Madras Agric. J., 2020; doi:10.29321/MAJ.2020.000358

RESEARCH ARTICLE

Genetic Diversity Analysis in Barnyard Millet Germplasm (*Echinochloa frumentacea* L.)

Vanniarajan C* and Chandirakala R
*Department of Plant Breeding & Genetics, AC & RI,Tamil Nadu Agricultural University, Madurai – 625 104.

Received : 07th June, 2020
Revised : 15th June, 2020
Accepted : 28th June, 2020

ABSTRACT

A study was conducted to evaluate the genetic diversity studies among fifty-three genotypes of Barnyard millet in Randomized Block Design with two replications. Diversity analysis for qualitative traits by Dice Similarity Co-efficient and UPGMA method grouped the genotypes into eight clusters indicating the presence of enough diversity among the genotypes. The clustering of the genotypes based on the quantitative characters was done by using the D^2 method. Out of 12 clusters formed, the maximum inter-cluster distance was found between cluster V and cluster VIII, indicating high diversity between the genotypes present in these two clusters. Cluster VIII included the genotypes, which showed significantly higher mean for grain yield and maximum value for many of the yield contributing traits and grain yield/ plant. The clustering pattern also indicated that the genotypes are grouped irrespective of their geographical origin.

Keywords: *Barnyard millet; Genetic diversity and Metroglyph analysis*

INTRODUCTION

Among the millets, Barnyard millet (*Echinochloa* sp.) is the fastest growing millet and produces a crop in 6 weeks from sowing to maturity. The plant has attracted some attention as a fodder crop in the United States and Japan (Nirmalakumari and Vetriventhan, 2009). Barnyard millet is nutritious and is an appropriate food for patients intolerant to gluten, causing celiac disease. Barnyard millet is rich in nutrients and essential amino acids. The protein, calcium, and iron content of the *Echinochloa* spp. is found to be comparable to or greater than that of major cereals (Mandelbaum *et al*., 1995). The flour is mixed with other minor millet flours and used as unfortified weaning (UW) mix for the malnourished infants (Anju Thathola *et al*., 2002). The crop is less susceptible to pests and diseases and is a valuable fodder plant. The wide distribution of the crop across the world indicates the presence of greater diversity in the crop. The assessment and utilization of this genetic variability are very much essential in plant breeding. So understanding the pattern of diversity and the genetic structure of gene pools is critical for effective management and the use of germplasm resources. Progress in plant breeding depends on the identification of new sources of genetic variation for beneficial traits in such a way that a combination of alleles produces progenies with superior performance. Diversity is in both qualitative and quantitative characters will have a direct or indirect effect on the yield of the crop. In the present study, the genetic diversity existing in the fifty-three genotypes, including checks, were analyzed based on both qualitative and quantitative traits.

MATERIAL AND METHODS

Fifty genotypes were evaluated for the study, among which 14 were collected from Coimbatore, 10 from Madurai, and 26 from Bengaluru. Two National checks *viz.,* VL172 and VL29 were used along with Co(kV) 2 as local check-in Randomized Block Design (RBD) with two replications. The list of genotypes used in the study is given in Table 1. Sowing was done with a spacing of 30 x 10cm with a row length of 3m. The data on sixteen quantitative traits and fifteen qualitative traits were recorded based on the descriptors of barnyard millet (Bioversity International, 1983). Fifteen quantitative characters were taken from five uniform plants, selected from each of the replication. Observations on three physiological characters and four biochemical characters were recorded on five plants chosen randomly. Genetic diversity based on qualitative traits was studied based on nine multistate characters. These multistate traits were converted into binary codes by the additive coding method of Sneath and Sokal (1973) and were subjected to cluster analysis based on Dice's (1945) similarity coefficient and Un-weighted Paired Group Arithmetic Average (UPGMA) clustering method. Genetic diversity based on quantitative traits was done using

*Corresponding author's e-mail: vanniarajan.c@tnau.ac.in

D^2 analysis by Mahalanobis (1936). For determining the group constellation, a relatively simple criterion by Rao (1952) was followed.

RESULTS AND DISCUSSION

The diversity in germplasm collection is essential for both plant breeders and germplasm curators to optimize the use of the variability available (Ekta Sharma *et al*., 2014).

Genetic diversity for qualitative traits

Qualitative traits are widely preferred for the characterization of the germplasm because they are relatively less influenced by the environment, unlike that of quantitative phenotypic traits. They form discrete phenotypic classes and therefore are a highly useful tool in classifying germplasm and can be predominantly assessed visually and by novice evaluators. Grouping of genotypes to different clusters based on the Dice Similarity Co-efficient and UPGMA method for qualitative traits revealed that the genotypes were grouped into seven different clusters (Table 2).

A maximum number of genotypes (16) were grouped in cluster V. The genotypes of this cluster had medium culm branching, slender shaped lower raceme, erect growth habit, and straw white to grey+ straw white grain color. In contrast, cluster III had eleven genotypes that had the phenotype like medium culm branching, pigmented cylindrical compact inflorescence, erect growth habit, light grey to grey colored seed.

The accessions *viz.,* ACM 313, and ACM 295 were separately grouped in clusters II and IV, which may be due to its distinct phenotypic appearance. The genotype ACM 313 had pigmentation on the stem, leaf and inflorescence, medium culm branching (upper two to four nodes produce inflorescences), cylindrical-shaped, open type panicle with straight lower raceme. The culture *viz.,* ACM 295 had erect growth habits with high culm branching. The plant was non pigmented with cylindrical compact inflorescence and had light grey colored seeds. Genotypes in cluster VI and VII had low culm branching (upper one to four nodes rarely branched) with pigmented inflorescence, while cluster VIII had open type panicle with medium culm branching. Most of the high yielding genotypes were found to be grouped under cluster I, all of which had non pigmented compact inflorescence with erect growth habit and medium culm branching. Cluster mean values for different characters in genotypes based on D^2 values are given in Table 3.

The abundance of erect growth habits may be due to the selection of these types by farmers for ease of spotting the weed under the crop and other management practices (Upadhaya *et al*., 2007).

Table 1. List of genotypes used in the study

Sl. No.	Genotypes	
1	ACM 294	
2	ACM 295	
3	ACM 296	
4	ACM313	
5	ACM331	
6	ACM333	
7	MA1	Madurai (10)
8	MA10	
9	ACM 334	
10	ACM335	
11	TNEF192	
12	TNEF193	
13	TNEF196	
14	TNEF197	
15	TNEF198	
16	TNEF199	
17	TNEF200	
18	TNEF201	
19	TNEF202	
20	TNEF203	
21	TNEF204	Coimbatore (14)
22	TNEF205	
23	TNEF206	
24	TNEF207	
25	GECH1	
26	GECH3	
27	GECH5	
28	GECH6	
29	GECH8	
30	GECH10	
31	GECH12	
32	GECH13	
33	GECH15	
34	GECH16	
35	GECH18	
36	GECH25	
37	GECH27	
38	GECH525	
39	GECH759	
40	GECH440	
41	GECH403	
42	GECH41	Bengaluru (26)
43	GECH204	
44	GECH209	
45	GECH426	
46	GECH351	
47	GECH758	
48	GECH768	
49	GECH746	
50	GECH779	
51	VL 172	National Check (2)
52	VL29	
53	CO2	Local Check (1)

Similarly, the majority of the genotypes had cylindrical-shaped, compact inflorescence with medium culm branching. The difference observed across locations, genetic variation among genotypes collected, and preferential adaptation of species can

be influenced by altitude gradients that comprise of an assemblage of environmental factors such as climatic and other edaphic factors (Ohsawa and Ide, 2008).

The above information about qualitative character distribution in barnyard millet germplasm will have an idea to assist in selecting high yielding genotypes. Diversity based on qualitative characters is not given much importance when the aim of the breeding program is for yield improvement. Hence diversity based on quantitative characters was thought as a feature of productivity that could be measured quantitatively (Heng-Shing Lin *et al.,* 2012).

Table 2. Distribution of 53 genotypes into different clusters for qualitative characters

Cluster	Number of genotypes	List of genotypes
C I	10	ACM294, MA10, ACM296, ACM333, ACM334, ACM335, GECH758 ,VL 172, VL29, GECH41
C II	1	ACM 295
C III	11	ACM295, ACM331, TNEF197 TNEF203, TNEF205 GECH13, GECH15, GECH16, GECH18, GECH440, GECH41, GECH768, GECH779
C IV	1	ACM313
C V	16	TNEF192, TNEF193, TNEF196, TNEF198, TNEF199, TNEF200, TNEF206, TNEF207, GECH3, GECH5, GECH10, GECH759, GECH204, GECH746, GECH1, GECH6
C VI	6	MA1, TNEF201, TNEF204, GECH12, GECH25, GECH209
C VII	6	GECH27, GECH525, GECH403, GECH426, GECH351, CO2
C VIII	2	TNEF202, GECH8

Genetic diversity for quantitative traits

D2 statistics, a concept developed by Mahalanobis (1936) is an important tool to plant breeders to classify the genotypes into different groups based on the genetic divergence between them. The basic idea behind the formation of clusters is to get the intra and inter-cluster distances, which serve as the index for the selection of genetically divergent parents for hybridization programs.

Based on D^2 analysis, fifty-three genotypes were grouped into twelve clusters indicating wide diversity in the experimental material for the majority of the characters (Table 4). Cluster X had the maximum number of twenty-six genotypes, while cluster II, III, IV, V, VI, XI, and XII had two genotypes each. The genotypes in each cluster were found to be similar for some of the phenotypic and quantitative traits.

Table 3. Cluster mean values for different characters in barnyard millet genotypes based on D^2 values

CLUSTERS	DFF	PHT	FLL	FLW	LOI	PAB	LLR-R	LLR-L	NOR	LOP	NON	STG	NOT	TWT	FYD	GYD
C I	**68.00**	148.90	**28.71**	3.04	19.61	3.65	2.58	**1.86**	56.36	1.66	**9.62**	3.71	9.00	2.81	35.85	24.57
C II	54.00	141.40	23.10	2.50	22.40	4.60	3.10	2.80	47.50	1.65	7.65	2.50	5.50	2.90	17.29	9.58
C III	52.00	138.83	22.10	2.35	19.70	3.80	2.55	2.15	37.50	2.10	8.00	2.75	5.25	2.90	19.56	11.10
C IV	55.00	119.90	21.30	2.00	16.10	3.85	**2.50**	2.05	35.00	1.95	6.05	1.20	5.50	3.35	15.98	8.38
C V	54.00	115.45	21. 35	2.25	16.20	**5.50**	3.15	**2.85**	**32.33**	4.15	**5.40**	**0.80**	4.50	3.07	18.27	9.50
C VI	**51.00**	120. 46	21.40	2.65	20.25	4.80	3.15	2.65	46.20	4.40	6.30	1.50	5.00	2.85	23.89	12.25
C VII	54.25	127.02	21.10	2.31	18.07	**2.91**	2.50	2.12	41.45	3.77	7.97	2.04	**9.37**	2.90	19.91	11.78
C VIII	62.25	**154.69**	28.55	2.87	22.22	3.72	2.80	2.17	47.04	1.70	8.80	2.95	6.62	3.15	41.45	26.23
C IX	55.00	136.27	23.85	2.90	20.90	4.58	3.15	2.75	**58.83**	**1.55**	7.30	**3.84**	4.75	**3.45**	**45.32**	**29.15**
C X	53.26	132.54	21.74	2.36	19.41	4.20	2.90	2.32	43.13	3.12	6.91	2.14	5.56	2.99	22.18	12.20
CXI	52.00	151.27	23.93	**3.26**	**22.66**	4.10	3.00	2.56	53.00	2.70	7.30	3.73	**4.33**	3.26	41.46	26.40
C XII	**51.00**	**118.15**	**20.40**	**1.65**	**15.90**	3.95	**3.20**	2.50	33.87	**4.60**	6.80	1.00	9.00	**2.50**	**13.77**	**7.410**

The number bolded are highest and lowest cluster mean values.

Cluster I had genotypes that scored significantly higher mean value than the grand mean for single plant yield. These genotypes were also found to be the late-maturing type, which had taken more days for 50% plants to flower. Late maturing genotypes recorded high mean single plant yield. This finding was supported by Arunachalam and Vanniarajan (2012). The genotypes also had higher mean value for yield contributing traits like plant height (134.5-161.4cm), flag leaf length (27.2-29.6), flag leaf width (2.8-3.5), stem girth (31.-4.31), fodder yield (39.5-53.4), harvest index and relative water content.

Cluster II, VI, and XII included the genotypes which had significantly higher mean value for traits like panicle breadth, length of lower raceme right, and lower raceme left, which were non-significantly correlated with grain yield. Consequently, the grain yield/plant of the genotypes was found to be very meager. On the contrary, the genotypes in the later cluster had a higher percentage of relative

water content. Cluster III contained two genotypes from different locations, which had very poor yield contributing traits performance and thus the grain yield. On the contrary, the proline content of these genotypes was significantly higher, which indicated the ability of these genotypes to tolerate the drought and other abiotic stresses.

Table 4. Inter and intra D square cluster distances

	C I	C II	C III	C IV	C V	C VI	CVII	C VIII	C IX	CX	C XI	CXII
C I	14.195	29.140	23.953	28.395	36.506	28.291	26.666	21.230	27.396	29.041	24.409	33.273
C II		**7.971**	**12.398**	14.557	19.009	17.207	27.560	35.951	37.197	21.345	34.567	21.956
C III			8.309	13.069	22.320	16.512	22.668	30.371	31.962	19.711	29.216	20.509
C IV				8.395	17.005	15.994	24.153	33.852	34.929	20.454	33.973	18.267
C V					8.435	14.222	32.806	**40.619**	39.039	24.278	38.131	22.367
C VI						9.518	26.073	31.974	30.029	19.891	28.403	19.253
C VII							**27.503**	32.162	36.007	27.901	33.803	24.661
C VIII								24.388	22.860	33.577	22.437	38.069
C IX									11.246	33.831	15.971	39.602
C X										25.192	32.187	24.411
CXI											18.722	38.410
C XII												17.545

The number bolded are highest and lowest inter and intra cluster distances

Cluster IV included the genotypes with early maturity, high test weight, and high relative water content, while genotypes in cluster V were also early maturing containing a significant amount of relative water content and the highest amount of proline. The grain yield of the genotypes in these two clusters is very low. Cluster VII included the genotype recording maximum values for traits like peduncle length, number of tillers, and minimum values for inflorescence length, panicle breadth, and number of the raceme.

Table 5. Distribution of 53 barnyard millet genotypes into different clusters based on D^2 values for quantitative traits

Cluster	Number of genotypes	Name of the genotypes
C I	5	ACM294, ACM 295, ACM 296, ACM 334, MA10
C II	2	GECH6, GECH41
C III	2	TNEF205,GECH16
C IV	2	TNEF202, GECH440
C V	2	TNEF201, TNEF 207
C VI	2	TNEF200, GECH 10
C VII	4	ACM 313,ACM331, GECH5, GECH204
C VIII	4	ACM333, MA 10, GECH403, GECH426
C IX	2	Co2, GECH 758
C X	23	ACM335, TNEF204, TNEF203, TNEF199, TNEF198, TNEF192, TNEF193, TNEF196, TNEF197, TNEF206, GECH 1, GECH 8, GECH 3, GECH12, GECH13, GECH15, GECH18, GECH25, GECH27, GECH525, GECH759 , GECH 209, GECH351
C XI	3	GECH768, VL172, VL29
C XII	2	GECH746, GECH779

Cluster VIII included the genotypes, which showed significantly higher mean for grain yield and maximum value for many of the yield contributing traits and grain yield/plant. All these genotypes recorded a high number of days for 50% flowering, which ranged between 54 and 72 days. It had significantly higher values for plant height, flag leaf length, flag leaf width, harvest index, relative water content, and fodder yield.

Cluster X contained a maximum number of twenty-three genotypes of different locations. All these genotypes were found to be low yielders and had significantly higher mean value for non-yield contributing traits. Few of the genotypes were found to have a significantly higher value for fodder yield than the grand mean. Cluster XI contained genotypes developed at Bengaluru in conjunction with the two national checks VL12 and VL29, while the local check Co2 was placed in cluster IX along

with GECH758. It indicated that these genotypes were similar to the respective checks concerning yield and other yield attributing traits.

The tendency of genotypes occurring in cluster cutting across distant places suggested that the geographical isolation was not only the factor causing the genetic diversity (Harsh Mehta *et al*., 2005). The study also revealed that the genotypes in cluster I and cluster VIII can perform well under normal climatic conditions. So the genotypes in these two clusters could be efficiently used for the hybridization program to get higher heterosis. Even though the genotypes in cluster III and cluster V were poor performers, they were found to have increased proline and relative water content, which were very important physiological parameters to be considered while selecting genotypes for stress conditions.

A comparison of these morphological clusters revealed that maximum intra-cluster distance (D=27.503) was observed in cluster VII (Table 5). This implied that these clusters had genotypes with varied genetic architecture (Karad *et al*., 2013). While the maximum inter-cluster centroid distance (D=40.619) was observed between the cluster V and cluster VIII. Contribution of the characters towards diversity indicated that the characters viz., grain yield followed by fodder yield contributed much to the variability exhibited by the genotypes (Table 6), which was supported by the findings of Dhanalakshmi *et al.* (2019). It had previously been suggested that genetic drift and selection in different environments could produce greater diversity, indicating that genetic makeup of genotypes falling in these clusters might be entirely different from one another. The pattern of distribution of genotypes to different clusters also indicated that the genotypes were clustered irrespective of their geographical origin. The superior most diverse genotypes identified based on phenotypic traits could be utilized in the breeding program to improve and to widen the genetic base of barnyard millet for the selection of superior lines. Genotypes with multiple superior traits could be utilized for the simultaneous transfer of multiple traits/genes in crop improvement.

CONCLUSION

The superior most diverse genotypes identified based on phenotypic traits could be utilized in a breeding program to improve and to widen the genetic base of barnyard millet for the selection of superior lines. Genotypes with multiple superior traits could be utilized for the simultaneous transfer of multiple traits/genes in crop improvement.

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