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Research Note

Genetic diversity studies for yield and physiological traits using principal component analysis in little millet

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Abstract
Principal Component Analysis (PCA) was conducted to assess the genetic variability among 50 little millet genotypes based on yield and physiological traits. Results revealed six principal components with an Eigen value more than one, which accounted for 74.25% of the total variability. PC 1 contributed the most towards the total variability at 27.98%, while PC 2, PC 3, PC 4, PC 5, and PC 6 contributed 12.90%, 11.19%, 8.93%, 7.08%, and 6.14% respectively. Days to 50 per cent flowering, grain yield plot^{-1}, harvest index, leaf area index at both panicle and 15 days after panicle initiation, specific leaf weight at 15 days after panicle initiation, and main panicle weight were the foremost contributors to genetic diversity among the studied genotypes. The biplot diagram revealed that WV-167, BL-6, TNPsu-174 and GPUL-2 were the most diverse genotypes, with high yield potential compared to other entries. GPUL-1 and DLM-186 are likely to be drought resistance due to lower relative membrane injury (%). Hybridization among these genotypes could result in transgressive segregants with desirable traits for yield and physiological characteristics.

Keywords: Little millet, Principal Component Analysis, Physiological traits, Yield traits

Little millet (*Panicum sumatrense L* Roth. Ex. Roemer and Schultes*)* is a highly self-pollinated C_4 tetraploid crop (2n = 4x =36 AABB), belonging to Poaceae family and subfamily Pancoiedae (Rachie, 1975). It is utilized as a multipurpose crop with food, fodder and bio-energy values (Joshi *et al*., 2021). Moreover, it has better agronomic characters such as rapid growth with a short duration, good growth and performance in both drought and waterlogged conditions (Patil *et al*., 2021). The crop is gaining much attention in recent years owing to its high climate-resilient in adapting to the diverse agro-climatic zones and having nutrient-rich components compared to other major cereal food crops (Vetriventhan *et al.,* 2020).

Little millet is cultivated in an area of 0.26 m.ha with a production of 0.12 mt, globally (Bhat *et al*., 2018), while it was grown in an area of 2.34 lakh hectares with an annual production of 1.42 lakh tonnes and productivity of 544 kg ha^{-1} in India(Vaishali *et al*., 2021). Karnataka, Tamil Nadu, Odisha, Madhya Pradesh, Chhattisgarh, Jharkhand, Andhra Pradesh, Uttarakhand, Maharashtra and Gujarat are the major little millet growing states in India. In Andhra Pradesh, little millet is cultivated in an area of 7000 ha with a production of 3000 t and productivity of 354 kg ha^{-1} (Venkata Ratnam *et al*., 2019).

Little millet grain has numerous nutritional benefits owing to its high micronutrient and dietary fiber content, as well as a low glycemic index (GI), which has potential health benefits (Chandel *et al*., 2014). Low-GI carbohydrate diets can help in preventing obesity, diabetes, and cardiovascular disease (Brand-Miller *et al*., 2009). Little millet is known for its ability to survive under severe water-deficit and other abiotic stresses. It also shows remarkable recovery upon mitigation of stresses (Govind in 2009). This resilience makes little millet a promising crop for cultivation in areas with erratic rainfall and other adverse environmental conditions.

However, the existence of genetic diversity and genetic relationships amid the genotypes is a foremost pre-requisite for any crop improvement programme (Selvi *et al*., 2014 and Geethanjali *et al.,* 2023). Multivariate analysis is an arithmetical tool for measuring the degree of divergence between genotypes. Principal component analysis (PCA) and cluster analysis were very important multivariate techniques in selecting parents for plant breeding programme. Principal Component Analysis (PCA) has been used frequently for genetic diversity studies in several crops such as rice (Bharadwaj *et al*., 2001 and Francis and Packiaraj, 2020) and wheat (Hailu e*t al*., 2006), etc. Further, PCA allows the grouping of the entries and is an accurate marker of differences between genotypes and PCA scores are vital in drawing conclusions for selecting promising genotypes.Previous research on little millet has not extensively explored the relationship between physiological characters such as leaf area index, specific leaf weight, and chlorophyll content with grain yield (Samundeswari *et al.,* 2018). Furthermore, there is a lack of data on the impact of stress on functional characters such as chlorophyll content and relative membrane injury, which can be used to screen germplasm for stress tolerance. Therefore, the aim of this study is to investigate the physiological traits of little millet and their association with grain yield, as well as the effect of stress on chlorophyll content and relative membrane injury. This study may useful in identifying stress-tolerant genotypes of little millet.

A total of 50 little millet germplasm accessions collected from diverse regions of India (**Table 1**) were used for evaluation in the study. These genotypes were planted in a Randomized Block Design (RBD) with three replications at Agricultural Research Station (ARS), Perumallapalle during *Kharif,* 2023. Each genotype was grown in a plot of two rows measuring 3 meters in length with proper spacing to ensure optimal crop growth. Yield and yield attributing characters such as grain yield per plot (kg/ha), plant height (cm), number of productive tillers plant^{-1}, panicle length (cm), main panicle weight, and test weight (g) were recorded at the harvest stage. Days to 50% flowering and maturity were also recorded at their respective growth stages. The harvest index was calculated using the formula given by Huhn (2008). The study also included analysis of physiological traits such as Leaf Area Index (LAI) (LICOR model-3100) using the formula by Williams (1946), and specific leaf weight (SLW) using the formula by Pearce *et al.* (1968) and expressed as mg cm^{-2}. Additionally, SCMR (SPAD Chlorophyll Meter Reading) and relative injury (%) were recorded at the panicle initiation stage in little millet genotypes, following the protocol described in earlier studies (Strain, 1971).

LAI = Leaf area plant^{-1} / Ground area occupied by the plant
SLW= Leaf dry weight/ Leaf area
Harvest Index = Economic yield / Biological yield × 100

Where, Economic yield = Grain yield (g)
Biological yield = Total plant yield (g)

Leopold *et al.* (1981) calculated the cell content leakage by following formula.

$$\text{Leakage (\%)} = \frac{I_a}{F_a} \times 100$$

where,
I_a = Initial absorbance
F_a = Final absorbance

The collected data was analyzed using standard statistical procedures outlined by Panse and Sukhatme (1961). The statistical analysis for principal component was conducted using the statistical software, R version 4.2.3.

Analysis of Variance (ANOVA) for yield and physiological traits, revealed highly significant differences among the little millet genotypes for all the studied characteristics, indicating sufficient variation in the experimental material (**Table 2**). These findings are consistent with earlier research by Sasamala *et al.* (2015), Selvi *et al*. (2015) and Jyothsna *et al*. (2016). Mean performance of the little millet genotypes in terms of yield, yield attributes and physiological traits are presented in **Table 3 and Table 4**.

Extent of variation among genotypes was reflected by high values of mean and range (Rahangdale *et al*., 2019). Days to 50 per cent flowering in the current study was observed to range from early flowering (45 days, RLM-238) to late flowering (82 days, GPUL-3) with a general mean of 58 days. As result, genotypes, RLM-37 and RLM-367 (46 days) were the earliest to flower and can be utilized in the hybridization programme as donor parents to develop short duration varieties. The conclusions agreed with the earlier reports of Bhavana (2018). The plant height varied from shortest (95.0 cm, GPUL-2) to tallest (143.7 cm, CO-2) with an overall mean value of 121.15 cm. Important yield influencing character is the productive tillers plant^{-1} and it was noticed to be minimum in DhLtMV-21-1 (5.1) and maximum in TNPsu-183 (9.4) with an overall average of 7. Maximum panicle length was recorded in DhLtMV–10-2 (32.80 cm) and lowest in IIMRLM-7012 (21.47 cm) with mean value of 26.86 cm. Main panicle weight was maximum in IIMRLM-7012 (4.57g) and minimum in BL-2 (1.17 g) with an average value of 1.85 g. The test weight ranged from 1.50 g (DhLtMV-28-4) to 2.91 g (KADIRI-1) with an overall mean of 2.24 g. The lowest fodder yield plot^{-1} was noted in DhLtMV-39-1 (0.78 kg) and highest in WV-126 (1.84 kg) with an average value of 1.15 kg. Grain yield plot^{-1} was observed to range from 0.08 kg (DhLtMV-21-1) to 0.31 kg (TNPsu-183). A perusal of the results also revealed significantly higher grain yield plot^{-1} in a few genotypes *viz*., WV-125 (0.31kg), WV-167(0.29kg), TNPsu-170

Table 1. List of pedigree details of 50 little millet genotypes

S. No.	Genotypes	Pedigree	Centre
1	BL-2	CO-2 × OLM-56	Jagdalpur
2	BL-4	CO-2 × TNAU-97	Jagdalpur
3	BL-8	CO-2 × OLM-56	Jagdalpur
4	BL-41-3	Paiyur-2 × TNAU-97	Jagdalpur
5	BL-150	Paiyur-2 × DLM-369	Jagdalpur
6	CO-2	Pure line selction	Coimbatore
7	DhLtMV-10-2	CO-4 × Paiyur-2	Hanumanamatti
8	DhLtMV-14-1	CO-2 × TNAU-110	Hanumanamatti
9	DhLtMV-21-1	CO-2 × TNAU-26	Hanumanamatti
10	DhLtMV-28-4	CO-2 × TNAU-26	Hanumanamatti
11	DhLtMV-36-3	CO-4 × Paiyur-2	Hanumanamatti
12	DhLtMV-39-1	CO-4 × Paiyur-2	Hanumanamatti
13	DLM-8	Pure line selection from Local Germplasm	Dindori
14	DLM-14	Pure line selection from Local Germplasm	Dindori
15	DLM-89	Pure line selection from Local Germplasm	Dindori
16	DLM-95	Selection from Local Germplasm	Rewa
17	DLM-103	Pure line selection from Local Germplasm	Dindori
18	DLM-186	Selection from Local germplasm of Rewa	Dindori
19	DhLt-28-4	CO-2 × TNAU-26	Waghai
20	GPUL-1	JK-8 × Peddasame	Banglore
21	GPUL-2	Pure line selection from Peddasame	Banglore
22	GPUL-3	JK-8 × Peddasame	Banglore
23	GPUL-4	JK-8 × Peddasame	Banglore
24	GPUL-5	JK-8 × Peddasame	Banglore
25	GV-2-1	Mutant of Gujarat Vari-1	Waghai
26	IIMRLM-7012	Selection from IPMR-699	IIMR, Hyderabad
27	IIMRLM-7162	Selection from GPMR-1153	IIMR, Hyderabad
28	KADIRI-1	Selection from Kadiri local	Bangalore
29	KOPLM-53	IPS from local germplasm	Kolhapur
30	Nallasama	Selection from Local	ARS, Perumallapalle
31	OLM-217	Selection from Udayagiri Local	Berhampur
32	OLM-233	Selection from L55	Berhampur
33	RLM-37	Selection from Local germplasm No. 37	Rewa
34	RLM-238	Selection from Local germplasm of Rewa	Rewa
35	RLM-367	Selection from Local germplasm No. 367	Rewa
36	TNAU-152	Paiyur-1 × PM-29	Coimbatore
37	TNAU-159	TNAU-81 × TNAU-25	Coimbatore
38	TNAU -160	TNAU-91 × MS-4729	Coimbatore
39	TNPsu-167	CO-2 × TNAU-26	Coimbatore
40	TNPsu-170	CO-4 × IPM-113	Coimbatore
41	TNPsu-171	CO-2 × TNAU-28	Coimbatore
42	TNPsu-174	CO-2 × IPM-113	Coimbatore
43	TNPsu-183	CO-2 × MS-4729	Athiyandal
44	TNPsu-186	MS-507 × MS-1211	Athiyandal`
45	WV-125	Local collection from Waghai	Waghai
46	WV-126	Local collection from Dangas	Waghai
47	WV-167	Local selection from Subir	Waghai
48	BL-6	Paiyur-1 × OLM-29	Jagdalpur
49	JK-8	Selection from Local germplasm	Rewa
50	OLM-203	Pure line selection from Lakshmipur local	Berhampur

Table 2. Analysis of variance for yield, yield components and physiological traits in little millet

S. No.	Characters	Mean sum of squares		
		Replications (df:2)	Genotypes (df:49)	Error (df:98)
1	Days to 50% flowering	17.92	326.55**	9.13
2	Days to maturity	27.12	318.24**	9.71
3	Plant height	63.88	304.67**	16.17
4	Number of productive tillers plant^{-1}	0.17	6.53**	0.45
5	Panicle length	1.91	28.46**	3.03
6	Main panicle weight	0.005	0.71**	0.051
7	Thousand seed weight	0.06	0.30**	0.02
8	Fodder yield plot^{-1}	0.02	0.23**	0.02
9	Grain yield plot^{-1}	0.005	0.006**	0.001
10	LAI (Panicle initiation stage)	0.12	0.96**	0.05
11	LAI (15 days after panicle initiation stage)	0.20	0.95**	0.08
12	SCMR (Panicle initiation stage)	5.78	36.23**	3.41
13	SCMR (15 days after panicle initiation stage)	4.28	24.50**	3.12
14	SLW (Panicle initiation stage)	0.004	0.0003*	0.00004
15	SLW (15 days after panicle initiation stage)	0.00001	0.0002*	0.00003
16	Relative injury (15 days after panicle initiation stage)	11.18	94.47**	10.44
17	Harvest index	30.79	98.43**	14.27

*Significant at 5 % level ** Significant at 1 % level

(0.25kg) and IIMRLM-7162(0.25kg) when compared to the high yielding check, BL-8 (0.22 kg). Maximum harvest index was observed in BL-41-3 (29.50), whereas the minimum in BL-4 (8.25) with an overall mean value of 18.58 (%). Based on the above, five genotypes were identified as promising ones compared to the check (BL-8) are illustrated in **Table 5**.

The focus of the study was to assess the physiological traits and their correlation with grain yield. By analyzing the physiological traits of crop plants at various growth stages, we aimed to gain insights into their adaptations and crop characteristics (Samundeswari *et al*., 2018). Leaf area, in particular, plays a crucial role in determining the rate of photosynthesis in plants. Optimal development of leaf area is essential for effective interception of light energy and facilitates higher dry matter production. Nonetheless, LAI was recorded highest in TNPsu-186 while Nallasama showed lowest value at both panicle initiation and 15 days after panicle initiation of crop. SPAD ranged from 45.46 (BL-8) to 32.66 (DhLtMV-39-1) at panicle initiation, while BL-41-3 (37.19) showed highest and CO-2 (23.71) recorded lowest mean values at 15 days after panicle initiation. SLW was noted maximum in DhLt-28-4 (0.06 g cm^{-2}), while RLM -367 displayed the lowest value (0.02 g cm^{-2}) at panicle initiation stage. Similarly, TNPsu -186 registered maximum (0.06 g cm^{-2}) and WV-167 displayed the minimum SLW (0.01 g cm^{-2}) at 15 days after panicle initiation. SLW is one of the physiological traits in plant growth analysis and is the ratio of dry weight to its land area. The SLW is often considered as an indirect measure of leaf expansion. Similar results were reported earlier by Samundeswari *et al*. (2018) and Bhavana (2018).

Genotypes with lower relative injury possess high drought tolerance as it maintained high thermo tolerance apart from moisture stress tolerance (Bhavana, 2018). In the study, low membrane injury was found in the genotypes *viz.,* GPUL-1, DLM-186, GV-2-1, GPUL-3 and BL-41-3 at 15 days after panicle initiation, implying their tolerance to high heat stress and hence they can be utilized for drought resistance breeding in future. The genotypes, TNPsu-183, WV-167 IIMRLM-7162, DhLtMV-10-2 and DhLtMV-14-1 showed better performance for the yield attributing traits *viz*., days to maturity, number of productive tillers plan^{-1}, plant height, main panicle weight, thousand seed weight and harvest index (**Table 6**), while the genotypes, TNPsu-183, WV-167, BL-8 and BL-41-3 were recorded higher grain yield associated with more chlorophyll content, leaf area index at both panicle and 15 days after panicle initiation and relative injury. Hence, these genotypes can be used for potent donor sources for breeding of high yielding along with drought resistant lines.

Principal Component Analysis: The Eigen values, variability and cumulative variability of the six principal components (PC) which helps in understanding the

Table 3. Mean performance of little millet genotypes for yield and yield attributing traits

S.No	Genotypes	DFF	DM	PH	NPT	PL	MPW	TSW	FYPP	GYPP
1	BL-2	57	86	126.3	5.8	32.47	1.17	2.37	1.00	0.15
2	BL-4	58	88	108.3	5.8	23.30	1.88	2.09	1.79	0.15
3	BL-8	49	79	124.0	8.0	32.28	1.67	2.58	0.83	0.22
4	BL-41-3	61	90	120.7	6.6	25.40	1.80	2.09	0.87	0.22
5	BL-150	54	84	132.7	8.0	26.87	1.63	2.46	0.97	0.24
6	CO-2	61	91	143.7	6.2	25.00	2.12	2.52	0.93	0.21
7	DhLtMV-10-2	55	82	138.0	9.0	32.80	1.73	2.39	1.22	0.23
8	DhLtMV-14-1	61	93	122.7	9.2	29.43	2.69	2.21	1.22	0.23
9	DhLtMV-21-1	65	95	134.4	5.1	24.67	1.75	1.82	0.82	0.08
10	DhLtMV-28-4	54	86	123.0	7.8	26.00	1.65	1.50	0.98	0.18
11	DhLtMV-36-3	57	89	114.3	8.0	27.47	1.73	2.53	0.95	0.19
12	DhLtMV-39-1	48	79	114.3	6.0	27.30	1.74	2.34	0.78	0.18
13	DLM-8	51	80	118.7	7.1	27.93	1.40	2.27	1.35	0.22
14	DLM-14	56	85	115.0	9.1	23.90	1.58	2.15	1.24	0.22
15	DLM-89	56	84	127.7	9.2	25.40	2.12	1.88	1.45	0.21
16	DLM-95	66	96	116.3	7.4	23.00	1.72	1.71	1.26	0.18
17	DLM-103	48	80	135.0	8.4	30.50	1.55	2.50	1.09	0.22
18	DLM-186	52	82	119.3	8.3	25.67	1.73	2.81	0.79	0.19
19	DhLt-28-4	52	87	126.7	7.4	24.97	1.64	2.41	0.87	0.22
20	GPUL-1	77	107	96.3	7.2	23.30	1.93	2.27	1.45	0.14
21	GPUL-2	74	104	95.0	4.4	24.17	1.83	1.73	1.36	0.19
22	GPUL-3	82	110	119.3	4.2	27.90	1.74	2.42	1.37	0.15
23	GPUL-4	49	79	126.3	6.3	26.00	2.77	2.26	1.25	0.21
24	GPUL-5	48	78	124.0	9.1	24.23	1.46	1.91	0.93	0.15
25	GV-2-1	78	108	111.7	5.2	21.73	1.88	1.81	1.16	0.15
26	IIMRLM-7012	61	95	114.3	5.6	21.47	4.57	1.94	1.32	0.22
27	IIMRLM-7162	60	90	126.0	9.2	26.73	2.09	2.47	1.05	0.25
28	KADIRI-1	64	93	127.3	8.0	29.87	2.12	2.91	0.85	0.20
29	KOPLM-53	72	102	116.7	7.2	26.97	2.36	2.28	1.64	0.15
30	Nallasama	49	77	114.0	7.2	27.97	1.32	2.18	1.08	0.15
31	OLM-217	79	109	112.3	8.0	24.93	1.84	1.78	1.79	0.18
32	OLM-233	51	77	115.6	9.3	24.83	1.65	2.31	1.51	0.20
33	RLM-37	46	76	112.6	7.1	23.70	1.69	2.17	0.81	0.14
34	RLM-238	45	78	114.0	8.1	28.03	1.70	2.26	1.54	0.18
35	RLM-367	46	77	106.3	7.2	25.23	1.61	2.09	0.82	0.16
36	TNAU-152	54	86	128.6	6.3	28.77	1.94	2.34	0.86	0.18
37	TNAU-159	54	84	138.3	7.0	32.23	1.51	2.14	0.86	0.18
38	TNAU-160	53	81	122.6	7.1	26.30	1.62	2.59	0.93	0.21
39	TNPsu-167	69	99	125.6	7.3	23.87	1.74	2.15	1.03	0.22
40	TNPsu-171	52	82	127.3	9.2	32.00	1.55	2.35	1.35	0.20
41	TNPsu-170	51	82	127.0	7.0	31.77	2.14	2.42	1.37	0.25
42	TNPsu-174	52	81	129.4	8.1	30.80	1.53	2.70	1.13	0.24
43	TNPsu-183	63	93	123.3	9.4	32.47	1.71	2.56	1.43	0.31
44	TNPsu-186	52	82	127.2	6.0	25.50	2.07	2.50	1.07	0.19
45	WV-125	80	110	128.6	5.5	27.20	1.77	1.71	1.04	0.31
46	WV-126	72	105	123.3	7.0	27.17	2.22	1.83	1.84	0.21
47	WV-167	46	77	120.0	9.3	24.53	1.69	2.08	0.95	0.29
48	BL-6	53	82	116.7	8.2	29.77	2.07	2.84	0.95	0.25
49	JK-8	50	80	125.7	9.1	26.80	1.67	2.37	1.08	0.19
50	OLM-203	75	105	98.3	8.0	22.23	1.97	1.88	1.22	0.10
	Mean	**58.33**	**88.34**	**121.15**	**7.22**	**26.86**	**1.85**	**2.24**	**1.15**	**0.20**

DFF : Days to 50% flowering; DM : Days to maturity; PH : Plant height (cm); NPT : Number of productive tillers per plant; PL : Panicle length (cm); MPW : Main panicle weight (g); TSW : Thousand seed weight (g); FYPP : Fodder yield plot^{-1} (kg); GYPP : Grain yield plot^{-1} (kg)

Table 4. Mean performance of little millet genotypes for physiological traits

S. No.	Genotypes	LAI (PI)	LAI (15 DA PI)	SCMR (PI)	SCMR (15 DA PI)	SLW (g cm^{-2}) (PI)	SLW (g cm^{-2}) (15 DA PI)	RI (%) (15 DAPI)	HI
1	BL-2	0.95	1.18	33.05	27.12	0.03	0.02	32.30	15.51
2	BL-4	1.23	1.96	34.28	34.01	0.04	0.04	29.58	8.25
3	BL-8	0.50	0.86	45.47	36.96	0.04	0.04	26.18	26.14
4	BL-41-3	0.53	0.73	37.05	37.19	0.03	0.03	25.95	29.50
5	BL-150	1.08	2.64	42.92	33.62	0.04	0.03	27.25	27.00
6	CO-2	1.18	1.33	36.45	23.71	0.03	0.03	28.54	19.06
7	DhLtMV-10-2	0.52	1.01	37.44	34.24	0.05	0.04	31.65	21.98
8	DhLtMV-14-1	1.12	1.70	43.57	27.86	0.03	0.03	31.07	19.46
9	DhLtMV-21-1	1.17	1.85	43.23	29.20	0.05	0.03	32.30	10.99
10	DhLtMV-28-4	1.82	2.35	37.67	32.45	0.04	0.03	27.55	18.15
11	DhLtMV-36-3	1.25	1.52	36.15	29.28	0.05	0.03	33.30	20.00
12	DhLtMV-39-1	1.26	1.51	32.66	28.05	0.03	0.04	29.29	23.87
13	DLM-8	1.70	1.87	33.44	27.38	0.05	0.04	37.58	17.20
14	DLM-14	1.58	1.95	36.09	27.84	0.02	0.02	32.79	19.21
15	DLM-89	1.22	1.59	35.81	30.30	0.04	0.02	35.38	14.09
16	DLM-95	1.76	2.18	35.31	28.66	0.03	0.02	36.37	14.13
17	DLM-103	2.08	2.38	38.97	28.96	0.03	0.02	33.56	18.71
18	DLM-186	0.67	1.01	36.63	28.38	0.05	0.03	22.40	20.52
19	DhLt-28-4	1.25	2.12	37.22	29.34	0.06	0.04	32.04	26.66
20	GPUL-1	2.33	2.49	42.65	27.33	0.03	0.03	21.06	9.75
21	GPUL-2	1.34	1.84	33.37	28.79	0.04	0.03	32.36	13.81
22	GPUL-3	1.29	2.01	43.80	25.32	0.03	0.03	25.17	10.82
23	GPUL-4	0.60	1.22	36.59	28.29	0.05	0.05	30.40	17.00
24	GPUL-5	2.05	2.09	34.86	32.69	0.03	0.02	37.63	16.42
25	GV-2-1	1.30	2.09	33.62	29.40	0.05	0.04	25.82	13.55
26	IIMRLM-7012	1.93	2.15	41.70	29.86	0.04	0.03	40.22	20.80
27	IIMRLM-7162	2.21	2.35	41.78	29.62	0.02	0.02	23.44	23.58
28	KADIRI-1	0.78	1.35	36.17	27.61	0.05	0.03	27.28	23.16
29	KOPLM-53	2.15	2.09	33.83	27.44	0.05	0.02	37.04	9.11
30	Nallasama	0.45	0.53	40.83	26.98	0.02	0.02	35.38	14.41
31	OLM-217	1.32	1.80	42.25	33.27	0.05	0.03	38.64	10.97
32	OLM-233	0.79	1.01	41.57	31.14	0.03	0.02	35.13	13.53
33	RLM-37	0.91	1.23	33.10	27.60	0.02	0.03	27.05	16.02
34	RLM-238	0.69	0.92	35.80	27.08	0.03	0.03	34.69	13.61
35	RLM-367	0.75	0.96	35.12	28.06	0.02	0.02	33.67	25.98
36	TNAU-152	0.80	1.34	35.86	28.18	0.04	0.04	40.50	23.45
37	TNAU-159	1.74	2.45	34.89	33.26	0.03	0.04	28.68	20.52
38	TNAU-160	1.47	1.96	40.57	31.64	0.02	0.04	36.37	21.69
39	TNPsu-167	1.35	1.85	36.83	26.65	0.03	0.03	37.23	20.18
40	TNPsu-171	0.83	1.12	35.20	28.11	0.02	0.02	49.07	14.66
41	TNPsu-170	1.81	2.17	39.12	32.22	0.03	0.03	33.99	16.46
42	TNPsu-174	1.61	2.39	35.30	32.48	0.03	0.03	28.61	20.84
43	TNPsu-183	2.07	2.26	33.54	29.22	0.02	0.03	44.54	26.48
44	TNPsu-186	2.34	2.66	34.79	28.95	0.04	0.06	39.58	17.63
45	WV-125	2.27	2.33	37.68	25.62	0.03	0.03	32.15	28.50
46	WV-126	2.01	2.57	37.56	26.03	0.03	0.02	27.70	13.02
47	WV-167	1.21	1.79	43.52	29.32	0.02	0.01	37.84	28.56
48	BL-6	0.45	1.06	34.25	30.01	0.04	0.02	32.94	26.62
49	JK-8	0.76	1.06	35.34	28.17	0.02	0.02	29.48	18.17
50	OLM-203	0.97	1.91	36.18	32.56	0.06	0.03	28.41	9.10
	Mean	**1.31**	**1.72**	**37.42**	**29.55**	**0.04**	**0.03**	**32.34**	**18.58**

LAI (PI) : Leaf area index at panicle initiation stage; LAI (15 DAPI) : Leaf area index at 15 days after panicle initiation stage; SCMR (PI) : SPAD chlorophyll meter reading at panicle initiation stage; SCMR (15 DA PI) : SPAD chlorophyll meter reading at 15 days after panicle initiation stage; SLW (PI) : Specific leaf weight panicle initiation stage (g cm^{-2}); SLW (15 DAPI) : Specific leaf weight at 15 days after panicle initiation stage (g cm^{-2}); RI : Relative injury at 15 days after panicle initiation stage (%); HI : Harvest index (%).

Table 5. List of top little millet five genotypes for grain yield, yield attributing and physiological traits

S. No.	Genotypes	Grain yield plot^{-1} (kg)	Traits
1	TNPsu-183	0.31	Number of productive tillers, panicle length, thousand seed weight, fodder yield plot^{-1}, specific leaf weight at 15 days after panicle initiation and harvest index
2	WV-167	0.31	Days to 50% flowering, days to maturity, number of productive tillers, panicle length, SPAD chlorophyll meter reading at panicle initiation and harvest index
3	IIMRLM-7162	0.25	Days to 50% flowering, number of productive tillers, main panicle weight, thousand seed weight, SPAD chlorophyll meter reading at both panicle initiation and 15 days after panicle initiation, leaf area index and relative injury at 15 days after panicle initiation and harvest index
4	DhLtMV-10-2	0.23	Plant height, number of productive tillers, panicle length, thousand seed weight, SPAD chlorophyll meter reading and relative injury at 15 days after panicle initiation
5	DhLtMV-14-1	0.23	Number of productive tillers, Main panicle weight, test weight, Fodder yield, SCMR (PI & 15 DAPI) and RI (15 DAPI)
6	BL-8 (Check)	0.21	Thousand seed weight, SCMR (PI & 15 DAPI) and leaf area index

SCMR : SPAD Chlorophyll meter reading; PI : Panicle initiation ;15 DAPI : 15 days after panicle initiation

Table 6. Superior little millet genotypes identified for yield, yield attributing and physiological traits

S.No	Characters	Superior genotypes identified
1	Days to 50% flowering	RLM-238, RLM-37, RLM-367, WV-167 and DhLtMV-39-1
2	Days to maturity	RLM-37, RLM -367, OLM-233, WV-167 and RLM-238
3	Plant height (cm)	CO-2, TNAU-159, DhLtMV-10-2, DLM-103 and DhLtMV-21-1
4	Number productive tillers plant^{-1}	TNPsu-183, WV-167, OLM-233, DhLtMV-14-1 and DLM-89
5	Panicle length (cm)	DhLtMV–10-2, BL-2, TNAU-159, TNPsu-170 and TNPsu-171
6	Main panicle weight(g)	IIMRLM-7012, GPUL-4, DhLtMV-14-1, KOPLM-53 and WV-126
7	Thousand seed weight (g)	KADIRI-1, DLM-186, TNAU-160, TNPsu-174 and BL-8
8	Fodder yield plot^{-1} (kg)	WV-126, BL-4, OLM-217, KOPLM-53, RLM-238 and OLM-233
9	Grain yield plot^{-1} (kg)	TNPsu-183, WV-125, WV-167, TNPsu-170 and IIMRLM-7162
10	Leaf area index at PI	TNPsu-186, BL-8, BL-41-3 and DhLtMV-10-2
11	Leaf area index at 15 (DAPI)	TNPsu-186, BL-41-3. BL-8, RLM-238 and RLM-367
12	SCMR at PI	BL-8, GPUL-3, DhLtMV-14-1, WV-167 and DhLtMV-21-1
13	SCMR at 15 (DAPI)	BL-41-3, BL-8, DhLtMV-10-2, BL-150 and OLM-217
14	SLW (g cm^{-2}) at PI	DhLt-28-4, DhLtMV-36-3, DhLtMV-10-2 and GPUL-4
15	SLW (g cm^{-2}) at 15 (DAPI)	TNPsu-183, BL-4, DhLtMV-39-1, DLM-8 and TNAU- 152
16	Relative injury (%) at 15 (DAPI)	GPUL-1, DLM-186, GV-2-1, GPUL-3 and BL-41-3
17	Harvest index (%)	BL-41-3, WV-167, WV-125, BL-150 and DhLt-28-4

SCMR : SPAD Chlorophyll meter reading; SLW : Specific leaf weight; PI : Panicle initiation ;15 DAPI : 15 days after panicle initiation

contribution of each component to the total variance of the dataset are presented in **Table 7**. The scree plot (**Fig. 1**) provides a visual representation of the percentage of variation contributed by each PC and this plot helps in identifying the significant PCs and their contribution to the overall variability of the dataset (Upadhyay *et al*., 2022). It was identified that the first six principal components accounted for 74.25% of the total variability in the data set. This indicates that the primary characteristics of the data set are represented by these six components. The first principal component (PC1), was found to contribute 27.98% to the total variability. Days to maturity (0.379),

Table 7. Eigen values, variability and cumulative variability of principal components in little millet genotypes

Principal components	Eigen value	Variability (%)	Cumulative variability (%)
PC 1	4.75	27.98	27.98
PC 2	2.19	12.90	40.89
PC 3	1.90	11.19	52.08
PC 4	1.51	8.93	61.02
PC 5	1.20	7.08	68.10
PC 6	1.04	6.14	74.25
PC 7	0.96	5.68	79.93
PC 8	0.90	5.30	85.24
PC 9	0.67	3.97	89.21
PC10	0.54	3.18	92.39
PC11	0.45	2.69	95.09
PC12	0.33	1.99	97.09
PC13	0.30	1.78	98.87
PC14	0.13	0.79	99.66
PC15	0.03	0.17	99.66
PC16	0.02	0.14	99.98
PC17	0.00	0.01	100.00

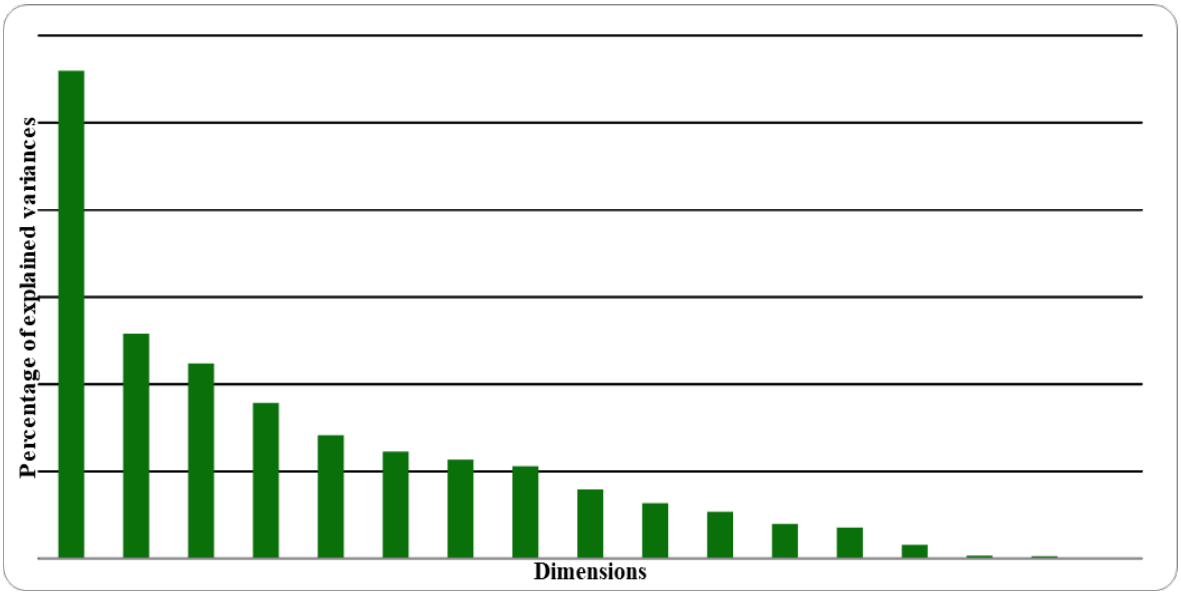

Fig. 1. Scree plot diagram using principal components of little millet genotypes

days to 50 per cent flowering (0.371), leaf are index at 15 days after panicle initiation (0.233), leaf are index at panicle initiation (0.215), fodder yield (0.238) and main panicle weight (0.157), represented the maximum variance in this component. Hence, the selection of lines and traits from this PC will be useful. PC 2 contributed to 12.90 per cent of the total variance. The characters such as, LAI at 15 days after panicle initiation (0.465), leaf are index at panicle initiation (PI) (0.437), grain yield plant^{-1} (0.396), relative injury (0.303), plant height (0.261), days to maturity (0.171), panicle length (0.168), days to 50 (%) flowering (0.167), and SPAD chlorophyll content at panicle initiation stage (0.104) described the highest loadings in this component. PC 3 was illustrated by 11.19 per cent contribution toward the total variability. The traits namely, SPAD chlorophyll content at panicle initiation (0.458), 15 days after panicle initiation (DAPI) (0.429), specific leaf weight at PI (0.304), main panicle weight (0.230), plant height (0.178) and test weight (0.150) recorded maximum variance in this component. The principal component (PC 4) contributed to 8.93 per cent of the total variance. The traits, fodder yield (0.409), number of productive tiller (0.389), SLW at 15 days after panicle initiation (0.325), SPAD chlorophyll content (0.242) and plant height (0.121), described the highest loadings in this component. The principal component (PC 5) was illustrated by 7.08 per cent contribution toward the total variability. The characters, SPAD chlorophyll content at 15 DAPI (0.373), number of productive tillers per plant (0.308), SPAD at PI (0.285), main panicle weight (0.255), harvest index (0.230), fodder yield (0.217) and grain yield plant^{-1} (0.169), recorded maximum variance in this component. The sixth principal component (PC 6) contributed 6.14 per cent to the total variability. The characters, SLW at 15 days after panicle initiation (0.275), SPAD at 15 DAPI (0.265), relative injury (0.215) and leaf are index at 15 DAPI (0.123) and PI (0.117), represented the maximum variance in this component. Per cent contribution of traits towards the existing variability presented in **Fig.2**. The maximum contributing traits towards the genetic diversity of the little millet genotypes are identified as, days to 50 per cent flowering, productive tillers plant^{-1}, grain yield plant^{-1}, fodder yield, leaf area index, specific leaf weight, main panicle weight, relative injury and days to maturity. Similar results were noted earlier by Vaishali *et al*. (2021) for days to 50 per cent flowering and days to maturity, grain yield plant^{-1}, harvest index and productive tillers and Trivedi, *et al.* (2018) for SPAD chlorophyll content. Further, biplot diagram is a graphical representation of the interaction between the various characters and genotypes that performed better for specific traits (Mahendran *et al*., 2015). Biplot diagram provides a visualization of the distribution and nature of diversity among the genotypes and yield-contributing traits, allowing for a better understanding of the relationships between them (Singh *et al*., 2020). The length of each vector in the biplot diagram represents the contribution of the corresponding trait to the total divergence, with longer vectors indicating a greater contribution. Similar inferences were drawn by Christina *et al*. (2021).

In this study, the biplot (**Fig.3**) illustrates the distribution and nature of diversity for the genotypes and yield contributing traits along the PC1 and PC2 axes. grain yield plant^{-1} was observed to be the character with the maximum vector length which indicates its significant contribution to the total divergence. This was followed by harvest index, LAI, days to 50 per cent flowering, days to maturity, SLW, main panicle weight and fodder yield. The angle between the vectors is also important, with an acute angle (<90°) indicating a positive correlation, an obtuse angle (>90°) indicating a negative correlation, and a right angle (90°) indicating no correlation (Christina *et al*., 2021). The biplot diagram provides a clear visualization of the inter-relationships among the various characters and genotypes, allowing for the identification of those with the greatest potential for use in future breeding programs for improved yield and other desirable traits.

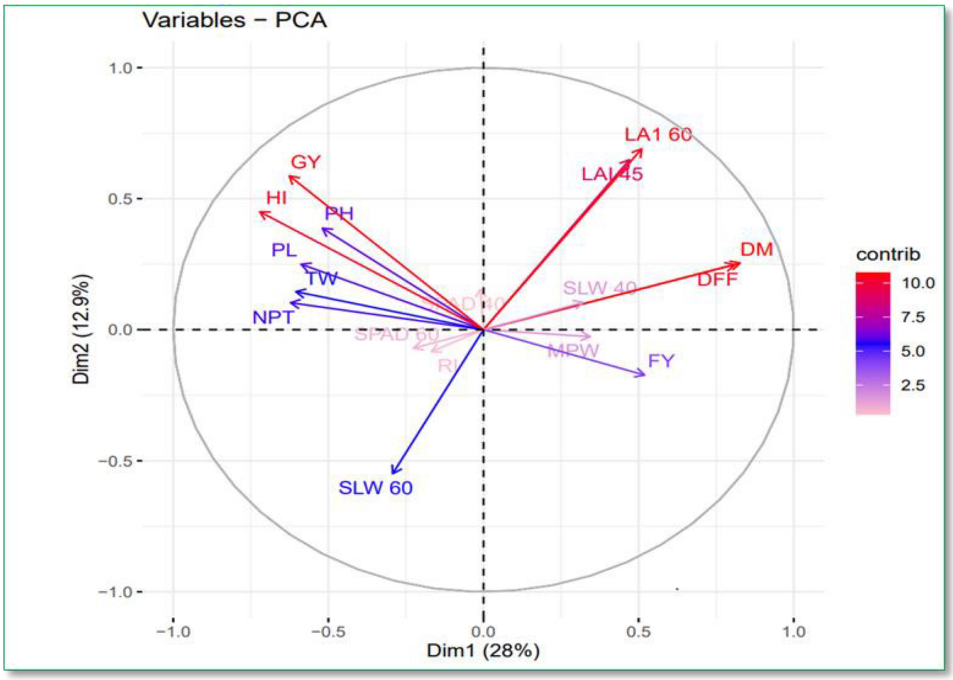

Fig. 2. Per cent contribution of traits towards the existing variability

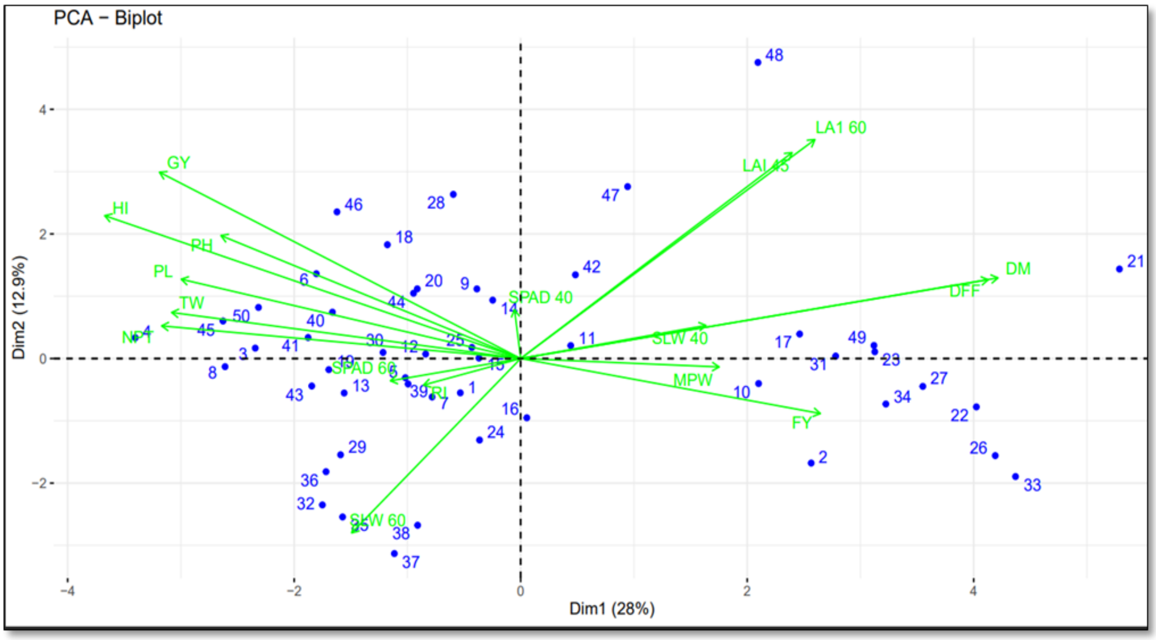

Fig. 3. Biplot diagram showing the dispersion of Little millet genotypes across PC1 and PC2

Table 8. Factor loadings for different characters of first three principal components in little millet genotypes

S. No.	Character	PC 1	PC 2	PC 3	PC4	PC5	PC6
1	Days to 50 percent flowering	0.371	0.167	0.131	0.055	-0.300	0.030
2	Days to maturity	0.379	0.171	0.132	0.039	-0.260	0.021
3	Plant height	-0.238	0.261	0.178	0.121	-0.179	-0.103
4	Number of productive tillers plant^{-1}	-0.285	0.069	-0.064	0.389	0.308	-0.138
5	Panicle length	-0.269	0.168	0.090	0.161	-0.382	-0.341
6	Main panicle weight	0.157	-0.017	0.230	-0.038	0.255	-0.461
7	Test weight	-0.277	0.097	0.150	0.083	-0.311	-0.210
8	Fodder yield	0.238	-0.117	0.046	0.409	0.217	-0.451
9	Grain yield plant^{-1}	-0.286	0.396	-0.000	-0.031	0.169	-0.115
10	LAI (Panicle initiation stage)	0.215	0.437	-0.261	0.198	0.135	0.117
11	LAI (15 days after panicle initiation stage)	0.233	0.465	-0.201	0.117	0.142	0.123
12	SCMR (Panicle initiation stage)	-0.004	0.104	0.458	0.242	0.285	0.265
13	SCMR (15 days after panicle initiation stage)	-0.103	-0.047	0.429	-0.096	0.373	0.190
14	SLW (Panicle initiation stage)	0.147	0.070	0.304	-0.503	0.055	-0.219
15	SLW (15 days after panicle initiation stage)	-0.134	-0.371	-0.069	0.325	-0.118	0.275
16	Relative injury (15 days after panicle initiation stage)	-0.077	0.303	-0.004	-0.266	0.018	0.215
17	Harvest index	-0.330	-0.056	-0.492	-0.272	0.230	-0.266

The PCA analysis provide insights into the genetic diversity of the little millet genotypes, which can be useful in developing new varieties that are more productive and adaptable to changing environmental conditions. Notably, genotypes such as TNPsu-183 and WV-167 emerged as promising genotypes for future breeding programs due to their high yield and yield attributing characters. Future research should focus on leveraging these insights for targeted hybridization and selection efforts.

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