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Cross Transferability of SSR Markers from Finger Millet, Pearl Millet and Rice to Indian Little Millet and their Genetic Diversity Analysis

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ABSTRACT: Little millet (*Panicum sumatrense*) is nutrient rich, highly resilient to climate, abiotic and biotic stresses in comparison to other cultivated cereals. Lack of genomic information of little millet severely limits the use molecular markers in the study of genetic variability and crop improvement. Development of molecular markers for genetic diversity analysis of little millet is required for conservation of germplasm *ex-situ*. This study was conducted to evaluate the genetic diversity of 33 little millet landraces accessions collected from different agro-climatic zones of Madhya Pradesh and few samples from Chhattisgarh state using simple sequence repeat (SSR) markers. About 15 SSR markers were selected from reported markers of finger millet, pearl millet and rice. Among 15 SSR markers, 9 markers showed polymorphism. Totally 30 polymorphic alleles were detected with 44.71% percentage of polymorphism. The averages of gene diversity, polymorphic information content (PIC) and major allele frequency were 0.29, 0.26 and 0.78 respectively. The range of genetic distance value of 33 little millet lines was 0.0-0.6. DINKUT20160830-6 landrace was found to be most diverse based on cluster analysis. Findings of this study thus demonstrate the utility of SSR primers of same family crops in assessing genomics relationships in little millet. This study aid in development and use of SSR marker in little millet germplasm conservation and utilization in crop improvement programs effectively.

Keywords: Little millet, SSR markers, landraces, polymorphism, genetic diversity, *Panicum sumatrense*.

INTRODUCTION

The little millet (*Panicum sumatrense* Roth ex Roemer and Schultes) belongs to the family of Poaceae and is a tetraploid (2n=4x=36) minor millet crop. The crop is commonly known as kutki, sawa, samai and samalu (Padulosi *et al.,* 2009). The place of origin of little millet is Indian subcontinent (Hu *et al.,* 2009). It is grown all over India to a limited extent up to altitudes of 2100 m, but it is less important elsewhere. The seeds of little millet are smaller in comparison to other common millets. It is an annual herbaceous and self-pollinating crop. It is a rich source of fiber, fat, carbohydrates, and protein and phytochemicals including phenolic acids, flavonoids, tannins, and phytate (Pradeep and Guha 2011). The crop is superior or at par with other cultivated cereals nutritionally as well as medicinally. Its grains are beneficial for diabetic and patients of cardio–vascular diseases. Tribal and poor farmers cultivate this crop in low fertile soils with low or no cash input for the purpose of food and feed. In comparison to other cereal crops, it has an excellent rejuvenating capacity. Seeds of this millet is extremely small but easy to harvest and highly nutritious. They can be cooked or ground into a powder and used for making cakes etc. They can also be eaten as sprouts and used in salads. The flowers are used as a food colouring in ceremonial maize bread (Shweta *et al.,* 2018).

In India, during 2016 the crop was cultivated in an area of 255.5 thousand hectare with production of 119.9 thousand tons and productivity of 469 kg/ha. India occupies first rank in the world for production of little millet and provides 0.1% contribution to total millets production. India is major production country of little millet contributing 100% of world production of little millet (Bhat *et al.,* 2018; IIMR estimates based on FAO/DES – GOI data). Major little millet growing states in the country are Andhra Pradesh, Chhattisgarh, Madhya Pradesh, Orissa, Tamil Nadu, Karnataka, Jharkhand and Gujarat. The area of production of this crop is 51.54 thousand hectares with productivity of 525.5 kg per hectare in Madhya Pradesh (www.landrecords.mp.gov.in). Major little millet growing districts of Madhya Pradesh are Dindori, Mandla, Chindwara, Balaghat, Seoni, Anuppur, Betal, Singrauli, Umaria, Sidhi, Shahdol, Jabalpur, Narsinghpur, Raisen and Khandwa. The grain of little millet can be stored under ordinary storage conditions for several years without any harm by stored grain

pests. Being eco-friendly, the crop is suitable for fragile and susceptible agro-ecosystems (Kumar *et al.,* 2017).

The major goal of genetic diversity assessment is crop improvement. Among various molecular markers, at present AFLP, RAPD, ISSR and SSR are being used to assess the genetic diversity of different crop species (Tripathi *et al.,* 2013). Our knowledge of the genetic diversity and gene pool structure of little millets is very limited. Consequently, to facilitate the development of these cereals, lots of studies need to be carried out to determine their genetic diversity and to define their gene pools for utilization in selection and breeding. Genetic diversity studies among some little millet accessions have previously been carried out by using randomly amplified polymorphic DNA (RAPD) by M'Ribu and Hilu (1994); Tiwari *et al.* (2018). However, very limited studies have been reported of use of SSR primers for little millet diversity analysis (Ali *et al.,* 2017; Desai *et al.,* 2021). SSR markers have been used successfully to evaluate genetic diversity among several millet species, including barnyard millet (Manimekalai *et al.*, 2018), pearl millet (Singh *et al.,* 2018; Kumar *et al.*, 2020; Bougma *et al.*, 2021) proso millet (Cho *et al.*, 2010; Rajput and Santra 2016; Han *et al.,* 2020), foxtail millet (Kim *et al.,* 2012; Zhao *et al.,* 2012) and finger millet (Babu *et al.,* 2014; Prabhu *et al.,* 2018; Lule *et al.*, 2018; Bwalya *et al.*, 2020; Joshi *et al.*, 2020; Brhane *et al.*, 2021; Wang *et al.,* 2021). Similarly, several studies of genetic diversity analysis using SSR have been reported for rice cereal which belong to same family poaceae (Singh *et al.,* 2016; Becerra *et al.,* 2017; Rashmi *et al.*, 2017; Jasim Aljumaili *et al.,* 2018; Pathaichindachote *et al.*, 2019; Verma *et al.*, 2019; Suvi *et al.*, 2020). Little millet is perhaps the least studied of the small millet species and other cereals. To bridge this gap, present study was undertaken to analyze polymorphism and cross transferability of SSR markers finger millet, pearl millet and rice into little millet.

MATERIALS AND METHODS

Thirty-three accessions of little millet were obtained from different agro-climatic zones of Madhya Pradesh like Rewa, Chindwara and Dindori and few samples from Chhattisgarh state (Table 1). Seeds were grown in greenhouse to collect the fresh leaves samples for genomic DNA isolation. Genomic DNA was isolated using protocol (Saghai-Maroof *et al.,* 1984) and quantified using Nanodrop (Jenway Genova Nano) by measuring the absorbance at 260nm and 280nm and quality was checked by horizontal submarine gel electrophoresis on 0.8% agarose gel. DNA samples (2 µl) were loaded in each well in agarose gels along with the 1µl λ Hind III digested DNA ladder, in 0.8% gel concentration a separate well and run at 80V for 60 min.

Table 1: List of little millet landraces and location of collection site.

Sr. No.	Name of Accession	Collection site	Geographical location		District
			Latitude	Longitude	
1.	DINKUT20160830-3	Shivri-1	N 22,50,35	E 81,14,57	Dindori
2.	DINKUT20180219-2	Aunrai-2	N 22,56,36	E 81,4,37	
3.	DINKUT20160830-1	Shivri-3	N 22,50,35	E 81,14,57	
4.	DINKUT20160830-7	Sherajhar-4	N 22,35,17	E 81,19,16	
5.	DINKUT20160830-6	Sherajhar-5	N 22,35,17	E 81,19,16	
6.	DINKUT20160830-8	Shivri-6	N 22,50,35	E 81,14,57	
7.	DINKUT20180315-1	Shivri-7	N 22,50,35	E 81,14,57	
8.	DINKUT20180315-6	Dindori-8	N 22,56,36	E 81,4,38	
9.	DINKUT20180315-2	Shivri-9	N 22,50,35	E 81,14,57	
10.	DINKUT20180219-1	Fadki-10	N 22,59,59	E 80,57,28	
11.	DINKUT20180315-3	Shivri-11	N 22,50,35	E 81,14,57	
12.	DINKUT20180315-4	Shivri-12	N 22,50,35	E 81,14,57	
13.	DINKUT20180830-2	Aunrai-13	N 22,56,36	E 81,4,370	
14.	DINKUT20180315-5	Shivri-14	N 22,50,35	E 81,14,57	
15.	DINKUT20160830-5	Shivri-15	N 22,50,35	E 81,14,57	
16.	DINKUT20160830-4	Khaparipani-16	N 22,39,20	E 81,16,41	
17.	DINKUT20180219-3	Padariya-17	N 22,3,26	E 78,56,17	
18.	JBPKUT20180315-7	Kundam-18	N 23,13,7	E 80,21,3	Jabalpur
19.	REWKUT20171125-4	Pokhra-19	N 34,48,21	E 82,21,54	Rewa
20.	REWKUT20171126-3	Pokhra-20	N 34,48,21	E 82,21,54	
21.	REWKUT20171125-9	Amwa-21	N 34,48,21	E 82,21,54	
22.	REWKUT20171126-4	Amwa-22	N 34,48,21	E 82,21,54	
23.	REWKUT20171125-1	Charhai-23	N 34,48,21	E 82,21,54	
24.	CHHKUT20171127-3	Pipariya-24	N 22,3,26	E 78,56,17	Chhindwara
25.	CHHKUT20171127-1	Pipariya-25	N 22,3,26	E 78,56,17	
26.	CHHKUT20171127-8	Ghugarlakalan-26	N 22,3,26	E 78,56,17	
27.	CHHKUT20171127-7	Ghugarlakalan-27	N 22,3,26	E 78,56,17	
28.	CHHKUT20171127-6	Ghugarlakalan-28	N 22,3,26	E 78,56,17	
29.	BETKUT20171128-6	Lahas-29	N 21,54,4	E 77,53,45	Betul
30.	Chhattisgarh-1	Chhattisgarh	N 21,16,11	E 81,35,59	Chhattisgarh
31.	Chhattisgarh-2	Chhattisgarh	N 21,16,11	E 81,35,59	
32.	Chhattisgarh-3	Bastar-1	N 19,4,0.12	E 82,1,59	
33.	Chhattisgarh-4	Bastar-2	N 19,4,0.12	E 82,1,59	

Cross amplification of finger millet, pearl millet and rice SSR markers. Cross-species amplification of little millet landraces were performed using 20 finger millet and pearl millet and 15 rice SSR markers. The finger millet and pearl millet SSR markers were obtained from earlier studies (Arya *et al.*, 2013; Lee *et al.*, 2017; Yadav *et al.*, 2014) whereas rice SSR markers were obtained from the study (Chakravarthi and Naravaneni 2006). The polymerase chain reactions (PCR) were performed in 10µL reaction volume containing 1 µL of 1X buffer having 2.5mM$MgCl_2$, 10Pm primer, 0.2µL of 200 µM dNTPs, 0.2 µL of 1 U of *Taq* DNA polymerase, and about 50 ng of template DNA. The PCR amplification was performed with initial denaturation for 4 min at 94°C followed by 35 cycles of 30 s at 94°C, 1.0 min of annealing temperature at 55°C, extension of 2.0 min at 72°C, with a final extension of 5 min at 72°C, and hold at 4°C. The electrophoresis was done by using the 2.5 percent agarose gel concentration. Gels were stained with ethidium bromide and visualized using Bio Imaging System (Vilber Laurmet).

Statistical analysis. Data were analyzed to obtain genetic distance table among different isolates by using power marker 3.25 version software (Liu and Muse 2005). A dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Averages (UPGMA).

Table 2: List of SSR primers used for fingerprinting.

	Primer	Sequence (5'-3')	TB	PB	PP
	GB-FM-70	**F**-GAAGGTGGGAACCGTCTC	2	0	0
		R-ACCCAGCTCATGAAAGCC			
	UGEP12	**F**-ATCCCCACCTACGAGATGC	5	4	80
		R-TCAAAGTGATGCGTCAGGTC			
	GB-FM-53	**F**-TTCAGAATCCGTTCGTGC	3	2	66.67
		R-CTGCCTTTGAATAGTTCACCA			
	UGEP26	**F**-ATGGGGTTAGGGTTCGAGTC	1	0	0
		R-TGTCCCTCACTCGTCTCCTC			
	Xcump004	**F**-GCATTGATGTGCCAATCG	1	0	0
		R-ACCCGGGTCTGGTTAGACTT			
	RM275	**F**-GCATTGATGTGCCAATCG	1	0	0
		R-CATTGCAACATCTTCAACATCC			
	RM475	**F**-CCTCACGATTTTCCTCCAAC	2	1	50
		R-ACGGTGGGATTAGACTGTGC			
	RM250	**F**-GGTTCAAACCAAGCTGATCA	7	6	85.72
		R-GATGAAGGCCTTCCACGCAG			
	RM280	**F**-ACACGATCCACTTTGCGC	4	3	75
		R-TGTGTCTTGAGCAGCCAGG			
	RM152	**F**-GAAACCACCACACCTCACCG	6	5	83.34
		R-CCGTAGACCTTCTTGAAGTAG			
	RM413	**F**-GGCGATTCTTGGATGAAGAG	2	1	50
		R-TCCCCACCAATCTTGTCTTC			
	RM21	**F**-ACAGTATTCCGTAGGCACGG	4	4	100
		R-GCTCCATGAGGGTGGTAGAG			
	RM474	**F**-AAGATGTACGGGTGGCATTC	1	0	0
		R-TATGAGCTGGTGAGCAATGG			
	RM11	**F**-TCTCCTCTTCCCCCGATC	1	0	0
		R-ATAGCGGGCGAGGCTTAG			
	RM19	**F**-CAAAAACAGAGCAGATGAC	5	4	80
		R-CTCAAGATGGACGCCAAGA			
Total			45	30	-
Mean			3	2	44.71%

Legends: TB= Total bands, PB = Polymorphic bands, PP = Polymorphism percentage

RESULTS AND DISCUSSION

In the present investigation, 33 landraces of *P. sumatrense* were obtained from different agro-climatic zones of Madhya Pradesh like Rewa, Chindwara and Dindori and few samples from Chhattisgarh to detect polymorphism using fifteen SSR as genetic marker. Similarly, SSR markers have been used for genetic diversity analysis in twenty finger millet germplasm in Sri Lanka (Wakista *et al.*, 2017). Arya *et al.* (2013) analyzed diversity among sixty-seven finger millet accessions in Bangalore, India and Kumar *et al.* (2020) evaluated the genetic diversity of 17 important Indian pearl millet inbred genotypes and one popular hybrid 9444. Bougma *et al*. (2021) used 20 pairs of SSR markers to compare and analyze the genetic diversity of 86 landrace populations of Burkina Faso. Manyasa *et al*. (2014) collected 340 finger millet accessions from Kenya, Tanzania and Uganda and 15 minicore accessions by using 23 SSR markers to detect polymorphism. Brhane *et al*. (2021) analyzed genetic diversity among 55 landrace accessions and 5 cultivars of Ethiopian finger millet using 3 genomic and 7 novel EST derived markers. Jasim Aljumaili *et al.* (2018) analyzed genetic diversity among the 50 aromatic rice accessions from Peninsular Malaysia, Sabah, and

Sarawak with 3 released varieties as a control using the 32 SSR markers. Verma *et al.* (2019) assessed genetic diversity among 114 rice genotypes of North East India by using 65 SSR markers. Pathaichindachote *et al.* (2019) collected 167 Thai and exotic rice accessions for evaluation of genetic diversity SSR markers.

Initially 35 primers (20 primers from millets and 15 primers from rice genome) were screened among 33 landraces of *P. sumatrense.* Among them 15 primers (5 from millet and 10 from rice, Table 2) were successfully amplified and given sharp and clear banding pattern. The band size of amplified markers ranged from 110-700 bp. 110 bp band size was obtained with primer UGEP26, Xcump004, RM413 and 700bp band was obtained with primer RM 280. Maximum numbers of bands scored were 7 in case of primer RM250 while minimum number of band was 1 produced by UGEP26, Xcump004, RM275, RM474 and RM11 primers. Out of 45 bands amplified, 30 were found to be polymorphic with 44.71% percentage of polymorphism and rest 15 bands were found to be monomorphic. Average numbers of band per primer was 3 while, average numbers of polymorphic band per primer was 2. Out of 15 SSR primers, polymorphic alleles were presented with nine primers such as UGEP12, GB-FM-53, RM475, RM250, RM280, RM152, RM413, RM21 and RM19. In our study, primer GB-FM-53 was found to be polymorphic with PIC value 0.1125. While Lee *et al*. (2017) observed that primer GB-FM-53 was polymorphic with 0.717 PIC value in their study. Among rice primers RM475, RM250, RM280, RM152, RM413, RM21 and RM19 were found to be polymorphic with PIC values 0.2784, 0.6033, 0.4988, 0.5256, 0.3180, 0.4556 and 0.4802 respectively. Primer RM21 showed 100% polymorphism. Similarly, Khumeshwar (2018) found 100% polymorphism in primer RM21 but also observed polymorphism in all primers (RM474, RM11, RM475, RM250, RM280, RM152, RM413, RM21 and RM19).

Genetic diversity of specific locus was evaluated by polymorphic information content (PIC) values. The higher the PIC values, the higher the probability that polymorphism will exist. The range of PIC for polymorphic allele was 0.11 (GB-FM-53) to 0.62 (UGEP12) with an average of 0.26. A study conducted by Wakista *et al.* (2017) to assess genetic diversity of 20 finger millet accessions of Sri Lanka using ten SSR markers reports the same range of PIC values from 0.00 to 0.69 and the average PIC value was 0.26. Ramakrishnan *et al.* (2016) assessed genetic diversity of 128 Indian and non-Indian genotypes of finger millet using 87 genomic SSR markers and reported PIC values ranging from 0.32 to 0.64 with an average PIC value of 0.44. Major allele frequency was calculated among all SSR markers. The highest major allele frequency was 1.000 (GBFM70, UGEP26, Xcump004, RM275, RM474, RM11) and lowest value was 0.4242 (UGEP12). Average major allele frequency was found to be 0.7818. Gene diversity ranges from 0.000 (GB-FM70, UGEP26, Xcump004, RM275, RM474 and RM11) to 0.6850 (UGEP12) with an average value of 0.2931. Eight primers showed (UGEP12, RM475, RM250, RM280, RM152, RM413, RM21 and RM19) more gene diversity than the average value and UGEP12 showed the highest. Wakista *et al.* (2017) observed range of gene diversity values from 0.00 to 0.73 with an average value of 0.29. Five SSR markers (UGEP5, UGEP12, UGEP15, UGEP24 and UGEP68) showed more gene diversity than the average value and UGEP24 showed the highest. Ramakrishnan *et al.* (2016) observed gene diversity values ranged between 0.02-0.35 with a mean value of 0.14.Genetic distance values for 33 little millet lines were calculated and a dendrogram was generated by UPGMA cluster analysis based on genetic distance values using Power marker version 3.25 (Liu and Muse 2005). The cluster analysis grouped little millet landraces into two main groups *i.e.* A and B named as minor and major groups respectively. Minor group contained only one landrace namely DINKUT20160830-6 which belongs to Dindori district and site of collection is Shivri. Major group contained 32 landraces and this group is further divided into 2 subgroups *i.e.*, C and D. The group C contained 17 landraces and group D contained 15 landraces. Group C is further divided into subgroups E and F. Subgroup E contained 10 landraces namely DINKUT20160830-7, DINKUT20180830-2, DINKUT20160830-8, DINKUT20180219-1, DINKUT20160830-1, DINKUT20180315-6, DINKUT20180315-1, DINKUT20180315-3, DINKUT20180315-2 and DINKUT20180315-4 and subgroup F contained 7 landraces namely DINKUT20160830-5, DINKUT20180315-5, JBPKUT20180315-7, REWKUT20171126-3 REWKUT20171125-9, DINKUT20180219-3 and REWKUT20171125-9. The group D is further divided into 2 subgroups *i.e.*, G and H. Subgroup G had 4 landraces namely DINKUT20160830-3, DINKUT20160830-4, DINKUT201819-2, REWKUT20171126-4 while subgroup H contained 11 landraces namely CHHKUT20171127-8, CHHKUT20171127-1, CHHKUT20171127-3 Chattisgarh-3, Chattisgarh-4, REWKUT20171125-1, BETKUT20171128-6, Chattisgarh-2, CHHKUT20171127-7, Chattisgarh-1 and CHHKUT20171127-6 (Fig. 1). This cluster analysis revealed that the most diverse landrace of little millet was DINKUT20160830-6 found separately in minor group. Similar clustering was reported by Tiwari *et al.* (2018) while working on molecular diversity analysis among thirty-two genotypes of little millet based on RAPD data.

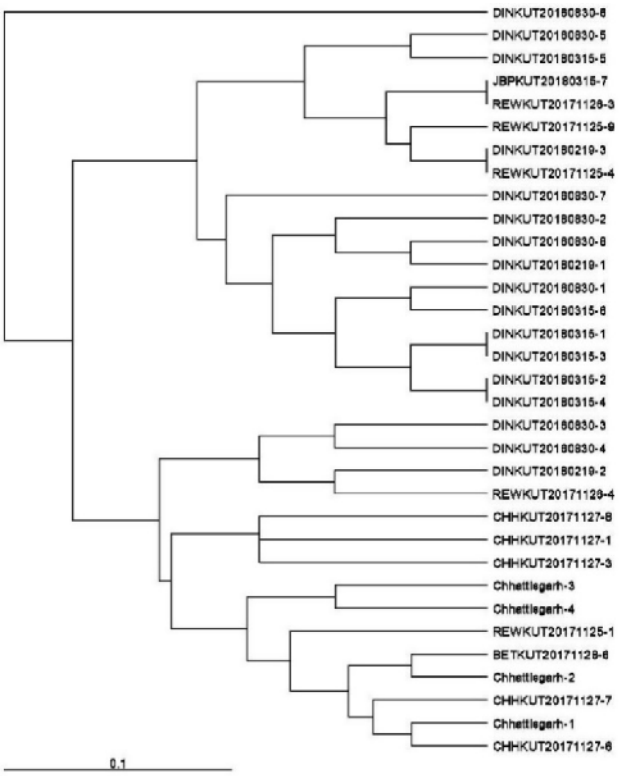

Fig. 1. Genetic diversity analysis among thirty-three genotypes of little millet based on SSR data.

Genome cross-transferability. Little millet genomics is less explored in comparison with other cereal crops like, wheat, rice and maize and small millets such as finger millet and foxtail millet. The success of transferability of SSR markers rely on the genetic or evolutionary closeness among the species examined. In the present study, transferability of SSR markers was detected in 15 (42.9%) out of 35 SSRs tested. 20 primers were from other millet crops *i.e*., finger millet and pearl millet and 15 primers from rice were screened. Out of 20 only 5 primers of other millets *viz.*, GB-FM-70, UGEP12, GB-FM-53, UGEP26 and Xcump004 and 10 primers of rice *viz.*, RM275, RM475, RM250, RM280, RM152, RM413, RM21, RM474, RM11 and RM19 were successfully amplified. This study shows the inter-generic transfer of SSR primers within the same family of crops. Genome of little millet is transferable with rice and other millets like finger millet and pearl millet. However few studies have been reported for cross- transferability in little millet. Earlier, Ali *et al.*, (2017) using 22,961 EST sequences of switch grass (*Pancium virgatum*) developed 48 species transferable EST-SSR markers for little millet. Later Desai *et al.* (2021) developed eSSR markers of little from transcriptome and 39 markers were successfully amplified in other minor millet crops. Whereas, several studies have been reported in other millet crops. Arya *et al.* (2009) evaluated transferability of the EST-SSRs among three varieties of pearl millet which were developed in this study for finger millet. Amplification was detected in 11 (64.7%) out of 17 SSRs tested. Kumari *et al.* (2013) reported cross-genera amplification of about 106 selected primer pairs of foxtail millet at an average of ~88% in eight millets and four non-millet species. Rajput *et al*. (2014) tested 548 SSR of switch grass on eight proso millet genotypes. Out of these, 339 SSR markers were amplified in proso millet. This showed that 62% of the switch grass SSR markers were transferable to proso millet. Similarly, Babu *et al.* (2018) used 64 genomic SSRs of finger millet and maize, only 39 (61%) were amplified across the barnyard millet genotype. Krishna *et al.* (2018) reported the cross-genome transferability of 101 SSR markers of finger millet and 26 SSR markers of foxtail millet in 8 other millets. Out of these, 33 finger millet and 2 foxtail millet SSR markers showed 100% cross-genome transferability in other millets. The possible reason of the transferability of SSR markers is that these markers share common features across distantly related species that has been demonstrated in previous studies (Varshney *et al.*, 2005).

CONCLUSION

The genetic diversity among different landraces of little millet was analyzed by using SSR markers, which are effective and reliable tools for this type of analysis. These findings not only highlight the capacity of the SSR technique but also helps in the selection of diverse little millet landraces for conservation and crop improvement. This study shows the inter-generic transfer of SSR primers within the same family of crops. Genome of little millet is transferable with rice and other millets like finger millet and pearl millet.

Diverse parents can be used for the improvement of millet genotypes and rice also because; the study confirms the suitability of gene transfer among rice, finger millet and pearl millet. The SSR markers identified herein can be applied in different hereditary studies together with association mapping in this crop and in related millet crops. Hence, these selected SSR markers can be helpful for molecular breeding in this underutilized millet crop.

FUTURE SCOPE

For more reliable genetic diversity analysis, SSR primers from little millet genome should be designed in future research works because in present study, primers from other millet crops (finger millet, pearl millet) and rice were used for genetic diversity analysis of little millet crop. Information obtained should be utilized in crop improvement programme of little millet. For obtaining more data, a greater number of SSR primers and other molecular markers such as SNP should be applied for generating fingerprints. This study shows that little millet genome is similarity with crops rice, finger millet and pearl millet so, several beneficial genes which are present in little millet may be transferred to rice, finger millet and pearl millet.

Conflict of Interest. None.

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