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Research Article

Varietal characterization and categorization of barnyard millet (*Echinochloa frumentacaea* L.) varieties, cultivars and mutants for varietal identification

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Abstract
The goal of this study is to evaluate the major characteristics of barnyard millet (*Echinochloa frumentacaea* L.) varieties, cultivars and mutants to analyse morphological characters on the basis of descriptors with the objective to identify the key diagnostic characters of the genotypes. A group of 11 barnyard millet genotypes were evaluated in randomized block design with three replications to characterize based on their morphological characters *viz.,* Plant growth habit, the number of basal tillers, Culm branching, Plant height (cm), Lodging, Flag leaf length, Flag leaf width, Flag leaf attitude, Inflorescence colour, Stigma colour, Peduncle length (cm), Lower raceme length (cm), Lower raceme thickness, Lower raceme branching, Seed shattering and Seed shape. This experiment revealed the diverse characteristics of germplasms and indicated that the morphological variations exist in collected germplasm is due to the variation in genetic makeup and could be better utilized for field functionaries, certification officers, seed production officers and seed growers for regulating quality of the seed. These differences in morphological traits were helpful in recognition of individual barnyard millet cultures.

Keywords
Barnyard millet, DUS, Morphological characterization, Varietal identification.

INTRODUCTION

Barnyard millet *(Echinochloa frumentacaea* L.*)* is native of Eurasia, the area of cultivation ranges from 50°N to 40°S latitude and it is one of the oldest domesticated millets in the semiarid tropical regions of Asia and Africa. Two main species, *Echinochloa frumentacea* (Indian barnyard millet) and *Echinochloa esculenta* (Japanese barnyard millet) are being cultivated and grown as cereals. In India, barnyard millet was cultivated in about 146,000 ha with production of 151,000 tons during 2016 (Bhat *et al.,* 2018). The barnyard millet contains about 65% carbohydrate, majority of which is in the form of non starchy polysaccharide and dietary fibre. In those areas where climatic and edaphic conditions are unsuitable for rice cultivation, barnyard millet is considered as a staple cereal. Despite enormous potential, the crop has not gained the popularity among masses and is still believed to be poor man's food. In India, its cultivation is confined to Tamil Nadu, Andhra Pradesh, Karnataka and Uttar Pradesh (Channappagoudar *et al.,* 2008).

Morphological characterization of seed, seedling and plant would usually be considered for varietal identification. The concept of DUS was fundamental for the categorization of the variety as a unique creation. The varietal identification and parietal purity evaluation is a significant parameter for the released cultivars. Cultivars are usually known on the basis of morphological differences of seed, seedling and mature plant. Practically, a variety must prove Distinct, Uniform and Stable (DUS) variations in the characters. With the foreword of Indian legislation Protection of

Plant Varieties and Farmers Rights Act (PPV and FRA, 2001), the release of new crop varieties is possible only if it is distinct (D) from other varieties, uniform (U) in their characteristics and generally stable (S) over the years (DUS). Thus, there is an essential need to look for rapid and reliable methods of varietal identification. Qualitative characters are considered as morphological markers in the identification of landraces of rice, because they are less influenced by the environmental changes (Raut, 2003). It is concluded that DUS guidelines can be effectively used for the morphological characterization of genotypes which later on is useful in the process of testing for DUS of the respective genotypes which can be used for developing new varieties.(Gandamala Raghu *et al.,* 2018) Similar results with regards to plant growth habit, pigmentation, culm branching and branching of lower racemes were reported by (Joshi *et al.*, 2015) and similar variation for various qualitative traits in kodo millet reported by (Nirubana *et al.,* 2019), in chilli by (Padma *et al.,* 2017) and in barnyard millet by Renganathan *et al.,* (2017).

The diverse characteristics of germplasms and indicated that the morphological variations exist in collected germplasm due to variation in genetic makeup could be better utilized for field functionaries, certification officers, seed production officers and seed growers for regulating quality of the seed. By these variations and differences are found among the genotypes will be useful for seed certification officers during field inspection and certification. For identification of varieties through morphological characters and conduct of GOT, the plant and seed characters need to be studied and documented. Since, a variety attains acceptance only when farmers get genetically pure seeds of high standards its simple, economical and do not require any sophisticated laboratory techniques. With these view, the present study was made to assess the qualitative characterization and categorization of barnyard millet varieties, cultivars and mutants.

MATERIALS AND METHODS

A total of 11 barnyard millet genotypes which includes two TNAU varieties (MDU 1, CO (KV) 2), two Japanese varieties(PRJ1, PRB 903), six cultivars (ACM 15 343, ACM 15 353, IEC 350, IEC356, TNEF 317, TNEF 318) and one mutant (M 38) were obtained from Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai and the research was carried out in the Department of Seed Science and Technology at B block, AC&RI, Madurai during August 2019.

Eleven genotypes were evaluated in three replications using Randomized Block Design (RBD) with the plot size of 3× 3 m with inter and intra row spacing as 30 × 15 cm respectively. The suggested agronomical and plant protection packages were followed. The observations were recorded on 5 randomly selected plants for each character in each replication at different crop growth stages. The morphological characters such as plant growth habit, the number of basal tillers, culm branching, plant height (cm), lodging, flag leaf length (cm), flag leaf width (cm), flag leaf attitude, inflorescence colour, stigma colour, peduncle length(cm), lower raceme length(cm), lower raceme thickness, lower raceme branching, seed shattering and seed shape were recorded.

The qualitative morphological traits observed were subjected to cluster analysis. The association between genotypes was evaluated by calculating the similarity matrix coefficient for pairwise comparisons based on the morphological characters. The data were subjected to analysis by using NTSYSpc version 2.02i (Rohlf, 1998). Similarity matrix was prepared with similarity coefficient using Simqual. The Un weighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method of the clustering subroutine SAHN was used to construct the dendrogram.

RESULTS AND DISCUSSION

The identification of barnyard millet genotypes was based on the morphological characteristics. These are highly useful to set up the distinctness, uniformity and stability of the cultivars. Based on the variations in morphological characteristics, it was attempted to group the 11 barnyard millet genotypes and to identify each and every genotypes. The results of 16 qualitative traits were studied and presented in (Table.1).The characters like plant growth habit, the number of basal tillers, culm branching, plant height (cm), lodging, flag leaf length (cm), flag leaf width (cm), flag leaf attitude, inflorescence colour, stigma colour, peduncle length (cm), lower raceme length (cm), lower raceme thickness, lower raceme branching, seed shattering and seed shape showed greater variations. Based on the plant growth habit, the genotypes were grouped into three categories as erect, decumbent and prostrate **(Fig. 1)**. Based on this variations, the 9 genotypes (MDU 1, CO (KV)2, ACM 15 353,TNEF317, TNEF 318, IEC 356, M 38, PRJ 1 and PRB 903.) were erect and two (ACM 15 343 and IEC 350) were decumbent in nature. Based on the number of basal tillers, the genotypes were categorized as low (<4), medium (4-7) and high (>7). The two genotypes (PRJ 1 and PRB 903.) are grouped as low, seven (MDU 1, CO (KV)2, ACM 15 343, TNEF317, TNEF 318, IEC 350, IEC 356) as medium tillering genotype and the other two (ACM 15 353, M 38.) as high tillering crop. Based on the culm branching, no difference was found among the 11 genotypes since culm branching was present in all genotypes **(Fig. 2)**.

Barnyard millet exhibited variability in the height of plants; the genotypes were grouped into three categories as semi dwarf, tall and very tall. Based on this variations, the genotypes were grouped in semi dwarf with two (PRJ 1 & PRB 903), three were tall (TNEF 317, IEC 350 and IEC 356) and the other six (MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF 318 and M 38) grouped under very tall category. Variation in lodging is grouped as present or absent. Based on this character, five genotypes (CO

Table 1. Morphological characterization and Categorization of eleven barnyard millet genotypes

S. No	Characters	States	Varieties	Score
1	Plant growth habit	Erect	MDU 1, CO (KV)2, ACM 15 353,TNEF317, TNEF 318, IEC 356, M 38, PRJ 1 and PRB 903.	1
		Decumbent	ACM 15 343 and IEC 350	5
		Prostrate	-	7
2	Number of basal tillers	Low (<4)	PRJ 1 and PRB 903.	3
		Medium(4-7)	MDU 1, CO (KV)2, ACM 15 343, TNEF317, TNEF 318, IEC 350, IEC 356.	5
		High (>7)	ACM 15 353, M 38.	7
3	Culm branching	Absent	-	1
		Present	MDU 1, CO (KV)2,ACM 15 343, ACM 15 353,TNEF317, TNEF 318, IEC 350, IEC 356, M 38, PRJ 1 and PRB 903.	9
4	Plant height (cm)	Dwarf (< 40)	-	3
		Semi dwarf (41.0-80.0)	PRJ 1 & PRB 903.	5
		Tall (80.1-120.0)	TNEF 317, IEC 350 and IEC 356.	7
		Very Tall (>120.0)	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF 318 and M 38.	9
5	Lodging	Absent	MDU 1, ACM 15 343, ACM 15 353,TNEF317, IEC 350 and IEC 356.	1
		Present	CO (KV)2, TNEF 318, M 38, PRJ 1 and PRB 903.	9
6	Flag leaf length (cm)	Short(>15)	PRJ 1 and PRB 903.	3
		Medium (15.0-30.0)	MDU 1, ACM 15 343, IEC 350 and M 38	5
		Long(15.1-45.0)	CO (KV)2, ACM 15 353,TNEF317, TNEF 318 and IEC 356.	7
		Very long (>45.0)	-	9
7	Flag leaf width (cm)	Narrow(<2.0)	PRJ 1 and PRB 903.	3
		Medium (2.0-3.0)	CO (KV)2, IEC 356, M 38,	5
		Wide(>3.0)	MDU 1, ACM 15 343, ACM 15 353,TNEF317, TNEF 318 and IEC 350	7
8	Flag leaf attitude	Erect	ACM 15 353.	3
		Semi erect	-	5
		Horizontal	PRJ 1.	7
		Semi drooping	MDU 1, CO (KV)2, ACM 15 343,TNEF317, TNEF 318, IEC 350, IEC 356, M 38 and PRB 903.	9
9	Inflorescence colour	Green (RHS NO 149A)	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF 317, TNEF 318, M 38, PRJ 1 and PRB 903.	1
		Light purple (RHS NO 58 A)	IEC 350.	5
		Dark purple (RHS NO 59A)	IEC 356.	7
10	Stigma colour	White	MDU 1, ACM 15 353 and M 38.	1
		Purple	-	2
		Dark purple	CO (KV)2, ACM 15 343, TNEF 317, TNEF 318, IEC 350 and IEC 356.	3
11	Peduncle length (cm)	Short (<10.0)	-	1
		Medium (10.0-20.0)	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF317, TNEF 318, IEC 350, IEC 356, M 38, PRJ 1 and PRB 903.	3
		Long (>20.0)	-	5
12	Lower raceme length	Short (<3.0)	-	3
		Medium (3.0-5.0)	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF317, TNEF 318, IEC 350, M 38, PRJ 1 and PRB 903.	5
		Long (>5.0)	IEC 356	7
13	Lower raceme thickness	Slender	CO (KV)2, ACM 15 343, TNEF 318, IEC 350, IEC 356, M 38, PRJ 1 and PRB 903.	3
		Thick	MDU 1, ACM 15 353, TNEF317.	7
14	Lower raceme branching	Absent	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF317, TNEF 318, IEC 350, IEC 356, PRB 903	1
		Present	M 38 and PRJ 1.	9
15	Seed shattering	Absent	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF317, TNEF 318, IEC 356, M 38 and PRJ 1.	1
		Present	IEC 350.	9
16	Seed shape	Concave	MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF317, TNEF 318, IEC 350, IEC 356, M 38 and PRJ 1	1
		Oval	-	2

(KV)2, TNEF 318, M 38, PRJ 1 and PRB 903) genotypes have lodging capacity due to lower culm thickness and the other six genotypes (MDU1, ACM 15 343, ACM 15 353, TNEF317, IEC 350 and IEC 356) possess absence of lodging. With reference to flag leaf length, the genotypes were considered as short, medium, long and very long. Two genotypes (PRJ 1 & PRB 903) were short, four genotypes *viz.,* MDU 1, ACM 15 343, IEC 350 and M 38 were medium and five genotypes (CO (KV)2, ACM 15 353,TNEF317, TNEF 318 and IEC 356) were long. The differences were found in flag leaf width also and based on this 2 genotypes (PRJ 1 & PRB 903) were narrow, 3 genotypes (CO (KV)2, IEC 356, M 38) genotypes were medium and the other 6 genotypes (MDU 1, ACM 15 343, ACM 15 353,TNEF317, TNEF 318 and IEC 350) were wide in nature. Similar characterization was also done by Tiwari *et al.,* (2017) and Neeru *et al*., (2017) in Indian mustard.

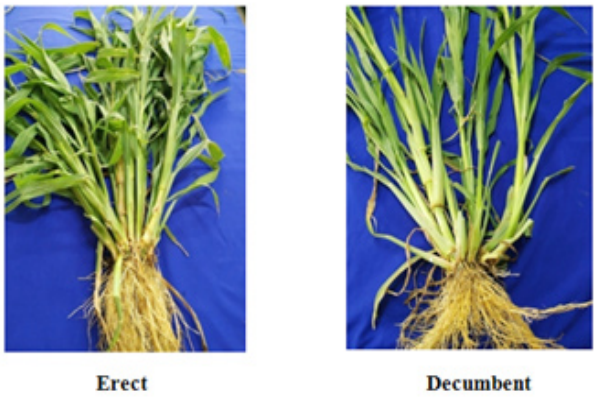

Fig 1. Plant growth habit

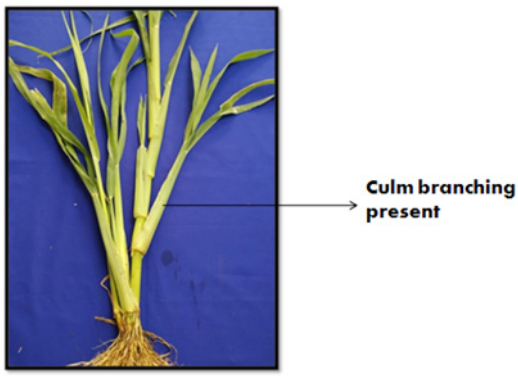

Fig 2. Culm branching

The study related to flag leaf attitude, the genotypes were classified as erect, semi erect, horizontal and semi drooping **(Fig. 3).** Among the 11 genotypes, only one culture (ACM 15 353) showed erect flag leaf, one (PRJ 1) showed horizontal type and other genotypes (MDU 1, CO (KV)2, ACM 15 343,TNEF317, TNEF 318, IEC 350, IEC 356, M 38 and PRB 903.) comes under semi drooping type. With reference to inflorescence colour **(Fig. 4)**, one genotype (IEC 350) exhibited light purple, one (IEC 356) showed dark purple and the other nine genotypes (MDU 1, CO (KV)2, ACM 15 343, ACM 15 353, TNEF 317, TNEF 318, M 38, PRJ 1 and PRB 903) exhibited green colour. The variations were found in colour of the stigma as white and dark purple **(Fig. 5)**. Out of 11 genotypes, 3 (MDU 1, ACM 15 353 and M 38) were white, 6 genotypes ((CO (KV) 2, ACM 15 343, TNEF 317, TNEF 318, IEC 350 and IEC 356) were dark purple and the other 2 genotypes (PRJ 1 and PRB 903) stamen and stigma colour was not visible. Based on the peduncle length, no difference was found and all the 11 genotypes possessed medium peduncle length. Similar characterization and grouping of genotypes based on plant morphological characters were made by Gediya *et al.,* (2018) and Janghel *et al.,*(2020) in chickpea Chaudari *et al.,* (2019) in castor and Bhoot *et al.,* (2019) in sesame.

Fig 3. Flag leaf attitude

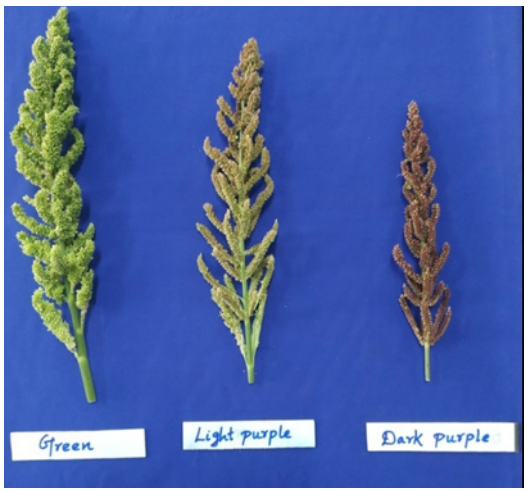

Fig 4. Inflorescence colour

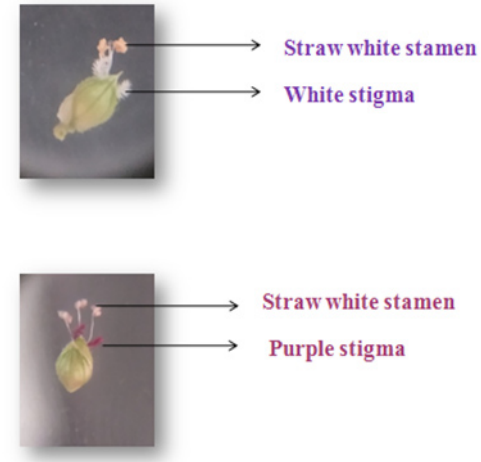

Fig 5. Stigma colour

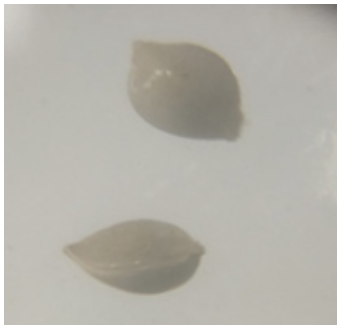

Fig 7. Seed shape

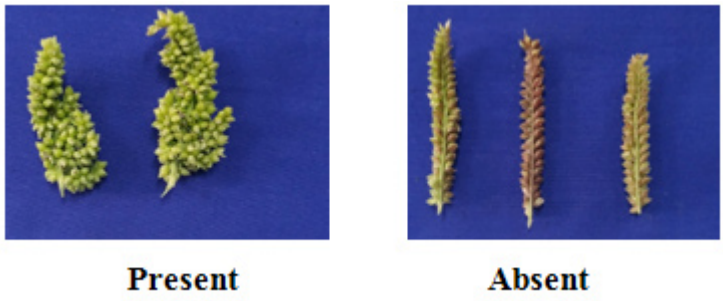

Fig 6. Lower raceme branching

The observations on lower raceme length revealed that the barnyard genotypes were grouped into 3 categories as short, medium and long. Only one culture (IEC 356) comes under long and other 10 cultures (MDU 1, CO (KV)2, ACM 15 343, ACM 15 353,TNEF317, TNEF 318, IEC 350, M 38, PRJ 1 and PRB 903) grouped under medium category. Two groups slender and thick **(Fig. 6)** were made based on the lower raceme thickness. Within 11 genotypes, three (MDU 1, ACM 15 353, TNEF317) showed thick raceme and remaining nine genotypes (CO (KV)2, ACM 15 343, TNEF 318, IEC 350, IEC 356, M 38, PRJ 1 and PRB 903) showed slender raceme. Differences were also found in lower raceme branching. Among these nine cultures (MDU 1, CO (KV)2, ACM 15 343, ACM 15 353,TNEF317, TNEF 318, IEC 350, IEC 356, PRB 903) showed the presence of lower raceme branching and in M 38 and PRJ 1 had no branching . Based on seed shattering, presence or absence of seed shattering was observed and only one (IEC 350) culture recorded the shattering of seeds and other nine had no shattering of seeds. Concave and oval are two groups of seed shapes were observed. But there is no variations was found among the genotypes and fall under concave shape **(Fig. 7)**. There was no seed set was observed in PRB 903 culture due to an unfavourable climatic conditions.

The cluster analysis of 11 genotypes for 16 qualitative characters using UPGMA method resulted in grouping of genotypes into different clusters at 56% similarity level **(Fig 8)**. The similarity matrix coefficient ranged from 56% to 94% with an average of 75%. At 56 % similarity, two clusters alone formed Cluster I includes the 8 genotypes *viz.,* MDU 1, ACM 15 343, TNEF 317, CO (KV)2, TNEF 318, ACM 15 353, IEC 356 and IEC 350 . Cluster II includes three genotypes M 38, PRJ 1 and PRB 903. At 94% similarity, CO (KV)2 and TNEF 318 alone comes under a single cluster and other cultures were solitary in nature. Under 75% similarity level, six clusters were formed (Table 2). Cluster I consists of 5 genotypes MDU 1, ACM 15 343, TNEF 317, CO (KV)2, TNEF 318. ACM 15 353 fall under Cluster II. Cluster III with IEC 356 genotype, IEC 350 and M 38 comes under Cluster IV and V respectively. Cluster VI includes PRJ 1 and PRB 903. Cluster II, III, IV and V produces solitary clusters, which indicates that the wide variations were found among these genotypes. Hence the diverse genotypes can be easily identified.

Table 2. Constituents of VI clusters in barnyard millet genotypes for 16 qualitative characters truncating the tree 75% similarity level

Cluster	Number of genotypes	Constituent genotypes
I	5	MDU 1, ACM 15 343, TNEF 317, CO(KV)2, TNEF 318
II	1	ACM 15 353
III	1	IEC 356
IV	1	IEC 350
V	1	M 38
VI	2	PRJ 1 and PRB 903

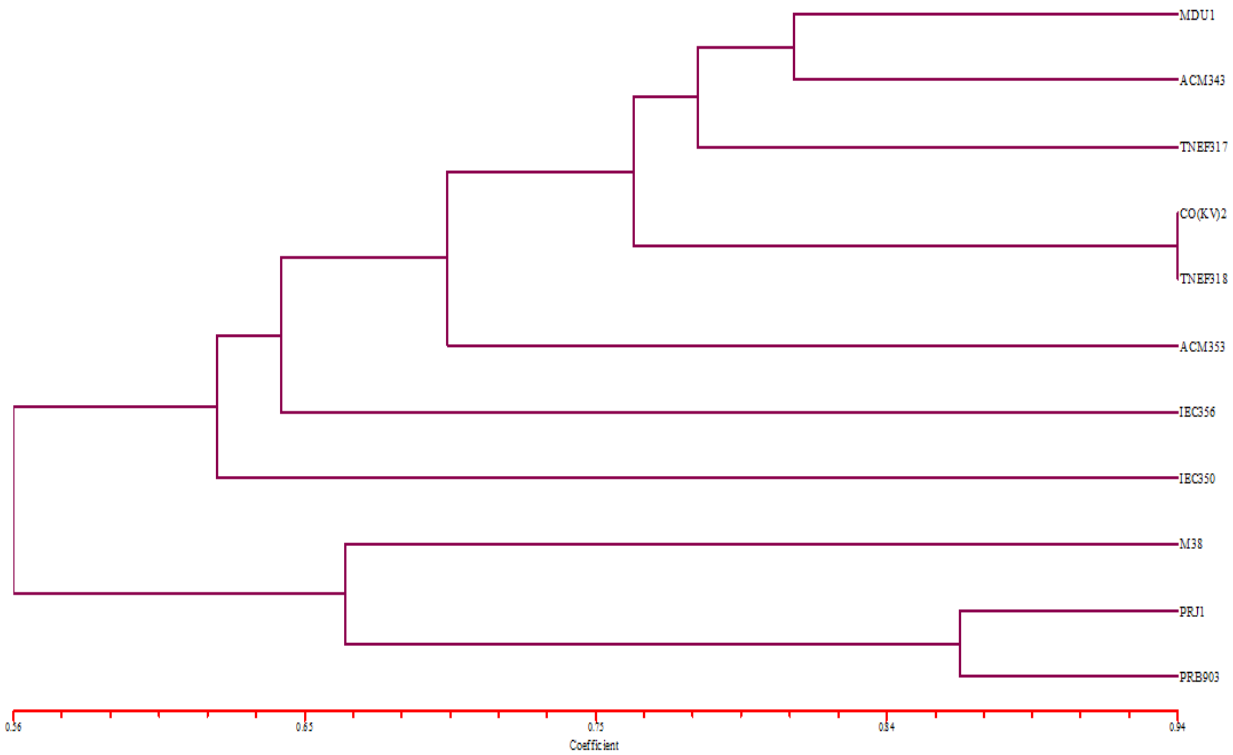

Fig. 8 Dendrogram representing the grouping of eleven barnyard genotypes formed through UPGMA based on morphological markers

Each separate line indicates a cluster
Mean genetic dissimilarity – 0.75

It could be concluded that a total of 11 barnyard millet genotypes can be successfully distinguished by its morphological characters (Table1). Among these, 16 morphological characters, were found as important for its varietal identification. These qualitative characters showed stable and discrete variations that are not influenced by environment hence these characters served as reliable source for identification of a variety. This study may be provided an ideal knowledge to carry out DUS testing in barnyard millet genotypes. A detailed morphological description of plants and seeds should therefore be prepared.

REFERENCES

Bhat, B. V., Tonapi, V. A., Rao, B. D., Singode, A., and Santra, D. (2018). Production and utilization of millets in India. *International Millet Symposium and The 3rd International Symposium on Broomcorn Millet (3rd ISBM),* eds. D. K. Santra and J. J. Johnson, 24–26.

Bhoot HV, Sharma LK, Kulkarni GU, Umesh Ravat and Sahdev Rathva.(2019). Characterization of sesame genotypes through morphological characters. *Journal of Pharmacognosy and Phytochemistry.* **8(3)**: 3132-3138.

Channappagoudar B.B., Hiremath, S. M., Biradar, N. R., Koti, R. V. and Bharamagoudar,T. D. (2008). Influence of morpho-physiological and biochemical traits on the productivity of barnyard millet. *Karnataka Journal of Agricultural Sciences*, **20(3)**:477-480

Chaudhari, B.A., M.P. Patel, J.A. Patel, R.R. Makwana, A.M. Patel and Patel, M.K. (2019). The Characterization of Castor (*Ricinus communis* L.) Genotypes for Morphological Traits. *Int.J.Curr.Microbiol.App.Sci,* **8(05)**: 2482-2492. [Cross Ref]

Gandamala Raghu, Arun Kumar Chaurasia, K. Rohan Reddy, Bineeta M. Bara and Nithyananda, N. (2018). DUS Characterization of Bottle Gourd [*Lagenaria siceraria* (Mol.) Standl.] Genotypes. *Int.J.Curr. Microbiol.App.Sci,* **7(10)**: 680-686. [Cross Ref]

Gediya L. N., Dipak A Patel, Deep Kumar, Vekariya KJ and Nayak JJ. (2018). Characterization of chickpea genotypes based on morphological traits. *Journal of Pharmacognosy and Phytochemistry*, **7(4):** 3008-3015.

Guidelines for the conduct of tests for Distinctiveness, Uniformity and Stability Barnyard millet (*Echinocloa frumentaceae* (Roxb.) Link) by PPV&FRA 2001. https://www.plantauthority.gov.in/pdf/final-Barnyard%20millet.pdf

Janghel, D. K., Krishan Kumar, R. Sunil and Chhabra, A. K. (2020). Genetic Diversity Analysis, Characterization and Evaluation of Elite Chickpea (*Cicer arietinum* L.) Genotypes. *Int.J.Curr.Microbiol.App.Sci,* **9(01)**: 199-209. [Cross Ref]

Joshi, R., Jain, A., Chauhan, S., and Singh, G. (2015). Research Article Characterization of barnyard millet (*Echinochloa frumentacea* (Roxb.) Link.) landraces for agro-morphological traits and disease resistance. *Electronic Journal of Plant Breeding,* **6(4),** 888-898.

Kuraloviya M., Vanniarajan C., Vetriventhan. M., Babu. C., Kanchana. S and Sudhagar. R (2019). Qualitative characterization and clustering of early-maturing barnyard millet (*Echinochloa spp*.) germplasm. *Electronic Journal of Plant Breeding*, **10 (2)**: 535-541. [Cross Ref]

Madhvi Tiwari, Bhushana Babu V, Nachiket Kotwaliwale, Rajendra Hamad and Kara Singh. (2017). Identification of Indian mustard (*Brassica juncea* L.) varieties by DUS testing using morphological characters. *Journal of Oilseed Brassica,* **8 (1)** : 57-61

Neeru, Thakral N. K., Ram Avtar and Hari Kesh (2017). Characterization and categorization of Indian mustard genotypes for agro-morphological traits. *Journal of Applied and Natural Science,* **9 (2)**: 805 – 811. [Cross Ref]

Nirubana,V., Ravikesavan, R., and Ganesamurthy, K. (2019). Characterization and clustering of kodo millet (*Paspalum scrobiculatum* L.) genotypes based on qualitative characters. *Electronic Journal of Plant Breeding,* **10(1),** 101-110. [Cross Ref]

Padma, J., S. Anbu and Sivasubramaniam, K. (2017). Efficacy of Morphological Characters for Varietal Identification of Chilli. *Int.J.Curr.Microbiol.App.Sci,* **6(2)**: 690-700. [Cross Ref]

Raut, V. M. (2003). Qualitative genetics of Soyabean-a review. Soybean Research, 1: 1-28.

Lokesh Gour, R.K. Dubey, S.K. Singh and Sharad Tiwari. (2018). Morpho-Diversification Study of Indigenous Gene-Pool of *Pisum sativum* L. at Madhya Pradesh, India. *Int.J.Curr.Microbiol.App.Sci.,* **7(03):** 2533-2542. [Cross Ref]

Renganathan, V., Vanniarajan, C., Nirmalakumari, A., Raveendran, M., and Thiyageshwari, S. (2017). Cluster analyses for qualitative and quantitative traits in barnyard millet *Echinochloa Frumentacea* germplasm. *The Bioscan,* **12(4),** 1813-1817