International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume: 09, Issue: 05 "September-October 2023"

MORPHOLOGICAL CHARACTERIZATION AND DIVERSITY ANALYSIS OF PROSO MILLET (*Panicum miliaceum* L.*)* GERMPLASM IN BANGLADESH

*[1]M. F. Khatun, [2]M. S. Islam, [1]N. Jahan, [1]Q. M. Ahmed, [3]M. R. Islam, [1]N. Pervin and [1]N. Jahan

[1*]Senior Scientific Officer, Plant Genetic Resources Centre,
Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh.

[2]Senior Scientific Officer, Soil Science Division,
Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh.

[3]Scientific Officer, Regional Agricultural Research Station,
Bangladesh Agricultural Research Institute, Barishal-8211, Bangladesh.

*Corresponding Author

DOI: https://doi.org/10.51193/IJAER.2023.9501

Received: 29 Aug. 2023 / Accepted: 13 Sep. 2023 / Published: 28 Oct. 2023

ABSTRACT

Characterization and variability analysis of germplasm is a prerequisite for the improvement of high-yielding variety. Thus, a study was executed with ninety-seven proso millet accessions to characterize and assess their variability using multivariate analysis. The study showed a wide range of variations in the qualitative and quantitative characteristics of proso millet accession. Of the qualitative characters, growth habits, sheath pubescence, ligule pubescence, inflorescence shape, degree of lodging, and seed color showed distinct variation. The highest coefficient of variation was obtained in grain yield (43.2%) within the quantitative traits. The total variation of principal components was 18.8, 13.6, 11.6, 10.8, and 10.3% in the PC1, PC2, PC3, PC4, and PC5, respectively. The overall variability in the number of secondary inflorescence branches, plant height, grain yield, days to flowering 50%, and sheath length of flag leaf has been recorded as the most relevant contributing factors regarding the diversity of the accession in PC1. In addition, the clustering analysis of ninety-seven proso millet accessions was able to group them into five clusters based on ten quantitative characters, while the maximum number of accessions (27) were contained in cluster V. In conclusion, the existing variability of the proso millet accession can attract plant breeders to adopt certain recipient accessions for breeding programs.

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Keywords: Proso millet; accession; morphological characterization; variability; multivariate analysis

1. INTRODUCTION

Proso millet *(Panicum miliaceum* L.) is the sixth most important cereal grain in the world, which belongs to the *Poaceae* family [1,2]. It was first introduced in Manchuria and Europe about 3,000 years ago after that it transferred to India [3]. It is commonly known as broomcorn millet, common millet, hog millet, and Russian millet [4]. It is a short-duration (60-100 days) self-pollinated plant [5]. The seed has multiple variations i.e., the white cream, yellow, red, orange, black, and brown colors that can easily grow against heat and moisture stress conditions [3]. Compared to the other cereals, proso millet may represent a valuable crop, because of its higher leaf area index, nitrogen use efficiency, and radiation use efficiency than that of C3 cereals. The nutritive values such as minerals and protein content (>14%) are higher than that of wheat and rice by 11.8% and6.8%, respectively [6]. Mostly, proso millet is produced for food, feed, forage, and fuel purposes, while feeding birds and cattle in Western and Asian countries [7]. Lately, proso millet has received attention in the food industries in Europe and North America for its flavor, light color, gluten-free quality, and potential health benefits [8]. Among cereal crops, millets frequently produce low yields and can easily grow on low-fertility soil. Anyway, it is considered the most suitable crop for sustainable agriculture and food security [9].

Genetic improvement of any crop requires the availability of varied genetic resources. In addition to the availability of genetic resources, their characterization is critical and the starting point for crop development programs [10]. Morphological characteristics are thought to be the most basic sign for measuring the genetic variation of species. Traditionally, morphological characteristics have typically been employed to classify species; nonetheless, they are often sufficient to accurately categorize a single entry to the species level in an ex-situ collection [11]. This form of characterization requires a substantial investment in terms of facilities and time as well as technical experience to assess traits, such as pollen shape and flower morphology [12]. Multivariate techniques are effective for the characterization, evaluation, and categorization of the collected germplasm. Multivariate analysis information can be used for different germplasm identification, which has explained traits for crossing, and for developing an effective agricultural development program.

Many studies have been found on different millet germplasm using multivariate techniques, principal component analysis (PCA), and cluster analysis for a breeding program. For example, Mumtaz *et al.* [13] discovered a strong correlation between grain yield, panicle length, brix value, days to 50% anthesis, and 1000-grain weight of sorghum germplasm that contributed to total genetic diversity. Furthermore, in foxtail millet germplasm [13], yield plant-1, basal tillers

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plant-1, and peduncle length showed a highly significant and positive correlation, whereas yield showed a negative correlation with plant height and days to 50% flowering. Besides, the multivariate analysis of 55 finger millet genotypes contributed about 66.54% of the total variability [14]. On the other hand, Salini *et al.* [3] characterized 364 proso millet germplasm in terms of genetic variability, diversity, correlation, and path analysis. Recently, several researchers have shown interest in studying the proso millet, such as studied correlation analysis with different traits of proso millet. [16] reported on the nutritional and health benefits of proso millet. Salini *et al.* [3] Evaluated three hundred and sixty-four proso millet germplasm for breeding purposes.

The Bangladesh Agricultural Research Institute has collected proso millet germplasm from home and abroad and conserved 199 for intensive research. In this regard, Uddin *et al.* [5] analyzed the genetic diversity of limited germplasm of proso millet concerning phenotypic character. Rahman *et al.* [17] adapted proso millet germplasm in char area limitedly. To date, a huge number of proso millet germplasm remains out of characterization. The study of proso millet accessions would have the potential for innovative use, and genetic improvement by multivariate analysis in underdeveloped countries including Bangladesh. To the best of my knowledge, not enough literature has been found to proso millet improvement due to being a minor crop. The diversity study of the proso millet accessions can find out the high-yielding and other agronomic traits against a stressful environment. Besides, it will contribute to the food demands of the rising population as a climate-resilient crop. Thus, the study has been executed to characterize the collected accessions by using agro-morphological parameters and also identify genetically potential germplasm for the improvement of proso millet variety.

2. MATERIALS AND METHODS

The experiment was conducted in the experimental field at the Plant Genetic Resources Centre of Bangladesh Agricultural Research Institute (BARI), Gazipur during the winter season of 2019-20. The location of the site was 23°59'61" N latitude and 90°24'81" E longitude at an elevation of 16 m above sea level. The experiment included ninety-seven accessions for characterization which were collected in different parts of the country. The study was laid out according to the augmented design. Seeds were sown on 27 November 2019 following in continuous line sowing system. The unit plot size was 5m× 3m. The recommended fertilizer doses were applied at the rate of 10 t ha^{-1} decomposed cowdung, N, P, and K at the rate of 42, 15, and 18 $kgha^{-1}$, respectively. The full doses of decomposed cowdung, TSP, MoP, and one-third urea were applied during final land preparation. The rest of the urea was applied in the three equal splits at 20, 45, and 60 days after sowing. The diseases and insect infestation were not observed over the growing period. Weeding was done 15 days after sowing while the irrigations were applied at the

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20^{th}, 45^{th}, and 60^{th} days of the plant. The qualitative (10) and quantitative (10) data were recorded in various stages according to the prescribed descriptors by the International Board for Plant Genetic Resources (IBPGR) for proso millet [18]. Data were collected from one square meter area of each accession. The range, mean, standard deviation, and coefficient of variation were calculated in Office Excel 2016. The principal component analysis (PCA), cluster analysis, and intra and inter-cluster distance were analyzed by using the software R [19].

3. RESULTS AND DISCUSSION

3.1 Qualitative characters

Qualitative characteristics significantly indicate the variability of germplasm. The evaluation of the diversity of the tested germplasm identified ten important qualitative characters (Table 1). Of the qualitative characters' growth habits, sheath pubescence, ligule pubescence, inflorescence shape, degree of lodging, and seed color had distinct variations while plant pigmentation, leaf blade pubescence, inflorescence compactness, and shattering of inflorescence had no variation. In the growing period, three types of plant growth habits(erect, erect geniculate, and prostrate) were observed in the overall germplasm. The highest plant growth habit was found erect type (72.16%) while erect geniculate (18.56%), and prostrate (12.37%). Sheath pubescence and legule pubescence showed similar variations. The sheath pubescence was observed strongly (57.73%) and medium sheath pubescence (42.26%) in germplasm. According to [20], the dense pubescent in the plant leaf sheath and legule is particularly effective in reducing water loss and protecting photosynthetic regions from UV-B radiation. Moreover, the photon-based chlorophyll content of a pubescent leaf is lower than that of a glabrous or less pubescent leaf [21]. In the case of inflorescence, three categories of inflorescence shape were found among the germplasm. The various shape of inflorescence was recorded in proso millet germplasm such as arched shape (75.26%), globose-elliptic (20%), and diffused (4%). Similarly, the race ovatum proso millet cultivar showed more or less compact inflorescences under the ovate shape and somewhat bent [22]. Besides, the degree of lodging is an important characteristic of millet crops. In this regard, 28% of the studied proso millet accessions showed slight lodging and the others were observed as extensive (37.11%) and medium lodging (34.02%).

The maximum variation was found in seed color among the germplasm after drying. The seed colors were found in dark brown, brown, yellowish-brown, and light brown by 48.45%, 29.90%, 15.64%, and 2.06%, respectively and the few (4.12%) were in olive dark, tan, gray, olive green color. The seed coat color may have an important role in seed germination, water uptake, seedling growth as well and seed persistence in the soil. In this sense, Khan *et al.* [23] reported that dark-pigmented seeds germinated more slowly, while white-pigmented seeds germinated the

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fastest and one of the black-pigmented collections took the longest (108 h). Moreover, colored seed coats are heavier and thus likely thicker than light-hued seed coats [23].

Table 1: Qualitative variation of different characters in proso millet accession

Character	Descriptor state	No. of accession	Accession (%)
Growth habit	Erect	70	72.2
	Erect geniculate	18	18.6
	Prostrate	12	12.4
Plant pigmentation	Not pigmented	97	100
Leaf blade pubescence	Essentially glabrous	97	100
Sheath pubescence	Strongly pubescence	56	57.7
	Medium pubescence	41	42.3
Ligule pubescence	Strongly pubescence	59	60.8
	Medium pubescence	38	39.2
Inflorescence shape	Arched	73	75.3
	Globose-elliptic	20	20.6
	Diffused	4	4.12
Compactness of inflorescence	Open	97	100
Shattering of inflorescence	No Shattering	97	100
Degree of lodging	Extensive	36	37.1
	Medium	33	34.0
	Slight	28	28.9
Seed color	Dark brown	47	48.5
	Brown	29	29.9
	Yellowish-brown	15	15.6
	Others(Olive dark, tan, grey, olive green)	4	4.12
	Light brown	2	2.06

3.2 Quantitative characters

Quantitative characters are also important indicators to assess the variability of germplasm. The range, mean, standard deviation, and coefficient of variation (%) of the quantitative data of proso millet are presented in Table 2. The ten quantitative traits were studied which all showed remarkable variations. The plant height ranged from 62.2 to 113.8 where BD-1418 and BD-1336 were recognized as short stature plants. The number of basal tillers ranged from 7.8 to 30.4, while the average value was 13.8. The average sheath length of the flag leaf was 8.90 cm, ranging from 6.1 to 11.3 cm. The inflorescence peduncle length ranged from 10.2 to 21.5 cm, with a mean of 15.1 cm. Likewise, the inflorescence length ranged from 19.1 to 30.3 cm, with

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an average of 24.4 cm. The number of primary inflorescence branches ranged from 3.4 to 8.4 with an average of 6.08. Days to 50% flowering ranged from 49 to 72 days. In our observations, Bd-780, BD-783, and BD-1446 showed comparatively early flowering. The 1000 seed weight ranged from 4.2 to 6.2 g, with 5.4 g being the average. The highest grain yield range was found 7.2 to 113g with an average of 47.3 g. In this regard, the accessions viz; BD-761, BD-772, BD-1386, BD-1387, BD-1418, and BD-1336 were identified as high yielding than others. The highest coefficient of variation (43.2%) was obtained in grain yield which was followed by the number of the basal tiller (27.4%) and number of secondary inflorescence branches (24.3%) while the lowest coefficient of variation (5.56%) obtained in 1000 seed weight (g) which was followed by days to 50% flowering (8.57%). Among 10 quantitative characters, the highest variation represents grain yield m-2, the number of basal tillers, and plant height (cm). The findings were corroborated with [3].

Table 2: Quantitative variation of different characters in proso millet accession

Character	Range	Mean	Standard Deviation	Coefficient of variation (%)
Plant height (cm)	62.2-113.8	90.4	11.5	12.7
Number of the basal tiller	7.8-30.4	13.8	3.77	27.4
Sheath length of flag leaf (cm)	6.1-11.3	8.90	0.96	10.8
Length of the peduncle (cm)	10.2-21.5	15.1	2.57	17.0
Length of inflorescence (cm)	19.1-30.3	24.4	2.68	10.9
Number of primary inflorescence branches	3.4-8.4	6.08	0.95	15.6
Number of secondary inflorescence Branches	11.9-47	27.0	6.58	24.3
Days to flowering 50%	49-72	60.1	5.15	8.57
1000 seed weight (g)	4.2-6.2	5.4	0.03	5.56
Grain yield m^{-2}	7.2-113	47.3	20.4	43.2

3.3 Principal component analysis

Table 3 shows the percentage of variation explained by the first five principal components as well as the vector loadings for each agronomic attribute and PC. The principal component analysis is the technique to determine, which plant attributes are responsible for the majority of the observed variation among a set of genotypes [14]. PCA gives information on the independent impact of a specific characteristic on total variance, with each eigen vector coefficient indicating the degree of contribution of each original variable to which each principle component is connected [24]. According to principal component analysis, the top five eigenvectors explained approximately 65.1 percent of the total variation of the germplasm's quantitative attributes. The

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first principal component (PC1) accounted for 18.8% of the total variation and that was heavily influenced by the number of secondary branches inflorescence, plant height, grain yield m-2, and flag leaf sheath length. The second principal component (PC2) accounted for 13.6 percent of the variability and had a high contributing factor loading from the sheath length of the flag leaf and the number of primary inflorescence branches. The third principal component (PC3) accounted for 11.6 percent of the total variability has originated from the length of inflorescence, sheath length of flag leaf, number of primary branches inflorescence, number of the basal tiller, and days to 50% flowering. The fourth (PC4) and fifth (PC5) principal components accounted for 10.8 and 10.3 percent of variation and the lowest positive loading was found from plant height and length of the peduncle, number of the basal tiller, number of secondary branches inflorescence, respectively. The findings suggest that grain yield m-2, peduncle length, number of secondary inflorescence branches, plant height, inflorescence length, and number of basal tillers could be used to differentiate germplasm entries. PC1 had the highest positive loading among the principal components (PC), indicating their importance.

Table 3: Principal components analysis showing the contribution of 10 characteristics of proso millet accession

Characters	PC1	PC2	PC3	PC4	PC5
Plant height (cm)	0.499	-0.164	-0.002	0.195	-0.173
Number of the basal tiller	-0.116	-0.530	0.077	-0.224	0.341
Sheath length of flag leaf (cm)	0.418	0.296	0.381	-0.054	0.278
Length of the peduncle (cm)	-0.038	-0.231	-0.077	-0.316	0.582
Length of inflorescence (cm)	0.022	-0.163	0.449	-0.281	-0.563
Number of primary inflorescence branches	0.021	0.268	0.101	-0.773	-0.007
Number of secondary inflorescence branches	0.532	-0.030	-0.236	0.005	0.114
Days to flowering 50%	0.148	-0.671	0.030	0.050	-0.200
Grain yield m^{-2}	0.485	0.013	-0.294	0.093	-0.051
1000 seed weight(g)	-0.142	0.039	-0.699	-0.341	-0.263
Eigenvalue	1.88	1.36	1.16	1.08	1.03
% of variation	18.8	13.6	11.6	10.8	10.3
Cumulative variation (%)	18.8	32.4	44	54.8	65.1

3.4 Scree plot analysis

A scree plot is a line graph representing the eigen values of factors or major components in a multivariate study. The scree plot is used to determine the number of factors to keep in an exploratory factor analysis or the number of principal components to keep in a principal

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component analysis. In this study, the scree plot (Figure 1) represented the eigen values on the y-axis and the PC component on the x-axis. PC1 provided the maximum Eigen value (1.88), followed by PC2 (1.36), and PC3 (1.16). This indicated that the level of the curve means that the principal components from PC6 to PC10 had no remarkable responsibility for genetic diversity, which was mentioned in sorghum [13]. The results of this study consisted of finger millet germplasm [12]. Moreover, Selvi *et al.* [25] disclosed a similar variation in little millet by principal component analysis.

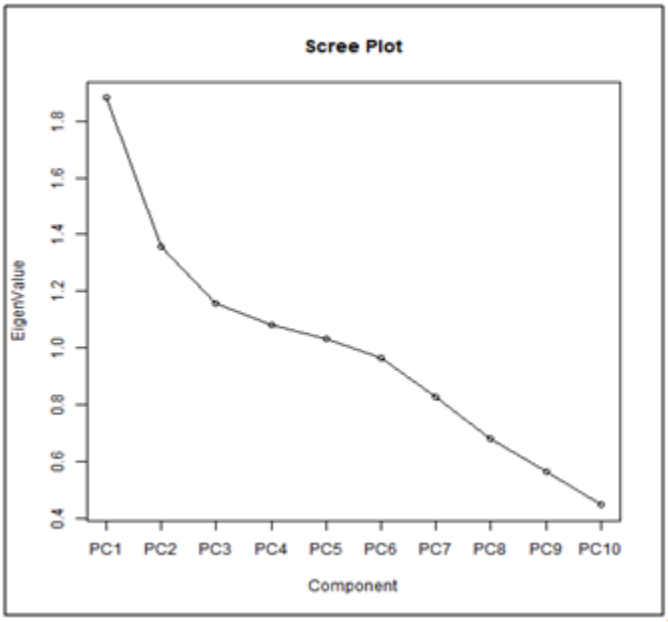

Figure 1: A cluster scree plot showing the contribution pattern of genetic diversity

3.5 Biplot

Biplot analysis is usually used to evaluate the components that have the greatest influence on genotypic variations. The highest value implies that the character has the most effect on the overall variations. The graph in the bi-plot indicates accessions (Figure 2). It describes the interaction of traits across genotypes [26]. Biplot may also be used to determine plant gene expression [27]. Furthermore, biplot analysis revealed the trait profiles of the genotypes, particularly those located far from the origin, and the results indicated a correlation between traits and genotypes. A positive correlation was represented by the acute angle between two traits, whereas a negative correlation was represented by the obtuse angle between two traits [26].

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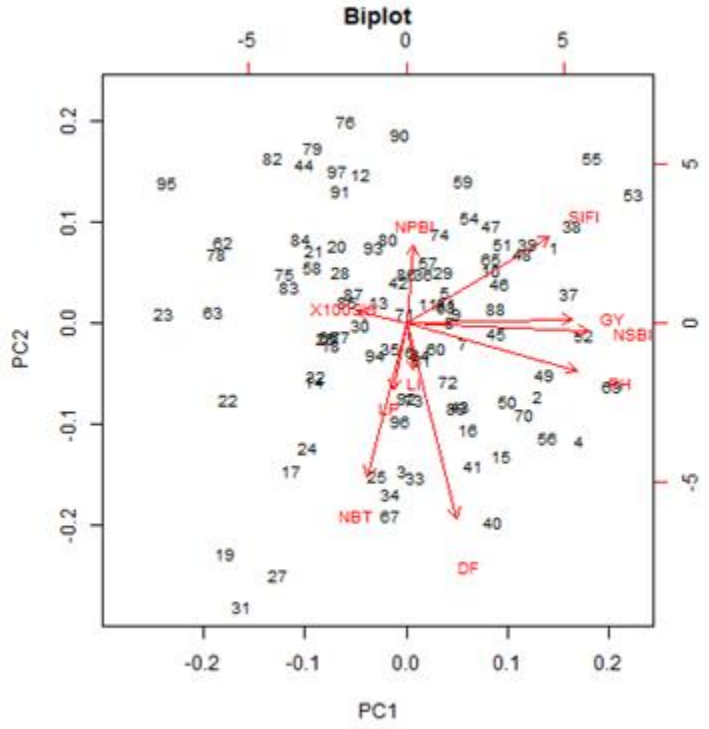

Figure 2: Formation of Biplot based on the PC1 and PC2 values

3.6 Cluster analysis

Among the multivariate techniques, cluster analysis has proven to be particularly useful in selecting genotypes for a breeding program that meets a plant breeder's objectives. This study used Ward's Hierarchical Clustering method. The studied germplasm was separated into five unique groups (Table 4). The dendrogram was used to represent the clustering pattern (Figure 3). Cluster V had the most accessions which consisted of 27 accessions, followed by cluster I (26) and cluster II (24).Cluster III included 14 accessions, while cluster IV contained 6accessions (Table 4). Similarly, Uddin *et al.* [5] identified VIII clusters from 119 proso millet genotypes. Patil *et al.* [28] investigated the genetic diversity of 65 finger millet accessions classified into five clusters. Based on quantitative features, 1312 foxtail millet germplasm was classified into 37 clusters [24].

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Table 4: Distribution of used proso millet accessions into five distinct clusters

Clusters	Number of accessions	Constituent accessions
Cluster I	26	BD-758, BD-762, BD-767, BD-769, BD-770, BD-771,BD-789, BD-790, BD-1338, BD-1395, BD-1400, BD-1411, BD-1412, BD-1414, BD-1421, BD-1422, BD-1423, BD-1424, BD-1425, BD-1431, BD-1433, BD-1435, BD-1437, BD-1438, BD-1442, BD-1447
Cluster II	24	BD-759, BD-760, BD-761, BD-763, BD-764, BD-765, BD-768, BD-772, BD-774, BD-795, BD-797, BD-1331,BD-1371,BD-1372, BD-1379, BD-1383, BD-1387, BD-1388,BD-1397, BD-1401, BD-1402, BD-1406, BD-1409, BD-1410
Cluster III	14	BD-766, BD-1365, BD-1366, BD-1370, BD-1384, BD-1385, BD-1386, BD-1390,BD-1391, BD-1394, BD-1396, BD-1398, BD-1407, BD-1416
Cluster IV	6	BD-775, BD-777, BD-784, BD-785, BD-787, BD-792
Cluster V	27	BD-776, BD-780, BD-781, BD-782, BD-783, BD-786,BD-791, BD-793, BD-1378, BD-1382, BD-1399, BD-1403, BD-1405, BD-1408, BD-1413, BD-1415, BD-1417, BD-1418, BD-1419, BD-1420, BD-1426, BD-1428, BD-1430, BD-1336, BD-1439, BD-1446, BD-1448

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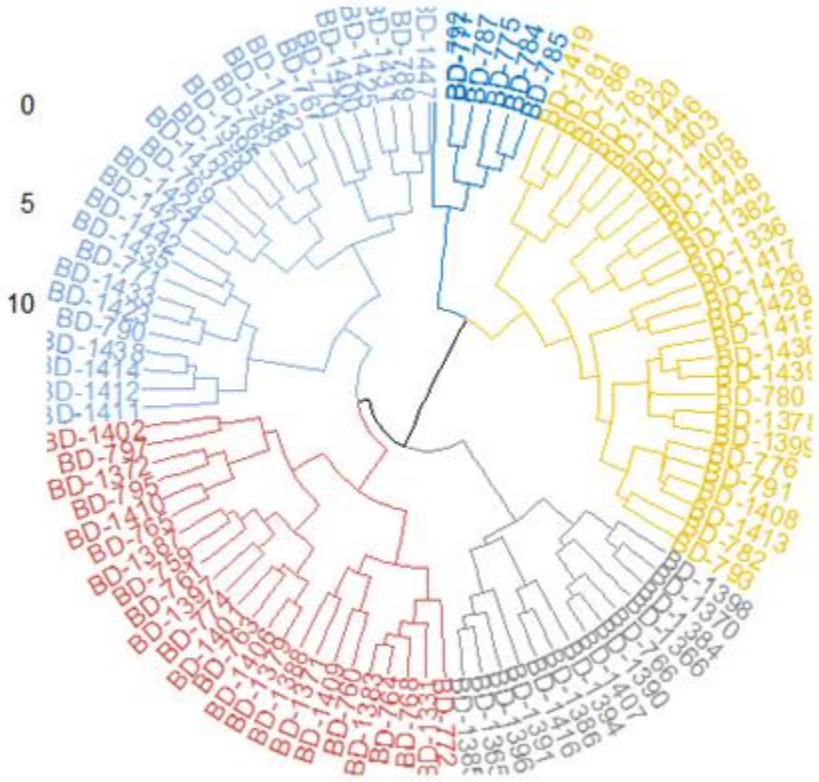

Figure 3: Dendrogram of proso millet accession based on quantitative characters obtained by cluster analysis while the colors; blue, yellow, ash, red, and light blue represented the groups cluster IV, V, III, II, and I, respectively.

Table 5 and Figure 4 show the intra and inter-cluster distances of five clusters. Cluster II had the greatest intra-cluster distance (6.77), followed by cluster V (6.47), cluster I (6.34), and cluster III (6.16), while cluster IV had the shortest intra-cluster distance (4.91), indicating that Genotypes are closely associated. On the other hand, clusters III and IV had the maximum inter-cluster distance (8.29) between them followed by clusters I and IV (8.25), clusters III and V (8.17), which revealed the highest degree of genetic divergence which could be used in the inter varietal breeding process to achieve high yielding recombinants. Cluster I and III had the shortest inter-cluster distance (6.82) followed by cluster I and II (6.92), and cluster II and cluster III (6.95), indicating that these cluster groupings were less divergent and that crossing between them could not produce strong offspring (F1 progenies). The inter-cluster distance varied from 6.82 to 8.29. Anyway, all inter-cluster distances were larger than intra-cluster distances, indicating more variation among the genotypes. In our study, the majority of used germplasm was conventional which showed a significant variation among them. The highest intra-cluster value was 6.77, while the largest inter-cluster value was 8.82, implying that different clusters' germplasm

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differed. Besides, it proposed that the genotypes are related to cluster pairs isolated by a significant inter-cluster distance as mentioned [29,30].

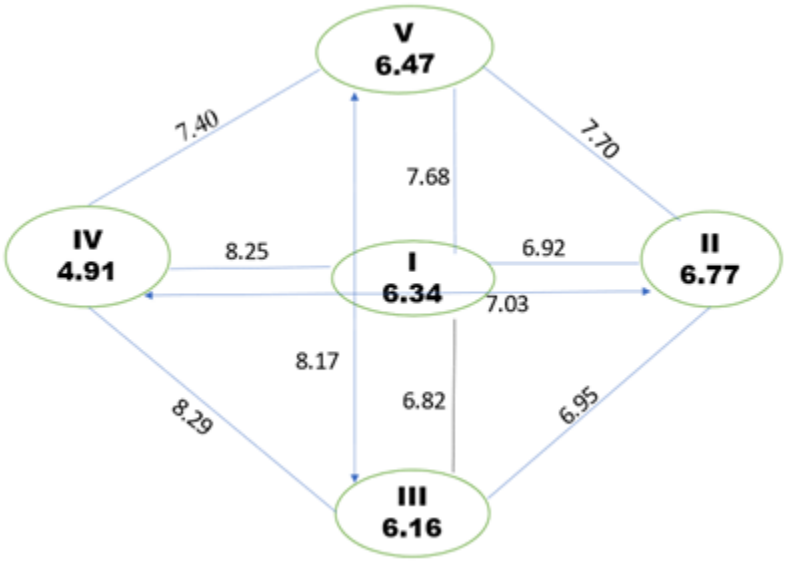

Figure 4: The diagram shows intra-cluster distance (circle value) and distance among the cluster members (between the circles)

Table 5: Intra and inter-cluster distances of proso millet accession

Cluster	I	II	III	IV	V
Cluster I	**6.34**				
Cluster II	6.92	**6.77**			
Cluster III	6.82	6.95	**6.16**		
Cluster IV	8.25	7.03	8.29	**4.91**	
Cluster V	7.68	7.70	8.17	7.40	**6.47**

Legend: Bold values are shown as intra-cluster distances while non-bold values are shown as inter-cluster distances

The cluster mean of the ten quantitative traits is presented in (Table 6). All the cluster mean showed differences among ten characters. Cluster I had the highest mean value for the number of primary inflorescence branches (6.24), followed by cluster III (6.16). In case of cluster II, the maximum mean value was found in the length of inflorescence (25.6 cm), followed by cluster I (25.3 cm) and cluster III (25 cm). The maximum mean value is also found in plant height (100.2 cm), which belongs to cluster II. The maximum mean values for grain yield m-2 (64.2 g), 1000 seed weight (5.7g), number of secondary inflorescence branches (34.9), and sheath length of flag leaf (9.63cm) were documented in cluster III. In addition, the maximum mean value was provided in cluster IV for the number of the basal tiller (19.8) and days to 50% flowering (68

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days) which indicates late flowering. On the contrary, cluster IV had the lowest average value for days to 50% flowering (55 days) which indicates earliness. Cluster V provided the maximum mean value for the length of the peduncle (18.5 cm) which was trailed by cluster I and II. Similarly, Wolie *et al.* [10] chose cluster mean as an appropriate genotype breeding tool because genotypes from various clusters can be hybridized to develop desired traits with increased heterotic potential [31].

Table 6: Cluster mean value of different characters of proso millet accessions

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Plant height(cm)	90.1	100.2	96.1	83.7	76.9
Number of the basal tiller	13.8	12.3	12.6	19.8	11.4
Sheath length of flag leaf (cm)	9.1	8.3	9.63	7.72	8.12
Length of the peduncle (cm)	15.0	15.2	14.5	14.5	18.5
Length of inflorescence (cm)	25.3	25.6	21.7	23.0	21.8
Number of primary inflorescence branches	6.24	5.98	6.16	5.67	5.10
Number of secondary inflorescence branches	26.2	28.8	34.9	21.7	19.8
Days to flowering 50%	58	67	59	68	55
1000 seed weight(g)	5.2	5.6	5.7	5.4	4.9
Grain yield m-2(g)	46.9	45.9	64.2	32.5	33.8

4. CONCLUSION

The germplasm of proso millet showeda wide range of variability. The PCA and cluster analysis identified suitable proso millet germplasm based on qualitative and quantitative traits. Most of the qualitative traits showed distinct variations among the accessions. The maximum variation was recorded in seed color, inflorescence shape, and degree of lodging while the accessions under PCA1 showed the positive contribution of traits in the proso millet germplasm. The mean value of cluster III was most suitable in terms of days to 50% flowering, number of secondary inflorescence branches, sheath length of flag leaf, plant height, and grain yield. The yield performance was superior in BD-761, BD-772, BD-1386, BD-1387, BD-1418, and BD-1336 while the accessions like; Bd-780, BD-783 and BD-1446showed early flowering. Moreover, the accessions of BD-1418 and BD-1336 were identified as the short-stature plant. However, the identified prominent germplasm with suitable traits could be utilized as a guideline for the next breeding program to improve the desired character as well as release the suitable proso millet variety.

5. ACKNOWLEDGEMENT

The authors are grateful to the Plant Genetic Resource Center (PGRC), Bangladesh Agricultural Research Institute (BARI), Gazipur, for donating the proso millet seed material. The authors also thank the Director General of BARI in Gazipur, Bangladesh for enhancing all the support to conduct the research.

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