Genetic diversity analysis of proso millet (*Panicum miliaceum* L.) in relation to phenotypic characters

M. Shalim Uddin[1], Md. Golam Azam[2*], Shamim Ara Bagum[3] and Md. Abdul Hakim[4]
[1]Plant Genetic Resource Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2]Plant breeding division, Bangladesh Agricultural Research Institute, Chattogram-4225, Bangladesh
[3]Seed technology division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[4]Plant breeding division, Bangladesh Wheat and Maize Research Institute, Dinajpur-5200, Bangladesh
[*]Corresponding authors email: kbdrashedbari@gmail.com, kbdrashed@bari.gov.bd

***Abstract*- Genetic divergence study is very essential for the selection of genetically diverse parents from existence germplasm for conducting successful hybridization program. An investigation with one hundred nineteen genotypes of proso millet was carried out in Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh to study the nature and magnitude of genetic divergence following Mahalanobis D^2 statistics. The experiment was accompanied following Randomized Completely Block Design (RCBD) which replicated thrice. The 119 genotypes of proso millet were assembled into eight clusters. Among the eight clusters, cluster VI was found to be largest having 27 germplasm followed by cluster V having 20 germplasm. While the minimum number of germplasms was observed in cluster II noted as 7. High degree of genetic diversity was revealed by the genotypes of cluster III and cluster IV. Cluster III was appropriate for filling period, height of plant, weight of seed per panicle, yield of straw /plant and yield of grain /plant. Cluster VIII is suitable for early flowering and short duration proso millet variety. Cluster III is best suited for the development of dwarf variety. Weight of seed/ panicle (g) and flag leaf area (cm2) contributed most towards genetic diversity of proso millet. Analysis confirmed the lack of association between geographic origin and hereditary assortment, as germplasm from the unalike area clustered into same groups and the germplasm of alike area were congregated into different clusters. Therefore, plant breeder should assess their material for genetic diversity and should not purely depend on their geographical origin.**

***Keywords* - Genetic distance; harvest index; flag leaf area; plant breeding; cluster group**.

I. INTRODUCTION

Proso millet (*Panicum miliaceum* L.) is the mankind's utmost antique cultivated crops. There are evidence that its farming was started in China before 5000 BC; from there it was transferred to India and through the Eurasian grasslands went to Eastern Europe about 4000 years before [1]. In defiance of its long history in farming, it has inadequate genetic representation. Proso millet normally is considered as an allotetraploid, in which there are 2n=4x=36 chromosomes [2]. There were some genotypes of proso which have 72 chromosomes [3]. It is a self-pollinated C_4 crop. Proso is a comparatively short-duration crop which water requirement is very low. Hence, it can be perfectly cultivated during dry, hot, and short summer seasons. The life cycle is about 60–100 days [4]. Proso millet grain contain about 58.1–77.9% starch of the whole grain mass [5]. In Bangladesh proso millet is cultivated in both Rabi and Kharif seasons in all sorts of soil as single or mixed crop. But the main area is mostly flood prone riverbeds and marginal lands. To get maximum output from marginal land having minimum fertility, proso millet can be considered as best suited crop than major cereal crops produces low outcome for sustaining agriculture and food security [6]. It can produce higher yield even under punitive growing environments such as India and Sub-Saharan and West Africa, particularly where typical rainfall is usually recorded as below 500 mm and having sandy and slightly acidic type soil [7]. Among the world's greatest vital cereal grains, the rank of millet is six, supporting over and above one-third of the world's inhabitants [7, 8]. The largest millet producers are Asian and African countries (Figure 1). Millions individuals in China, Japan, Africa, and India obtained their energy and protein from millets, and especially for the people who are breathing in hot and arid parts of the universe [6, 9]. The person attacked by coeliac disease usually use proso millets product in their diets. People are trying to utilize it as the basis to manufacture new food due to its high protein composition and some specific constituents which are beneficiary to health [10].

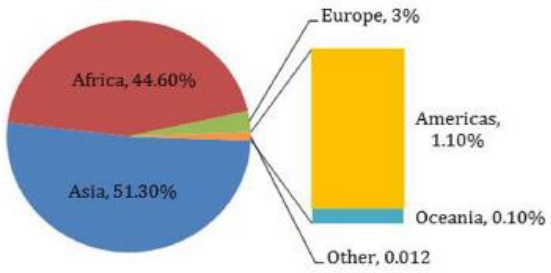

Figure 1: Worldwide millet production by region, 2014 [11].

Variability in plant genetic resources (PGR) offers chance for plant breeders to produce new and upgraded cultivars with desired traits. Natural genetic variability has been exposed within the crop species to fulfill existence food necessity from the very beginning of agriculture. Now it is being highlighted to produce excess food for continuous growing population of the world. Conservation biology is a science deal with the fortune of populations, which are distinct and recognized by their genetic constituency. This unique genetic constitution not only differentiates them from other populations, but also documented their ability to acclimate to changing environments

and, potentially, to yield new species. Many conservationists would debate that the preservation of genetic diversity is the foundational basis of all conservation labors because genetic diversity is obligatory for evolutionary acclimatization, is the key to the long period existence of any species The selection of suitable diverse parents in order to get huge frequency of transgressive segregants is the prerequisite for the successfully completion of a hybridization program for many crops including proso millet. The proso millet becomes more versatile due to its acclimatization to a varied range of geographical region and agro-ecological diversity. Besides the accessibility of genetic assets, their characterization is important for effective exploitation in crop development programs especially for quality improvement [12]. Extensive genetic corrosion is triggered due to the use of modern cultivars and hybrids [13] or by a lessening in crop farming. Thus, the conservation of germplasm is very important to expand the genetic basis of crop. It would be more effective if the selection of desire plant types was done on the basis of divergence analysis. Therefore, the present study was conducted to determine the variability/genetic divergence among the prosomillet germplasms or lines which can be used in plant breeding program.

II. Materials and Methods

The experiment was conducted at Bangladesh Agricultural Research Institute (BARI), Gazipur to evaluate genetic diversity of 119 genotypes of proso millet. Proso millets germplasm were collected from Plant Genetic Resources Center, BARI, Gazipur-1701. The experimental site is located at the center of Modhupur Tract, AEZ-28 ($24^0$29 N latitude and $90^0$26 E longitude) having an altitude of 8.2 m from the ocean level. The climatic condition of the experimental plot was subtropical in nature characterized by heavy rainfall from June to September and scarcity of water in winter and mean rainfall is around 2200 mm per year. The soil was silty clay loam in nature and pH 6.5 in the surface. The experiment was accompanied following Randomized Completely Block Design (RCBD) which replicated thrice. The experiment was conducted in the plot having 3 m x 2 m size. Seeds were cured with the help of Provex @ 2g/kg to eradicate seed borne diseases. Treated seeds were continuously sown in a line and the line was 30 cm apart from each. Thinning of plant was completed maintaining 6 cm distance from each other after few days of plant germination. N-P-K fertilizers were applied at the rate of 45-30-20 kg/ha respectively. The half of the urea and all other fertilizers were provided during final land preparation. After thinning of plant the half of the residual part of urea was given to the land. The remaining part of the urea was applied at seedling stage before flowering. The land was irrigated each time of fertilizer application. Hand weeding was practiced to reduce the weed infestation at seedling stage. The growth and yield subsidizing data were recorded from five randomly selected plant. Data were documented on 50% flowering days, maturity days, filling period, height of plant (cm), no. of tiller/plant, flag leaf area (cm^2), panicle length (cm), weight of seeds/ panicle (g), yield of straw/ plant (g), harvest index, yield of grain/plant (g).

Flag leaf area was estimated by succeeding formula [14].

1) Flag leaf area (cm^2) = flag leaf length (cm) x flag leaf width (cm)

Harvest index was obtained from the formula [15].

2) $$HI = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

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

The records were subjected to analysis via Genstat 4.2 statistical package.

III. Results and Discussion

Significant difference was found for all the traits from the analysis of variance indicates that there was a considerable amount of hereditary variability among the genotypes. Therefore diversity analysis was performed to find out the diversity of the genotypes. One hundred nineteen germplasms were grouped into eight different clusters. The distributions of germplasm are presented in Table 1. Among the eight clusters, cluster VI was found to be largest having 27 germplasm followed by cluster V having 20 germplasm. While the minimum number of germplasms was observed in cluster II noted as 7. Genotypes within the same cluster have less genetic diversity. Hence breeding would be rewarding if crossing is performed between the germplasm of different clusters rather than same cluster. Different researchers grouped genotypes of different crops into different clusters such as in prosomillet [16], little millet [17], foxtail millet [18, 19]. The clustering design disclosed that germplasms of dissimilar topographical parts were categorized in one group and also the genotypes of identical topographical zone were not only assembled into similar cluster but also in different cluster signifying that there was no recognized association between topographical divergence and genetic divergence. Similar investigation based on D^2 statistic was also accomplished by different contributors in different crops [20, 21, 22]. The genetic drift and selection in diverse location could produce broader diversity than geographical distance [23].

The intra and intercluster distance of eight clusters are presented in Table 2. The maximum intra cluster distance was attained from cluster IV tailed by cluster VI and cluster II demonstrating variances in genotypes inward cluster. Slightest intra cluster distance was obtained from cluster VIII and I representing that the genotypes are closely related to each other. The genotypes in cluster III and cluster IV due to highest inter cluster distance between them, revealed maximum degree of genetic divergence and thus could be utilized under inter varietal breeding process (transgressive breeding) for attaining

high yielding recombinants. Similar results can be found if cross is accompanied between genotype in cluster III and VI, cluster III and VII, cluster II and IV, cluster I and IV, cluster III and V and cluster IV and VI. Many researchers opined that plant of most dissimilar cluster may be utilized as parental materials in crossing programme to produce cultivars capable of producing more yield [21, 22].The minimum inter cluster distance was found between cluster VI and VIII followed by cluster IV and VIII presenting these cluster groups were relatively less diverge in nature and crossing between them cannot yield strong offspring (F1 progenies). Similar statement was documented by several researchers [24, 25, 26].

The cluster means of the various characters are exhibited in Table 3. Differences in cluster means was found mostly from all the traits. Cluster I had the maximum mean values for number of tiller per plant. Cluster II and cluster VII had higher means values for 50% flowering days. High mean values for filling period, height of plant, weight of seed per panicle, yield of straw per plant and grain yield per plant were documented in cluster V. Cluster VII provided highest mean value for flag leaf area (cm^2) and panicle length (cm). Highest mean value for harvest index obtained from cluster IV. Cluster VIII is suitable for early flowering and short duration proso millet variety. Cluster III is best suited for the development of dwarf variety. Therefore, these group of plants may be preferred for transporting the desirable qualities through hybridization technique. Genotypes selection should be done based on cluster mean for the good bleeding of genetic potential [22].

Relative involvement of traits to the total divergence in proso millets was presented in table 4. Among the studied characters weight of seed/ panicle contributed most towards the genetic divergence followed by flag leaf area. The minimum contribution towards the genetic divergence was recorded from yield of grain/ plant followed by height of plant. Grain yield plant-1, 1000 grain weight, productive tillers plant-1 and 50 percent flowering days subsidized towards the genetic divergence in proso millet [27]. Fifty percent flowering days and yield of grain per plant contributed maximum towards genetic divergence in finger millet [28].

Table 4. Relative contribution of the 11 characters to the total divergence in proso millets

Sl. no.	Characters	Vector I	Vector II
1	50% flowering days	0.04	-0.27
2	Maturity days	0.05	-0.13
3	Filling period	-0.01	0.23
4	Height of plant (cm)	-0.30	-0.02
5	No. of tiller/plant	0.06	-0.05
6	Flag leaf area (cm^2)	0.01	0.01
7	Panicle length (cm)	-0.07	-0.05
8	Weight of Seed/panicle (g)	0.03	0.02
9	Yield of straw/plant (g)	0.09	-0.02
10	Harvest index	7.05	-2.78
11	Yield of grain /plant	-0.43	-0.07

IV. CONCLUSION

Germplasms from cluster III and cluster IV can be selected for plant breeding program as they displayed high degree of genetic diversity. Genotypes from cluster III can be used in breeding to get desired characters such as height of plant, weight of seed per panicle, yield of straw per plant and yield of per plant. Cluster VIII is suitable for early flowering and short duration proso millet variety. Cluster III is best suited for the development of dwarf variety. In this study it was found that weight of seed/ panicle (g) and flag leaf area (cm^2) contributed most towards genetic diversity of proso millet. However this study can be used as a guideline for future breeding program to release prosomillet variety.

ACKNOWLEDGMENT

We are indebted to the Plant Genetic Resource Center, BARI, Gazipur for providing the seed material o of Proso millet. Our gratitudes are also extended to Director General, BARI, Gazipur, Bangladesh for enhancing the all types of logistic help to conduct the research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

[1] Lágler, R., G. Gyulai, M. Humphreys, Z. Szabó, L. Horváth, A. Bittsánszky and L. Heszky. (2005). Morphological and molecular analysis of common millet (P. miliaceum) cultivars compared to an aDNA sample from the 15th century (Hungary). Euphytica, 146(1-2):77-85.

[2] Zeller, F. J. (2000). Utilization, genetics and breeding of small-seeded Proso millets (Panicum miliaceum L.). Journal of Applied Botany, 74(5/6):182-186.

[3] Wanous, M. K. (1990). Origin, taxonomy and ploidy of the millets and minor cereals. Plant Varieties & Seeds, 3(2):99-112.

[4] Baltensperger, D. D. (2002). Progress with proso, pearl and other millets. Trends in new crops and new uses, 100-103.

[5] Yang, Q., P. Zhang, Y. Qu, X. Gao, J. Liang, P. Yang and B. Feng.(2018). Comparison of physicochemical properties and cooking edibility of waxy and non-waxy proso millet (Panicum miliaceum L.). Food chemistry, 257: 271-278.

[6] Amadou, I., M. E. Gounga and G. W. Le.(2013). Millets: Nutritional composition, some health benefits and processing-A review. Emirates Journal of Food and Agriculture, 501-508.

[7] Changmei, S. and J. Dorothy. (2014). Millet-the frugal grain. International Journal of Scientific Research and Reviews, 3(4):75-90.

[8] Verma, V. and S. Patel. (2012). Nutritional security and value added products from finger millets (ragi). Journal of Applicable Chemistry, 1(4): 485-489.

Table 1: Distribution of 119 entries of proso millet into nine clusters

Clusters	No. of entries	Genotypes with original place of collection
I	13	PMB-10, PMB-11, PMB -19, PMB -20, PMB -22, PMB -52, PMB -70, PMB -86, PMB -89, PMB -121, PMB -157, PMB -160, PMB -259
II	7	PMB-454, PMB-48, PMB-54, PMB-55, PMB-161, PMB-216, PMB-273
III	8	PMB-7, PMB-43, PMB-56, PMB-58, PMB-59, PMB-123, PMB-124, PMB 258
IV	11	PMB-51, PMB 62, PMB 64, PMB-65, PMB-67, PMB-77, PMB-80, PMB-91, PMB-130, PMB-166, PMB-8403
V	20	PMB-42, PMB-69, PMB-101, PMB-102, PMB-104, PMB-106, PMB-108, PMB-134, PMB-139, PMB-141, PMB-142, PMB-146, PMB-147, PMB-149, PMB-170, PMB-208, PMB-250, PMB-251, PMB-279, PMB-285
VI	27	PMB-6, PMB-30, PMB-31, PMB-34, PMB-44, PMB-49, PMB-68, PMB-71, PMB-72, PMB-76, PMB-79, PMB-90, PMB-93, PMB-96, PMB-113, PMB-114, PMB-117, PMB-119, PMB-125, PMB-128, PMB-266, PMB-275, PMB-276, PMB-278, PMB-284, PMB-293, PMB-294
VII	14	PMB-32, PMB-39, PMB-49, PMB-109, PMB-140 PMB-63, PMB-122, PMB-132, PMB-143, PMB-151, PMB-164, PMB-249 , PMB-287, PMB-2532
VIII	19	PMB-2, PMB-92, PMB-100, PMB-110, PMB-129, PMB-135, PMB-167, PMB-169, PMB-186, PMB-189, PMB-252, PMB-281, PMB-282, PMB-283, PMB-286, PMB-288, PMB-289, PMB-290, PMB-292

Table 2: Inter cluster distance (D^2) in 119 genotypes of Proso millet

0Cluster	I	II	III	IV	V	VI	VII	VIII
I	**1.40**							
II	3.97	**1.70**						
III	3.13	4.55	**1.65**					
IV	7.41	8.53	11.30	**1.90**				
V	3.21	4.50	7.18	3.18	**1.48**			
VI	6.70	5.79	10.45	7.20	4.82	**1.78**		
VII	5.34	7.50	8.84	4.45	3.16	3.10	**1.58**	
VIII	4.07	6.55	7.45	3.07	4.33	2.56	6.44	**1.40**

Table 3: Cluster mean values for yield related traits of proso millets

Cluster no.	50% flowering days	Maturity	Filing period	Height of plant (cm)	No. of tiller/ Plant
I	70	110	50	5812	10.92
II	77	110	43	58.29	7.86
III	69	106	48	46.38	8.00
IV	67	106	49	60.00	5.82
V	68	109	51	78.35	8.00
VI	71	109	49	67.76	7.48
VII	77	109	42	75.19	10.40
VIII	64	103	50	70.42	7.42

Table 3: continued

Cluster no.	Flag leaf Area (cm^2)	Panicle length (cm)	Wt. of Seed/ panicle (g)	Yield of straw/ Plant	HI (%)	Yield of grain /plant (g)
I	10.81	20.23	1.06	6.42	51	6.56
II	12.85	21.00	1.21	6.37	50	5.72
III	8.71	17.56	1.36	3.74	52	4.37
IV	11.39	21.50	1.33	3.68	57	4.76
V	14.59	23.74	1.65	10.98	49	10.48
VI	13.00	21.96	1.26	5.70	56	7.12
VII	14.97	24.85	1.31	8.10	50	7.90
VIII	13.69	23.21	1.37	6.31	56	8.09

[9] Rachie, K. O. (1975). The millets: importance, utilization and outlook. Hyderabad: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

[10]Kalino0vá, J. (2007). Nutritionally important components of proso millet (Panicum miliaceum L.). Food, 1:91-100.

[11]Habiyaremye, C., J. B. Matanguihan, J. D'Alpoim Guedes, G. M. Ganjyal, M. R. Whiteman, K. K. Kidwell and K. M. Murphy.(2017). Proso millet (Panicum miliaceum L.) and its potential for cultivation in the Pacific Northwest, US: A review. Frontiers in plant science, 7:1961.

[12]Patel, S.N., H. E. Patil, H.M. Modi and T.J. Singh. (2018). Genetic Variability Study in Little Millet (Panicum miliare L.) Genotypes in Relation to Yield and Quality Traits. International Journal of Current Microbiology and Applied Sciences, 7(6): 2712-2725.

[13]Villa,T.C.C., N. Maxted, M. Scholten and B. Ford-Lloyd .(2005). Defining and identifying crop landraces. Plant Genetic Resources, 3(3): 373-384.

[14]Mokhtarpour, H., C. B. Teh, G. Saleh, A. B. Selamat, M. E., Asadi and B. Kamkar .(2010). Non-destructive estimation of maize leaf area, fresh weight, and dry weight using leaf length and leaf width. Communications in Biometry and Crop Science, 5(1): 19-26.

[15]Hühn, M. (1990). Comments on the calculation of mean harvest indices. Journal of Agronomy and Crop Science, 165(2-3): 86-93.

[16]Akhtar, A., V. Kumar and B. C. Saha.(1996). Genetic divergence in proso millet (Panicum miliaceum L.). Journal of Applied Bilology, 6(2):9-11.

[17]Arunachalam, V., R. Rengalakshmi and M. K. Raj. (2005). Ecological stability of genetic diversity among landraces of little millet (Panicum sumatrense) in south India. Genetic Resources and Crop Evolution, 52(1): 15-19.

[18]Nirmalakumari, A. and M. Vetriventhan. (2010). Characterization of foxtail millet germplasm collections for yield contributing traits. Electronic Journal of Plant Breeding, 1(2):140-147.0

[19]Shingane, S. N., (2012). Assessment of genetic and molecular diversity for different trait in foxtail millet. Ph.D. Theis, Mahatma Phule Krishi Vidyapeeth, Rahuri.

[20]Dhanapal, A. P., B. Selvi and M. Govindaraj. (2008). Variability and multivariate analysis in Finger Millet (Eleusine coracana) germplasm for yield characters. Crop Research, 36: 218-223.

[21]Dinesh, K., T. Vikrant, B. B. Ramesh and P. Sukram. (2010). Genetic Diversity in Finger Millet (Eleusinecoracana L.). Crop Improvement, 37(1): 25-28.0

[22]Wolie, A., T. Dessalegn and K. Belete. (2013). Heritability, variance components and genetic advance of some yield and yield related traits in Ethiopian collections of finger millet (Eleusine coracana (L.) Gaertn.) genotypes. African Journal of Biotechnology, 12(36).

[23]Patel, J. and N. Patel. (2012). Genetic divergence in Vigna radiata (L.). Life Sciences and leaflets, 33-57.

[24]Karad, S. R., J. V. Patil and A. A. Kale. (2013). Study of genetic diversity in finger millet (Elesuine coracana L.) genotypes using RAPD markers. International Journal of Integrative Sciences, Innovationa and Technology, 2(4):31-36.

[25]Daniel, H., M. Mebrahtom and G. Tsige. (2011). Genetic divergence analysis on some bread wheat genotypes grown in Ethiopia. Journal of Central Europien Agriculture, 12(2): 344-352.

[26]Kahrizi, D., M. Maniee, R. Mohammadi and K. Cheghamirza. (2010). Estimation of genetic parameters related to morpho-agronomic traits of Durum Wheat (Triticum turgidum var. durum). Biharean Biologist, 4(2):93-97.

[27]Verulkar, K. K., L. C. Mohotkar and S. R. Karad. (2014). Genetic diversity studies in proso millet (Panicum miliaceum L.). Journal of Agriculture Research and Technology, 39(2): 228-232.

[28] Kumari, S. and S. K. Singh. (2015). Assesment of genetic diversity in promising finger millet Eleusine coracana (L.) Gaertn] genotypes. The bioscan, 10(2): 825-83.