, IIMR AVS49, IIMR AVS52, IIMR AVS53, IIMR AVS54, IIMR AVS55, IIMR AVS56, IIMR AVS58, IIMR AVS59, IIMR AVS60, IIMR AVS61, IIMR AVS62, IIMR AVS63, IIMR AVS64, IIMR AVS65, IIMR AVS66, IIMR AVS67, IIMR AVS70, IIMR AVS71, IIMR AVS72, IIMR AVS73, IIMR AVS74, IIMR AVS75, IIMR AVS77, Bulk-1, Bulk-2, Bulk-4, TSFB15-8							
Cluster II	1	IIMR AVS12							
Cluster III	6	IIMR AVS44, IIMR AVS45, IIMR AVS46, IIMR AVS47, IIMR AVS50, IIMR AVS51							
Cluster IV	1	IIMR AVS18							
Cluster V	1	IIMR AVS15							
Cluster VI	14	IIMR AVS1, IIMR AVS2, IIMR AVS8, IIMR AVS14, IIMR AVS16, IIMR AVS17, IIMR AVS19, IIMR AVS21, IIMR AVS22, IIMR AVS23, IIMR AVS27, IIMR AVS31, Foragen-raftar, Wonderleaf							
Cluster VII	1	IIMR AVS57							
Cluster VIII	1	IIMR AVS20							
Cluster IX	2	IIMR AVS3, IIMR AVS10							
Cluster X	1	IIMR AVS9							
Cluster XI	1	IIMR AVS7							

Table 3: Forage pearl millet lines' intra- (diagonal) and inter-cluster distances (D² values)												
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	
Cluster I	54.11	103.64	99.66	99.24	108.20	108.89	101.63	221.96	410.82	279.55	216.54	
Cluster II		0.00	248.23	22.51	30.37	64.15	162.92	112.83	257.88	106.06	143.34	
Cluster III			52.99	243.02	253.00	217.11	101.53	353.68	594.54	471.67	340.45	
Cluster IV				0.00	16.51	54.60	162.98	134.61	300.94	95.93	127.86	
Cluster V					0.00	70.60	201.02	183.18	378.44	136.54	158.95	
Cluster VI						86.59	142.31	149.60	310.38	145.75	152.21	
Cluster VII							0.00	179.26	367.89	310.38	286.00	

clusters. Since the pearl millet lines in these clusters can exhibit a high heterotic response, they are useful in a hybrid development programme because they can cross the designated lines to produce a large base population for the development of hybrids with desirable traits. Between clusters IV and V were reported smallest distance 16.51. This suggests that intercrossing (hybridization) between cluster III and IX, cluster III and X, cluster IX and XI, and cluster I and IX genotypes could lead to desirable transgressive segregants. Lakshmana et al. (2010), Govindaraj et al. (2011), Swamynatham et al., (2020) and Shashibhushan et al. (2022) conducted a similar study to

Cluster VIII

Cluster IX

Cluster X

Cluster XI

identify better and desirable genotypes for improving high yield and traits attributing yield in pearl millet lines.

0.00

71.62

52.30

130.38

242.68

0.00

304.31

458.76

120.18

0.00

3.5. Cluster means of the characters

The mean values of the clusters for the majority of the analysed parameters showed a broad range of variation, as shown in table 4. Green fodder yield at first cut was greatest in cluster III (8.80 kg), Green fodder yield in second cut was most significant in cluster XI (10.35 kg), and Regeneration ability was lowest in cluster X (0.68). Plant height had a maximum cluster mean in cluster III (182.42 cm) and a minimum in cluster X, while Days to 50% flowering were highest in cluster VII (69.00).

Tabl	Table 4: Pearl millet genotype cluster means for yield and yield-related variables											
S1. No.	Character	Clus- ter I	Clus- ter II	Clus- ter III	Clus- ter IV	Clus- ter V	Clus- ter VI	Clus- ter VII	Cluster VIII	Clus- ter IX	Clus- ter X	Clus- ter XI
1.	Days to 50% flowering	55.44	55.50	56.33	58.00	56.50	58.71	69.00	57.50	54.75	56.00	54.00
2.	Plant height (cm)	157.83	128.80	182.42	121.80	124.10	137.80	170.90	131.80	131.05	104.80	141.90
3.	Leaf length (cm)	55.94	50.00	67.55	52.50	57.30	57.64	57.40	58.30	50.90	53.90	54.20
4.	Leaf width (cm)	2.95	2.95	3.18	3.05	3.10	3.09	2.90	3.25	2.98	3.00	3.50
5.	No. of leaves tiller ⁻¹	7.03	5.50	7.90	5.70	5.80	6.29	7.10	6.00	5.40	5.70	6.30
6.	No. of tillers plant ⁻¹	5.66	5.00	6.62	4.90	5.10	5.46	5.80	5.50	4.55	4.40	5.10
7.	Internodal length (cm)	19.75	16.20	21.17	17.90	16.70	17.88	19.70	17.00	16.90	14.70	16.30
8.	Stem thickness (mm)	11.04	12.97	12.43	10.26	10.49	11.21	11.93	10.31	9.57	10.75	11.93
9.	Leaf to stem ratio	0.60	0.85	0.47	0.65	0.50	0.88	1.00	2.20	2.98	1.25	0.40

S1. No.	Character	Clus- ter I	Clus- ter II	Clus- ter III	Clus- ter IV	Clus- ter V	Clus- ter VI	Clus- ter VII	Cluster VIII	Clus- ter IX	Clus- ter X	Clus- ter XI
10.	Green fodder yield (kg plot ⁻¹) in first cut	6.24	5.45	8.80	5.19	4.62	4.90	8.25	6.98	4.19	6.15	5.00
11.	Green fodder yield (kg plot ⁻¹) in second cut	4.60	3.90	4.97	4.95	3.50	5.27	4.75	4.87	5.82	8.85	10.35
12.	Dry fodder yield (kg plot ⁻¹)	2.24	2.15	2.40	2.35	1.89	2.65	2.36	2.29	2.76	4.02	5.34
13.	Regeneration ability	0.54	0.63	0.46	0.56	0.59	0.56	0.52	0.62	0.64	0.68	0.58

The most important factor of genetic divergence was Plant height, which contributed 32.38%, followed by Leaf stem ratio (14.29%), Green fodder yield in first cut (13.52%), Internode length (8.02%), Number of leaves tiller⁻¹ (6.94%), Green fodder yield in second cut (6.79%) and Days to 50% flowering (6.36%). The remaining characteristics contributed less genetic variance, indicating that they had little genetic diversity. The list of feature benefits is shown in table 5. Comparable outcomes for pearl millet were demonstrated by Shanmuganathan et al. (2006) and Shashibhushan et al. (2022).

Table 5: Genetic variation in 81 forage pearl millet lines is largely influenced by 13 characteristics.

S1.	Characters	Times	Contribution
No.	Characters	ranked first	(%)
1.	Days to 50% flowering	206	6.36
2.	Plant height (cm)	1049	32.38
3.	Leaf length (cm)	87	2.69
4.	Leaf width (cm)	103	3.18
5.	Number of leaves tiller ⁻¹	225	6.94
6.	Number of tillers plant ⁻¹	17	0.52
7.	Internodal length (cm)	260	8.02
8.	Stem thickness (mm)	58	1.79
9.	Leaf to stem ratio	463	14.29
10.	Green fodder yield (kg plot ⁻¹) in first cut	438	13.52
11.	Green fodder yield (kg plot ⁻¹) in second cut	220	6.79
12.	Dry fodder yield (kg)	13	0.40
13.	Regeneration ability	101	3.12

4. CONCLUSION

Some traits exhibited high heritability associated with high genetic advance, indicating the prevalence of

additive action of genes in their passed down through generations and indicating the selection of these traits would be relatively effective. Between clusters greater intercluster distance was reported that indicates the presence of sufficient diversity among these clusters to make these diverse forage pearl millets more valuable for a hybridization breeding program.

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