

# Agro-morphological Characterization of Proso millet Germplasm for Utilization

## ABSTRACT

**Aims:** In this study, 634 accessions of proso millet were evaluated to assess phenotypic diversity using morpho-agronomic traits, aiming to identify specific donors for desired traits.

**Study design:** Mention the design of the study here.

**Place and Duration of Study:** During the Kharif season of 2021, a total of 634 proso millet germplasm accessions were characterized, alongside three control varieties, as part of the Institute Project (IIMR/CI/2021-2026/150) at ICAR-IIMR in Hyderabad.

**Methodology:** The experimental design employed was an augmented block design, and four check varieties, namely GPUP 8, TNAU 145, TNAU 164, and TNAU 202, were included.

**Results:** The results revealed significant variation across all the traits studied. Principal Component Analysis (PCA) demonstrated that the first three principal components accounted for 60% of the total variation. PC1 accounted for 27.3% of the variation, followed by PC2 (22.6%) and PC3 (10%). PC1 was primarily influenced by Leaf blade length (cm), Peduncle length (cm), Panicle length (cm), Plant height (cm), and grain yield (g plant<sup>-1</sup>). Furthermore, the study identified 21 accessions with superior performance in multiple traits (3-6 traits).

**Conclusion:** These accessions can serve as valuable genetic resources for improving yield and quality traits. The selected multi-trait donors can be directly released for cultivation, serving as a short-term breeding goal. This approach has the potential to enable farmers to achieve high remuneration by harvesting increased yields.

**Keywords:** Proso millet; germplasm characterization, PCA, trait donors

## 1. INTRODUCTION

Proso millet (*Panicum miliaceum*) is an ancient crop that was domesticated over 10,000 years ago in China [1]. Research indicates that proso millet was independently domesticated in multiple regions and has since spread across Europe and Asia. Throughout centuries of cultivation, the crop has adapted to diverse climatic conditions, leading to significant population differentiation. The germplasm collected from various regions is likely to harbor a wide range of genetic alleles. Key proso millet-growing countries, including Russia, China, Germany, the USA, and India, have collected and conserved germplasm in their gene banks, recognizing its importance as a foundational resource in crop breeding programs. Proso millet possesses valuable nutritional attributes, as it is rich in protein, starch, dietary fiber, and several trace elements such as magnesium, iron, and calcium [2]. Moreover, it has been associated with potential health benefits, including the prevention of arteriosclerosis, gastrointestinal cancer, coronary heart disease, and other ailments [3]. In recent years, there has been a growing interest in the use of millet, particularly in food applications as a gluten-free ingredient [4]. Proso millet is extensively cultivated in countries such as China, Korea, India, Pakistan, the United States, and Russia. It serves as a food source in South and Southeast Asian countries, while in the United States, it is predominantly used as bird feed [5].

The rapid and widespread adoption of proso millet during its early domestication can be attributed to its short growing duration and resilience to biotic and abiotic stress [6],[7]. Additionally, proso millet is rich in protein [8], and recent studies have reported the presence of high levels of antioxidants and secondary metabolites with health benefits [9],[10]. Analyses of the C4 pathway in proso millet have revealed its

33 greater efficiency in photosynthesis under lower gradients of transport metabolites and in varying light  
34 intensities [11]. Moreover, proso millet exhibits high water and nitrogen use efficiency [12].

35 Despite its desirable attributes, proso millet has become a contingent crop over time. However, it  
36 possesses immense potential in the food industry and health food sector, making it a suitable crop under  
37 the current climate change regime. The limited availability of information on the characterization of proso  
38 millet accessions in terms of yield, agronomic traits, and quality hampers its utilization and practicality.  
39 This tetraploid crop has demonstrated considerable genetic plasticity, enabling it to adapt to diverse  
40 geographical regions, ranging from sea level to altitudes of 3500 meters above sea level [13]. This  
41 geographical adaptation has resulted in significant crop diversity of proso millet worldwide. To fully  
42 harness the potential of this crop, it is crucial to undertake detailed characterization of these collections.

43 In India, proso millet is cultivated in several states, including Tamil Nadu, Maharashtra, Karnataka,  
44 Andhra Pradesh, Bihar, Madhya Pradesh, and Uttar Pradesh, covering an area of 0.41 lakh hectares and  
45 yielding 0.22 lakh tonnes of grains [14]. However, the crop area in Northeastern India is not accounted for  
46 in the present data. The primary challenge in proso millet cultivation lies in achieving higher yield levels.  
47 To address this issue, the Indian proso millet program has initiated a focused breeding program aimed at  
48 enhancing both yield and protein content in proso millet.

49 The National Bureau of Plant Genetic Resources (NBPGR) in New Delhi houses a total of 1007  
50 indigenous collections of proso millet. These collections serve as crucial genetic resources for the  
51 ongoing proso millet improvement program conducted at the ICAR-Indian Institute of Millets Research  
52 (ICAR-IIMR). ICAR-IIMR is mandated to act as a National Active Germplasm Site for all millet crops. It is  
53 an apex body in India for research and development in millets. At present, ICAR-IIMR holds a collection  
54 of 1556 accessions collected from various sources, nationally and internationally. This study was  
55 conducted with the following objectives to characterize 634 proso millet accessions based on important  
56 agronomic traits. To develop a database of traits that can be utilized by researchers in the proso millet  
57 improvement program. To identify trait-specific germplasm that can be used as trait donors by proso millet  
58 breeders. By achieving these objectives, the study aims to enhance the understanding of proso millet's  
59 genetic diversity and facilitate targeted breeding efforts for improved varieties.

60

## 61 **2. MATERIAL AND METHODS**

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### 63 **2.1 The experimental design and layout**

64 During the Kharif season of 2021, a total of 634 proso millet germplasm accessions were characterized,  
65 alongside three control varieties, as part of the Institute Project (IIMR/CI/2021-2026/150) at ICAR-IIMR in  
66 Hyderabad. The characterization process took place in the experimental fields of ICAR-Indian Institute of  
67 Millets Research (IIMR), located at geographical coordinates 17.3207°N latitude, 78.3959°E longitude,  
68 and an elevation of 476.5 meters above mean sea level.

69 The experimental design employed was an augmented block design, and four check varieties, namely  
70 GPUP 8, TNAU 145, TNAU 164, and TNAU 202, were included. The sowing of accessions took place in  
71 the third week of July, with a plot size of 1-meter row length and 60 cm distance between rows. Two  
72 weeks after thinning, the distance between plants was maintained at 10 cm. Fertilizers were applied at a  
73 rate of 80 kg/ha of nitrogen and 40 kg/ha of  $P_2O_5$  during the crop's growth, while all necessary agricultural  
74 practices were followed to ensure a healthy crop stand. Regular irrigation was provided to maintain  
75 adequate moisture levels, and measures were taken to protect the crop from weeds, pests, and diseases.

### 76 **2.2 Agro-morphological traits studied**

77 The study involved characterizing the germplasm for 11 quantitative traits, namely the number of basal  
78 tillers, days to 50% flowering, flag leaf blade length, flag leaf blade width, peduncle length, panicle length,  
79 panicle width, plant height, days to maturity, grain yield per plant, and 100-seed weight (Table 1). The trait

80 descriptors provided by the International Board for Plant Genetic Resources (IBPGR, 1985) were followed  
 81 to ensure consistency in trait assessment [15].

82 **Table 1. Quantitative traits and measurement units**

Organ	Trait	Abbreviation	Unit
Phenology	Days to 50% flowering	DAYS_FLW	days
	Days to maturity	DAYS_MAT	days
Plant	Number of basal tillers	BASAL_TILLERS	Number
	Plant height	PLANT_HT	cm
Leaf	Leaf blade length	LEAF_BLD_LEN	cm
	Leaf blade width	LEAF_BLD_WID	cm
Flower	Panicle length	PANICLE_LEN	cm
	Panicle width	PANICLE_WID	cm
Seed	100_Seed weight	100_SD_WT	g
	Grain yield	GRN_YLD_PLN	g plant <sup>-1</sup>

83  
 84 **2.3 Statistical analysis**

85 The analysis of variance (ANOVA) was conducted using the augmented randomized complete block  
 86 design (RCBD) package of R (version 3.4.2), following the method described by Aravind et al. [16].  
 87 Pearson's correlation coefficient (r) was employed to determine the relationships between the studied  
 88 traits. Factor analysis was performed based on the Pearson correlation matrix and Euclidean distances.  
 89 The latent roots or eigen values for all principal components were calculated and presented. To assess  
 90 diversity, the Shannon-Weaver diversity index (H) and principal component analysis (PCA) were  
 91 generated using the PAST4 software, as outlined by Hammer et al., [17].

92  
 93 **3. RESULTS**

94 **3.1 Descriptive statistics and diversity indices for 634 proso millet germplasm**  
 95 **accessions**

96 Table 2 presents the summary statistics of the 11 studied traits, including mean, standard error of mean,  
 97 standard deviation, range, and coefficient of variance. The results indicate a significant variability in all the  
 98 studied traits. The number of basal tillers ranged from 1 to 24, with an average of 6.9. The days to 50%  
 99 flowering varied from 36 to 60 days, with an average of 47.5 days. The flag leaf blade length measured at  
 100 the time of flowering ranged from 12.3 cm to 45.8 cm, while the flag leaf blade width had a mean value of  
 101 0.94 cm, ranging from 0.23 cm to 2.9 cm. The peduncle length exhibited a minimum value of 5.3 cm and  
 102 a maximum value of 15.7 cm, with an average of 2.83 cm. The panicle characteristics, such as panicle  
 103 length (mean = 25.9 cm; range = 12.4 cm – 46.7 cm) and panicle width (mean = 3.3 cm; range = 1.2 cm -  
 104 7.0 cm) were also exhibiting the greatest variability with extreme values.

105  
 106 **Table 2. Summary statistics of quantitative traits of 634 acc. of Proso millet germplasm**  
 107 **characterized during Kharif 2021**

SN	Trait	Mean	SEM	SD	CV	Minimum	Maximum
1.	Number of basal tillers	7.0	0.1	3.3	48.0	1.0	24.0
2.	Days to 50% flowering (days)	47.5	0.2	5.3	11.2	36.0	60.0
3.	Flag leaf blade length (cm)	23.3	0.2	4.1	17.6	12.3	45.9
4.	Flag leaf blade width (cm)	0.9	0.0	0.4	39.7	0.2	2.9
5.	Peduncle length (cm)	8.1	0.0	1.3	15.5	5.3	15.8
6.	Panicle length (cm)	25.9	0.2	4.0	15.3	12.4	46.7

7.	Panicle width (cm)	3.3	0.0	0.9	27.1	1.2	7.0
8.	Plant height (cm)	77.4	0.6	16.1	20.8	33.3	151.7
9.	Days to maturity (days)	75.4	0.3	6.4	8.5	59.0	89.0
10.	Grain yield (g/plant)	4.8	0.1	3.6	74.1	1.1	18.4
11.	1000-Seed weight (g)	0.5	0.0	0.1	16.7	0.1	0.8

108

SEM: Standard Error of mean, SD: Standard deviation, CV: coefficient of variation

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The plant height with a range of 33.3 cm to 151.6 cm, had a mean of 77.3 cm. The main yield and yield attributing traits like grain yield and 100 seed weight also showed the presence of a vast variability. On the one hand, the grain yield with an average of 4.7 g per plant ranged from a minimum of 1.1 g to a maximum of 18.4 g per plant, while on the other hand, 100 seed weight was ranging from 0.06 g to 0.82 g with a mean value of 0.46 g.

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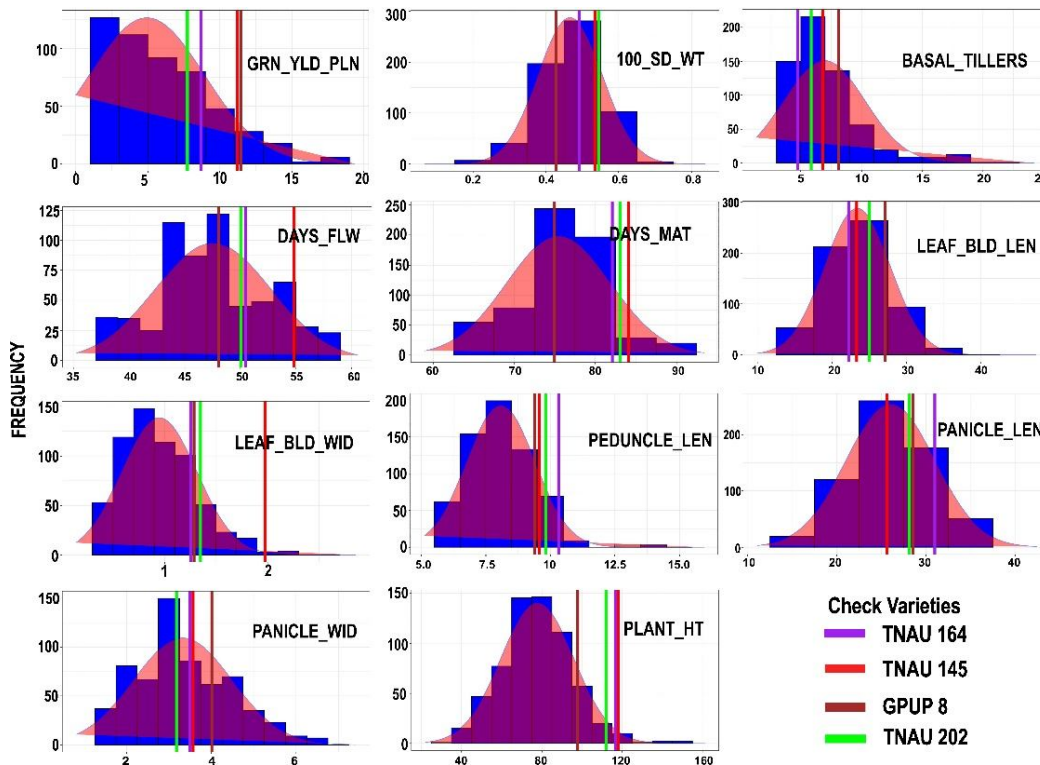
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The frequency distribution of proso millet accessions for various levels of studied traits along with the checks is shown in figure 1. The distribution of checks around the mean for almost all traits can be observed.



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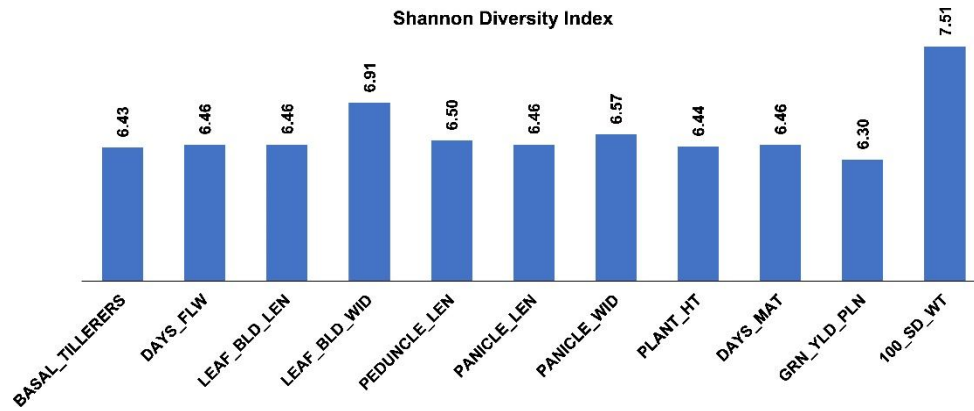
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**Fig 1. Histogram showing frequency distribution of studied quantitative traits in 634 Proso millet germplasm.**

The shannon-weaver diversity index for studied traits ranged from 6.3 for grain yield to a maximum of 7.51 for 100 seed weight (figure 2).



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**Figure 2. Diversity among 634 acc. of proso millet germplasm based on Shannon diversity index (H')**

127 **3.2 Analysis of variance**

128 Analysis of variance (ANOVA) using augmented design indicated the presence of significant ( $P < 0.05$ ) and  
129 non-significant ( $P > 0.05$ ) mean sums of squares for studied traits for different sources of variation (Table  
130 2). The block effects eliminating the treatments were non-significant ( $P > 0.05$ ) for all the traits studied. The  
131 treatment effects ignoring blocks were highly significant ( $P < 0.01$ ) for days to 50% flowering, days to  
132 maturity, and peduncle length indicating treatments had a significant effect on these traits, and the  
133 differences between the treatments were not due to chance.

134 The mean sums of squares due to germplasm versus checks were significant ( $P < 0.05$ ) for all traits except  
135 for number of basal tillers, and panicle width. The germplasm had a significant effect on all of the traits  
136 except for number of basal tillers, and panicle width. The differences between the germplasms were not  
137 due to chance.

138

139 **Table 3. Analysis of variance using augmented block design for 634 Proso millet germplasm**

Source	Df	NBT	DFF	DM	GY	LBL	LBW	PanL	PanW	PedL	PH	100SW
Block (eliminating Treatments)	7	4.86ns	7.46ns	19.1ns	17.86ns	12.3ns	0.06ns	39.8ns	1.19ns	0.62ns	296.2ns	0.01ns
Treatment (ignoring Blocks)	637	11.2 ns	28.94 **	42.58 **	13.88 ns	16.93 ns	0.16ns	16.15ns	0.81 ns	1.7 **	314.85 ns	0.01 ns
Check	3	16.43 ns	64.7 **	137.36 **	28.38 ns	36.94 ns	0.92 **	38.39 ns	0.94 ns	1.28 ns	706.12 ns	0.02 **
Germplasm	633	11.17 ns	28.31 **	40.72 *	12.64 ns	16.81 ns	0.14ns	15.8 ns	0.81 ns	1.57 **	259.2 ns	0.01 ns
Germplasm vs. Check	1	12.73ns	319.2 **	934.8 **	754.6 **	31.8 ns	8.3 **	171.2*	1.6 ns	85.64 **	34369**	0.03*
Error	21	8.87	8.63	17.77	31.74	16.95	0.09	31.43	0.72	0.53	306.19	0.0046
Check vs germplasm CD at 5%		7.34	7.24	10.4	13.89	10.15	0.76	13.83	2.09	1.8	43.15	0.17

140 Df degree of freedom, 100SW 100 seed weight (g), DFF Days to 50% flowering (days), LBL Leaf Blade Length (cm), LBW Leaf Blade Width (cm),  
 141 GY Grain Yield (g plant<sup>-1</sup>), NBT Number of basal tillers, PanL Panicle Length (cm), PanW Panicle Width (cm), PedL Peduncle Length (cm), PH  
 142 plant height (cm); ns P > 0.05; \* P <= 0.05; \*\* P <= 0.01  
 143

### 144 3.3 Principal Component Analysis

145 Principal component analysis (PCA) of the data recorded on characterization of 11 traits from 634 proso  
 146 millet germplasm accessions indicated that the first three principal components explained 60 % of the  
 147 variation (Table 4). The total of 11 principal components were estimated. The **eigen value** of PC1 was  
 148 3.0, while it was 0.24 for PC11. The eigen values of PC4-PC11 were less than 1, hence, only the first  
 149 three PCs were considered to assess the diversity. The first principal component (PC1) explained 27.3 %  
 150 of the variation, followed by PC2 (22.6 %), and PC3 (10 %). The important traits that were positively  
 151 related and contributed to the variation in PC1 are: Leaf blade length (cm), Peduncle length (cm), Panicle  
 152 length (cm), Plant height (cm) and grain yield (g per plant) (Table 5).

153 Days to 50 % flowering, days to maturity and leaf blade width are negatively related. These traits  
 154 contribute maximum to the variation explained by PC2. The leaf blade length and peduncle length are  
 155 positively related and contribute maximum variability to PC3.

156 **Table 4. Eigen values (Latent roots) and variability of non-rotated values of principal components**

Principle component	Eigen value	Proportion of variance Explained	Components cumulative proportion (%)
PC1	<b>3.006</b>	0.273	27.3
PC2	<b>2.489</b>	0.226	50
PC3	<b>1.104</b>	0.100	60
PC4	0.918	0.083	68.3
PC5	0.830	0.075	75.9
PC6	0.642	0.058	81.7
PC7	0.564	0.051	86.8
PC8	0.496	0.045	91.4
PC9	0.429	0.039	95.3
PC10	0.282	0.026	97.8
PC11	0.240	0.022	100

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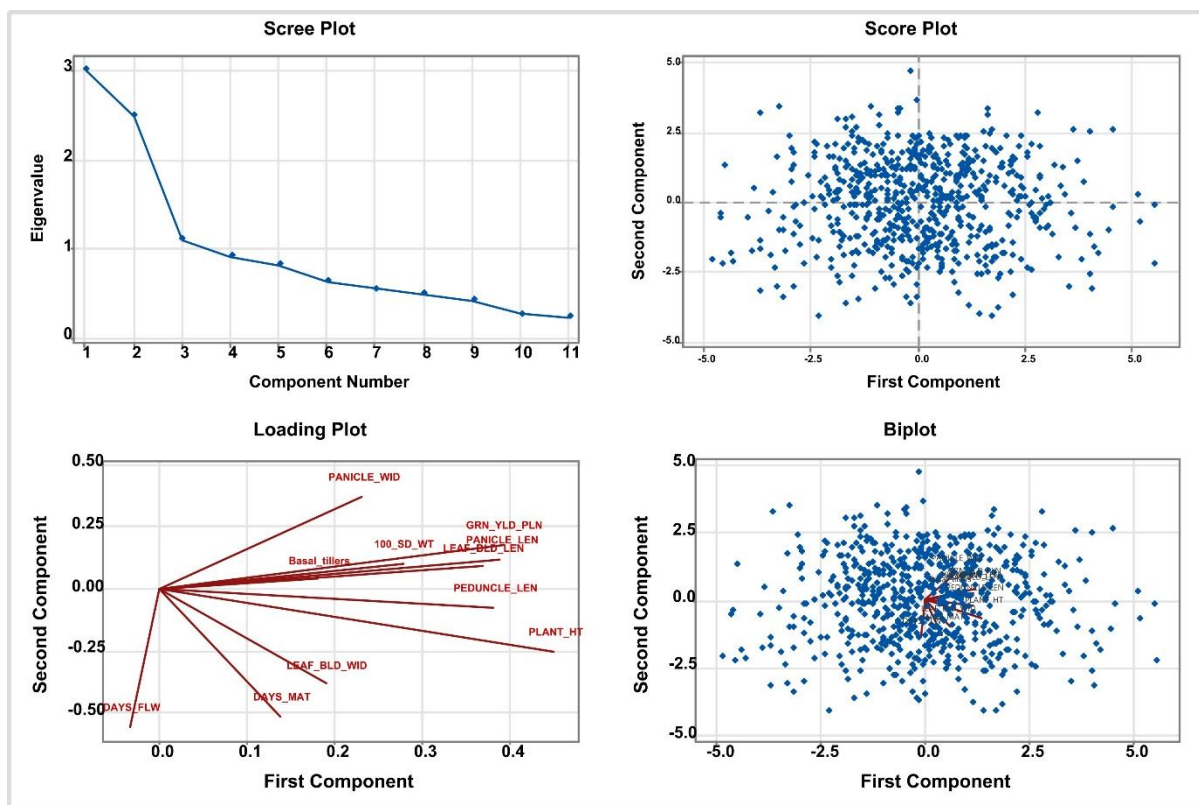
159 **Table 5. Non-rotated component loadings (values of principal component traits of Proso millet)**

Trait/Variable	PC1	PC2	PC3	PC4	PC5
1. BASAL_TILLERS	0.18	0.04	<b>-0.82</b>	-0.12	-0.02
2. DAYS_FLW	-0.04	<b>-0.56</b>	-0.08	0.18	-0.17
3. LEAF_BLD_LEN	<b>0.37</b>	0.09	-0.07	<b>-0.45</b>	0.09
4. LEAF_BLD_WID	0.19	<b>-0.38</b>	<b>0.30</b>	<b>-0.40</b>	0.08
5. PEDUNCLE_LEN	<b>0.38</b>	-0.07	<b>0.40</b>	-0.04	0.02
6. PANICLE_LEN	<b>0.39</b>	0.12	-0.06	0.10	<b>-0.59</b>
7. PANICLE_WID	0.23	<b>0.37</b>	0.19	0.19	<b>-0.46</b>
8. PLANT_HT	<b>0.45</b>	-0.26	-0.03	0.05	0.01
9. DAYS_MAT	0.14	<b>-0.52</b>	-0.15	0.25	-0.11
10. GRN_YLD_PLN	<b>0.39</b>	0.18	-0.09	-0.12	<b>0.40</b>
11. 100_SD_WT	0.28	0.10	0.03	<b>0.68</b>	<b>0.48</b>

160 Bold values indicate the traits which contributed maximum to principal component.

161

162 The distribution of traits among the principal components explains the existence of greater variability  
 163 among the studied accessions. The scree plot, score plot, loading plot, and biplot of principal component  
 164 analysis of 634 accessions of proso millet are shown in Figure 3. It is obvious from the scree plot that only  
 165 the first three components explained maximum of diversity existed in the studied germplasm lines.



166  
167 **Fig3. Scree plot, Score plot, Loading plot and biplot of principal component analysis of 554**  
168 **accessions of Proso millet.**

169 100SW 100 seed weight (g), DF Days flowering (days), FLL Flag Leaf Length (cm), FLW Flag Leaf Width  
170 (cm), GY Grain Yield (g plant<sup>-1</sup>), LReCL Lowest receme length (cm), NBT Number of basal tillers, PanL:  
171 Panicle Length (cm), PanW Panicle Width (cm), PedL Peduncle Length (cm), PH plant height (cm)

172  
173 **3.4 Frequency distribution of qualitative traits**  
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175 The frequency distribution of lines for qualitative traits showed that the dominant type among the  
176 populations was recorded (Table 6). In the qualitative data, the following traits were the most frequent:  
177 Erect growth habit (444 acc.), Absence of leaf sheath pigmentation (630 acc.), Sparse leaf sheath  
178 pubescence (333 acc.), Absence of ligule pubescence (639 acc.), Glabrous leaf blade pubescence (559  
179 acc.), Arched inflorescence shape (443 acc.), Presence of culm branching (378 acc.), Absence of lodging  
180 (677 acc.), Absence of seed shattering (661 acc.), Intermediate panicle compactness (303 acc.), Golden  
181 yellow grain color (470 acc.), and oval grain shape (379 acc.).

182  
183 **Table 6: The frequency distribution of qualitative traits of 634 acc. of Proso millet germplasm characterized under**  
184 **Institute Project (IIMR/CI/2021-2026/150) during Kharif 2021**

S.No	Category	Plant growth habit
1	Erect	444
2	Decumbent	239
3	Prostrate	1
	<b>Category</b>	<b>Leaf sheath pigmentation</b>
1	Absent	630
2	Present	54
	<b>Category</b>	<b>Leaf sheath pubescence</b>
1	Sparse	333
2	Glabrous	248
3	Strong	103
	<b>Category</b>	<b>Ligule pubescence</b>

S.No	Category	Plant growth habit
1	Absent	639
2	Present	45
	Category	Leaf blade pubescence
1	Glabrous	559
2	Sparse	107
3	Strong	18
	Category	Inflorescence shape
1	Arched	443
2	Diffused	214
3	Globose elliptic	27
	Category	Culm branching
1	Present	378
2	Absent	306
	Category	Lodging
1	Absent	677
2	Present	7
	Category	Seed shattering
1	Absent	661
2	Present	23
	Category	Panicle compactness
1	Intermediate	303
2	Open	282
3	Compact	99
	Category	Grain colour
1	Golden yellow	470
2	Grey	140
3	Light Grey	35
4	Dark grey	23
5	Cream	15
6	Grey	1
	Score/State	Grain shape
1	Oval	379
2	Elliptical	305

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### 186 3.5 Potential germplasm identified

187 There are 442 acc. of trait specific proso millet germplasm identified. In which, 82 acc. are multi-  
 188 trait specific germplasm for multi-trait specific germplasm for 2-6 traits. The list of 21 acc. identified  
 189 with multi-trait specific germplasm for 3-6 traits are given Table 7.

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191 **Table 7: Multi-trait specific Proso millet germplasm identified under Institute Project**  
 192 **(IIMR/CI/2021-2026/150) during Kharif 2021 from 634 acc. characterized.**

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S.No.	Crop Name	MGB No	IC No/ Other ID	Multi-trait
1	Proso millet	PRO 1185	IC0484157	Flag leaf blade length (>30.00 cm) Flag leaf blade width, (>2.00 cm) and Panicle length (>32.00 cm)
2	Proso millet	PRO 165	TNPM 471	Days to 50% flowering (>40.00 days), Grain yield (>15.00 g/plant and 1000-Grain weight (>5.50 g)
3	Proso millet	PRO 208	TNPM 516	Panicle length (>32.00 cm), Panicle width (>5.00 cm), Plant height (>120.00 cm) and Grain yield (>15.00 g/plant

S.No.	Crop Name	MGB No	IC No/ Other ID	Multi-trait
4	Proso millet	PRO 221	TNPM 529	Peduncle length (>11.00 cm), Panicle length (>32.00 cm) and Plant height (>120.00 cm)
5	Proso millet	PRO 226	TNPM 534	Panicle length (>32.00 cm), Plant height (>120.00 cm) and Grain yield (>15.00 g/plant)
6	Proso millet	PRO 302	TNPM 615	Days to 50% flowering (>40.00 days), Plant height (<50.00 cm) and Date of harvest (<65.00 days)
7	Proso millet	PRO 325	TNPM 638	Days to 50% flowering (>40.00 days), Flag leaf blade length (>30.00 cm), Panicle length (>32.00 cm), Panicle width (>5.00 cm)
8	Proso millet	PRO 330	TNPM 643	Days to 50% flowering (>40.00 days), Plant height (<50.00 cm) and Date of harvest (<65.00 days)
9	Proso millet	PRO 356	TNPM 669	Days to 50% flowering (>40.00 days), Plant height (<50.00 cm) and Date of harvest (<65.00 days)
10	Proso millet	PRO 359	<b>TNPM 672</b>	Number of basal tillers (>15.00), 1000-Grain weight (>5.50 g), Days to 50% flowering (>40.00 days), Plant height (<50.00 cm) and Date of harvest (<65.00 days)
11	Proso millet	PRO 38	TNPM 336	Days to 50% flowering (>40.00 days), Panicle length (>32.00 cm), Panicle width (>5.00 cm) and 1000-Grain weight (>5.50 g)
12	Proso millet	PRO 49	TNPM 347	Days to 50% flowering (>40.00 days), Panicle width (>5.00 cm), Plant height (<50.00 cm) and Date of harvest (<65.00 days)
13	Proso millet	PRO 50	<b>TNPM 348</b>	Days to 50% flowering (>40.00 days), Panicle width (>5.00 cm), Plant height (<50.00 cm), Date of harvest (<65.00 days) and 1000-Grain weight (>5.50 g)
14	Proso millet	PRO 593	<b>IC0483576</b>	Days to 50% flowering (>40.00 days), Flag leaf blade length (>30.00 cm), Peduncle length (>11.00 cm), Panicle length (>32.00 cm) and Date of harvest (<65.00 days)
15	Proso millet	PRO 618	<b>IC0483602</b>	Days to 50% flowering (>40.00 days), Plant height (<50.00 cm), Date of harvest (<65.00 days), Flag leaf blade length (>30.00 cm), Panicle length (>32.00 cm) and 1000-Grain weight (>5.50 g)
16	Proso millet	PRO 660	IC0483867	Days to 50% flowering (>40.00 days), Date of harvest (<65.00 days), Grain yield (>15.00 g/plant)
17	Proso millet	PRO 690	IC0484165	Number of basal tillers (>15.00), Flag leaf blade length (>30.00 cm) and Panicle length (>32.00 cm),
18	Proso millet	PRO 715	IC0484177	Number of basal tillers (>15.00), Panicle length (>32.00 cm) and 1000-Grain weight (>5.50 g)
19	Proso millet	PRO 791	IC0483718	Number of basal tillers (>15.00), Panicle length (>32.00 cm), Days to 50% flowering (>40.00 days) and Date of harvest (<65.00 days)
20	Proso millet	PRO 919	IC0483859	Number of basal tillers (>15.00), Days to 50%

S.No.	Crop Name	MGB No	IC No/ Other ID	Multi-trait
				flowering (>40.00 days) and Date of harvest (<65.00 days)
21	Proso millet	PRO 968	IC0483913	Days to 50% flowering (>40.00 days), Plant height (<50.00 cm) and Date of harvest (<65.00 days)

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## 4. DISCUSSION

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### 4.1 Field evaluation of germplasm lines

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Genetic characterization of a large set of available germplasm accessions conserved across the global gene banks is useful for researchers worldwide [12]. Phenotypic characterization helps breeders, geneticists, and botanists in identifying promising trait donors for use in crop improvement activities towards developing high-yielding, climate-resilient, and pest- and disease-resistant cultivars.

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In the present study, a high variability was observed for the studied traits, and trait-specific germplasm lines were identified for future use in the development of cultivars for higher grain yield and earliness. Earlier researchers have also reported higher variability in studied proso millet germplasm for morpho-agronomic traits [18],[19]. The observed high coefficient of variation within the studied accessions for a few traits explains the presence of high variability for these traits.

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### 4.2 Principal Component Analysis

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Principal component analysis was carried out to study the traits associated with the variability components and the accessions with specific traits contributing maximum towards the variability. Leaf blade length (cm), Peduncle length (cm), Panicle length (cm), Plant height (cm), and grain yield (g plant<sup>-1</sup>) were the important traits contributing to variability explained by **the principal component 1**. This indicates that the accessions which fall in this region of the score plot of PCA could be used as potential donors of these traits.

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On the other hand, the traits such as Days to 50 % flowering, days to maturity and leaf blade width were mainly contributing to **the principal component 2**. Hence, the accessions falling in this region may be used for the development of cultivars with early flowering and maturity in proso millet. The distinctly identified accessions through the PCA may be either used for hybridization programmes as parents or may be directly released for commercial cultivation after multi-location evaluation for stability of the genotype.

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The current study ascribed 60 % of the total variations observed across the 11 quantitative traits to the first three PCs with eigen values greater than one. The PCA result shows that the 11 quantitative traits examined were discovered the inherent uniqueness among the studied proso millet accessions. Similar kind of results were reported by [18] where the variability in **the principal component 1** was contributing to a maximum extent by days to 50% flowering, plant height, flag leaf blade length, flag leaf sheath length, inflorescence length, number of nodes per main stem, days to maturity, grain yield, and protein content. Similarly, [18] also reported that first three PCs contributed majorly to diversity in the studied accessions. In another study by [20], the PCA was conducted on 18 starch quality traits in 95 proso millet accessions. The first three principal components accounted for 83.3% of the variation.

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### 4.3 Potential germplasm

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Identification of the germplasm accessions by tagging trait/traits is an invaluable information to the researchers in tailoring the new varieties with improved performance. It will help in utilizing trait specific germplasm in breeding programs to dissect the traits at genetic/ molecular level. The present study has identified 21 potential accessions tagged with superior performance for more than one trait. The accession IC0483602 was identified as potential donor for six specific traits. The accessions, TNPM 672, TNPM 348 and IC0483576 were identified as potential donors of

240 more than five traits together in a single accession. The accessions are useful to develop lines with  
241 high panicle length, early flowering, 100 g weight etc.  
242 Trivedi *et al.*, 2017 [21] mentioned that trait-specific germplasm is important genetic material for  
243 crop improvement, climate resilience breeding, and improving food and nutritional security in a  
244 sustainable way. Similarly, the trait-specific accessions identified with two/three/four traits in the  
245 present study will serve as ready reckoner for the improvement of targeted traits in the crop  
246 improvement programs of barnyard millet as potential donor parents specifically for the traits like  
247 earliness and high grain yielding ability. Similar kind of results were reported by Upadhyaya *et al.*,  
248 2011 [22] in foxtail millet, 26 accessions with three desirable trait combinations were identified  
249 giving useful donor lines for grain yield, high iron, Zinc, and Calcium content, and early flowering.  
250 Khairwal *et al.*, in 2007 [23] identified trait-specific lines for vigor, extra early flowering, early  
251 maturity, plant height, high productive tillers, long panicle, thick panicle, and high grain yield and  
252 mentioned that the developed trait-specific gene-pools will serve as a core-subset for future  
253 breeding programs in pearl millet.

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#### 255 **4. CONCLUSION**

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257 The study revealed presence of significant genetic variation for the studied eleven quantitative  
258 traits in proso millet. The principal component analysis showed that the first three principal  
259 components accounted for 60% of the total variation. Additionally, the study also identified 21  
260 multiple traits (3-6 traits) donor accessions which may directly be used for proso millet  
261 improvement by breeders.

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#### 269 **COMPETING INTERESTS**

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271 The authors have no competing interests to declare that are relevant to the content of this article

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#### 273 **AUTHORS' CONTRIBUTIONS**

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275 Authors may use the following wordings for this section: 'M Elangovan' designed the study. 'K  
276 Venkatesh' performed the statistical analysis, wrote the protocol, and wrote the first draft of the  
277 manuscript. 'Avinash Singode' and 'Amasiddha B' edited the manuscript. 'A Sai Karthik' recorded  
278 field data. 'Sushil Pandey' and 'Chitra Devi Pandey' facilitated funding and reviewed the project.  
279 All authors read and approved the final manuscript."

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#### 280 **REFERENCES**

281

282 [1] Lu H, Zhang J, Liu K, Wu N, Li Y, Zhou K, Ye M, Zhang T, Zhang H, Yang X, Shen L,  
283 Xu D, Li Q. Earliest domestication of common millet (*Panicum miliaceum*) in East Asia  
284 extended to 10,000 years ago. *Proceedings of the National Academy of Sciences of*  
285 *the United States of America* 2009;106(18):7367–72.

285

286 <https://doi.org/10.1073/pnas.0900158106>

286

287 [2] Cho Y-I, Chung J-W, Lee G-A, Ma K-H, Dixit A, Gwag J-G, Park Y-J. Development  
288 and characterization of twenty-five new polymorphic microsatellite markers in proso  
289 millet (*Panicum miliaceum* L.). *Genes Genom* 2010;32(3):267–73.

288

289 <https://doi.org/10.1007/s13258-010-0007-8>

- 290 [3] Saleh ASM, Zhang Q, Chen J, Shen Q. Millet Grains: Nutritional Quality, Processing,  
291 and Potential Health Benefits: Millet grains.... *Comprehensive Reviews in Food*  
292 *Science and Food Safety* 2013;12(3):281–95. [https://doi.org/10.1111/1541-](https://doi.org/10.1111/1541-4337.12012)  
293 4337.12012
- 294 [4] Zhu F, Wang S. Physicochemical properties, molecular structure, and uses of  
295 sweetpotato starch. *Trends in Food Science & Technology* 2014;36(2):68–78.  
296 <https://doi.org/10.1016/j.tifs.2014.01.008>
- 297 [5] Cavers PB, Kane M. The Biology of Canadian Weeds: 155. *Panicum miliaceum* L.  
298 *Can J Plant Sci* 2016;96(6):939–88. <https://doi.org/10.1139/cjps-2015-0152>
- 299 [6] Gyulai G, Humphreys M, Lagler R, Szabo Z, Toth Z, Bittsanszky a., Gyulai F, Heszky  
300 L. Seed remains of common millet from the 4th (Mongolia) and 15th (Hungary)  
301 centuries: AFLP, SSR and mtDNA sequence recoveries. *Seed Science Research*  
302 2006;16(3):179–91. <https://doi.org/10.1079/ssr2006251>
- 303 [7] Gyulai F. THE HISTORY OF BROOMCORN MILLET ( *Panicum miliaceum* L .) IN  
304 THE CARPATHIAN-BASIN IN THE MIRROR OF ARCHAEOBOTANICAL REMAINS I  
305 . FROM THE BEGINNING UNTIL THE ROMAN AGE Archaeobotany or palaeo-  
306 ethnobotany is the D ÄEULGJLQJ ´UROH ZLWKLQ WKH V \ WWHP RI VFLH.  
307 *Columella - Journal of Agricultural and Encironmental Sciences* 2014;1(1):29–38
- 308 [8] Kalinova J, Moudry J. Content and Quality of Protein in Proso Millet (*Panicum*  
309 *miliaceum* L.) Varieties 2006. <https://doi.org/10.1007/s11130-006-0013-9>
- 310 [9] McSweeney MB, Seetharaman K, Ramdath DD, Duizer LM. Chemical and Physical  
311 Characteristics of Proso Millet (*Panicum miliaceum*)-Based Products. *Cereal*  
312 *Chemistry* 2017;94(2):357–62. <https://doi.org/10.1094/CCHEM-07-16-0185-R>
- 313 [10] Kim JK, Park SY, Yeo Y, Cho HS, Kim YB, Bae H, Park CH, Lee Dr. JH, Park Dr. SU.  
314 Metabolic profiling of millet (*Panicum miliaceum*) using gas chromatography-time-of-  
315 flight mass spectrometry (GC-TOFMS) for quality assessment. *Plant OMICS*  
316 2013;6(1):73–8
- 317 [11] Wang Y, Bräutigam A, Weber APM, Zhu XG. Three distinct biochemical subtypes of  
318 C4 photosynthesis? A modelling analysis. *Journal of Experimental Botany*  
319 2014;65(13):3567–78. <https://doi.org/10.1093/jxb/eru058>
- 320 [12] Habiyaemye C, Matanguihan JB, D’Alpoim Guedes J, Ganjyal GM, Whiteman MR,  
321 Kidwell KK, Murphy KM. Proso Millet (*Panicum miliaceum* L.) and Its Potential for  
322 Cultivation in the Pacific Northwest, U.S.: A Review. *Frontiers in Plant Science* 2017.  
323 <https://doi.org/10.3389/fpls.2016.01961>
- 324 [13] Baltensperger DD. Foxtail and proso millet. *Progress in New Crops: Proceedings of*  
325 *the Third National Symposium Indianapolis, Indiana, 22-25 October, 1996* 1996;(Haq  
326 1989):182–90
- 327 [14] Rajasekaran R, Francis N. Genetic and genomic resources for improving proso millet  
328 (*Panicum miliaceum* L.): a potential crop for food and nutritional security. *Nucleus*  
329 (*India*) 2021;64(1):21–32. <https://doi.org/10.1007/s13237-020-00331-2>
- 330 [15] IBPGR. Descriptors for *Panicum miliaceum* and *P. sumatrense*. Rome, Italy: IBPGR;  
331 1985
- 332 [16] Aravind J, Shankar SM, Wankhede DP, Kaur V. augmentedRCBD: Analysis of  
333 Augmented Randomised Complete Block Designs 2021.  
334 <https://doi.org/10.5281/ZENODO.4938491>
- 335 [17] Hammer Ø, Harper DA, Ryan PD, others. PAST: Paleontological statistics software  
336 package for education and data analysis. *Palaeontologia Electronica* 2001;4(1):9

- 337 [18]Vetriventhan M, Upadhyaya HD. Diversity and trait-specific sources for productivity  
338 and nutritional traits in the global proso millet (*Panicum miliaceum* L.) germplasm  
339 collection. *The Crop Journal* 2018;6(5):451–63.  
340 <https://doi.org/10.1016/j.cj.2018.04.002>
- 341 [19]Nithyashree K, Vijayalaxmi KG. Study on Physical Properties of Minor Millets. *IJECC*  
342 2023;156–62. <https://doi.org/10.9734/ijecc/2023/v13i11663>
- 343 [20]Li K, Zhang T, Narayanamoorthy S, Jin C, Sui Z, Li Z, Li S, Wu K, Liu G, Corke H.  
344 Diversity analysis of starch physicochemical properties in 95 proso millet (*Panicum*  
345 *miliaceum* L.) accessions. *Food Chemistry* 2020;324:126863.  
346 <https://doi.org/10.1016/j.foodchem.2020.126863>
- 347 [21]Trivedi AK, Arya L, Verma SK, Tyagi RK, Hemantaranjan A. Evaluation of barnyard  
348 millet diversity in central Himalayan region for environmental stress tolerance. *J Agric*  
349 *Sci* 2017;155(10):1497–507. <https://doi.org/10.1017/S0021859617000545>
- 350 [22]Upadhyaya HD, Ravishankar CR, Narasimhudu Y, Sarma NDRK, Singh SK,  
351 Varshney SK, Reddy VG, Singh S, Parzies HK, Dwivedi SL, Nadaf HL, Sahrawat KL,  
352 Gowda CLL. Identification of trait-specific germplasm and developing a mini core  
353 collection for efficient use of foxtail millet genetic resources in crop improvement.  
354 *Field Crops Research* 2011;124(3):459–67. <https://doi.org/10.1016/j.fcr.2011.08.004>
- 355 [23]Khairwal I, Yadav S, Rai K, Upadhyaya H, Kachhawa D, Nirwan B, Bhattacharjee R,  
356 Rajpurohit B, Dangaria C, others. Evaluation and identification of promising pearl  
357 millet germplasm for grain and fodder traits. *Journal of SAT Agricultural Research*  
358 2007;5(1):1–6  
359