Journal of Phytology 2020, 12: 29-34
doi: 10.25081/jp.2020.v12.6317
https://updatepublishing.com/journal/index.php/jp

Research Article

ISSN: 2075-6240

Characterization of little millet (*Panicum sumatrense*) varieties using morphological descriptors and SSR based DNA fingerprinting

Senthil Natesan[1]*, Gayathri Venugopalan[1], Selva Babu Selvamani[1], Madhumitha krishnamoorthy[1], Sarankumar Chandran[1] and Nirmalakumari Angamuthu[2]

[1]Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India, [2]Centre of Excellence in Millets, Athiyanthal, Tamil Nadu Agricultural University, Thiruvannamalai-606 603, Tamil Nadu, India

ABSTRACT

Little millet varieties are generally distinguished by morphological descriptors which are being used for seed certification and DUS characterization [1]. But in practical terms, these key differentiation descriptors between varieties of little millet are very fewer and hence difficult to differentiate germplasm accessions. Germplasm registration in NBPGR needs DNA fingerprint to show the uniqueness of germplasm in comparison to existing varieties. DNA fingerprinting is a better option to identify unique markers to differentiate the varieties. Available genomic resources are scarce since little millet is still considered to be an orphan crop. Therefore, markers from other cereal genomes such as maize, pearl millet and barnyard millet that are been utilized for DNA fingerprinting purpose with a clue of cereal synteny relationship. Twenty-one morphological descriptors studies revealed that the variety ATL 1 is different from the other varieties for more than 16 morphological characters studied. DNA fingerprinting is attempted in five genotypes of little millets such as BL6, ATL 1, TNPsu 176, Co (Samai) 4, Paiyur 2 using cereal SSR markers. Among the 25 maize SSR markers used two markers *viz.*, phi213984 and phi295450 scored polymorphism by the amplicon size of 310bp and 600bp respectively. From the 25 Pearl millet SSR markers used only one SSR marker found polymorphic at 305bp allele size for ATL 1 and Hence, SSR based DNA fingerprinting helped to differentiate ATL1, the newly released high yielding variety from other genotypes of little millets which can be used for varietal identification purpose.

KEYWORDS: Little millet, DNA Fingerprinting

Received: June 29, 2020
Revised: July 26, 2020
Accepted: August 01, 2020
Published: August 08, 2020

***Corresponding author:**
Senthil Natesan,
Email: senthil_natesan@tnau.ac.in

INTRODUCTION

Little millet (Panicum sumatrense) is also known as Indian millet. It is cultivated as a cereal across Nepal, India and Western Myanmar, and its center of origin is West Africa. The wild relative of little millet is *P.psilopodium*. It forms an important role in tribal agriculture in Eastern Ghats of India. Little millet is grown on temperate and tropical climate. Little millet is an annual tuffed grass with slender culms, soft leaves, inflorescence a panicle with erect hairy branches, spikelets in pairs with two glumes. The discovery of syntenic regions among the cereals which aids to identify useful alleles of important agro-morphological traits. An earlier study by Ali et al [2] developed 48 EST-SSR markers among 37 accessions of the little millets. Only limited reports are available on the genetic diversity of little millet germplasm, that too is based on a limited number of DNA markers [3, 4]. Thus the limited sequence information available in the little millets necessitates the search for the available markers from the maize, barnyard millet, and pearl millet due to the cereal millet synteny and also the availability of the SSR markers. The little millet husked grain is cooked as like rice and sometimes made into flour for different types of food preparations. The soft straw is palatable to cattle and the green plant has potentialities as a quick-growing fodder. The present investigation was conducted to carry out the comprehensive characterization of little millet genotypes based on Distinctness, Uniformity and Stability (DUS) characters for Protection of Plant Varieties and Farmers Rights Authority (PPVFRA) registration because of its property to identify the dissimilarity between the newly released and the existing genotypes as well as to distinguish the germplasm. Cereal crops exhibit cross-genera transferability, of DNA markers into many cereals such as maize,

pearl millet, barnyard millet. The present study was conducted with the objectives of fingerprinting the little millet varieties using the cross transferability of maize, pearl millet, barnyard millet genomic SSR markers.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Little millet germplasm was raised in Randomized Block Design (RBD) with two replications at Centre of Excellence in Millets, Athiyanthal during Kharif 2019. The little millet cultures in the advance stage of varietal trail along with released varieties viz., BL6, ATL1, TNPsu176, Co (Samai) 4, Paiyur2 are chosen for the DUS characterization and DNA fingerprinting work. The leaf samples were collected from the Center of Excellence in Millets, Athiyandal. The genomic DNA was isolated from 15 days old seedlings by the modified CTAB method [5]. The genomic DNA quantity and quality was checked in Nanodrop and also using 0.8% agarose gel electrophoresis.

Morphological Characters for DUS Testing

The 15 quantitative traits were recorded from five randomly selected plants from each replication during Kharif 2019 at the Centre of Excellence in millets, Athiyanthal, TNAU. All the five genotypes were characterized for 15 quantitative traits viz., Plant height (cm), Number of basal tillers, Culm branches, Blade length of flag leaf (cm), Blade width of flag leaf (cm), Sheath length of flag leaf (mm), Length of peduncle (mm), Peduncle exsertion (mm), Length of inflorescence (mm), Number of primary inflorescence branches, Number of nodes per primary axis of inflorescence, Number of secondary inflorescence branches, Length of fruit (mm), Width of fruit (mm) and days to flowering (days) were recorded. Observations were also recorded for 7 other qualitative traits viz., Growth habit, culm branches, Ligule pubescence, Degree of lodging at maturity, Inflorescence shape, compactness of inflorescence and Color of the apicules. In addition, nutritional quality, cooking quality and sensory evaluation were done among the three varieties viz., ATL1, Co (Samai) 4, Paiyur2.

PCR Amplification with Cereal Genomic SSR Markers

Amplification of little millet genotypes was performed using 16 barnyard millet, 25 maize and pearl millet genomic SSR markers (Table 1). Barnyard millet SSR markers are obtained from a previous study [6] is used to develop EST-SSRs. SSR primers were designed based on the sequences flanking the SSR motifs using the Primer Premier 6.0 software (Premier Biosoft International, Palo Alto, CA). The SSR loci containing ideal repeat units of 2-6 nucleotides only were selected. The standard based on the fixation of minimum SSR length was defined as five reiterations for each repeat unit. Complex SSR types and mononucleotide repeats were omitted. Maize SSR markers were obtained from the Maize GDB database (https://www.maizegdb.org/data_center/ssr). Pearl millet SSR primer pairs were taken from International Crop Research Institute for Semi-Arid Tropics (IPES, PMSP, ICMP).

Table 1: List of markers used for the polymorphic study

S.No	Barnyard Millet	Maize	Pearl Millet
1.	BMESSR5	umc1110	PMSP2220
2.	BMESSR28	umc2257	PMSP2086
3.	BMESSR27	umc2133	ICMP3081
4.	BMESSR37	umc2071	ICMP3022
5.	BMESSR31	umc1257	ICMP3018
6.	BMESSR34	umc1601	PMSP2204
7.	BMESSR21	umc1142	PMSP2203
8.	BMESSR7	umc1018	PMSP2210
9.	BMESSR25	umc2190	PMSP2207
10.	BMESSR36	umc1970	PMSP2223
11.	BMESSR29	umc1906	IPES0007
12.	BMESSR24	umc420	IPES0010
13.	BMESSR20	umc1018	IPES0034
14.	BMESSR23	umc1948	PMSP0088
15.	BMESSR19	umc2021	PMSP2216
16.	BMESSR3	umc2231	ICMP3022
17.		umc2023	ICMP3036
18.		umc1602	ICMP3035
19.		umc1066	ICMP3021
20.		bnlg1802	IPES0037
21.		phi213984	IPES0111
22.		phi125	IPES0010
23.		phi 295450	IPES0094
24.		phi087	PMSP2219
25.		phi280	PMSP2211

The polymerase chain reactions (PCR) were performed in 10μL reaction volume containing 7μL of the 1X Master mix, 0.6μL of primer (forward and reverse), 1.4μL of Sterilized water and about 200ng (1μL) of template DNA. The PCR temperature profile for maize genomic SSRs was initial denaturation at 94°C for 7 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing temperature at 55°C for 30 sec, extension at 72°C for 45 sec and a final extension at 72°C for 7 min and terminated by the hold at 10°C. The electrophoresis was done at 150V for 1.5 hr at room temperature by using the 3% agarose gel. Gels were added with ethidium bromide and visualized using GELSCAN 1312.

RESULTS AND DISCUSSION

Morphological Variations

Fifteen and seven quantitative and qualitative characters (Tables 2 and 3) were studied and revealed that the varieties selected for the study were significant for the morphological characters. Large variations were recorded in ATL 1 (125-135 cm) for the trait plant height (Figure 1 and Table 3). Whereas other varieties recorded from the range of 105-112cm. Similar results were studied by [7,8].Number of tassel branches were 8-10 which is on par with Paiyur 2 and BL 6 and 10-12 range of number of tassel branches was recorded in CO (Samai) 4 and TNPsu 176. 32-34cm, 1.5-1.7cm and 18-20cm of Blade length of flag leaf, Blade width of flag leaf and Sheath length of flag leaf were recorded uniquely for the variety ATL 1 and on the other hand, it was 21-28cm, 1.3-1.6 and 12-19 range for the other varieties respectively. Wide variations were recorded for the traits Length of the peduncle (21-23mm), Length of inflorescence (41-43), Number of primary inflorescence branches (20-25),

Table 2: Morphological characters for the quality traits of the little millet varieties

S.No	Descriptors	ATL 1	CO (Samai) 4	Paiyur 2	BL 6	TNPsu 176
1.	Growth habit (Decumbent (D)/ Erect (E))	D	E	E	E	E
2.	Culm branches (Present (P)/ Absent (A))	A	P	P	P	P
3.	Ligule pubescence (Strongly Pubescent (SP)/ Medium Pubescent (MP))	SP	MP	MP	MP	MP
4.	Degree of lodging at maturity Slight (S)/ Medium (M))	S	S	M	M	M
5.	Inflorescence shape (Arched (A)/Diffused (D))	A	D	D	D	D
6.	Compactness of inflorescence (Intermediate (I)/Open (O))	I	O	O	O	O
7.	Colour of apiculus (Yellow (Y)/Brown (B))	Y	B	B	B	B

Table 3: Morphological characters for the distinct quantitative traits of the Little millet varieties

S.No	Varieties	Plant Height (cm)			Days to Flowering (days)			Peduncle exersion (mm)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
1	ATL1	125	135	130.0*	55	60	57.5*	13	15	14
2	CO (Samai) 4	105	115	110.0	52	57	54.5	14	16	15
3	Paiyur 2	100	110	105.0	51	54	52.5	13	15	14
4	BL 6	105	110	107.5	53	58	55.5	13	15	14
5	TNPsu 176	107	112	109.5	52	57	54.5	14	16	15
Mean				112.40			54.9			14.4
SE				0.48			0.05			0.54
SD				0.68			0.08			0.77
CD				3.14			0.37			3.56

SE: Standard error, SD: Standard deviation, CD: Critical difference

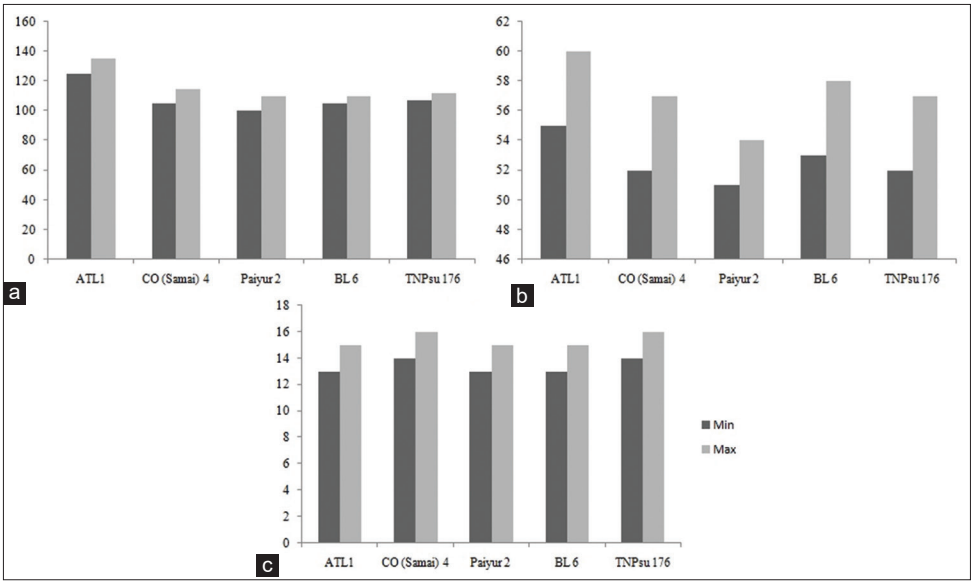

Figure 1: Morphological characters for Quantitative traits of the Little mille varirties. (a) Plant height (cm), (b) Days to flowering (days), (c) Peduncle exertion (mm)

Number of nodes per primary axis of inflorescence (27-31) and Number of secondary inflorescence branches (13-15) whereas it is reduced in the other varieties. The varieties *viz*., ATL 1, Paiyur 2 and BL 6 recorded 13-15 range of Peduncle exertion and the varieties viz., CO (Samai) 4 and TNPsu 176 recorded the range of 14-16 Peduncle exsertion (Figure 1). Length of the fruit and Width of the fruit showed a range from 4.2-4.5mm and 3.5-3.7mm for ATL 1 and it is on par with the other varieties. In case of 7 qualitative traits studied the traits, Compactness of inflorescence (Intermediate) (Figure 2), Colour of the apicules (Figure 3) (Yellow), Ligule pubescence (Strongly pubescent), Growth habit (Decumbent), Culm branches (Absent) and Inflorescence shape (Arched) was found distinct for ATL 1 and was different for the other varieties. Similar results in accordance with [9]. In addition characterization of grain quality such as nutritional quality, cooking quality and sensory evaluation sore was conducted among the three varieties *viz*., ATL 1, CO (Samai) 4 and Paiyur 2 which recorded that the newly released variety ATL 1 has higher characteristics for nutritional quality, cooking quality and sensory evaluation score among the other two varieties (Figure 4). [9]. DUS characterization is the practice done to record the distinct morphological characteristics existed in newly identified varieties and on similar varieties what are called 'common knowledge'. But the availability of DUS characters is limited, hence the certification agency use combination of key distinguishable characters like

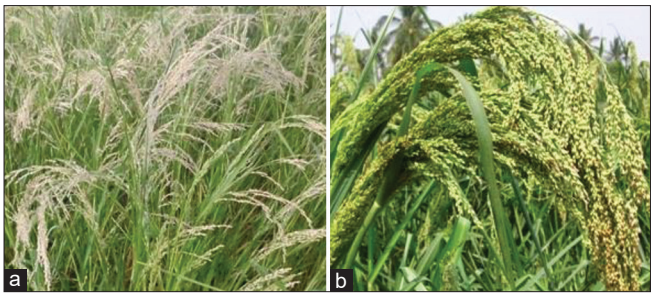

Figure 2: Infloresence of samai varieties. (a) CO (Samai) 4, (b) ATL 1

Figure 3: Grains of CO (Samai) 4 (a) and ATL 1(b)

plant height, panicle characters at the time of flowering, seed characters at the time of harvesting. Though the little millets had narrow diversity it is difficult to identify a trait specificity exists between the varieties. Hence the molecular markers are employed to fingerprint the DNA along with the DUS characters which could be usefull in varietal identification and also in the seed certification process (Science and Advice for Scottish Agriculture).

Screening of Little Millet Variety using SSR Markers

Amplification of little millet genotypes was performed using 16 barnyard millet, 25 maize and pearl millet genomic SSR markers listed in the (Table 1). Barnyard millet EST-SSR primers showed multiple allelic patterns for the range of annealing temperature 55°C to 60°C. Among 25 maize SSR markers, two markers viz., phi213984 and phi295450 showed polymorphism (Figure 4) between the little millet variety under study. The marker phi213984 showed unique 310bp amplicon in ATL 1 whereas, other varieties viz., BL6, TNPsu 176, Co (Samai) 4 and paiyur 2 showed 300bp amplicon size. 600 bp amplicon were uniquely found in ATL 1 and TNPsu 176 which is distinguishable from the other varieties. The maize SSR marker umc2021 and phi087 showed monomorphic with the amplicon size of 140bp and 150bp

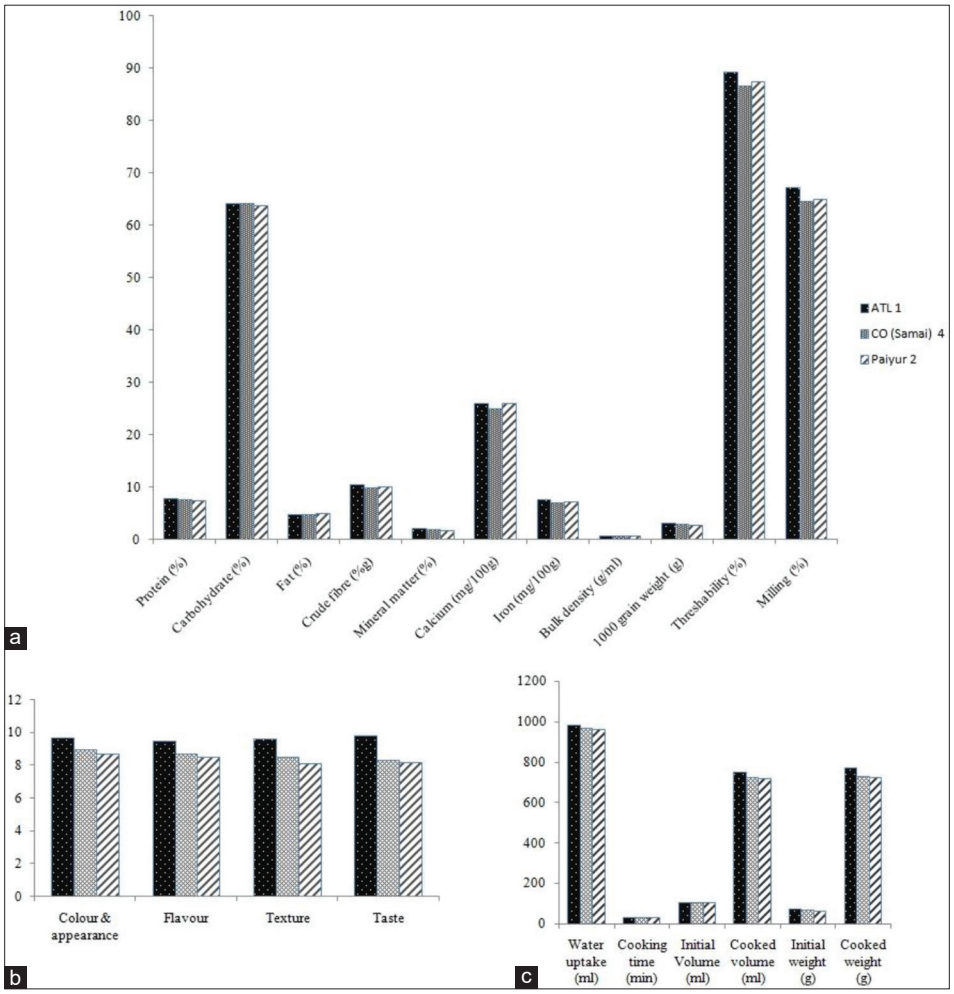

Figure 4: Characteristics of grain quality of ATL 1, CO (Samai) 4, Paiyur 2. (a) Nutritional quality, (b) Cooking quality, (c) Sensory evaluation score

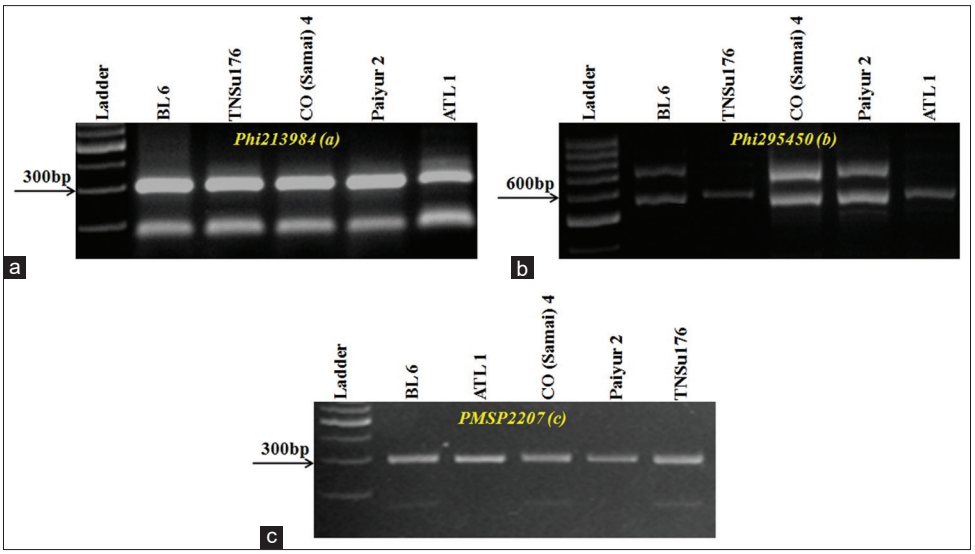

Figure 5: Screening of little millets using Maize SSR markers (a,b) and Pearl millet SSR marker (c)

respectively. Amidst 25 Pearl millet SSR markers, three markers viz., PMSP2216, PMSP2203, PMSP2223 showed monomorphic by the amplicon size of 150bp, 300bp, 190bp respectively. A unique amplicon size with 305bp was recorded in ATL 1 and BL 6 which is distinguishable from the other three varieties under study (Figure 5). These are in accordance with [10,11].

Utilization of three microsatellite markers in the analysis of varieties revealed a high level of genetic polymorphism which allowed unique banding pattern. Microsatellites are considered appropriate for variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently [12]. In this study, three SSR markers distinguished the ATL 1 from other ruling varieties. The set of SSR markers used here provides a positive assessment of the ability to produce unique DNA profiles of little millet germplasm. Maize and little millet might have originated from a common ancestor in Poaceae species [13], and hence the variation was introduced in the structural and regulatory sequences after species during speciation. Previously Ali et al, 2017 demonstrated the validation of switchgrass EST-SSR markers in assessing the relationship of the little millet genomic. Thus, this study could be utilized for identification of the diverse germplasms and also for fingerprinting in the future.

CONCLUSION

The morphological DUS descriptors can be effectively used for identification, documentation and grouping of varieties for registration of varieties under the PPVFRA. The morphological descriptors sometimes seem difficult to differentiate the closely related varieties and hence the DNA fingerprint data is given along with plant variety notification proposal which would provide great help to the plant breeders for registration of germplasm in NBPGR. Although DUS testing has been done precisely to provide diversity assessment at morphological level there remains a further scope to validate the findings through DNA markers.

ACKNOWLEDGMENT

The Core projects for research activities at Colleges and Research stations of TNAU- Phase-I (CPMB/CBE/PBT2012/CP004) funded by the Agricultural Department (AU), Government of Tamil Nadu (B-Agriculture Plan-27-University Research Scheme-NV-Core projects TNAU Phase-I) is kindly acknowledged and the financial aid supported by the TNAU research sub project CPBG/ATL/PBG/SMM/2014/002 - Genetic improvement of small millets for drought resistance and evolving high yielding varieties suitable for the Northeastern zone of Tamil Nadu. (Project period Aug 2014 – July 2019) supported by TNAU is also acknowledged.

REFERENCES

1. Smith S, Helentjaris T. DNA fingerprinting and plant variety protection. Genome mapping in plants. 1996; 10: 95-110.
2. Ali A, Choi YM, Hyun DY, Lee S, Kim JH, Oh S, Lee MC. Development of EST-SSRs and assessment of genetic diversity in little millet (*Panicum sumatrense*) germplasm. 2017;3:287-97.
3. Geervani P, Eggum BO. Nutrient composition [1]and protein quality of minor millets. Plant Foods for Human Nutrition. 1989; 39:201-208.
4. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucl. Nucleic Acids Research. 1980; 8: 4321–4325.
5. Nehra M, Kumar M, Vart D, Sharma RK, Choudhary M. DUS characterization and diversity assessment in pearl millet inbreds. Electronic Journal of Plant Breeding. 2016; *7*: 925-933.
6. Jia X, Zhang Z, Liu Y, Zhang C, Shi Y, Song Y Li Y. Development and genetic mapping of SSR markers in foxtail millet [*Setariaitalica* (L.) P. Beauv.]. Theoretical and applied genetics. 2009; 118: 821-829.
7. Chandra Shekara AC, Prasanna BM, Bhat SR, Singh BB. Genetic diversity analysis of elite pearl millet inbred lines using RAPD and SSR markers. Journal of plant biochemistry and biotechnology. 2007; 16: 23-28.
8. Panwar P, Nath M, Yadav VK, Kumar A. Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusinecoracana* L.). Journal of Genetics. 2010; 89: 121-133.
9. Nirmalakumari A, Salini K, Veerabadhiran P. Morphological characterization and evaluation of little millet (Panicumsumatrense Roth. ex. Roem. and Schultz.) germplasm. Electronic Journal of Plant Breeding. 2010; 1: 148-155.
10. Padmaja, Studies on variability, correlation, path analysis

and D2 analysis for yield and yield attributes in little millet (*Panicumsumatrense* Roth.) M.Sc. Thesis in Tamil Nadu Agricultural University; 1998.
11. Nozawa S, Nakai H, Sato YI. Characterization of microsatellite and ISSR polymorphisms among Echinochloa (L.) Beauv. spp. Japanese Breeding Research. 2004;6:87-93
12. Danquah EY, Hanley SJ, Brookes RC, Aldam C. and Karp A. Isolation and characterisation of microsatellites in Echinochloa (L.) beauv. spp. Molecular Ecology. 2002;2: 54-56.
13. Manimekalai M, Dhasarathan M, Karthikeyan A, Murukarthick J, Renganathan VG, Thangaraj K, Senthil N. Genetic diversity in the barnyard millet (*Echinocholafrumentacea*) germplasms revealed by morphological traits and simple sequence repeat markers. Current Plant Biology. 2018; *14*: 71-78.