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ORIGINAL ARTICLE

Cross-genera transferability of rice and finger millet genomic SSRs to barnyard millet (*Echinochloa* spp.)

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Abstract

Barnyard millet (*Echinochloa* spp.) is an important crop from nutritional point of view, nevertheless, the genetic information is very scarce. In the present investigation, rice and finger millet genomic SSRs were used for assessing cross transferability, identification of polymorphic markers, syntenic regions, genetic diversity and population structure analysis of barnyard millet genotypes. We observed 100% cross transferability for finger millet SSRs, of which 91% were polymorphic, while 71% of rice markers were cross transferable with 48% polymorphic out of them. Twenty-nine and sixteen highly polymorphic finger millet and rice SSRs yielded a mean of 4.3 and 3.38 alleles per locus in barnyard millet genotypes, respectively. The PIC values varied from 0.27 to 0.73 at an average of 0.54 for finger millet SSRs, whereas it was from 0.15 to 0.67 at an average of 0.44 for rice SSRs. High synteny was observed for markers related to panicle length, yield-related traits, spikelet fertility, plant height, root traits, leaf senescence, blast and brown plant hopper resistance. Although the rice SSRs located on chromosome 10 followed by chromosome 6 and 11 were found to be more transferable to barnyard millet, the finger millet SSRs were more polymorphic and transferable to barnyard millet genotypes. These SSR data of finger millet and rice individually as well as combined together grouped the 11 barnyard millet genotypes into 2 major clusters. The results of population structure analysis were similar to cluster analysis.

Keywords Cross-genera transferability · Barnyard millet · SSR markers · Population structure · Orthologs

Introduction

Barnyard millet (*Echinochloa* spp.) is one of the oldest domesticated millets, belongs to the sub-family panicoideae, family poaceae and consists of two main cultivated species viz, Japanese barnyard millet [*E. esculenta* (A. Braun) H. Scholz] and Indian barnyard millet (*E. frumentacea* Link). It is cultivated for food and feed purpose in Japan, Korea, the northeastern parts of China and India, Pakistan and Nepal (Yabuno 1987; Sood et al. 2015). It is a rich source of highly digestible proteins, dietary fibre with good amounts of soluble and insoluble fractions (Veena et al. 2005), and also found to be most effective in reducing blood glucose which is effective for diabetic patients (Ugare et al. 2014). Though, the crop has several advantages than other cereals like rice and wheat, genomic studies are very meagre even in comparison to other millets such as foxtail millet and finger millet. Very few nucleotide sequences (41) are available in the NCBI database for barnyard millet in comparison to other cereals, where there is a full genome sequence available for nearly 22 crops including foxtail millet (Hamilton

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and Buell 2012). Also, there are no genomic microsatellites available for barnyard millet.

Since, there is a conservation of gene sequences within the same plant family, comparative genomics plays important role by utilizing the synteny among the conserved regions of crops belonging to the same family (Moore et al. 1995; Gale and Devos 1998). The discovery of conserved syntenic regions in minor cereals with reference to the major cereals like rice, maize and wheat is very important to identify useful alleles of important agro-morphological traits. Evidence for similar conserved genomic relationships is already well documented in cereals like rice (Zhao and Kochert 1993) and wheat (Roder et al. 1995). Recently, there was a report that 90% of EST-based foxtail millet SSRs were transferable to barnyard millet (Kumari et al. 2013). Likewise Pandey et al. (2013) found 91% transferability of foxtail millet SSRs to barnyard millet germplasm. Microsatellites or simple sequence repeat (SSR) markers have been useful for molecular breeders and geneticists to link phenotype–genotype variations for marker-assisted selection of desired genotypes. The genomic microsatellites offer a few advantages like higher percentage of polymorphism over EST-based SSRs. Since, the development of new SSRs involve high cost of library screening and clone sequencing, the alternative strategy to identify the best suitable genomic SSRs in barnyard millet is through cross transferability from the related close species like finger millet and rice. With this aim, the present study was conducted on a set of 11 barnyard millet genotypes consisting of nine wild *E. crusgalli* species accessions, one cultivated *E. frumentacea* genotype and one accession of unknown species with the objective of (1) assessing cross transferability of finger millet genomic SSRs to barnyard millet and identification of polymorphic markers, (2) cross transferability of rice genomic SSRs to barnyard millet and identification of polymorphic markers, (3) comparison of efficiency of finger millet and rice genomic SSRs as molecular markers for barnyard millet (4) genetic diversity and population structure analysis of barnyard millet genotypes using polymorphic finger millet and rice genomic SSRs. The present study is the first report of this kind for identification of suitable polymorphic genomic SSRs through cross transferability of finger millet and rice genomic SSR markers.

Materials and methods

Plant materials and DNA extraction

For identification of cross-transferability of SSR markers, 11 barnyard millet accessions were used, which consisted of nine *E. crusgalli* species accessions, one cultivated *E. frumentacea* accession and one accession of unknown species, which were also used for molecular characterization and diversity analysis. The nine *E. crusgalli* accessions along with one unknown accession IEC 566 were obtained from ICRISAT, Hyderabad. Details of the accessions used in the present study are given in Table S1. Genomic DNA of different accessions of barnyard millet was isolated by standard method (Murray and Thompson 1980), quantified and analyzed by agarose gel electrophoresis (Sambrook et al. 1989). The genomic DNA was quantified on 0.8% agarose gel along with λ DNA for determining the quantity of DNA.

Cross amplification of finger millet and rice genomic SSR markers

For detecting cross-species amplification, a total of 120 rice and 32 finger millet genomic SSR markers spread throughout all the chromosomes of both the species were taken. The SSR markers were selected based on their polymorphic nature from the earlier reports and their uniform distribution across all the chromosomes. The finger millet genomic SSR markers were obtained from an earlier study (Dida et al. 2007), whereas rice genomic SSRs were obtained from the Gramene website (www.gramene.org).

SSR amplification and detection

Polymerase chain reactions (PCR) were performed in a 20 µL reaction volume containing 2 µL of 10X buffer having 15 mM $MgCl_2$, 0.2 µM of each forward and reverse primer, 2 µL of 2 mM dNTPs, 0.2 µL of 1 U of *Taq* DNA polymerase (Invitrogen, USA), and about 25 ng of template DNA. PCR amplification protocol was standardized for genomic SSRs. The reaction conditions for rice genomic SSRs are, initial denaturation of 4 min at 95° C followed by 40 cycles of 30 s at 94 °C, 30 s at annealing temperature 52 °C, extension of 1.0 min at 72 °C, with a final extension of 7 min at 72 °CC, and hold at 4 °C. Amplified products were visualized on 0.8% agarose gel and documented.

The scoring of the PCR amplicons was done based on the molecular size of the allele. The same allelic size scoring was used as input format for statistical analysis in Power Marker V3.0 (Liu and Muse 2005) and STRUCTURE v2.3.4 software (Pritchard and Wen 2003). The reaction conditions for finger millet genomic SSRs were followed as per Dida et al. (2007).

Data analysis

The dataset of SSR loci on 11 barnyard millet genotypes were used for diversity analysis using Power Marker V3.0 (Liu and Muse 2005) for estimating the polymorphism information content (PIC), gene diversity, allele frequency, most frequent and rare alleles. Unweighted pair group method

(UPGMA) was used to generate the tree using the CS Chord (Cavalli-Sforza and Edwards 1967) frequency matrix. The population structure among the finger millet genotypes was analyzed using STRUCTURE v2.3.4 (Pritchard and Wen 2003). The admixture model was used to identify the number of sub populations, and the number of sub groups (K) was determined by running the programme from $K1$ to $K10$ with five independent runs for each K value with a burn-in period of 10,000 and 100,000 MCMC replications after burn-in period. The optimum K value was determined using the Structure Harvester Software (Earl dent and VonHoldt Bridgett 2012). The syntenic regions were identified based on the information provided in the Gramene website (www.gramene.org) and earlier reports. Outcrossing rate (t) was calculated as $F = (He–Ho)/He$; $t = (1 - F)/(1 + F)$, Where F is fixation index, He is expected heterozygosity; Ho is observed heterozygosity.

Results and discussion

Cross transferability of finger millet genomic SSRs

Barnyard millet genomics is lagging far behind major cereal crops, like rice, maize, wheat and small millets like foxtail millet and to some extent finger millet. Until now very few reports on the transferability of EST-based SSR markers to barnyard millet species have been published (Kumari et al. 2013). In the present study, a set of 32 finger millet genomic SSRs markers were used to amplify DNA from eleven barnyard millet genotypes. All the SSR markers (100%) produced amplicons in all the barnyard millet genotypes. This high transferability indicated usefulness of genomic SSRs of finger millet in the study of barnyard millet genome. The transferability observed here is more than earlier reports with EST-SSRs (Kumari et al. 2013), SSRs (Pandey et al. 2013), miRNA markers (Yadav et al. 2008) and ILP-based markers (Muthamilarasan et al. 2014) across various millets. Kumari et al. (2013) studied the transferability of SSRs of foxtail millet to barnyard millet genotypes and found 90% transferability. Similarly, Yadav et al. (2014) found 80.9% transferability of sorghum SSRs in barnyard millet. Rajput et al. (2014) used the genomic SSRs of switch-grass in proso millet and found 62% transferability. Chandrashekhar et al. (2016) found 79% transferability of pearl millet SSR markers into finger millet. This high amount of transferability was observed since they used EST-based SSR markers, where they are expected to be more transferable than genomic SSR markers. Yadav et al. (2008) studied the transferability of sorghum, rice and wheat SSR markers into pearl millet and found that sorghum SSR markers were much more transferable than others. The list of the polymorphic SSRs with their chromosome number, and repeat motif is given in Table 1.

Gel pattern showing the polymorphism among the genotypes with four representative finger millet SSRs is presented in Fig. S1. Out of the 32 amplified markers, 29 (90.6%) markers were found to be polymorphic among the barnyard millet genotypes. This showed that high amount of synteny exists between finger millet and barnyard millet genome. Even, the finger millet genomic SSRs did not detect such a high polymorphism in the evaluation of finger millet germplasm earlier (Babu et al. 2014a; Bharathi 2011). A total of 21 (46%) out of 46 SSR loci were found polymorphic and the remaining 25 (54%) were monomorphic in finger millet (Babu et al. 2014a). Similar results were reported by Bharathi (2011), where only 20 out of the 30 genomic SSRs were polymorphic.

Cross transferability of rice genomic SSRs

Out of 120 rice genomic SSRs, 85 SSRs (71%) produced clear and scorable amplicons among all the barnyard genotypes indicating high synteny between the two genera. This transferability was more than our previous study, where only 50% of rice SSRs were found transferable to finger millet germplasm (Babu et al. 2017). These results, however, were similar to the earlier report of Yadav et al. (2014), where they found 72.1% transferability of genic rice SSR markers in two genotypes of barnyard millet. In our study, rice SSRs located on chromosome 10 followed by chromosome 6 and 11 were found to be more transferable to barnyard millet (Table 1). Yadav et al. (2008) also studied the transferability of sorghum, rice and wheat SSR markers to pearl millet and found that sorghum SSRs were comparatively more transferable than others. Higher transferability of rice SSRs showed that rice genome sequence data can act as a reference for barnyard millet genomics. Out of the 85 amplified markers, 41 (48.2%) markers were polymorphic among the genotypes. Among these 41 polymorphic SSRs, 38 SSRs consisted of di-repeat motifs (92%), and 3 SSRs consisted of tri repeat motifs (8%) (Table 1). These results were in close agreement with an earlier report (Rajput et al. 2014), where they also found di-repeats were the most frequent in proso millet. Similarly, Reddy et al. (2012) found dimeric repeats as the most frequent among all the repeats of EST-based SSRs in finger millet. Among the markers amplifying di-repeats, GA repeat motifs (10) were the most frequent followed by AC (8), and CT (6) repeats, which was similar to the earlier reports based on EST-based SSRs (Reddy et al. 2012; Babu et al. 2014b). Amplification pattern of four representative markers are presented in Fig. S1.

Results obtained in our study indicate that there might be high similarity between the barnyard millet and finger millet genome than between barnyard millet and rice. This may be because due to the fact that barnyard millet belongs to the subfamily, Panicoideae which is

Table 1 List of rice and finger millet polymorphic SSR markers found in Barnyard millet germplasm along with chromosome number and repeat motif

S. no.	Rice			Finger millet		
	Marker	Chromo no	Repeat motif	Marker	Chromo no	Repeat motif
1	RM209	11	(CT)18	UGEP 33	–	(TC)18
2	RM271	10	(GA)15	UGEP 57	–	(AG)16
3	RM10	7	(GA)15	UGEP 27	–	(GA)19
4	RM271	10	(GA)15	UGEP 22	–	(TC)29
5	RM202	11	(CT)30	UGEP 60	–	(GA)37
6	RM178	5	(GA)5 (AG)8	UGEP 52	–	(GA)16
7	RM447	8	(CTT)8	UGEP 56	–	(GT)12
8	RM1031	6	(AC)14	FM9	–	–
9	RM1032	1	(AC)14	UGEP 26	–	(CGG)7
10	RM1036	12	(AC)14	UGEP 21	–	(GA)16
11	RM1048	7	(AC)16	UGEP 18	–	(CT)12
12	RM1054	5	(AC)17	UGEP 90	6B	(CT)11/(CT)8
13	RM100	1	(GA)27	UGEP 102	10	(TG)17
14	RM1004	3	(AC)12	UGEP 81	–	(GT)12
15	RM1080	12	(AC)26	UGEP 110	–	(CT)12
16	RM1019	8	(AC)13	UGEP 53	2A	(AG)26
17	RM24928	10	(AT)12	UGEP 9	–	–
18	RM27957	12	(TC)10	UGEP 56	–	(GT)12
19	RM 24996	10	(TA20)	FM8	–	–
20	RM 2459	11	(AT)27	UGEP 6	5B	(GA)3TA(GA)9
21	RM 25031	10	(TA)41	UGEP 76	–	(CAG)7
22	RM 496	10	(TC)14	UGEP 11	–	(CT)12
23	RM 474	10	(AT)13	UGEP 5	–	(TC)12AC(TC)4
24	RM 38	8	(GA)16	FM5	–	–
25	RM 413	5	(AG)11	UGEP 04	–	–
26	RM 10076	1	(CT)27	FM10	–	–
27	RM 154	2	(GA)21	UGEP 1	5Ab	(TC)11
28	RM 307	4	(AT)14 (GT)21	UGEP 108	8A	(CTG)6(CAG)2
29	RM 501	–	–	FM6	–	–
30	RM 3408	6	(CT)17			
31	RM 241	4	(CT)31			
32	RM 222	10	(CT)18			
33	RM 552	11	(TAT)13			
34	RM 289	5	G11 (GA)16			
35	RM 423	2	(TTC)9			
36	RM 259	1	(CT)17			
37	RM 589	6	(GT)24			
38	RM 433	8	(AG)13			
39	RM 253	6	(GA)25			
40	OSR13	3	(GA)N			
41	RM439	6	(AAT)13			

phylogenetically very close to the finger millet subfamily Chloridoideae than rice subfamily Ehrhartoideae in the taxonomic classification. Similar results were found by Yadav et al. (2014) where they found high transferability of sorghum (Panicoideae) to barnyard millet, followed by finger millet (Chloridoideae).

Identification of putative orthologous regions

Comparative genetic mapping of cereal crops has shown that both gene content and/or order are largely conserved over the evolutionary history of the grasses (Gale and Devos 1998). The present study also resulted in identification of a few

putative syntenic regions between rice and barnyard millet genome. In the present study, we used rice SSRs which were reported to be linked to several traits as seen from the data available in Gramene website and earlier reports. It was found that high synteny exists for panicle length, seed weight or yield-related traits and spikelet characters. These were followed by plant height, root traits, seed dormancy and leaf senescence. Among the biotic stresses, synteny was found for blast and brown plant hopper (BPH) resistance. In case of quality parameters, amylose content was found to be in synteny with rice genome. The BPH (*Nilaparvata lugens*) is the most destructive pests of rice in Asian countries. Kim et al. (1994) found that antifeedants of Indian barnyard millet were resistant to *N. lugens*. It showed that barnyard millet genome contain BPH-resistant genes, which confer resistance to brown plant hopper. Our results also showed that high synteny between rice and barnyard millet was observed for the brown plant hopper resistance. The genotypes under the study (wild species) had more shattering character, which is governed by *sh4* homologue gene. The *sh4* homologue gene copies in barnyard millet were also reported in an earlier study of Aoki and Yamaguchi (2009). The present study also showed synteny for shattering character, which is one of the parameters of spikelets changed during domestication. The syntenic observations in the study give an indication that the focus on these common traits may help to identify the genes for these traits in barnyard millet through map-based cloning approaches.

Genetic variation of finger millet and rice SSR markers on barnyard millet genotypes

In case of finger millet 29 polymorphic SSRs were used for polymorphism studies among the 11 barnyard millet genotypes. These 29 polymorphic SSRs yielded 123 scorable alleles with a mean of 4.3 alleles per locus. The number of alleles generated ranged from two to a maximum of seven among the barnyard millet genotypes under study. The SSR marker FM9 found to contain maximum number of alleles (7), while four markers (UGEP60, UGEP56, UGEP21 and FM6) produced six alleles each. There were thirteen microsatellite markers which had alleles more than the average number (Table 2). In case of rice, though 41 polymorphic SSRs were found, but clear banding pattern and polymorphism was observed for 16 SSRs and hence 16 polymorphic rice SSRs were used for polymorphic studies among 11 barnyard millet genotypes. These 16 polymorphic SSRs yielded 54 scorable alleles with a mean of 3.38 alleles per locus. The number of alleles generated ranged from two to a maximum of six among the barnyard millet genotypes under study. The SSR marker RM 24957 found to contain maximum number of alleles, i.e., 6, followed by RM1004 (5). Six microsatellite markers had alleles more than the average number (Table 3). Bharathi (2011) studied a large set of finger millet genotypes and found alleles ranging from 7 to 25 at an average of 11.55 alleles per locus. This high number of alleles was due to very large collection of genotypes used in their study (nearly 900). Babu et al. (2014a) found two to a maximum of five alleles among the 190 finger millet genotypes. Nirgude et al. (2014) found 2–8 alleles with an average of 4.8 alleles per locus. The results obtained in this study were similar to earlier reports where we used the finger millet SSRs in finger millet (Babu et al. 2014a).

The PIC values of all the polymorphic loci of finger millet across the 11 barnyard millet genotypes varied from 0.27 to 0.73 at an average of 0.54 (Table 4). The maximum PIC value was observed with the marker UGEP 90 (0.73) followed by three markers UGEP 56, UGEP 104 and FM6 which had PIC of more than the 0.7 value. However, the lowest PIC value was observed for the SSR marker UGEP8 (0.27) followed by UGEP52 (0.29). Out of the 29 polymorphic SSR loci, sixteen SSR loci showed more than the average PIC value (0.54). Among the SSR loci, UGEP 90, UGEP 56, UGEP 104 and FM6 were noteworthy due to their relatively higher level of polymorphism. The PIC values of all the polymorphic loci of rice across the 11 barnyard millet genotypes varied from 0.15 to 0.67 at an average of 0.44 (Table 4). The PIC values observed in the present study were in close agreement with our earlier report (Babu et al. 2014a). The maximum PIC value was observed with the marker RM1080 (0.67) followed by RM 24957 (0.62). However, the lowest PIC value was observed for the SSR marker RM222 (0.15). Out of the 16 polymorphic SSR loci, nine SSR loci showed highest PIC value of more than the average (0.44). The PIC obtained in the study showed that the SSR markers were highly potential in identifying the genetic relationships.

Gene diversity (*He*) was in the range of 0.29–0.77 with an average value of 0.59 using finger millet SSRs. However, in case of rice SSRs, it was in the range of 0.17–0.71 with an average value of 0.50 (Table 3). Similar results were also obtained by Bharathi (2011), where she found *He* from 0.20 to 0.85. In our earlier report also we found the *He* in the range of 0.208–0.726 with an average value of 0.487 (Babu et al. 2014b). This value was higher than earlier reports based on RAPD markers (0.330) (Babu et al. 2007) and EST-SSRs (0.024–0.327) (Nirgude et al. 2014) in finger millet, The gene diversity was found to be highest with the SSR marker UGEP 90 (0.77) across the 11 barnyard millet genotypes, followed by UGEP 60 (0.75). A total of 17 SSR markers were observed to have more gene diversity than the average value (0.59). The SSR loci having more alleles, high gene diversity, also had highest PIC. This result indicated that PIC value is positively correlated to the number of alleles and the gene diversity (Varshney et al. 2001). In case of rice SSRs,

Table 2 The polymorphism details such as allele number, PIC, gene diversity and heterozygosity using finger millet SSRs

Marker	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC	Inbreeding coefficient	Expected band size	Observed band size
UGEP 33	0.44	4.00	0.67	0.44	0.61	0.38	216	200–900
UGEP 57	0.50	5.00	0.68	0.78	0.64	− 0.09	445	150–900
UGEP 27	0.50	4.00	0.65	0.56	0.59	0.20	247	100–400
UGEP 22	0.78	3.00	0.37	0.00	0.34	1.00	227	180–200
UGEP 60	0.33	6.00	0.75	0.89	0.71	− 0.13	240	160–400
UGEP 52	0.78	2.00	0.35	0.44	0.29	− 0.23	215	200–400
UGEP 56	0.33	6.00	0.75	0.78	0.71	0.02	162	150–1200
FM9	0.61	7.00	0.60	0.56	0.58	0.13	127	50–1200
UGEP 26	0.67	4.00	0.50	0.56	0.45	− 0.05	227	230–300
UGEP 21	0.67	6.00	0.53	0.22	0.51	0.62	225	150–300
UGEP 18	0.67	3.00	0.49	0.00	0.44	1.00	318	350–1200
UGEP 90	0.33	5.00	0.77	0.00	0.73	1.00	232	180–250
UGEP 102	0.78	3.00	0.37	0.22	0.34	0.45	184	110–200
UGEP 81	0.39	3.00	0.66	0.33	0.59	0.54	192	180–1000
UGEP 110	0.50	5.00	0.65	0.78	0.60	–0.13	192	150–800
UGEP 53	0.56	3.00	0.54	0.56	0.44	0.02	226	160–1200
FM8	0.72	4.00	0.45	0.33	0.42	0.31		300–400
UGEP 6	0.50	3.00	0.62	0.56	0.55	0.17	229	180–200
UGEP 76	0.44	4.00	0.67	0.00	0.61	1.00	169	50–150
UGEP 3	0.39	5.00	0.71	0.78	0.66	− 0.04	206	100–800
UGEP 8	0.83	3.00	0.29	0.11	0.27	0.65	297	800–1200
UGEP 11	0.44	5.00	0.67	0.89	0.62	− 0.27	153	140–400
UGEP 5	0.56	3.00	0.57	0.00	0.49	1.00	215	160–200
FM5	0.44	3.00	0.59	0.67	0.50	− 0.07		50–60
UGEP 104	0.33	5.00	0.76	0.22	0.72	0.74	189	150–400
UGEP 10	0.44	5.00	0.70	0.56	0.65	0.26	400	50–150
UGEP 1	0.78	3.00	0.37	0.00	0.34	1.00	233	200–250
UGEP 108	0.67	5.00	0.52	0.11	0.50	0.81	150	100–1200
FM6	0.44	6.00	0.73	0.56	0.70	0.30	237	150–500
Mean	0.55	4.24	0.59	0.41	0.54	0.35		
Min	0.33	2.00	0.29	0.00	0.27	− 0.27		
Max	0.83	7.00	0.77	0.89	0.73	1.00		

the gene diversity was found to be highest with the SSR marker RM1080 (0.71) across the 11 barnyard millet genotypes, followed by RM552 (0.66). The gene diversity present among the finger millet genotypes showed that markers used in the present study were highly polymorphic. The heterozygosity (*Ho*) ranged from 0.00 to 0.89 with an average of 0.41, indicating high heterozygosity in the barnyard millet genotypes using finger millet markers. The observed heterozygosity was found to be highest in the SSR marker UGEP 60 and UGEP 11 (0.89 each) followed by UGEP3 and UGEP110 (0.78 each) (Table 2). Using rice SSRs, average 'observed heterozygosity' (*Ho*) was 0.30 and ranged from 0.00 to 0.73 which showed that a wide range of heterozygosity was present in the finger millet genotypes. The observed heterozygosity was found to be highest in the SSR marker RM1004 (0.73) followed by RM 10076 (0.64 (Table 3). However, the lowest heterozygosity (0.00) was found with three markers. The heterozygosity observed among the selected barnyard millet genotypes was in congruence with the earlier reports in finger millet (Babu et al. 2014a; Bharathi 2011). The high amount of heterozygosity might be because of mutational rate exhibited in some of the SSR markers (Udupa and Baum 2001) and rare and unique alleles in the genotypes. Finger millet SSRs detected high out-crossing rate of 54 percent in comparison to rice markers (43%) which was in congruence to the results of observed heterozygosity. Detection of high heterozygosity by finger millet SSRs indicates that they are more efficient for gene diversity analysis of barnyard millet genotypes.

Table 3 The polymorphism details such as allele number, PIC, gene diversity and heterozygosity using rice SSRs

Marker	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC	Inbreeding coefficient	Expected band size	Observed band size
RM 209	0.50	4.00	0.63	0.45	0.57	0.32	134	400–1100
RM10	0.73	3.00	0.43	0.36	0.39	0.20	159	400–600
RM178	0.68	3.00	0.46	0.09	0.39	0.82	117	200–230
RM447	0.55	3.00	0.56	0.00	0.48	1.00	111	400–600
RM 1031	0.68	4.00	0.50	0.27	0.47	0.49	127	450–600
RM1036	0.73	4.00	0.43	0.36	0.39	0.21	146	220–400
RM1004	0.55	3.00	0.56	0.73	0.48	− 0.25	137	160–300
RM1080	0.41	5.00	0.71	0.55	0.67	0.28	206	100–1000
RM 24957	0.55	6.00	0.65	0.27	0.62	0.61	179	350–1100
RM24996	0.64	3.00	0.51	0.00	0.44	1.00	689	1000–1200
RM496	0.77	3.00	0.38	0.09	0.34	0.78	267	300–500
RM413	0.55	4.00	0.57	0.09	0.48	0.85	79	400–550
RM 10076	0.68	2.00	0.43	0.64	0.34	− 0.43	187	100–400
RM241	0.73	2.00	0.40	0.55	0.32	− 0.33	138	180–300
RM222	0.91	2.00	0.17	0.00	0.15	1.00	213	150–400
RM552	0.36	3.00	0.66	0.36	0.59	0.49	195	200–210
Mean	0.63	3.38	0.50	0.30	0.44	0.44		
Min	0.36	2.00	0.17	0.00	0.15	− 0.43		
Max	0.91	6.00	0.71	0.73	0.67	1.00		

Table 4 Comparison of polymorphism parameters of finger millet and rice microsatellites in barnyard millet genotypes

S. No	Genetic polymorphism parameters	Rice	Finger millet
1	Total markers used for amplification	120	32
2	Total amplified markers	85 (71%)	32 (100%)
3	Total polymorphic markers	41	29
4	Percentage of polymorphism	48.2%	90.6%
5	Mean allele number	3.38	4.24
6	Minimum allele number	2.00	2.00
7	Maximum allele number	6.00	7.00
8	Mean gene diversity	0.50	0.59
9	Minimum gene diversity	0.17	0.29
10	Maximum gene diversity	0.71	0.77
11	Mean heterozygosity	0.30	0.41
12	Minimum heterozygosity	0.00	0.00
13	Maximum heterozygosity	0.73	0.89
14	Mean PIC	0.44	0.54
15	Minimum PIC	0.15	0.27
16	Maximum PIC	0.67	0.73

Diversity and population structure analysis based on rice and finger millet SSRs

The genetic diversity analysis among the collection of 11 barnyard millet genotypes which consisted of accessions of wild species and cultivated genotypes was done using 29 and 16 polymorphic genomic SSRs of finger millet and rice, respectively. The dendrogram with 16 rice SSRs was generated through UPGMA analysis of Power Marker V3.25 software. These 16 SSR markers grouped the 11 barnyard millet genotypes into 2 major clusters (A, and B) based on the UPGMA analysis of Power Marker V3.25 software (Fig. 1a).

The cluster A comprised six genotypes, whereas B cluster consisted of five genotypes. The cluster A was further divided into two sub-groups A1 and A2. The sub-cluster A1 consisted of IEC409, IEC456, IEC563 and IEC428 genotypes, whereas A2 consisted of IEC331 and IEC539. Similarly, Dida et al. (2008) analyzed a set of 79 finger millet accessions using 45 SSR markers and were able to differentiate into two phylogenetic groups according to their geographic origin based on the power marker analysis. There was no clear clustering pattern in the group B. The genotypes IEC512 and IEC486 clustered together, whereas IEC566, IEC514 and VL172 did not cluster with any genotypes. The cultivated genotype which was released by ICAR-Vivekananda Institute of Hill Agriculture, Almora, i.e., VL172 also did not cluster with any of the wild species. The cultivated genotype VL 172 is expected not to cluster with any of the wild species involved in the present study as VL 172 is cultivated in India while others are in Japan. The average gene diversity existing among all the genotypes was high (50%), indicating existence of high levels of polymorphism among the barnyard millet. These results are in close agreement with the findings reported earlier (Babu et al. 2014c).

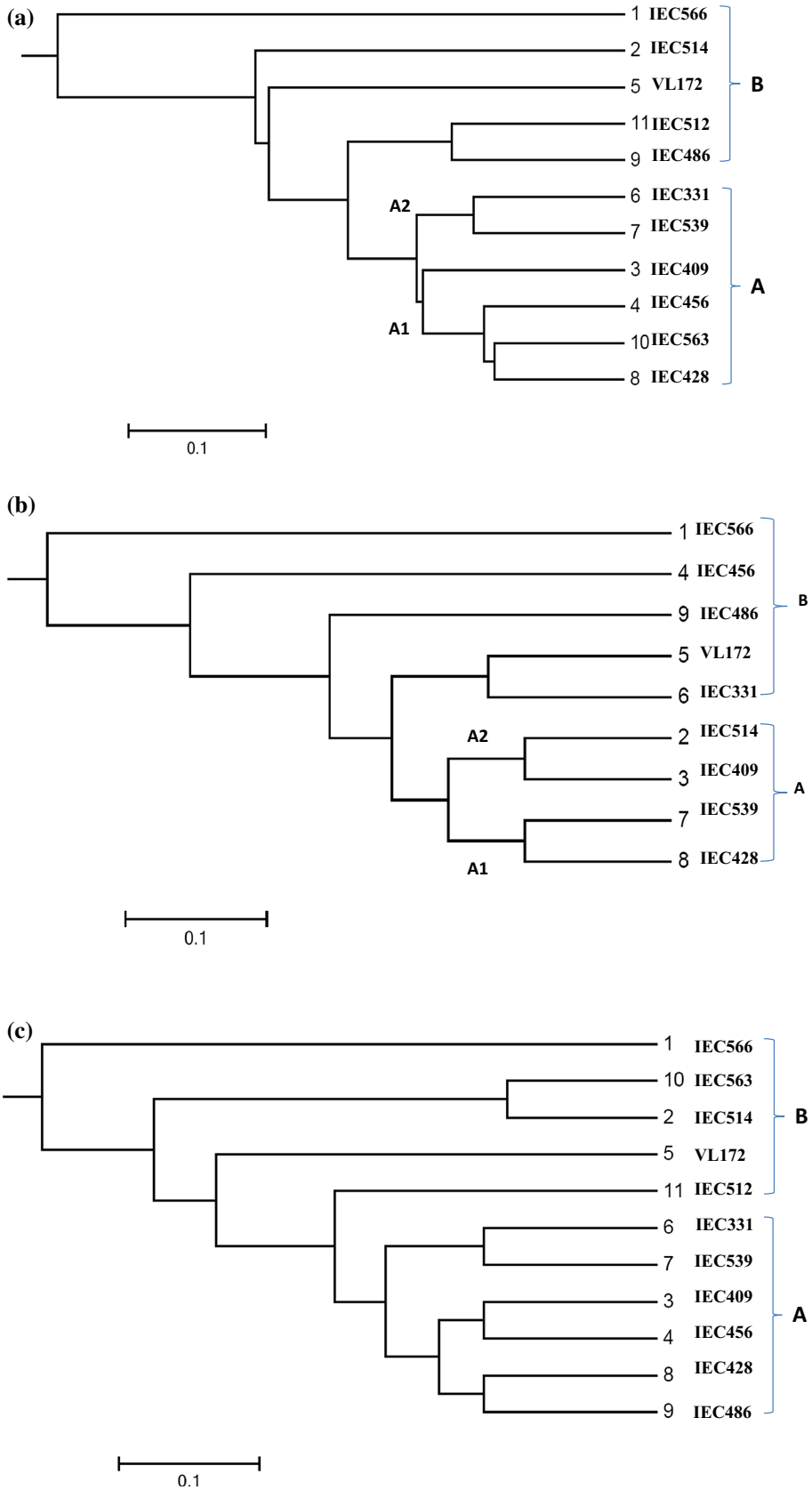

Fig. 1 The dendrogram generated from the UPGMA analysis among the barnyard millet genotypes using rice SSRs (**a**), finger millet SSRs (**b**) and combination of rice and finger millet SSRs (**c**)

As expected, the clustering pattern obtained by the finger millet genomic SSRs was different from dendrogram obtained from rice genomic SSRs. The 29 SSR markers grouped the 11 barnyard millet genotypes into 2 major clusters (A, and B) (Fig. 1b). The A cluster further divided into two sub clusters A1 and A2. The sub-cluster A1 consisted of two genotypes IEC539 and IEC428, whereas in the above dendrogram IEC539 formed a different cluster with IEC331. The sub-cluster A2 comprised IEC514 and IEC409, whereas in the rice-based dendrogram the genotypes IEC514 was grouped in the B cluster. The cultivated genotype VL172 was clustered with IEC331, however, it did not cluster with any genotypes in the rice SSR-based dendrogram. The cluster B comprised 5 accessions, of which IEC566, IEC456 and IEC486 did not cluster with any genotypes. In both the dendrograms, the IEC566 did not cluster with any genotype by forming a separate path.

However, when we used all the 29 finger millet and 16 rice SSR markers together for diversity analysis of 11 barnyard millet genotypes, the clustering pattern was mostly similar to the dendrogram generated by the rice SSR markers. The only exception was that the genotype IEC563 was found in cluster B, whereas in rice SSR-based dendrogram it was under cluster A. However, vice versa was the case for the genotype IEC486 (Fig. 1c).

The barnyard millet genotypes were evaluated for population structure using 29 finger millet and 16 rice SSR markers together. The dendrogram obtained from power marker software grouped the genotypes into two clusters. To know the exact structure among the barnyard millet genotypes, *K*'s from 1 to 8 (with five iterations) were run. The maximum *K* value was observed for $K = 2$ (Fig. 2a) which also suggested that there were two populations (Fig. 2b). Good correlations were found between population structure and genetic diversity analysis in differentiation of barnyard millet genotypes, where both grouped them into two groups. The structure analysis showed that eight genotypes were pure lines (no admixture) while two (IEC514, and IEC409) had admixture of alleles. The unknown species IEC 566 might contain similar alleles to that of VL172. This was also well supported by the dendrogram obtained from finger millet SSR markers. Though the grouping pattern observed was similar in both structure and cluster analysis, the bar plot of structure depicts estimated membership of each genotype in each of the populations and the admixtures could easily be identified better than the dendrogram in cluster analysis. The mean value of alpha was 0.079. The average distances (expected heterozygosity) between individuals in the same cluster were 0.613 and 0.3643 and the allele frequency [divergence among pops (net nucleotide distance), computed using point estimates of P] was 0.107 between clusters 1 and 2.

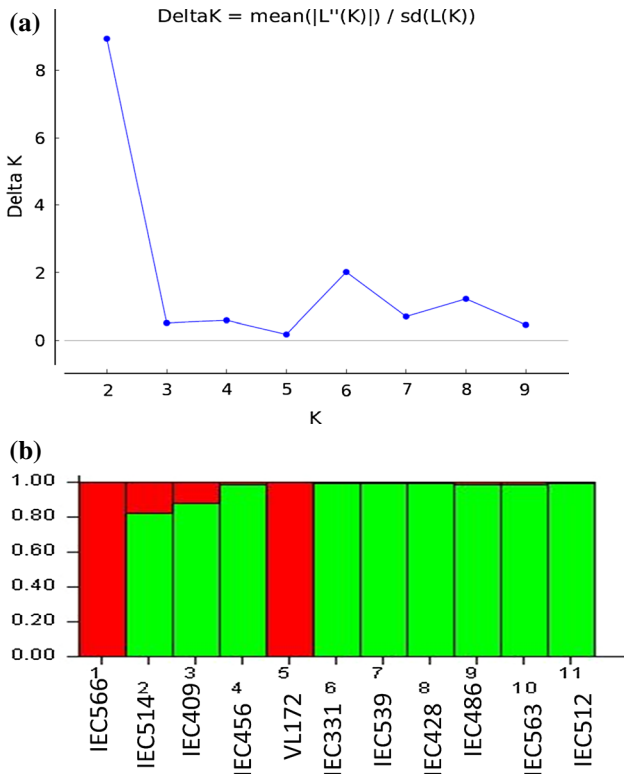

Fig. 2 **a** Representation of the appropriate sub-population number (*K*): sub population number (*K*) against delta *K* and the maximum *K* value observed at $K = 2$. **b** The structure of barnyard millet genotypes obtained from STRUCTURE software using finger millet and rice genomic SSR markers

Conclusion

The present study enriched the barnyard millet genomics by identifying the suitable polymorphic markers of rice and finger millet, which can be used for diversity analysis, cultivar identification and QTL mapping studies. The finger millet microsatellites were highly transferable, more polymorphic and also were able to differentiate and identify the diversity in the barnyard millet genotypes than rice microsatellite markers. Moreover, the study also identified the syntenic regions between rice and barnyard millet for traits like panicle number, spikelet fertility, BPH resistance, and seed dormancy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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