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Genetic Analysis of Proso Millet (Panicum Miliaceum L.) Genotypes for Morpho-Physiological and Biochemical Characters

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Abstract: This study examined 48 proso millet genotypes to evaluate genetic variability, diversity, and trait associations across 15 distinct characteristics. Traits such as yield per plant, peduncle length, leaf width, flag leaf length, and plant height exhibited substantial genetic variability with high heritability and genetic advance, indicating that selection for these attributes would be both efficient and effective. Genetic divergence analysis categorized the genotypes into five distinct clusters, demonstrating a broad range of genetic diversity. The clustering pattern revealed that geographical origin did not correspond with genetic diversity among the studied genotypes. Cluster IV contained the highest number of genotypes (15), while Cluster V had the fewest (2). The greatest inter-cluster distance was observed between Cluster I and Cluster V, suggesting that crossing genotypes from these clusters could potentially produce superior transgressive segregants in advanced generations. Among the traits analyzed, Plant Height (PH), Flag Leaf Length (FLL), Flag Leaf Width (FLW), Leaf Width (LW), and 1000 Seed Weight (TSW) exhibited the most significant direct impact on yield per plant (YPP), rendering them essential targets for breeding programs focused on yield enhancement. Furthermore, the correlation analysis corroborated these findings, reinforcing their importance in improving yield potential.

Keywords: Transgressive segregants, Coefficient of variability, Genetic advance, heritability, proso millet

INTRODUCTION

Proso millet (*Panicum miliaceum L.*) is recognized for its nutritional value and agronomic advantages. It serves as a rich source of carbohydrates (73.1 g), proteins (11.6 g), and essential micronutrients, including iron (2.9 mg), phosphorus (206 mg), and calcium (14 mg) per 100 g. Due to its short growth duration and drought tolerance, it plays a vital role in food security and fodder production, particularly in regions prone to drought and low soil fertility [11].

As a predominantly self-pollinated crop with occasional outcrossing, proso millet exhibits considerable genetic variability for key agronomic traits such as maturity period, grain yield, seed size, and nutritional content. This diversity presents an opportunity for breeding and genetic improvement. However, prolonged selection pressure over multiple generations has led to a reduction in genetic variation, necessitating the exploration of diverse genetic resources to enhance crop productivity [5].

Despite its economic and agronomic significance, proso millet remains largely underutilized in breeding programs, with limited systematic efforts directed toward its genetic enhancement [18]. Identifying and utilizing

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genetic variability within breeding populations is essential for effective selection strategies. Traits with high heritability and genetic advance are particularly important for achieving substantial genetic gains. Additionally, studying genetic diversity among elite germplasm can aid in the selection of superior genotypes and provide insights into their genetic relationships [8].

Since grain yield is a polygenic trait influenced by multiple genetic and environmental factors, analyzing genotypic correlation coefficients and path coefficients can elucidate key yield-contributing traits. In light of these aspects, this study aims to assess genetic parameters governing yield and its components in proso millet, enabling improved selection strategies and hybrid development. The research focuses on genetic variability, heritability, genetic advance, genetic divergence, trait correlations, and the direct and indirect effects of various traits on grain yield in proso millet germplasm.

MATERIALS AND METHODS

This research was conducted at the ICAR-Indian Institute of Millets Research, located in Hyderabad, Telangana State, during the *summer* of 2023. The experimental site is situated at a longitude of 78°23’36’’ East. A total of 48 proso millet genotypes were evaluated utilizing a randomized block design (RBD) with two replications. The experiment was conducted from January 2023 to May 2023. Each genotype was sown in a single-row plot measuring 3 meters in length, with a row spacing of 30 cm and 10 cm between plants. All genotypes were subjected to uniform agronomic practices throughout the study period.

Observations were recorded for 15 agronomic traits, including days to 50% flowering, days to maturity, plant height, flag leaf length, flag leaf width, leaf length, leaf width, number of leaves per plant, number of nodes per plant, peduncle length, panicle length, 1000-grain weight, protein percentage and yield per plant. Data were collected from five randomly selected plants per replication to ensure accuracy and consistency.

STATISTICAL ANALYSIS

The phenotypic and genotypic coefficients of variation (PCV and GCV) were estimated using the method outlined [6] and expressed as percentages. Heritability in a broad sense (h^2) was determined following [15] and categorized according to the classification system proposed by [12]. The genetic advance (GA) was also computed based on the formula described by [12] and expressed as a percentage of the mean.

GENETIC DIVERGENCE ANALYSIS

To assess genetic diversity among the 48 genotypes, Mahalanobis D^2 analysis was conducted following the methods [16]. Data transformation was performed using the pivotal condensation method [19]. All possible D^2 values were computed using the formula n(n-1)/2, considering pairwise differences in corresponding ‘Y’ values for two genotypes at a time. These D^2 values were analyzed utilizing the R statistical package.

Genotypes were categorized based on their genetic divergence using Tocher’s clustering method [22]. The inter-cluster genetic distance was determined by calculating the mean of all possible D^2 values within each cluster. The square root of the mean D^2 values was utilized to estimate the genetic distance between clusters. Clusters were classified based on inter-cluster distance, following the categorization [19]. The ranking procedure was implemented [22]. Each trait was ranked based on transformed correlated ‘Y’ values, and genotypes were ranked according to the method proposed by [27]

CORRELATION AND PATH COEFFICIENT ANALYSIS

The genotypic correlation coefficients were calculated to assess the association between yield and yield-related traits, as well as inter-trait relationships. The method described by [10] was employed to compute genotypic correlations, utilizing variance and covariance components as delineated by [1]

To elucidate trait contributions to yield, path coefficient analysis was conducted following the methodology of [7]. This analysis was utilized to partition genotypic correlation coefficients into direct and indirect effects. The classification of direct and indirect effects was executed according to the scale provided by [14]

RESULTS AND DISCUSSION

Understanding the genetic variability within a population is crucial for the success of any breeding program. The presence of high variability allows for better selection of promising genotypes and enables breeders to develop superior varieties. In the present study, the genotypic and phenotypic coefficients of variation (GCV and PCV) were estimated to quantify the extent of variability present in different traits among the evaluated proso millet genotypes. The highest GCV and PCV estimates were observed for yield per plant (GCV: 47.26%, PCV: 47.91%), peduncle length (GCV: 28.52%, PCV: 29.25%), leaf width (GCV: 27.58%, PCV: 30.12%), flag leaf length (GCV: 24.43%, PCV: 25.25%), and plant height (GCV: 22.16%, PCV: 22.31%) (Table 1). The high magnitude of both GCV and PCV for these traits suggests that they exhibit substantial genetic variation, which provides greater opportunities for genetic improvement through selection. These results are consistent with previous findings by [21] and [26] for yield per plant, [4] and [25] for flag leaf length, and [2, 3, 13], for flag leaf width. In contrast, moderate GCV and PCV values were observed for leaf length (GCV: 19.72%, PCV: 20.17%), panicle length (GCV: 16.06%, PCV: 17.46%), days to 50% flowering (GCV: 15.21%, PCV: 15.37%), 1000 seed weight (GCV: 11.91%, PCV: 12.54%), and days to maturity (GCV: 10.82%, PCV: 11.01%) (Table 1). The relatively lower variability for these traits suggests limited scope for improvement through selection alone, but moderate genetic variability still provides opportunities for targeted breeding strategies. These findings are supported by [2, 13] for 1000 seed weight and [26] for days to 50% flowering and maturity. The small difference between PCV and GCV for most traits indicates that these traits are less influenced by environmental factors, making selection based on phenotypic expression more reliable.

Heritability is a key indicator of how efficiently a trait can be passed from parent to progeny. It determines the effectiveness of selection-based breeding programs. High heritability along with genetic advance as percent of the mean (GA%) indicates that a trait is controlled primarily by additive gene action, making direct selection highly effective [24, 20]. In the present study, high heritability coupled with high genetic advance was recorded for plant height (98.61%, GA%: 45.33), days to 50% flowering (97.96%, GA%: 31.02), yield per plant (97.30%, GA%: 96.03), days to maturity (96.66%, GA%: 21.93), leaf length (95.65%, GA%: 39.74), peduncle length (95.04%, GA%: 57.28), flag leaf length (93.61%, GA%: 48.70), 1000 seed weight (90.13%, GA%: 23.30), and panicle length (84.62%, GA%: 30.44) (Table 2). These results suggest that these traits are primarily controlled by additive genetic effects, making them ideal targets for direct selection in breeding programs. Similar findings were reported by [21, 4] for yield-related traits, as well as for plant height [3, 24]

The Mahalanobis D^2 statistics [19] was used to assess genetic diversity among the 48 proso millet genotypes, classifying them into five distinct clusters (Table 3). Cluster I and Cluster IV contained the highest number of genotypes (13 and 15, respectively). Cluster II contained 6 genotypes, Cluster III had 12 genotypes, and Cluster V had 2 genotypes. The presence of a high number of clusters indicates significant diversity within the population, suggesting that crossing genotypes from diverse clusters will yield superior hybrids. The highest inter-cluster distance was observed between Cluster IV and Cluster V (14.407), followed by Cluster III and Cluster IV (13.718), suggesting that hybridization between these clusters may yield highly heterotic hybrids (Table 3). The lowest intra-cluster distance was observed in Cluster V (0.50), indicating genetic similarity among the genotypes in this cluster. These findings align with previous studies on proso millet [9], little millet, foxtail millet [17, 26]

Correlation analysis determines the relationship between traits, while path coefficient analysis reveals their direct and indirect effects on yield per plant. The study found that yield per plant had a significant positive correlation with plant height, flag leaf width, leaf width, days to 50% flowering, days to maturity, leaf length, peduncle length, and panicle length (Table 4). These findings suggest that improving these traits can lead to higher yield per plant, supporting similar reports [6, 23, 15]

Path coefficient analysis identified days to 50% flowering (4.415) and leaf width (1.329) as the most important contributors to yield per plant (Table 5). Other traits showing moderate direct positive effects included: Flag leaf length (0.924), Number of nodes per plant (0.875), 1000 seed weight (0.667), Number of basal tillers per plant (0.449), Total protein content (0.323), Peduncle length (0.226). However, days to maturity (-4.414), plant height (-0.097), flag leaf width (-0.570), leaf length (-0.675), number of leaves per plant (-0.538), and panicle length (-0.437) exhibited negative direct effects on yield per plant. The residual effect (0.6005) was low, indicating that most of the variation in yield per plant is explained by the selected traits.

CONCLUSION

In conclusion, the present study revealed that the traits yield per plant, peduncle length, leaf width, flag leaf length, and plant height recorded a high amount of genetic variability along

Table 1: Estimates of genetic variability parameters for 15 Morpho-physiological and biochemical character studied in proso millet (*Panicum miliaceum L.*)

Traits	*GCV (%)*	*PCV (%)*	*Heritability (%)*	*GA*	*GA as % of mean*
Days to 50% flowering	15.21	15.37	97.96	16.72	31.02
Days to maturity	10.82	11.01	96.66	16.65	21.93
Plant height (cm)	22.16	22.31	98.61	37.19	45.33
Flag leaf length (cm)	24.43	25.25	93.61	10.99	48.70
Flag leaf width (cm)	24.59	31.14	62.39	0.48	40.02
Leaf length (cm)	19.72	20.17	95.65	12.33	39.74
Leaf width (cm)	27.58	30.12	83.86	0.73	52.03
Number of leaves per plant	17.51	26.41	43.97	1.16	23.92
Number of nodes per plant	16.99	25.88	43.11	1.20	22.98
Number of basal tillers per plant	20.47	25.48	64.53	1.55	33.87
Peduncle length (cm)	28.52	29.25	95.04	6.18	57.28
Panicle length (cm)	16.06	17.46	84.62	7.64	30.44
Total protein percentage (%)	9.79	9.79	100	3.58	20.18
1000 seed weight (gm)	11.91	12.54	90.13	1.11	23.30
Yield per plant	47.26	47.91	97.30	17.82	96.03

Table 2: Distribution of 48 proso millet genotypes among different clusters on the basis of Mahalanobis D^2 – analysis

Cluster	*Number of genotypes*	*Genotypes*
1	13	IIMR-PM-10, IIMR-PM-113, IIMR-PM-114, IIMR-PM-165, IIMR-PM-166, IIMR-PM-170, IIMR-PM-190, IIMR-PM-29, IIMR-PM-34, IIMR-PM-35, IIMR-PM-6, IIMR-PM-9, HB1
2	6	IIMR-PM-110, IIMR-PM-143, IIMR-PM-183, IIMR-PM-186, IIMR-PM-59, IIMR-PRO-755
3	12	IIMR-PM-118, IIMR-PM-123, DHPM2769, IIMR10, IIMR137, IIMR163, IIMR188, IIMR225, IIMR7, TNAU145, TNAU164, TNAU202
4	15	IIMR-PRO-110, IIMR-PRO-173, IIMR-PRO-208, IIMR-PRO-221, IIMR-PRO-29, IIMR-PRO-348, IIMR-PRO-583, IIMR-PRO-671, IIMR-PRO-676, IIMR-PRO-688, IIMR-PRO-706, IIMR-PRO-737, IIMR-PRO-874, IIMR-PRO-875, IIMR-PRO-931
5	2	IIMR-PRO-795, IIMR-PRO-876

Table 3: Inter and Intra cluster distance

	Cluster I	*Cluster II*	*Cluster III*	*Cluster IV*	*Cluster V*
Cluster I	11.08	33.90	32.44	38.26	60.00
Cluster II	33.90	4.17	34.12	37.39	51.52
Cluster III	32.44	34.12	10.08	50.84	59.87
Cluster IV	38.26	37.39	50.84	13.07	43.70
Cluster V	60.00	51.52	59.87	43.70	0.50

Table 4: Mean values of 15 characters in 5 clusters

Characters	*CLUSTER*					
	I	*II*	*III*	*IV*	*V*	*Mean*
Days to 50% flowering	58.62	57	59.83	44.9	46.25	53.32
Days to maturity	80.77	79.08	81.88	66.83	68.25	75.36
Plant height (cm)	86.87	81.67	101.26	64.29	69.8	80.78
Flag leaf length (cm)	24.03	22.91	28.91	16.93	16.26	21.81
Flag leaf width (cm)	0.95	1.33	1.62	1.08	1.08	1.21
Leaf length (cm)	27.42	30.45	31.26	31.82	49.38	34.07
Leaf width (cm)	1.14	1.43	1.55	1.44	2.33	1.58
Number of leaves per plant	4.23	6	4.83	4.9	5.5	5.09
Number of nodes per plant	4.56	6.58	5.17	5.4	5.25	5.39
Number of basal tillers per plant	4.3	3.62	4.68	5.33	3.5	4.29
Peduncle length (cm)	11.89	10.95	13.63	8.03	6.82	10.26
Panicle length (cm)	25.52	25.68	27.69	22.68	23.35	24.98
Total protein percentage (%)	17.03	19.69	16.91	18.27	17.52	17.88
1000 seed weight (gm)	17.03	19.69	16.91	18.27	17.52	17.88
Yield per plant	4.89	4.63	5	4.57	5.2	4.86

with heritability and genetic advance. Hence, selection for these traits is simple and effective. The study on D^2 analysis revealed that the cluster mean for yield per plant was the highest in Cluster V, while Clusters I and IV exhibited significant genetic diversity. The high inter-cluster distance observed between these clusters indicates the potential for obtaining superior segregants through hybridization. Path analysis revealed that days to 50% flowering and leaf width were key traits that directly influenced yield per plant. The correlation study further confirmed that plant height, flag leaf width, leaf width, days to 50% flowering, days to maturity, leaf length, peduncle length, and panicle length had significant positive associations with yield, making them crucial selection criteria for genetic improvement.

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Table 5: Genotypic correlation coefficient among 15 Morpho-physiological and biochemical character studied in proso millet (*Panicum miliaceum L.*)

Characters	*DFF*	*DM*	*PH*	*FLL*	*FLW*	*LL*	*LW*	*NLPP*	*NNPP*	*NBTPP*	*PeL*	*PaL*	*TPC*	*TSW*	*YPP*
DFF	**1 ****	1.000**	0.573**	0.631**	0.275	-0.329*	-0.182	0.065	-0.060	-0.477**	0.600**	0.278	-0.204	0.186	0.119
DM		**1 ****	0.586**	0.642**	0.268	-0.331*	-0.185	0.078	-0.087	-0.467**	0.597**	0.266	-0.2	0.192	0.117
PH			**1 ****	0.730**	0.474**	-0.108	0.110	-0.238	-0.334*	-0.101	0.584**	0.353*	-0.262	0.340*	0.382**
FLL				**1****	0.533**	-0.035	0.048	-0.042	-0.268	-0.218	0.634**	0.423**	-0.221	0.189	0.291*
FLW					**1 ****	0.105	0.319*	0.027	0.327*	-0.145	0.469**	0.199	-0.174	0.349*	0.661**
LL						**1 ****	0.800**	0.341*	0.085	0.007	-0.240	0.051	0.014	0.027	0.155
LW							**1 ****	0.318*	-0.047	-0.108	-0.054	0.056	-0.074	0.108	0.404**
NLPP								**1 ****	0.599**	-0.160	-0.046	0.038	0.271	-0.211	-0.062
NNPP									**1 ****	-0.2885 *	-0.016	-0.042	0.292*	-0.258	-0.042
NBTPP										**1 ****	-0.165	-0.035	0.113	-0.331*	-0.228
PeL											**1 ****	0.692**	-0.121	0.109	0.259
PaL												**1 ****	0.051	0.157	0.104
TPC													**1 ****	-0.347*	-0.005
TSW														**1 ****	0.293*
YPP															1**

DFF - Days to 50 per cent flowering; DM – days to maturity; PH – plant height; FLL – flag leaf length; FLW – flag leaf width; LL – leaf length; LW – leaf width; NLPP – number of leaves per plant; NNPP – number of nodes per plant; NBTPP – number of basal tillers per plant; PeL – peduncle length; PaL – panicle length; TPC – total protein content; TSW – thousand seed weight; YPP – yield per plant.

Table 6: Path coefficient analysis showing direct effect of 15 Morpho-physiological and biochemical character studied in proso millet (*Panicum miliaceum L.*)

Characters	*DFF*	*DM*	*PH*	*FLL*	*FLW*	*LL*	*LW*	*NLPP*	*NNPP*	*NBTPP*	*PeL*	*PaL*	*TPC*	*TSW*	*YPP*
DFF	**4.415**	-4.416	-0.056	0.583	-0.157	0.222	-0.242	-0.035	-0.053	-0.214	0.136	-0.122	-0.066	0.124	0.1194 NS
DM	4.418	**-4.414**	-0.057	0.593	-0.153	0.224	-0.247	-0.042	-0.077	-0.210	0.135	-0.117	-0.065	0.128	0.1171 NS
PH	2.534	-2.588	**-0.097**	0.675	-0.271	0.073	0.147	0.128	-0.293	-0.046	0.132	-0.155	-0.085	0.227	0.3824 **
FLL	2.787	-2.835	-0.071	**0.924**	-0.304	0.024	0.065	0.023	-0.235	-0.098	0.143	-0.185	-0.071	0.126	0.2916 *
FLW	1.215	-1.186	-0.046	0.493	**-0.570**	-0.071	0.424	-0.015	0.287	-0.065	0.106	-0.087	-0.056	0.233	0.6614 **
LL	-1.454	1.463	0.010	-0.033	-0.060	**-0.675**	1.063	-0.184	0.075	0.003	-0.054	-0.023	0.005	0.018	0.1555 NS
LW	-0.806	0.820	-0.011	0.045	-0.182	-0.540	**1.329**	-0.171	-0.042	-0.049	-0.012	-0.025	-0.024	0.073	0.4047 **
NLPP	0.290	-0.348	0.023	-0.039	-0.015	-0.231	0.424	**-0.538**	0.524	-0.072	-0.011	-0.017	0.088	-0.141	-0.0628 NS
NNPP	-0.267	0.387	0.032	-0.248	-0.187	-0.058	-0.063	-0.322	**0.875**	-0.129	-0.004	0.018	0.094	-0.172	-0.0426 NS
NBTPP	-2.110	2.063	0.010	-0.202	0.083	-0.005	-0.144	0.086	-0.252	**0.449**	-0.038	0.016	0.037	-0.221	-0.228 NS
PeL	2.652	-2.637	-0.057	0.586	-0.268	0.162	-0.072	0.025	-0.014	-0.074	**0.226**	-0.303	-0.039	0.073	0.2599 NS
PaL	1.230	-1.176	-0.034	0.391	-0.114	-0.035	0.075	-0.020	-0.037	-0.016	0.157	**-0.437**	0.017	0.105	0.1042 NS
TPC	-0.901	0.883	0.025	-0.204	0.099	-0.010	-0.099	-0.146	0.256	0.051	-0.028	-0.023	**0.323**	-0.232	-0.0052 NS
TSW	0.824	-0.849	-0.033	0.175	-0.199	-0.019	0.145	0.114	-0.226	-0.149	0.025	-0.069	-0.112	**0.667**	0.293 *

DFF - Days to 50 per cent flowering; DM – days to maturity; PH – plant height; FLL – flag leaf length; FLW – flag leaf width; LL – leaf length; LW – leaf width; NLPP – number of leaves per plant; NNPP – number of nodes per plant; NBTPP – number of basal tillers per plant; PeL – peduncle length; PaL – panicle length; TPC – total protein content; TSW – thousand seed weight; YPP – yield per plant.

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