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COMPREHENSIVE DIVERSITY ASSESSMENT OF PROSO MILLET (*PANICUM MILIACEUM* L.) GERMPLASM ACCESSIONS

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ABSTRACT

Proso millet (*Panicum miliaceum* L.), also called common millet or broomcorn millet, is an ancient and essential crop cultivated extensively for food, animal feed, and fodder. Proso millet is primarily cultivated in China, Russia, India, and the United States. Despite the ICAR-All India Coordinated Research Project on Small Millets at the University of Agricultural Sciences, Bangalore safeguarding a vast collection of 611 proso millet germplasm accessions from around the globe, only a minuscule portion of this extensive repository has been rigorously leveraged for millet improvement initiatives. Our research aimed to dissect the genetic diversity within proso millet, with the goal of amplifying the utilization of these genetic resources in breeding programs and streamlining their management. A total of 213 germplasm accessions were characterized during *kharif* 2023 for the yield and yield-attributing traits. Analysis of variance confirmed notable differences across eight quantitative traits, influenced by genotype-environment interactions. Traits exhibited significant correlations and were influenced by genotype-environment interactions. Cluster analysis classified all the germplasm accessions into three main clusters. The observed variability and trait-specific sources offer valuable potential for advancing proso millet improvement. These findings will aid in selecting parental lines and developing selection indices for proso millet breeding programs.

Key words: Proso millet, Germplasm, Genetic diversity, Principal component analysis.

Introduction

Traditionally known as “coarse grains,” millets are now gaining recognition as “nutri-cereals” due to their exceptional nutritional value. Valued for their resilience against pests, diseases, and the harsh conditions typical of arid and semi-arid regions in Asia and Africa, millets are increasingly being hailed as “crops of the future” (Rao *et al.*, 2017). The most well-known small millets include finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), barnyard millet (*Echinochloa crusgalli*), and browntop millet (*Urochloa ramosa*).

After being largely overlooked for many years, these crops are now experiencing a renaissance in India’s agricultural industry. Their inherent drought tolerance and adaptability to challenging climates make them ideal

candidates for climate-resilient agriculture. Proso millet, one of the oldest cultivated cereals with a history extending over 10,000 years (Lua *et al.*, 2009), thrives in a variety of agro-ecological conditions. This allotetraploid crop, belonging to the Poaceae family and possessing a chromosome number of 36 ($2n=4x=36$), is also known by several names, including broomcorn millet, white millet, and Russian millet (Hamoud *et al.*, 1994).

China is considered a key center of diversity for proso millet (Vavilov, 1926), with Harlan (1975) suggesting its domestication occurred both in China and Europe. This hardy crop flourishes in regions with varying altitudes and requires minimal water, completing its growth cycle in just 60 to 90 days. Proso millet is cultivated across southern Europe, Afghanistan, Pakistan, India, Korea, northern China, and southeast Russia. In major producing countries like China, Russia, and India, proso millet serves

Table 1: Details of the source of proso millet germplasm accessions used in the study.

Source		Number of germplasm accessions
India	Karnataka	41
	Bihar	15
	NBPGR, New Delhi	56
	Andhra Pradesh	74
	Himachal Pradesh	16
	Gujarat	01
Japan	Japan	07
	India	3 control varieties
Total		213

both as a staple food and is also used in the production of alcoholic beverages (Das *et al.*, 2019; Santra and Rose, 2013). In India, it is grown on over half a million hectares, with Tamil Nadu, Karnataka, Andhra Pradesh, and Uttarakhand being the primary production states (Padmaja *et al.*, 2023).

The grain of proso millet is particularly valued for its high protein content, along with essential minerals, vitamins, and micronutrients such as zinc, copper, iron, and manganese (Gomeshe, 2017). Studies also show that the grain color is closely linked to its antioxidant properties, with darker grains containing significantly higher concentrations of phenols, free phenols, and flavonoid compared to lighter varieties (Li *et al.*, 2021).

A primary objective in proso millet cultivation is the creation of high-yielding cultivars that are resistant to lodging and grain shattering, traits essential for efficient direct harvesting. To achieve this, identifying germplasm that exhibits these desired characteristics is critical. Such germplasm can be utilized in breeding programs aimed at genetic improvement. Understanding the genetic diversity within proso millet is vital for the conservation and effective use of germplasm, particularly in developing new cultivars (Hu *et al.*, 2008). This genetic diversity, when coupled with thorough performance evaluations, serves as the foundation for advancing proso millet

cultivation.

The evaluation of agronomic traits in proso millet germplasm is key to optimizing its potential for crop improvement. Accordingly, the focus of this study was to assess the diversity within proso millet germplasm to boost grain production and overall yield. In total, 213 accessions were examined, with attention given to various yield-related traits. These traits were then analyzed for their interrelations, contributing to a broader understanding of diversity and providing a comprehensive evaluation of each accession.

Materials and Methods

Experiment details

National Active Germplasm Site at Project Coordinating Unit, ICAR-All India Coordinated Research Project (AICRP) on Small Millets, University of Agricultural Sciences, Bangalore conserves 611 proso millet germplasm accessions collected from across the world. This study utilized 213 proso millet germplasm accessions, comprising 210 unique accessions and 3 control varieties, sourced from collections in India and Japan (as detailed in Table 1). The experiment employed an augmented block design (Federer, 1961) with three standard checks (GPUP 21, GPUP 28 and GPUP 8). Each accession was planted in a single 3-meter-long row, with a spacing of 30 cm between rows and 10 cm between plants. During the cropping season, the site received 624 mm of rainfall. Standard agronomic practices were followed in order to ensure the health of the crop. The accessions were evaluated for yield and yield-related traits during the *kharif* 2023 season at the ICAR-AICRP on Small Millets, University of Agricultural Sciences, Bangalore, situated at 13°05' N latitude, 77°34' E longitude and an elevation of 924 meters above sea level.

Observations Recorded

For each accession, five randomly selected plants were observed, and the following traits were recorded: days to maturity, plant height (cm), number of productive

Table 2: Analysis of variance of the quantitative traits evaluated in 213 germplasm accessions.

Sources of Variance	DF	Days to maturity	Plant height (cm)	No. of productive tillers	Flag leaf blade length (cm)	Peduncle length (cm)	Inflorescence length (cm)	Test weight (g)	Grain yield per plant (g)
Treatment (Eliminating blocks)	212	19.311**	87.59**	0.66**	23.52**	20.24**	14.96**	0.078**	20.47**
Block (Eliminating treatments)	9	0.133	0.84	0.039	0.906	0.02	0.23	0	0.17
Residuals	18	0.344	0.89	0.044	2.584	0.249	0.137	0	0.63

Table 3: Summary statistics for various quantitative traits evaluated in 213 proso millet germplasm accessions.

Characters	Mean	Range		Variance	Standard deviation
		Min.	Max.		
Days to maturity	82.72	71.00	95.00	24.33	4.93
Plant height (cm)	94.51	69.20	139.60	133.59	11.55
No. of productive tillers	4.85	1.30	7.60	0.75	0.87
Flag leaf blade length (cm)	28.45	19.20	45.23	25.62	5.06
Peduncle length (cm)	15.27	07.90	36.80	24.41	4.94
Inflorescence length (cm)	23.44	15.70	36.20	20.49	4.52
Test weight (g)	5.71	5.00	6.70	0.081	0.28
Grain yield per plant(g)	14.31	4.10	29.33	17.80	4.21

tillers, flag leaf blade length (cm), peduncle length (cm), inflorescence length (cm), test weight (g), and grain yield per plant (g).

Statistical Analysis

Summary statistics were calculated using OPSTAT software. Analysis of variance (ANOVA) was performed for all eight quantitative traits (Federer and Raghavarao, 1975). Germplasm accessions were clustered using the Silhouette method, and principal component analysis (PCA) was conducted to assess the relative importance of various traits in capturing the variation within the entire germplasm collection. The analyses were performed using R software, version 4.0.2.

Results and Discussion

Variability in Proso Millet Germplasm Accessions

Significant variability was observed across the 213 proso millet germplasm accessions, as revealed by the analysis of variance, which indicated highly significant differences in all eight quantitative traits (Table 2). Detailed statistics for seven of the traits are shown in

Table 4: Correlation coefficient among quantitative traits evaluated in 213 proso millet germplasm accessions.

Characters	Days to maturity	Plant height (cm)	No. of productive tillers	Flag leaf blade length(cm)	Peduncle length (cm)	Inflorescence length (cm)	Test weight (g)	Grain yield per plant (g)
Days to maturity	1.000	0.301**	0.019	0.076	-0.148*	0.193**	0.071	0.167*
Plant height(cm)		1.000	-0.183**	0.122	0.190**	0.350**	0.234**	0.192**
No. of productive tillers			1.000	-0.194**	-0.419**	-0.211**	0.055	0.017
Flag leaf blade length(cm)				1.000	0.349**	0.611**	0.071	-0.032
Peduncle length(cm)					1.000	0.174*	0.040	0.047
Inflorescence length(cm)						1.000	0.124	0.064
Test weight (g)							1.000	0.038
Grain yield per plant(g)								1.000

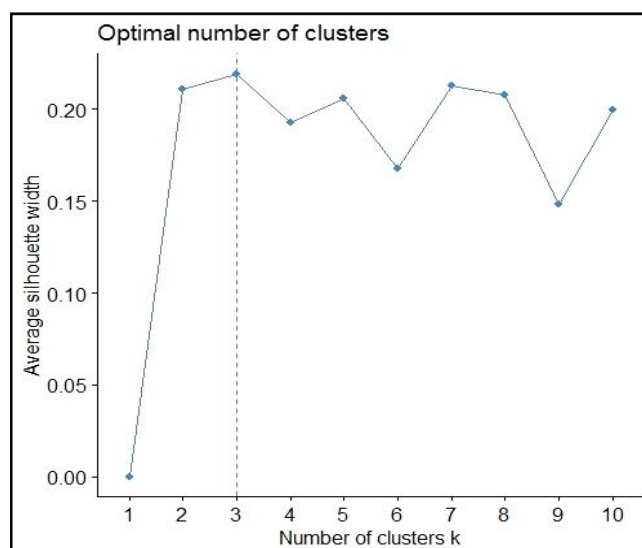
**Fig. 1:** Determination of optimum clusters by average silhouette method.

Table 3, covering the mean, range, and variance. Days to maturity ranged from 71 to 95 days, while plant height varied from 69.20 cm to 139.60 cm. Notably, accessions such as GPMS 399, GPMS 390, GPMS 367, and GPMS 365 exhibited early maturity (71 days) and dwarf characteristics, positioning them as strong candidates for developing short-duration, non-lodging varieties. Additionally, considerable variation was noted for traits like the number of productive tillers, flag leaf blade length, peduncle length, and inflorescence length. Among the accessions, GPMS 777 stood out for its high grain yield (29.33 g/plant) and production of 5-6 productive tillers. A majority of the high-yielding accessions were of Indian origin, highlighting the diverse genetic pool within the study material, which promises significant potential for future breeding programs. Previous studies also report substantial variability in various small millet species, including proso millet (Mani Vetriventhan and Upadyaya, 2018), finger millet (Reddy *et al.*, 2013; Ulaganathan *et al.*, 2013; Shinde *et al.*, 2014; Nagaraja *et al.*, 2023), barnyard millet (Nandini *et al.*, 2020), and little millet

Table 5: Clustering of 213 proso millet accessions into three clusters using 8 quantitative traits.

Cluster	No. of genotypes	Sl. No. and Name of the genotype									
Cluster I	38	1	GPMS 8	30	GPMS 152	47	GPMS 568	71	GPMS 795	87	GPMS 825
		7	GPMS 38	34	GPMS 165	56	GPMS 592	74	GPMS 807	90	GPMS 830
		8	GPMS 47	35	GPMS 170	64	GPMS 776	75	GPMS 808	96	GPMS 839
		13	GPMS 71	39	GPMS 214	65	GPMS 777	77	GPMS 813	106	GPMS 864
		14	GPMS 83	40	GPMS 215	66	GPMS 778	80	GPMS 816	107	GPMS 865
		18	GPMS 100	41	GPMS 223	68	GPMS 782	81	GPMS 817	176	GPMS403
		19	GPMS 106	42	GPMS 553	69	GPMS 792	82	GPMS 818		
		20	GPMS 108	46	GPMS 567	70	GPMS 794	85	GPMS 821		
Cluster II	100	17	GPMS 99	120	GPMS 217	142	GPMS458	162	GPMS390	184	GPMS431
		26	GPMS 117	121	GPMS460	143	GPMS457	163	GPMS367	185	GPMS435
		32	GPMS 163	122	GPMS442	144	GPMS408	164	GPMS365	186	GPMS429
		44	GPMS 555	123	GPMS438	145	GPMS406	165	GPMS409	188	GPMS423
		45	GPMS 556	124	GPMS437	146	GPMS448	166	GPMS369	189	GPMS420
		52	GPMS 580	125	GPMS433	147	GPMS393	167	GPMS413	190	GPMS417
		54	GPMS 582	126	GPMS397	148	GPMS454	168	GPMS432	191	GPMS450
		62	GPMS 600	127	GPMS407	149	GPMS376	169	GPMS398	192	GPMS400
		63	GPMS 773	128	GPMS391	150	GPMS453	170	GPMS426	195	GPMS410
		67	GPMS 779	129	GPMS405	151	GPMS467	171	GPMS445	198	GPMS427
		89	GPMS 828	130	GPMS399	152	GPMS468	172	GPMS401	199	GPMS415
		91	GPMS 832	131	GPMS419	153	GPMS421	173	GPMS396	200	GPMS456
		93	GPMS 836	132	GPMS446	154	GPMS370	175	GPMS368	201	GPMS436
		94	GPMS 837	133	GPMS463	155	GPMS388	177	GPMS434	202	GPMS441
		98	GPMS 842	134	GPMS459	156	GPMS384	178	GPMS461	205	GPMS449
		99	GPMS 846	135	GPMS392	157	GPMS387	179	GPMS465	206	GPMS451
		101	GPMS 848	136	GPMS379	158	GPMS418	180	GPMS412	207	GPMS452
		102	GPMS 850	137	GPMS381	159	GPMS440	181	GPMS404	208	GPMS469
		114	GPMS 873	138	GPMS389	160	GPMS466	182	GPMS443	209	GPMS464
		118	GPMS 880	141	GPMS411	161	GPMS416	183	GPMS430	210	GPMS422
Cluster III	75	2	GPMS 16	25	GPMS 116	55	GPMS 591	92	GPMS 833	117	GPMS 878
		3	GPMS 26	27	GPMS 125	57	GPMS 593	95	GPMS 838	119	GPMS 908
		4	GPMS 28	28	GPMS 126	58	GPMS 594	97	GPMS 840	139	GPMS385
		5	GPMS 36	29	GPMS 129	59	GPMS 596	100	GPMS 847	140	GPMS373
		6	GPMS 37	31	GPMS 159	60	GPMS 598	103	GPMS 851	174	GPMS378
		9	GPMS 48	33	GPMS 164	61	GPMS 599	104	GPMS 856	187	GPMS424
		10	GPMS 53	36	GPMS 171	72	GPMS 800	105	GPMS 858	193	GPMS414
		11	GPMS 56	37	GPMS 210	73	GPMS 801	108	GPMS 866	194	GPMS377
		12	GPMS 64	38	GPMS 213	76	GPMS 810	109	GPMS 867	196	GPMS402
		15	GPMS 88	43	GPMS 554	78	GPMS 814	110	GPMS 868	197	GPMS371
		16	GPMS 89	48	GPMS 576	79	GPMS 815	111	GPMS 869	203	GPMS444
		21	GPMS 109	49	GPMS 577	83	GPMS 819	112	GPMS 870	204	GPMS447
		22	GPMS 111	50	GPMS 578	84	GPMS 820	113	GPMS 871	C1	GPUP 21
		23	GPMS 112	51	GPMS 579	86	GPMS 824	115	GPMS 874	C2	GPUP 28
		24	GPMS 114	53	GPMS 581	88	GPMS 827	116	GPMS 877	C3	GPUP 8

(Narasimhulu *et al.*, 2024). This reinforces the ample genetic variation present, offering great opportunities for breeders to select genotypes with desirable traits for further crop improvement.

Trait Association

To explore the relationships between grain yield and other quantitative traits, Pearson's correlation coefficients were calculated for eight traits across 213 proso millet

Table 6: Comparison of the quantitative trait means between the three clusters.

Cluster Number	Days to maturity	Plant height (cm)	No. of productive tillers	Flag leaf blade length(cm)	Peduncle length (cm)	Inflorescence length (cm)	Test weight (g)	Grain yield per plant (g)
Cluster I	85.132	113.355	4.750	27.825	16.179	24.883	5.829	16.674
Cluster II	81.330	85.306	5.155	26.673	13.664	21.010	5.640	13.402
Cluster III	83.360	97.472	4.516	31.150	16.975	26.175	5.770	14.348

germplasm accessions (Table 4). The analysis revealed that days to maturity had a strong positive correlation with plant height, inflorescence length, and grain yield per plant, while it showed a significant negative correlation with peduncle length. Additionally, traits such as peduncle length, inflorescence length, test weight, and grain yield per plant were positively correlated with plant height. A negative correlation was found between the number of productive tillers and traits like flag leaf blade length, peduncle length, and inflorescence length. Conversely, flag leaf blade length exhibited a positive correlation with both peduncle length and inflorescence length. Based on these findings, days to maturity and plant height directly influence seed yield, making them key traits for selecting high-yielding proso millet genotypes. Similar associations were reported by Yazdizadeh *et al.*, (2020).

Cluster Analysis of Quantitative Traits

Using the average silhouette method, the proso millet accessions were categorized into three distinct clusters based on their yield and related traits (Fig. 1). The distribution of accessions across these clusters is outlined

in Table 5, with Cluster II containing the highest number (100) of accessions, followed by Cluster III. Table 6 provides the cluster means for all eight quantitative traits. Cluster I exhibited higher values for traits such as grain yield per plant, plant height, days to maturity, and test weight compared to the other clusters. The inter-cluster distance was greatest between Cluster I and Cluster II (28.89), compared to Cluster I and Cluster III (16.56), highlighting the broader genetic diversity between Clusters I and II (Table 7). These observations align with those of previous studies by Uddin *et al.*, (2005), Khatun *et al.*, (2023), Anilkumar *et al.*, (2022), and Santhosh kumar *et al.*, (2023), who found similar patterns in different small millet species, including finger millet (Nagaraja *et al.*, 2023), little millet (Narasimhulu *et al.*, 2024), and foxtail millet (Nandini *et al.*, 2018). Researchers have suggested that genotypes from the most distinct clusters can serve as valuable parental material for breeding programs, aiming to produce high-

Table 7: Inter cluster distance among the three clusters obtained.

Cluster No	Cluster I	Cluster II	Cluster III
Cluster I	0.000	28.893	16.560
Cluster II	28.893	0.000	14.531
Cluster III	16.560	14.531	0.000

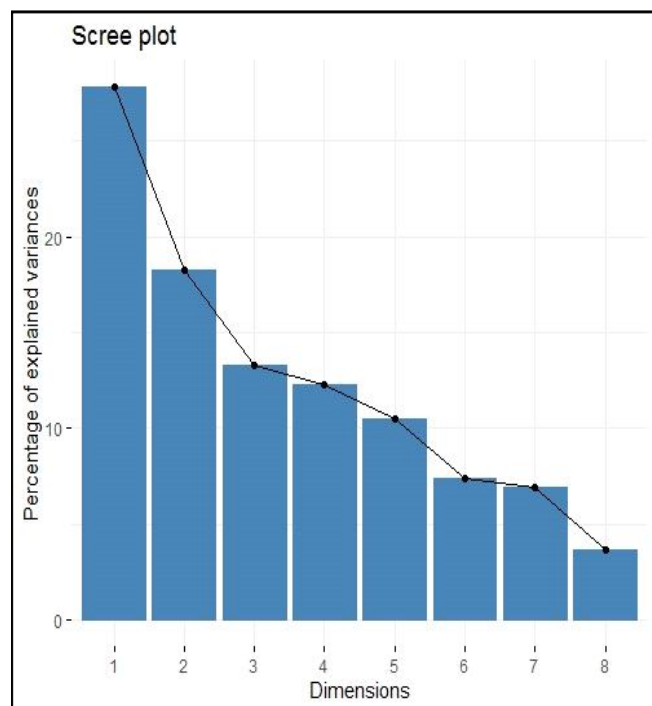
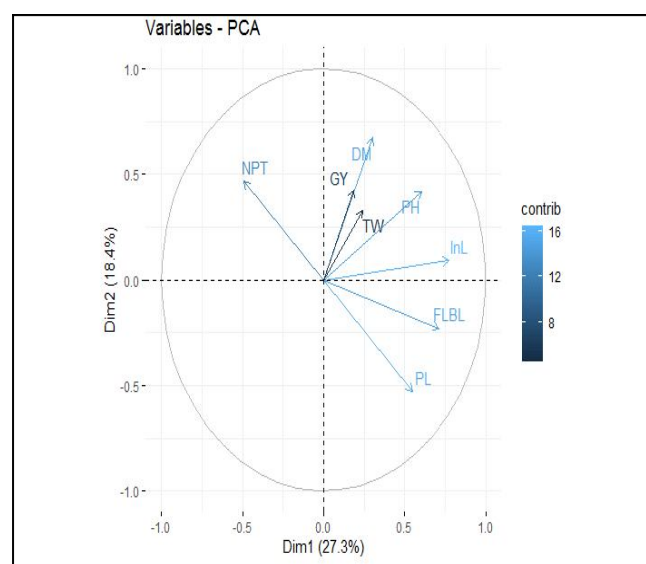
**Fig. 2:** Percentage of explained variances of eight principal components.**Fig. 3:** Contribution of variables in principal component 1

Table 8: Principal component analysis showing the contribution of morphological traits to the total variation among the proso millet accessions.

Communality	PC1	PC2	PC3	PC4	PC5
Eigen value	2.225	1.458	1.066	0.980	0.837
Proportion of variance	0.278	0.182	0.133	0.122	0.105
Cumulative variance (%)	0.278	0.460	0.594	0.716	0.821
Days to maturity	0.185	0.557	0.048	-0.365	-0.389
Plant height (cm)	0.403	0.351	-0.258	0.150	-0.305
No. of productive tillers	-0.343	0.363	0.395	0.080	0.423
Flag leaf blade length(cm)	0.480	-0.183	0.442	-0.140	0.296
Peduncle length(cm)	0.378	-0.418	-0.348	0.173	0.113
Inflorescence length(cm)	0.520	0.063	0.375	-0.163	0.130
Test weight (g)	0.169	0.293	0.120	0.851	0.050
Grain yield per plant (g)	0.118	0.368	-0.552	-0.192	0.676

yielding cultivars. For optimal genetic improvement, selection of genotypes should be guided by the cluster mean (Wolie *et al.*, 2013).

Principal Component Analysis (PCA):

Principal Component Analysis (PCA) is a statistical technique used to reduce a complex dataset into its principal components, highlighting the most important variables (Fig. 2). In this analysis, the first five principal components played a crucial role in differentiating the proso millet accessions, as detailed in Table 8. Together, these five components explained 82% of the total variance in the dataset. The first principal component (PC1) accounted for 27.80% of the variation and primarily separated the accessions based on four traits: plant height,

flag leaf blade length, peduncle length, and inflorescence length. Notably, flag leaf blade length (loading of 0.480) and inflorescence length (loading of 0.520) were the most influential traits within PC1. The second principal component (PC2), which explained 18.20% of the variance, was largely driven by plant height, number of productive tillers, test weight, days to maturity, and grain yield per plant. The remaining components, PC3, PC4, and PC5, contributed 13.30%, 12.20%, and 10.50% of the total variance, respectively. These results are consistent with findings from Rajput *et al.*, (2024), Salini *et al.*, (2010) on proso millet, and Nandini *et al.*, (2020) on barnyard millet. Similarly, Narasimhulu *et al.*, (2024) found that PC1 explained a significant portion of the variance in little millet, driven by traits such as days to 50% flowering, days to maturity, productive tillers, 1000-grain weight, and grain yield.

Trait-Specific Sources and Agronomic Performance

Key agronomic traits such as grain yield, plant height, days to maturity, and inflorescence length are central to advancing proso millet breeding. Grain yield per plant across all accessions ranged from 4.01g to 29.33g, with an average yield of 14.31g. The days to maturity for high-yielding accessions ranged between 77 and 94 days, with most accessions exhibiting tall growth, except for the dwarf accession GPMS 436. The study identified specific accessions with valuable traits for early maturity, high grain yield, compact stature, and long inflorescence, as detailed in Table 9.

Conclusions

This study has highlighted substantial genetic diversity within proso millet germplasm, offering significant potential for breeding initiatives. The results underscore the importance of traits such as plant height and days to maturity in developing proso millet varieties that are both high-yielding and resilient to climate challenges.

Table 9: Trait specific sources and their agronomic performance.

Sl.No	Early maturity		High grain yield per plant (g)		Dwarf accessions		Long inflorescence	
	Genotypes	Days to maturity	Genotypes	Grain yield	Genotypes	Plant height(cm)	Genotypes	Inflorescence length (cm)
1	GPMS397	71	GPMS 818	22.67	GPMS419	69.20	GPMS 109	36.20
2	GPMS373	71	GPMS 830	23.00	GPMS 873	72.30	GPMS 596	36.20
3	GPMS376	71	GPMS 874	23.00	GPMS 217	75.60	GPMS 16	35.40
4	GPMS370	71	GPMS 165	23.33	GPMS416	75.60	GPMS 111	34.70
5	GPMS384	71	GPMS 816	24.00	GPMS409	76.20	GPMS 215	34.50
6	GPMS398	71	GPMS 825	24.33	GPMS436	78.60	GPMS 56	33.60
7	GPMS401	71	GPMS 807	26.00	GPMS446	79.80	GPMS 117	33.20
8	GPMS396	71	GPMS 795	27.00	GPMS450	80.00	GPMS 126	33.20
9	GPMS378	71	GPMS436	28.20	GPMS397	80.80	GPMS 114	33.16
10	GPMS369	72	GPMS 777	29.33	GPMS 836	81.00	GPMS 129	33.10

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