Environment and Ecology 41 (3B): 1788—1798, July—September 2023
Article DOI: https://doi.org/10.60151/envec/CEQT4858
ISSN 0970-0420

Morpho-Molecular Genetic Diversity Analysis of Little Millet (*Panicum sumatrense*) using Yield Attributing Traits and ISSR Markers to Evaluate its Performance as a Summer Crop

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Received 7 October 2022, Accepted 16 June 2023, Published on 21 August 2023

ABSTRACT

The knowledge of genetic diversity among the genotypes and its relationship plays a vital role for genetic improvement in crop breeding programs. The present study was conducted to assess the performance of 18 little millet genotypes in local conditions of Bihar and analyze the genetic diversity by using morphological traits and 16 ISSR markers. The field experiment was conducted in RBD design in the month of April 2020, to evaluate the performance of the plant as a summer crop. ANOVA analysis revealed the presence of substantial amount of variability among 18 genotypes. The maximum mean sum of square values was recorded for plant height followed by flag leaf length within the genotypes. The descriptive statistical analysis showed that plant height followed by length of flag leaf contributed the most towards the divergence. A total of 117 alleles including 43 unique alleles and 74 shared alleles were obtained by amplification with ISSR primers. All the primers were highly polymorphic and produced amplified product with a size ranged between 354-2315 bp. The primers UBC 807 followed by HB 10 and UBC 814 were found to be the most effective in detecting genetic diversity. The dendrogram based on Dice's similarities coefficient, grouped the genotypes into five clusters. The genotypes WV-168 and WV-115 followed by WV-122 and WV-124 were found to have greatest variability. The present study identified genotypes which can be grown as summer crops. The diversified genotypes among the population were also identified, which could be utilized as parents for breeding program.

Keywords *Panicum sumatrense*, ANOVA, Yield attributing traits, Dendrogram, ISSR markers, Summer millet.

INTRODUCTION

Little millet, binomially known as *Panicum sumatrense* [Roth. ex. Roem. and Schult.] belongs to the family Poaceae and species *Panicum* which has two subspecies namely *sumatrense* (cultivated type) and *psilopodium* (wild progenitor) (de Wet *et al.* 1983). The little millet originated in South East Asia. It is widely distributed and cultivated around India and was domesticated along the hilly sides of Eastern Ghats. It is largely cultivated by the tribal people in marginal areas and known by various vernacular

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names in different parts as *samai* in Tamil, *kutki* in Hindi and *gajro* in Gujarati (Hiremath *et al.* 1990).

Little millet is a self-pollinating tetraploid crop (2n = 4x = 36) (Wanous 1990) which can grow in wide range of environmental conditions like dry, semi-arid to sub-humid regions. It is believed to withstand both extreme drought and water logging conditions. It is thus grown on light red soil along hilly sides as rainfed crop not as an irrigated crop (Mall and Tripathi 2016). It is one of the reliable catch crops for its earliness and resistance to adverse environmental conditions. It also shows very less incidence of disease as compared to other minor millets. The crop grows well in the soil conditions where other commercial crops cannot be grown profitably. It is rich in antioxidant property and can be used as raw material in nutritionally enriched food formulations (Nazni and Shobana 2016). Gluten free little millet has become a good choice for people affected with celiac disease or allergies and intolerance of wheat (Saturni *et al.* 2010). The low glycemic content of little millet helps to prevent type 2 diabetes, obesity and cardiovascular diseases (Mall and Tripathi 2016). It is also rich in phytochemicals like phenolic compounds, phytic acid, flavonoids and tannins (Pradeep and Guha 2011). Due to its superior nutritional qualities, little millets are used for human consumptions as well as feed and fodder for cattle (Roopa *et al.* 2013).

Little millet is one of the least exploited crops among the minor millet and had not gained much attention among the researchers and farmers. Thus, the knowledge and availability of the germplasm is very much limited (Selvi *et al.* 2015). There are 473 little millet germplasm accessions held by ICRISAT genebank as global collection and 1889 germplasm accessions by ICAR-NARS as national collection. But the performance of these accessions in different environmental conditions and their genetic diversity information has yet to be explored. The assessment of the genetic diversity is a prerequisite and plays a vital role for any crop development program. The genetic variability is considered as the reservoir for plant breeders to develop improved varieties during crop improvement program (Anuradha *et al*. 2017, Patel *et al*. 2018, Venkataratnam *et al.* 2019). Genetic diversity among the population helps to identify the superior parents with high amount of variability for various traits. Genetic divergence can be evaluated by using morphological traits and molecular marker system. Since the morphological traits are highly influenced by the developmental stages and environmental conditions (Nirmalakumari *et al.* 2010, Manimozhi Selvi *et al*. 2015) and molecular marker-based evaluation are not influenced by the any of environmental conditions, evaluation of genetic diversity based on morphological and molecular markers gains advantage in assessing the genetic diversity.

There are a variety of marker system which are used to assess the diversity present in a group. The choice of molecular marker depends on the genomic information available for the crop and the purpose of the experiment. Since, the little millet is the least studied among all the minor millets, very limited genomic and molecular information is available for the crop. As a result, the molecular marker specific for the crop are not very abundant. Hence, random molecular markers like RAPD or ISSR, become marker of choice for genetic diversity study in the crop. Among these two, ISSR marker becomes the marker of choice for analysing the genetic diversity of little millet due to its abundance, high reproducibility and high polymorphism. Also, since no available SSR markers are there for little millet, ISSR markers become a marker of choice for preliminary genetic diversity study.

ISSR is a region in the genome flanked by microsatellite sequence which can be amplified using 18-25 bp long primer. It is a multi-locus dominant marker which uses a single arbitrary primer for amplification of unknown sequence (Ng and Tan 2015). It has facilitated their use in area of genetic diversity analysis, phylogenetic inference, genome mapping, gene tagging and evolutionary biology in wide range of crops. Hence, it preferred as an ideal genetic marker for organisms whose genomic information is unknown. The knowledge of genetic information of any crop is useful during *in-situ* and *ex-situ* conservation and future crop improvement program. Hence, the potentiality and genetic divergence among the little millet genotypes need to be explored in order to strengthen the availability of genetic resources. The present investigation was carried out to evaluate the

Table 1. List of little millet genotypes included in the morphological study.

Sl. No.	Little millet genotypes	Source
1	WV-108	Gujarat
2	WV-110	Gujarat
3	WV-115	Gujarat
4	WV-119	Gujarat
5	WV-122	Gujarat
6	WV-124	Gujarat
7	WV-146	Gujarat
8	WV-155	Gujarat
9	WV-156	Gujarat
10	WV-159	Gujarat
11	WV-164	Gujarat
12	WV-167	Gujarat
13	WV-168	Gujarat
14	TNPS-171	Gujarat
15	TNPS-173	Gujarat
16	TNPS-167	Gujarat
17	JK-18	Gujarat
18	OLM-203	Gujarat

performance of the selected little millet genotypes in local conditions of Bihar to grow it as a summer crop and evaluate the genetic diversity using yield attributing traits and ISSR markers.

MATERIALS AND METHODS

The experiment was conducted in the agricultural farm located at Tirhut College of Agriculture, Dholi, Bihar which is situated at 25.98°N and 85.60°E at 52.18 m above MSL. The experiment material consisted of 18 little millet genotypes (Table 1) obtained from the Hill Millet Research Station, Navsari Agricultural University, Gujarat. For evaluation and characterization, the genotypes were grown in RBD design with three replications by following standard agronomic practices. Each genotype was grown in 6 lines of 3 m length with 22.5×10 cm spacing in April 2020.

The observations were recorded for randomly selected five plants in each plot for six quantitative traits viz., plant height (cm), number of tillers per plant, flag leaf length (cm), flag leaf width (cm), number of productive tillers/plant and days to 50% flowering as per the descriptors for *Panicum sumatrense* (IBPGR 1985). Five plants were randomly selected from each plot in replications and the average of the entry was used for the statistical analysis. One way ANOVA test, given by Ronald Fisher (Fisher 1925), was followed to analyze the influence of various factors. The mean values of the genotypes were subjected to descriptive test to estimate the maximum, minimum, mean, standard deviation, critical difference, standard error of mean, standard error of difference and coefficient of variation.

Table 2. ISSR primers used for amplification of genomic DNA.

Primer name	Repeat motif	Sequence 5' → 3'	Melting temperature (C°)	Annealing temperature (C°)
UBC807	$(AG)_8T$	AGAGAGAGAGAGAGAGT	50	44
UBC808	$(AG)_8C$	AGAGAGAGAGAGAGAGC	52	48
UBC809	$(AG)_8G$	AGAGAGAGAGAGAGAGG	52	48
UBC810	$(GA)_8C$	GA GAGAGAGAGAGAGAC	52	48
UBC811	$(GA)_8T$	GAGAGAGAGAGAGAGAT	50	42
UBC814	$(CT)_8A$	CT CTCTCTCTCTCTCTA	50	40
UBC815	$(CT)_8G$	CT CTCTCTCTCTCTCTG	52	48
UBC816	$(CA)_8T$	CA CACACACACACACAT	50	42
UBC817	$(CA)_8A$	CACACACACACACACAA	50	40
UBC829	$(TG)_8C$	TGTGTGTGTGTGTGTGC	52	42
UBC864	$(ATG)_6$	ATGATGATGATGATGATG	48	40
UBC866	$(CTC)_6$	CTCCTCCTCCTCCTCCTC	60	50
UBC873	$(GACA)_4$	GACAGACAGACAGACA	48	40
UBC880	$(GGAGA)_3$	GGAGAGGAGAGGAGA	48	40
HB10	$(GA)_8CC$	GAGAGAGAGAGACC	56	50
ISSR17	$CAG(CA)_8$	CAGCACACACACACACACA	58	50

Table 3. Analysis of variance of 6 characters in 18 little millet genotypes. *Significant at p=0.05.

Traits	Mean sum of squares		
	Replication df=2	Genotype df=17	Error df=34
Plant height (in cm)	65.33	1039.38*	41.18
Number of tillers per plant	06.47	0138.56*	02.15
Flag leaf length (in cm)	21.07	0191.07*	06.66
Flag leaf width (cm)	00.02	0000.24*	00.03
Number of productive tillers/plant	02.00	0050.51*	01.18
Days to 50 % flowering	02.80	2576.05*	07.82

The molecular characterization was done by isolation of genomic DNA and its subsequent amplification using ISSR primer. The genomic DNA was extracted from the young leaves of 15 days old plant by using CTAB method as described by Doyle and Doyle (1990) and reported by Ferdous *et al.* (2012) with modifications. The concentration and purity of the DNA in each sample, was detected using Benchtop TM Lab systems BT-Nano-200 Nano Spectrophotometer. The DNA was diluted to obtain a concentration of 30 ng and the PCR was done using ISSR primers.

A total of 16 ISSR primers (Table 2) were used for the amplification of 18 little millet genotypes. The primers used in the study have been used for the diversity analysis of finger millet, foxtail millet and pearl millet but not little millet. The amplification was performed in the volume of 15 µl reaction mix containing 2 µl of template DNA (30 ng), 0.5 µl of Taq DNA polymerase (1 unit), 1.2 µl of primer (5 pmol), 3 µl of dNTPs (1mM), 1.5 µl of $MgCl_2$ (10 mM), 3 µl of 5× PCR Buffer and 3.8 µl of water (Protease and Nuclease free). The amplification was carried out in 96-well plate thermal cycler (Eppendorf, Germany), by using the reaction conditions, initial denaturation at 95°C for 4 min followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 40-50°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. Agarose gel electrophoresis using 2% gel in 0.5× TBE was used to separate the amplified products and the size of amplified product was estimated by using 1 kb DNA ladder.

In each sample, the bands were scored for the presence by denoting '1' for the presence and '0' for the absence. The binary matrix was prepared as discrete variables for further analysis. The polymorphism per cent was determined in terms of percentage between unique alleles and total alleles for all the 16 ISSR primers by using formula :

$$\text{Polimorphism percent} = \frac{\text{No. of unique alleles}}{\text{Total no. of alleles}} \times 100$$

The PIC value was calculated for the identification of polymorphic and informative markers using the formula given by Reddy *et al.* (2009) as

$$PIC = \sum(1-Pi^2)/n$$

Where n is the number of band positions ana-

Table 4. Summary of statistics on different yield contributing traits of 18 little millet genotypes.

Particulars	Plant height (in cm)	Number of tillers per plant	Flag leaf length (in cm)	Flag leaf width (cm)	Number of productive tiller/plant	Days to 50% flowering
Minimum	072.10	14.00	36.70	1.13	01.00	099.00
Maximum	140.83	38.33	68.10	2.00	12.00	162.67
Mean	103.06	25.52	54.94	1.67	07.25	139.82
SD	018.62	06.80	07.99	0.28	04.11	029.31
CD	010.69	02.44	04.30	0.26	01.81	004.66
SE (m)	003.70	00.80	01.49	0.09	00.63	001.61
SE (d)	005.24	01.00	02.11	0.13	00.89	002.28
CV	006.23	05.75	04.70	9.26	14.97	002.00

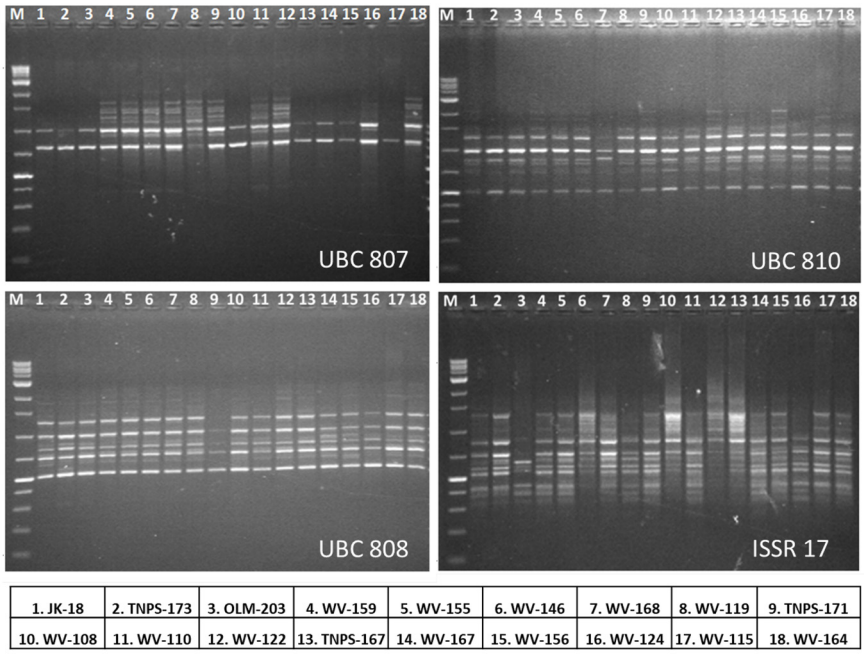

Fig. 1. ISSR primer based amplification pattern of targeted genomic region of 18 *Panicum sumatrense* genotypes.

lyzed in the set of accessions, and Pi is the frequency of i^{th} pattern. The PIC value of primers was used to determine the effectiveness of the individual primer in detecting the genetic diversity between the genotypes of little millet. The genetic associations among genotypes were analyzed by calculating the similarity coefficient (Dice 1945) for pair-wise combinations based on the proportions of shared bands produced

Table 5. Mean performances of 18 little millet genotypes for different yield attributing traits.

Genotypes	Plant height (in cm)	Number of tillers per plant	Flag leaf length (in cm)	Flag leaf width (cm)	Productive tiller/plant	Days to 50% flowering
WV-108	117.67	17	59.83	1.97	7	159.33
WV-110	101.9	28.33	61.5	2	10.33	160
WV-115	98.1	17.33	47.77	1.17	10.67	99.33
WV-119	73.3	37.33	36.7	1.17	9	99
WV-122	77.07	19.67	46	1.93	9.67	162.33
WV-124	72.1	14	49.9	1.83	9	99
WV-146	97.77	25.67	60.07	1.8	10.67	162
WV-155	118.53	27	57.63	1.87	7.67	99.33
WV-156	109.1	28	62.13	1.97	7.33	159.67
WV-159	104.83	25.33	59.4	1.6	8.33	160
WV-164	115.63	19	47.2	1.67	12	159
WV-167	108.33	21.67	62.57	1.63	11	157.33
WV-168	77.6	26.33	68.1	1.8	1	100.33
TNPS-171	140.83	38.33	49.4	1.8	1	156.33
TNPS-173	97.1	33	46.8	1.13	1	162.33
TNPS-167	122.63	28.33	57.13	1.53	1	162.67
OLM-203	113.47	22.33	56.8	1.57	11.67	158
JK-8	109	30.67	59.9	1.5	2	100.67

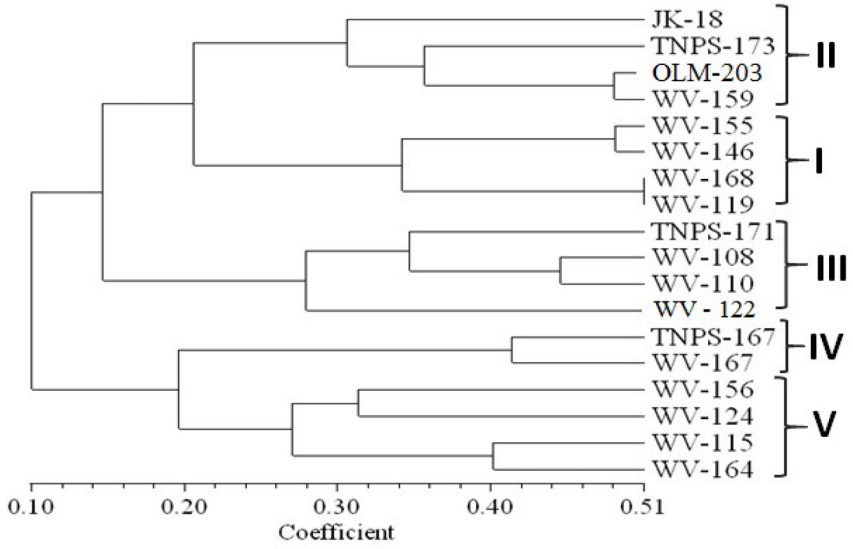

Fig. 2. Dendrogram based on the Dice similarity coefficients for 16 ISSR primers among 18 little millet genotypes.

by primers. The similarity coefficient was calculated by the formula :

$$\text{Similarity coefficient} = \frac{2a}{(2a+b+c)}$$

Where, a=Number of bands between J^{th} and K^{th} genotypes, b=Number of bands present in J^{th} genotype but absent in K^{th} genotype, c =Number of bands absent in j^{th} genotype but present in K^{th} genotype. The cluster analysis was performed with the help of NTSYS-pc software (Rohlf 2002). The SAHN clustering method based on Dice similarity coefficients was used for tree building. Tree diagram was constructed based on similarities coefficient values obtained by UPGMA.

Table 6. Analysis of 16 ISSR primers for the polymorphism per cent and polymorphism information content (PIC).

Sl. No.	Primer	Amplicon size range (bp)	Amplicon size difference (bp)	No. of unique alleles	No. of alleles	PP (%)	PIC
1	UBC 807	772-1882	1110	6	8	75.00	0.989
2	UBC 808	535-1484	0949	2	8	25.00	0.978
3	UBC 809	354-1260	0906	5	13	38.46	0.979
4	UBC 810	493-2315	1822	3	8	37.50	0.970
5	UBC 811	547-1605	1058	3	13	23.07	0.967
6	UBC 814	912-1390	0478	2	8	25.00	0.982
7	UBC 815	516-2000	1484	3	10	30.00	0.969
8	UBC 816	840-0906	0066	00	2	0000	0.969
9	UBC 817	942-1136	0194	01	05	20.00	0.957
10	UBC 829	775-1472	0697	00	2	0000	0.984
11	UBC 864	787-1062	0275	02	5	40.00	0.897
12	UBC 866	733-1916	1183	2	10	20.00	0.984
13	UBC 873	915-1650	0735	3	7	42.85	0.967
14	UBC 880	512-1129	0617	3	9	33.33	0.977
15	HB 10	514-1312	0798	05	9	55.55	0.987
16	ISSR 17	366-1425	1059	3	11	27.27	0.957
	Total			43	117		

Table 7. Dice similarity coefficients for pairwise combination of 18 little millet genotypes based on 16 ISSR primers.

	JK-18	TNPS-173	GLM-203	WV-159	WV-155	WV-146	WV-168	WV-119	TNPS-171
TNPS-173	0.3556								
GLM-203	0.3400	0.4167							
WV-159	0.2268	0.3011	0.4854						
WV-155	0.1782	0.2474	0.3364	0.4615					
WV-146	0.1600	0.1667	0.2642	0.3495	0.4860				
WV-168	0.0808	0.1263	0.1905	0.2353	0.3396	0.4952			
WV-119	0.0930	0.0976	0.1087	0.1798	0.2151	0.3261	0.5055		
TNPS-171	0.0971	0.0808	0.1284	0.1509	0.1636	0.2018	0.2593	0.4211	
WV-108	0.1348	0.1176	0.1474	0.1087	0.1250	0.1263	0.1489	0.1975	0.3673
WV-110	0.1702	0.0889	0.1200	0.1443	0.1386	0.1200	0.1818	0.2093	0.3301
WV-122	0.0825	0.1075	0.0971	0.0600	0.1154	0.0777	0.0980	0.1798	0.2075
TNPS-167	0.1031	0.1290	0.1359	0.1200	0.1346	0.0777	0.1373	0.1573	0.1509
WV-167	0.0682	0.1429	0.1064	0.1538	0.1263	0.0638	0.0645	0.1000	0.1443
WV-156	0.1277	0.0667	0.0600	0.0619	0.0990	0.1200	0.0606	0.0698	0.0583
WV-124	0.0899	0.0706	0.0632	0.0435	0.0625	0.0632	0.0426	0.0494	0.0408
WV-115	0.0879	0.0690	0.0619	0.0851	0.0408	0.0825	0.0208	0.0964	0.0800
WV-164	0.0690	0.0964	0.0215	0.0889	0.0851	0.0645	0.0870	0.1013	0.0833

Table 7. Continued.

	WV-108	WV-110	WV-122	TNPS-167	WV-167	WV-156	WV-124	WV-115
TNPS-173								
GLM-203								
WV-159								
WV-155								
WV-146								
WV-168								
WV-119								
TNPS-171								
WV-108								
WV-110	0.4494							
WV-122	0.2609	0.3711						
TNPS-167	0.1304	0.2062	0.3800					
WV-167	0.1687	0.1591	0.1758	0.4176				
WV-156	0.0899	0.1489	0.1237	0.2474	0.3409			
WV-124	0.0476	0.0674	0.0217	0.0652	0.1446	0.3146		
WV-115	0.0698	0.1099	0.1489	0.2128	0.2118	0.2637	0.3721	
WV-164	0.0488	0.1149	0.0444	0.0889	0.2469	0.2529	0.1951	0.4048

RESULTS AND DISCUSSION

ANOVA analysis

The availability of variation in the genetic resources provides the breeders an opportunity to develop improved cultivars with preferred traits. The ANOVA test is one of the effective tools to analyze the genetic diversity among the population at genetic level and also to estimate the relative contribution of various traits towards total divergence. The test was performed in the present study to quantify the magnitude of genetic divergence between the 18 little millet genotypes. The maximum mean sum of square values was recorded for days to 50% flowering (2576.05) followed by plant height (1039.38) (Table 3). All the mean sum of square values of genotypes are highly significant at table value p=0.05 which indicates the presence of substantial amount of variability for the 18 little millet genotypes under present study. Similar level of significance had been observed by Nirmalakumari *et al.* (2015) and Katara *et al*. (2019) during the diversity analysis of little millet. The genetic diversity study using morphological traits have

Table 8. Cluster composition of 18 little millet genotypes.

Sl. No.	Clusters	Number of genotypes in each cluster	Cluster members
1	Cluster I	4	WV-155, WV-146, WV-168 and WV-119
2	Cluster II	4	JK-18, TNPS-173, OLM-203 and WV-159
3	Cluster III	4	TNPS-171, WV-108, WV-110 and WV-122
4	Cluster VI	2	TNPS-167 and WV-167
5	Cluster V	4	WV-156, WV-124, WV-115 and WV-164

proved to be useful for determining the divergence among the little millet genotypes under different conditions as indicated by the works of Arunachalam *et al.* (2005), Nirmalakumari *et al*. (2010), Manimozhi Selvi *et al.* (2015); Anuradha *et al.* (2017), Patel *et al*. (2018) and Venkataratnam *et al.* (2019). Similar methodology was successfully followed and reported by Katara *et al.* (2019) with 30 little millet genotypes under different sown conditions.

Descriptive statistics

The mean values of the genotypes were subjected to descriptive study including maximum, minimum, mean, standard deviation, critical difference, standard error of mean, standard error of difference and coefficient of variation (Table 4). The considerable amount of variation was observed for the six quantitative traits (Table 5). Among the studied morphological traits, days to 50 % flowering followed by plant height contributed the most towards genetic divergence. The plant height ranged from 72.10 (WV-124) to 140.83 (TNPS-171) with the mean of 103.06. The length of flag leaf ranged from 36.70 cm (WV-119) to 68.10 cm (WV-168) with the mean of 54.94 cm. Number of tillers per plant ranged from 14 (WV-124) to 38.33 (TNPS-171) with the mean of 25.52. The number of productive tillers/plants ranged from 1 (WV-168, TNPS-171, TNPS-173, TNPS-167) to 12 (WV-164) with mean of 7.25. The width of flag leaf ranged from 1.13 cm (TNPS-173) to 2 cm (WV-110) with mean of 1.67 cm.

The days to 50 % flowering ranged from 99.00 (WV-119, WV-124) to 162.67 (TNPS-167) with a mean of 139.82. The little millet is considered a short duration crop and days to 50% flowering mostly takes 60-70 days (Nirmalakumari *et al.* 2010). But the genotypes evaluated in the present study showed delayed flowering. They also showed less seed setting as compared to *kharif* sown plants. Since it is the first study carried out to evaluate the performance of the plant as a summer crop in plains, these findings indicate that the little millets are photosensitive plants and have average performance as a summer crop in Bihar. However, six genotypes, WV -115, WV -119, WV -124, WV -155, WV -168 and JK-8 showed normal flowering and can be used for cultivation in local conditions of Bihar.

Molecular characterization

All the 16 ISSR primers produced polymorphic amplification product in the 18 genotypes except HB 10, UBC 814 and UBC 817, which were not able to produce amplification products in few genotypes (Table 6, Fig. 1). The size of amplification product size ranged from 354 bp to 2315 bp. A total of 117 alleles alongwith 43 unique alleles and 74 shared alleles were detected. The number of alleles detected ranged from 2 to 13 (Table 6). The lack of amplification by some primers can be a result of experimental error (Kumar *et al.* 2006, Kumari *et al*. 2011, Wei *et al.* 2012). Nevertheless, the formation of polymorphic, amplified product shows high transferability of the primers used in the study. The findings support the hypothesis that ISSR primer has high transferability ability in detecting the polymorphism (Boczkowska and Tarczyk 2013, Ng and Tan 2015). The applicability of the primer UBC 810 for finger millet and foxtail millet has also been reported by Kelkar *et al.* (2017) and Wei *et al.* (2012).

Polymorphism per cent and polymorphism information content

The polymorphism per cent and polymorphism information content of 16 ISSR primers was calculated to determine the effectiveness of the individual primer

in detecting the genetic diversity between the little millet genotypes (Table 6). All the primers evaluated in the study produced unique alleles except UBC 816 and UBC 829. The polymorphism per cent ranged from 20% for the primers UBC 866 and UBC 817 to 75% for the primer UBC 807. The primer UBC 807 was identified as the best for its ability to detect unique alleles. Since, ISSR markers can be effectively used for DNA fingerprinting studies if they produce polymorphic, unique bands (Ng and Tan 2015), UBC 807 was also identified as the best primer for fingerprinting studies in little millet. The studies for diversity assessment of foxtail millet and finger millet by Wei *et al*. (2012) and Animasaun *et al.* (2015) respectively, also reported the better ability of primer UBC 807. However, the primers UBC 816 and UBC 829, which produced unique alleles for foxtail millet, was not effective for little millet (Wei *et al.* 2012). On the contrary, primer UBC 810 produced amplification products during the present analysis but did not produce amplification product in foxtail millet (Wei *et al*. 2012).

The polymorphism information content values for all the 16 primers were high and greater than 0.890 (Table 6). The value of PIC ranged from 0.897 for the primer UBC 864 to 0.989 for the primer UBC 807 with an average of 0.969. Hence the primer UBC 807 was determined to be the best in its effectiveness to detect the genetic diversity among the 18 little millet genotypes followed by HB 10 and UBC 814. Similarly, the primer UBC 807 produced high polymorphism informative content in pearl millet, foxtail millet and finger millet as reported by Yadav *et al.* (2007), Gupta *et al.* (2010), Kelkar *et al.* (2017) and Wei *et al. (*2012). The primers used in the present were found to be highly polymorphic. The polymorphic primers are very effective for diversity analysis of any crop plant (Gilande *et al.* 2015, Kelkar *et al.* 2017, Lee *et al.* 2017, Tiwari *et al.* 2018, Rajput *et al.* 2019). Most of ISSR primers included in the study were also found to be highly polymorphic in pearl millet, foxtail millet and finger millet. Since these primers have not been used earlier for characterization of little millet genotypes, the results are considered to be important. The primers can also be employed for genetic diversity analysis of other little millet genotypes.

Dice's similarity coefficient

Polymorphism was recorded by scoring the bands and binary matrix as discrete variables were formed. The Dice's similarity coefficients were computed to determine the relativeness among the 18 little millet genotypes. The similarity coefficient value ranged from 0.0208 to 0.5055 (Table 7). The similarity value was observed highest between the genotype pair WV-168 and WV-119 (0.5055). The lowest similarity was observed between the genotype pair WV-168 and WV-115 (0.0208). The higher the similarity coefficient values, greater will be the similarity between the pairs. The similarity coefficient value was less than 0.4 for the most of the genotype pairs, which indicates the presence of high amount of genetic variability between the genotypes of the little millet evaluated in the study.

Dendrogram analysis

The phylogenetic relationship among the 18 little millet genotypes was determined by using UPGMA dendrogram analysis. The genotypes were divided into two major groups. Group 1 was further subdivided into three clusters namely cluster I, cluster II and cluster III (Table 8, Fig. 2). Each cluster in the group 1 has 4 genotypes. The cluster I was formed by four genotypes namely WV-155, WV-146, WV-168 and WV-119. The cluster II was formed by four genotypes namely JK-18, TNPS-173, OLM-203 and WV-159. The cluster III was formed by four genotypes namely TNPS-171, WV-108, WV-110 and WV-122. Group 2 was further subdivided into two clusters namely cluster IV and cluster V. The cluster IV was formed by two genotypes namely TNPS-167 and WV-167. The cluster V was formed by four genotypes namely WV-156, WV-124, WV-115 and WV-164. The 18 genotypes of little millets were grouped into 5 clusters at 40 phenon level (Table 8). The dendrogram analysis revealed the presence of diversification among the little millet genotypes. The substantial level of polymorphism was found between the little millet populations. Similar results have been found by Rajput *et al.* (2019) in little millet.

Based on the Dice's Similarity Coefficient matrix and cluster analysis, the genotypes pair WV-168 and

WV-119 followed by WV-146 and WV-168 was found to have greater genetic similarity compared to other genotypes in the population. The genotypes pair WV-168 and WV-115 followed by WV-122 and WV-125 was found to have greater diversity. The identified genotypes can be used as parents in hybridization programs by the plant breeders for the development of more promising cultivars.

All the little millet genotypes investigated in the present study successfully germinated in the local conditions of Bihar. Although most of the genotypes showed delayed flowering and maturity, some genotypes (WV -115, WV -119, WV -124, WV -155, WV -168 and JK-8) were identified which can be sown as summer crops in Bihar. The genotypes exhibited a wide range of variation. Different agronomical practices need to be adopted for the cultivation of the crop as a summer crop in Bihar, to enable timely flowering and fruiting. From the study, ISSR markers were found to be highly efficient for the assessment of genetic relationship at molecular level. Based on the Dice's Similarity Coefficient matrix and cluster analysis, the substantial level of variability was found between the little millet populations. The genetic diversity analysis concluded that genotypes WV-168 and WV-115 followed by WV-122 and WV-124 have greater variability than other genotypes. In the view of future crop improvement programme, plant breeders can utilize the above diverse genotypes for the development of the most promising varieties with desirable traits to support local farmers of Bihar and to shift in little millet cultivation. This is the first report of the assessment of the performance of the little millet genotypes in plains like Bihar and the results can help in finalizing crop breeding strategies for the crop in the region.

ACKNOWLEDGMENT

The authors express their acknowledgement to Dr. Rajendra Prasad Central Agricultural University for providing infrastructural and financial support for carrying out the research work.

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