

Cross transferability of finger millet and maize genomic SSR markers for genetic diversity and population structure analysis of barnyard millet

B. Kalyana Babu^{1,*}, C. Rashmi and Salej Sood

ICAR-Vivekananda Parvatiya Krishi Anusanthan Sansthan, Almora 263 601, Uttarakhand

(Received: December 2017; Revised: June 2018; Accepted: July 2018)

Abstract

The genomic information available in barnyard millet is very scarce though it is a rich source of highly digestible proteins and dietary fibre with good amounts of soluble and insoluble fractions. In the present investigation, 64 maize and finger millet genomic SSRs were used for cross transferability analysis among barnyard millet cultivated and wild species for identification of polymorphic markers, syntenic regions, genetic diversity and population structure analysis. Out of the 64 SSRs, only 39 (61%) were amplified across the barnyard millet genotypes. The PIC values of all the polymorphic loci for 24 barnyard millet genotypes varied from 0.25 to 0.73 at an average of 0.35. Based on the values of different parameters i.e., PIC values (>0.7), gene diversity (>0.6), inbreeding coefficient (>0.27), the SSR loci bnlg2323 and bnlg2123 were observed to be highly polymorphic. Polymorphism comparison of maize and finger millet SSRs revealed that maize microsatellites were highly transferable, more polymorphic and were able to distinguish barnyard millet genotypes clearly. Results of population structure and genetic diversity analysis were similar in differentiating the barnyard millet genotypes into two groups. The structure analysis showed that all genotypes were pure lines (no admixture) while two (IEC514, and IEC409) had mixture of alleles from other genotypes of population which is depicted from the SSRs used in the study. The present study enriched the barnyard millet genetic resources by identifying suitable polymorphic markers of maize and finger millet for diversity analysis, cultivar identification and marker assisted breeding programmes.

Key words: Cross transferability, barnyard millet, SSR markers, population structure, orthologs

Introduction

Barnyard millet (Echinochloa sp.) is one of the oldest

domesticated millets, belongs to the sub-family Panicoideae and family Poaceae. The genus has two main cultivated species viz., Japanese barnyard millet (E. esculenta (A. Braun) H. Scholz) and Indian barnyard millet (E. frumentacea Link). The barnyard millet crop is mainly grown for food and feed purpose in Japan, Korea, North-Eastern parts of China, India, Pakistan and Nepal (Yabuno 1987; Sood et al. 2015a). It is a rich source of highly digestible proteins, dietary fibre with good amounts of soluble and insoluble fractions (Veena et al. 2005) and has been found to be most effective in reducing blood glucose showing its benefits for diabetic patients (Ugare et al. 2014). Though the crop has several advantages than major cereals like rice and wheat, the genomics studies are very meagre. In the present next generation sequencing era very few nucleotide sequences (41) are available in the NCBI database for the barnyard millet crop in comparison to other cereals (https:// www.ncbi.nlm.nih.gov/), where there is a full genome sequence available for nearly 22 crops including foxtail millet (Hamilton and Buel 2012).

Since there is conservation of gene sequences within the same plant family, comparative genomics play an important role by utilizing the synteny among the conserved regions of crops belonging to same family (Moore et al. 1995; Gale and Devos 1998). The discovery of syntenic regions aids to identify useful alleles of important agro-morphological traits. Evidence for similar conserved genome relationships are already well developed in cereals like rice (Zhao and Kochert 1993) and wheat (Roder et al. 1995). It has been shown

*Corresponding author's e-mail: kalyan_biotek@yahoo.co.in

¹Present address: ICAR-Indian Institute of Oil Palm Research, Pedavegi 534 450, West Godavari, Andhra Pradesh

Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by indianjournals.com; www.isgpb.org

recently that 90% of EST based SSRs of foxtail millet 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. Recently, Wallace et al. (2016) identified 10,816 SNPs across 65 accessions of E. colona, and 8217 SNPs across 22 accessions of E. crus-galli through genotyping by sequencing analysis. The SNPs are more polymorphic than SSR markers, but require sequencing costs. The SSR markers are more user friendly with low cost and are also poly-allelic in nature. Microsatellites or simple sequence repeat (SSR) markers have been useful for molecular breeders and geneticists to link phenotypic and genotypic variation and also popular because of their abundance and amenability to high throughput screening. The genomic microsatellites offer advantages over EST based SSRs particularly higher percentage of polymorphism, however EST-SSRs are more reliable in MAS since allelic polymorphism may be more accurately linked with phenotypic variation. Since, development of SSRs is guite cumbersome as it involves high cost of library screening and clone sequencing; the alternative strategy is to identify the best suitable genomic SSR markers through transferability from the related close species like finger millet and maize. Availability of rich source of diverse germplasm is a prerequisite for any crop improvement programmes like barnyard millet. At the international level, Consultative Group on International Agricultural Research is maintaining a large collection of 2365 accessions, whereas International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad contains 743 active and 487 base collections from nine countries (Upadhyaya et al. 2008). The National Centre for Genetic Resources Conservation, Colorado maintains 306 accessions from 33 countries. In India, National Bureau of Plant Genetic Resources (NBPGR) has 1718 accessions while All India coordinated small millets improvement project has 985 accessions (Sood et al.

2015a). Genetic diversity among the barnyard millet accessions helps in systematic identification of germplasm for better vigour, sources for agronomically important traits such as higher yield, nutritional parameters, and various biotic and abiotic stresses resistance etc. Only few reports are available on the genetic diversity of barnyard millet germplasm, that too are based on limited number of markers (Nozawa et al. 2004; Danquash et al. 2002). To bridge this gap, the present study was conducted with the objectives of 1) cross transferability of finger millet genomic SSR markers into barnyard millet and identification of polymorphic markers, 2) cross transferability of maize genomic SSR markers into barnyard millet and identification of polymorphic markers, 3) comparison of transferability of finger millet and maize genomic SSR markers and 4) genetic diversity and population structure analysis of barnyard millet genotypes using polymorphic finger millet and maize genomic SSRs.

Materials and methods

Plant materials and DNA extraction

Twenty-four barnyard millet genotypes were selected to find out the cross species transferability and polymorphism of finger millet and maize SSR markers. The 24 genotypes contained 16 accessions of barnyard millet global core germplasm, 6 advanced breeding lines and 2 selections from exotic wild germplasm. The criterion for selection of germplasm was to include probable progenitors of both cultivated species which represent 16 accessions, and both cultivated species for which eight advanced breeding lines and varieties were selected. The list of genotypes used in the present study is given in Table 1. The genomic DNA was isolated from two weeks old seedlings by standard CTAB method (Murray and Thomson 1980). The genomic DNA quantity and quality was checked in nano drop (USA) and also using uncut lamda DNA standard on 0.8% agarose gel electrophoresis (Maniatis et al. 1989).

 Table 1.
 The list of barnyard millet genotypes used in the study along with origin and species

Name of the species	Accession number	Country of origin
Echinochloa frumentacea or E. colona	IEC751*, IEC381, IEC521, IEC675, IEC178, IEC706*, IEC350, IEC60, IEC196, IEC265	India Unknown*
E. esculenta	IEC552, PRB903, PRJ1	Japan
E. frumentacea	IEC374, VL232, VL234, VL207, VL29, VL224, VL172, EC788*	India Unknown*
E. esculenta or E. crus-galli	IEC517, IEC511, IEC436	Japan

Cross amplification of finger millet and maize genomic SSR markers

Cross-species amplification of barnyard millet genotypes were performed using 46 maize and 18 finger millet genomic SSR markers. The finger millet genomic SSR markers were obtained from an earlier study (Dida et al. 2007), whereas maize genomic SSRs were obtained from the maizegdb website (www.maizegdb.org). Eight genotypes of Barnyard millet were initially used to determine the transferability of maize and finger millet SSRs. The polymerase chain reactions (PCR) were performed in 20 µL reaction volume containing 2 µL of 10X buffer having 15 mM MgCl2, 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. The PCR amplification protocol was standardized for genomic SSRs. The reaction conditions for maize genomic SSRs were initial denaturation for 4 min at 95°C followed by 40 cycles of 30 s at 94°C, 30 s of annealing temperature at 52°C, extension of 1.0 min at 72°C, with a final extension of 7 min at 72°C, and hold at 4°C. The electrophoresis was done at 100 volts for 3 h at room temperature by using the 2.5 percent agarose gel concentration. The reaction conditions for finger millet genomic SSRs were followed as per Dida et al. (2007). Gels were stained with ethidium bromide and visualized using Bio Imaging System (SynGene, USA).

Data analysis

The data set of SSR loci on 24 barnyard millet genotypes were used for diversity analysis using Power Marker V3.0 (Liu and Muse 2005) to estimate 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 (1967) frequency matrix. The population structure analysis of barnyard millet genotypes was done 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 K2 to K10 with five independent runs for each K value with a burn-in period of 100000. The optimum value of K value was determined by using the structure harvester (Earl and vonHoldt 2012).

Results and discussion

Cross transferability polymorphism of finger millet and maize genomic SSRs

Barnyard millet genomics is far behind major cereal crops namely, rice, maize and wheat and small millets such as foxtail millet and finger millet. Till now very few reports are available on the transferability of EST based SSR markers to barnyard millet (Kumari et al. 2013), however no reports are for genomic SSRs transferability. In the present study, a set of 64 finger millet and maize genomic SSR markers were used to amplify 24 barnyard millet genotypes. Out of 64 SSRs, 39 (61%) were amplified across the barnyard millet genotypes. These 39 SSRs were further considered for molecular characterization. This high amount of transferability was a good sign of utilizing the genomic SSRs of maize and finger millet to barnyard millet genetic diversity studies. Similar reports were obtained earlier 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). Kumari et al (2013) found transferability of foxtail millet with barnyard millet, rice and maize at 90.6%, 89% and 84%, respectively. Shambhavi et al. (2014) found 80.9% cross transferability of sorghum SSRs in barnyard millet. Rajput et al. (2014) used the genomic SSRs of switch-grass into proso millet and found 62% transferability. These results showed the existence of high similarity between the barnyard millet and finger millet genome than with foxtail millet and rice.

Out of the 39 amplified markers, 32 (82%) were found to be polymorphic across the barnyard millet genotypes. The genomic DNA of the 24 barnyard millet accessions were amplified using 39 SSR markers and yielded 101 scorable alleles, out of which 94 alleles were found to be polymorphic, while 7 SSRs were monomorphic. The number of alleles generated with polymorphic primers ranged from 2 to 6 with a mean of 2.6 alleles per locus. The SSR markers bnlg2323, umc2071, bnlg2123 were found to have maximum number of alleles (6) followed by umc2364 (5). The high amount of polymorphism observed will be useful for genetic diversity studies of barnyard millet germplasm. The gel electrophoresis showing the polymorphism among the barnyard millet genotypes with maize and finger millet SSR markers is shown in Fig. 1. The identified polymorphic markers can be effectively used in genotyping mapping populations for construction of linkage maps, diversity studies, generation of comparative maps between barnyard



Fig. 1. The banding pattern of 24 barnyard millet genotypes using maize primer umc2030 (1a), and finger millet primer ugep110 (M-100bp ladder, Lanes 1-24 represents barnyard millet genotypes

millet and finger millet, and identification of QTLs. The 32 polymorphic SSRs used for genetic diversity studies among the 24 barnyard millet genotypes yielded 94 scorable alleles with a mean of 2.9 alleles per locus. Earliar reports indicated that a large set of finger millet genotypes showed more number of alleles alleles in the range of 7 to 25 at an average of 11.55 alleles per locus. This high number of alleles was due to a large collection of genotypes used in their study (nearly 900). Babu et al. (2014a) found two to a maximum of five alleles among the finger millet genotypes. Nirgude et al. (2014) found 2-8 alleles with an average of 4.8 alleles per primer. In the present study, three unique alleles and rare alleles from five SSR markers viz., bnlg2123, umc2364, umc2071, bnlg2323 and mmc0231 were observed. The alleles with frequencies of at least 99% considered as common and alleles with frequencies of less than 1% considered as rare alleles. In addition to the total number of alleles and unique alleles, the number of rare alleles obtained indicated the diversity at a given SSR locus.

The PIC values of all the polymorphic loci across the 24 barnyard millet genotypes varied from 0.25 to 0.73 at an average of 0.35. The PIC demonstrates the informativeness of SSR markers and their potential to detect differences among the accessions based on their genetic relationships. Very high PIC values were observed in the present study which was in close agreement with earlier reports (Babu et al. 2014a; Bharathi 2011). Bharathi (2011) reported the PIC range from 0.196 to 0.834. The PIC obtained in the present study showed that the SSR markers were quite informative in identifying the genetic relationships. Out of 32 primers, bnlg2323, bnlg2123 primers showed highest PIC values of 0.73 and 0.72, respectively. The lowest PIC value was observed in primer umc2101 (0.25). The number of SSR loci based on PIC values

with more than the average was 25 in number. Among them, bnlg2323, umc2071, and bnlg2123 SSR loci were noteworthy due to their relatively higher level of polymorphism.

Genetic diversity analysis using finger millet and maize SSR markers

Gene diversity (He) was in the range of 0.17 (ugep27) to 0.77 (bnlg2323) with an average value of 0.41. In earlier reports also we found the He in the range of 0.208 to 0.726 with an average value of 0.487 (Babu et al. 2014b). This value was higher than earlier reports based on RAPD markers in finger millet (0.330) (Babu et al. 2007) and based on EST-SSRs by Nirgude et al. (2014), where they found very less gene diversity (0.024-0.327). The expected heterozygosity was found to be highest with the SSR marker bnlg2323 (0.77) across the 24 barnyard millet genotypes, followed by bnlg2123 (0.76). The gene diversity present among the barnyard millet genotypes showed that markers used in the present study were more polymorphic. However, the lowest gene diversity was found in the SSR marker ugep27 (0.17). A total of 26 SSR markers were observed to have more gene diversity than the average value (0.41). These results indicated that PIC values positively correlated to the number of alleles and the gene diversity (Varshney et al. 2001).

The heterozygosity (Ho) was observed with an average of 0.50 and ranged from 0.05 to 1.00 which showed a wide range of heterozygosity in the barnyard millet genotypes. The observed heterozygosity was found to be highest in the SSR markers ugep110, ugep22, umc2364, mmc0231, dupssr19 and umc1012 (1.00) (Table 2). 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). However, the lowest heterozygosity (0.05) was found with umc1407 marker. The high amount of heterozygosity observed might be due to diverse genotypes used in the study. This high amount of heterozygosity might also be due to mutations in some of the SSR markers (Udupa and Baum 2001). As a result, many of the markers which displayed heterozygous nature had a large number of SSR units (Dje et al. 2000). Based on the above SSR analysis and considering the PIC values (> 0.7), gene diversity (>0.6), inbreeding coefficient (>0.27), two SSR loci bnlg2323, and bnlg2123 were observed to be highly polymorphic. These SSRs would be suitable for genetic diversity and mapping studies.

Marker	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC	Inbreeding coefficient
ugep52	0.52	3	0.60	0.00	0.53	1.00
ugep110	0.50	2	0.50	1.00	0.37	-1.00
ugep57	0.57	2	0.48	0.85	0.36	-0.73
ugep27	0.90	2	0.17	0.19	0.15	-0.08
ugep22	0.50	2	0.50	1.00	0.37	-1.00
ugep76	0.73	2	0.38	0.00	0.31	1.00
FM10	1.00	1	0.00	0.00	0.00	NaN
umc2364	0.38	5	0.69	1.00	0.63	-0.41
bnlg2123	0.34	6	0.76	0.73	0.72	0.05
umc2030	0.47	3	0.63	0.94	0.55	-0.46
umc1230	0.63	2	0.46	0.72	0.35	-0.55
bnlg1267	0.52	3	0.61	0.95	0.54	-0.54
mmc0231	0.50	4	0.65	1.00	0.60	-0.50
umc2071	0.45	6	0.72	0.80	0.69	-0.07
umc1052	0.50	2	0.50	0.00	0.37	1.00
bnlg2323	0.33	6	0.77	0.77	0.73	0.01
umc1148	1.00	1	0.00	0.00	0.00	NaN
umc1030	0.52	2	0.49	0.28	0.37	0.44
dupssr23	0.70	4	0.47	0.30	0.43	0.38
mmc0371	0.45	4	0.60	0.91	0.52	-0.47
umc2328	1.00	1	0.00	0.00	0.00	NaN
umc1407	0.97	2	0.05	0.05	0.05	0.00
umc1805	0.52	3	0.59	0.68	0.51	-0.12
umc1804	0.52	2	0.49	0.95	0.37	-0.90
bnlg1325	0.66	4	0.51	0.38	0.47	0.27
umc1075	0.70	2	0.41	0.58	0.32	-0.39
dupssr19	0.50	2	0.50	1.00	0.37	-1.00
mmc0282	0.70	2	0.42	0.50	0.33	-0.16
bnlg1346	0.71	3	0.44	0.28	0.40	0.38
mmc0081	1.00	1	0.00	0.00	0.00	NaN
umc1012	0.50	2	0.50	1.00	0.37	-1.00
umc2101	0.81	2	0.30	0.20	0.25	0.33
umc1506	0.79	3	0.34	0.41	0.30	-0.19
umc1054	1.00	1	0.00	0.00	0.00	NaN
umc2163	0.56	2	0.49	0.00	0.37	1.00
umc1858	0.52	2	0.49	0.94	0.37	-0.89
bnlg1600	0.60	3	0.49	0.79	0.39	-0.58
umc1941	1.00	1	0.00	0.00	0.00	NaN
umc1155	1.00	1	0.00	0.00	0.00	NaN
Mean	0.65	2.59	0.41	0.50	0.35	-0.17

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

Comparison of polymorphism of maize and finger millet SSRs

Comparison of maize and finger millet SSR polymorphism studies on barnyard millet genotypes revealed that maize microsatellites were highly transferable, more polymorphic and also were also able to differentiate and identify more diversity among the barnyard millet genotypes than finger millet microsatellite markers (Table 3). The high

 Table 3.
 Comparison of polymorphism parameters of finger millet and maize microsatellites in barnyard millet genotypes.

S.No.	Genetic polymorphism parameters		Maize	F	-inger millet
1	Total markers used for amplification		46		18
2	Total amplified markers	32	(70%)	7 ((39%)
3	Total polymorphic markers		26		6
4	Percentage of polymorphism		81.2%		85.7%
5	Mean allele number		3.38		4.24
6	Minimum allele number		1.00		1.00
7	Maximum allele number		6.00		3.00
8	Minimum gene diversity		0.00		0.00
9	Maximum gene diversity		0.77		0.60
10	Minimum heterozygosity		0.00		0.00
11	Maximum heterozygosity		1.00		1.00
12	Minimum PIC		0.25		0.16
13	Maximum PIC		0.73		0.53

transferability existed between barnyard millet (Panicoideae) and maize (Poaceae) may be due to their physiological relationships. The study also revealed that maize genome sequence can be used as reference map for identification of homologs and orthologs for traits found to be syntenic among maize, finger millet and barnyard millet. The polymorphism information content generated from maize SSRs was found to be more than finger millet SSR markers.

Cluster analysis

The genetic diversity analysis among the collection of 24 barnyard millet genotypes which consisted of land races and cultivated genotypes were done using 32 polymorphic genomic SSRs of finger millet and maize. The dendrogram with 32 SSRs was generated through UPGMA analysis of Power Marker V3.25 software. These 32 SSR markers grouped the 24 barnyard millet genotypes into two major clusters (A and B) based on the UPGMA analysis of Power Marker V3.25 software (Fig. 2). The clustering of the barnyard



Fig. 2. The rooted dendrogram generated from the UPGMA analysis among the barnyard millet genotypes using combination of maize and finger millet SSRs

millet genotypes was largely based on their geographical origin. The cluster A contained genotypes IEC436, IEC552, PRB903, IEC511, IEC517 and PRJ1 all belongs to Echinochloa esculenta group and were of Japanese origin. The cluster B comprised of Indian origin genotypes viz., IEC706, IEC60, IEC381, IEC675, IEC178, IEC350, IEC196, IEC751, IEC52, IEC265, VL29, VL224, VL207, VL234, VL232, VL172, IEC788, IEC374 and belong to Echinochloa frumentacea group. This clustering of the genotypes was found to be according to the geographic origin and similarity of races involved in breeding of these genotypes (Table 1). 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. The cluster B consisted of three sub clusters which were close to each other. In cluster B, genotypes IEC60, IEC706 were closely related to each other and formed a small cluster. Likewise, IEC178, IEC350, and IEC196, were very close to each other, which may be due to that all the genotypes belongs to similar geographic origin (India). The barnyard millet released varieties as well

as elite lines *viz.*, VL224, VL207, VL232, VL172, VL29 and VL234 formed a separate cluster but were close to IEC788, IEC52, IEC265, IEC751, IEC374 and IEC381. This grouping may be due to common parentage involved in the development of improved barnyard millet genotypes. Since the germplasm starting with VL number are all advanced breeding lines or varieties belonging to *E. frumentacea* species, these IEC number lines are also either *E. frumentacea* or *E. colona* which is the progenitor of *E. frumentacea*.

Population structure analysis based on maize and finger millet SSRs

The barnyard millet genotypes were evaluated for population structure using 32 SSR markers together. The dendrogram obtained from power marker software grouped the genotypes into two clusters. The barnyard millet genotypes representing popular varieties of India and exotic accessions from different regions were evaluated for estimation of population structure using a panel of 32 SSR loci. For estimation of the exact population structure (k), Ks from 1 to 10 (with 10 interaction) were ran and LnP(D) value was used to group all the genotypes. The maximum "k value was observed for k = 2, the inferred ancestry at k= 2 suggested that barnyard millet were grouped into two subpopulations (Fig. 3). Similarly, power marker



Fig. 3. Representation of the appropriate subpopulation number (K): Sub population number (K) against delta K and the maximum K value observed at K = 2

software was able to differentiate them into two clusters. From the obtained multiple and single bar population structure, genotypes were divided into two sub-populations *i.e.*, exotic (Japan) and Indian germplasm (Fig. 4). The admixture percentage among



Single bar population structure

Fig. 4. The structure of barnyard millet genotypes obtained from STRUCTURE software using finger millet and maize genomic SSR markers

the barnyard millet germplasm under the study was very negligible, which denotes that all were pure line except two genotypes IEC675, IEC196. The population 1, consisted of genotypes IEC381, IEC675, IEC178, IEC350, IEC706, IEC196, IEC374, VL232, VL234, VL207, VL29, VL224, VL172, IEC521, IEC751, IEC788, IEC60 and IEC265 and population 2 consisted of IEC517, PRB903, PRJ1, IEC511, IEC436, and IEC552 genotypes. All the genotypes under population 1 belonged to India and comprised of E. frumentacea group. Likewise, genotypes under population 2 were mostly of Japan origin and belong to E. esculenta group. The grouping pattern observed was similar in both the genetic diversity and population structure analysis. Similar grouping of barnyard millet whole global core germplasm into two groups based on species diversity have been reported earlier through agro-morphological evaluation (Sood et al. 2015b) and SNP data (Wallace et al. 2015). Since, there are no molecular markers available in barnyard millet for assessing the genetic diversity, so this study provides a set of polymorphic markers which can be utilized for the same. In the present study we used varieties along with wild species to know the relationship at species level also. The structure pattern, species of similar origin were grouped together. The mean value of alpha was 0.0421, and the ancestry-inferred cluster proportions of the membership of the sample were 0.746 and 0.254. The average distances (expected heterozygosity) between individuals in the same cluster were 0.0358 (cluster 1) and 0.4024 (cluster 2) and the allele frequency [divergence among pops (net nucleotide distance), computed using point estimates of p] was 0.1083 between clusters 1 and 2.

Authors' contribution

Conceptualization of research (BKB, SS); Designing of the experiments (BKB); Contribution of experimental materials (SS); Execution of field/lab experiments and data collection (BKB, CR); Analysis of data and interpretation (BKB, CR, SS); Preparation of manuscript (BKB, SS).

Declaration

The authors declare no conflict of interest.

Acknowledgement

The authors thank Indian Council of Agricultural Research for support of the project.

References

- Babu B. K., Senthil N., Michael Gomez S., Biji K. R., Rajendraprasad N. S., Satheesh Kumar S., and Chandra Babu R. 2007. Assessment of genetic diversity among finger millet [*Eleusine coracana* (L.) Gaertn.] accessions using molecular markers. Genet. Res. and Crop Evol., **54**: 399-404.
- Babu B. K., Agrawal P. K., Dinesh Pandey, Jaiswal J. P. and Anil Kumar. 2014a. Association mapping of agromorphological characters among the global collection of finger millet genotypes using genomic SSR markers. Mol. Biol. Rep., 41(8): 5287-5297.
- Babu B. K., Dinesh Pandey, Agrawal P. K., Salej Sood and Anil Kumar. 2014b. In-silico mining, type and frequency analysis of genic microsatellites of finger millet [*Eleusine coracana* (L.) Gaertn.]: a comparative genomic analysis of NBS–LRR regions of finger millet with rice. Mol. Biol. Rep., **41**(5): 3081-3090.
- Babu B. K., Agrawal P. K., Dinesh Pandey and Anil Kumar. 2014d. Comparative genomics and association mapping approaches for *opaque2* modifier genes in finger millet accessions using genic, genomic and candidate gene based SSR markers. Mol. Breed., 34: 1261-1279.
- Danquah E. Y., Hanley S. J., Brookes R. C., Aldam C. and Karp A. 2002. Isolation and characterisation of microsatellites in *Echinochloa* (L.) *beauv.* spp. Mol. Ecol. Notes, **2**: 54-56.
- Dida M. M., Srinivasachary R. S., Bennetzen J. L., Gale M. D. and Devos K. M. 2007. The genetic map of finger millet, *Eleusine coracana*. Theor. Appl. Genet., **114**: 321-332.
- Dida M. M., Wanyera N., Harrison Dunn M. L. N., Bennetzen

J. L. and Devos K. M. 2008. Population structure and diversity in finger millet (*Eleusine coracana*) germplasm. Trop. Plant Biol., doi: 10.1007/s12042-008-9012-3.

- Dje Y., Heuertz M., Levfebvre C. and Vekemans X. 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. Theor. Appl. Genet., **100**: 918-925.
- Earl Dent A. and M. vonHoldt Bridgett. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Cons Genet. Res., 4(2): 359-361.
- Gale M. D., and Devos K. M. 1998. Comparative genetics in the grasses. Proc. Nat. Acad. Sci. USA, **95**: 1971-1974.
- Kumari K., Muthamilarasan M., Misra G., Gupta S., Subramanian A., et al. 2013. Development of eSSR-Markers in Setaria italica and Their Applicability in Studying Genetic Diversity, Cross-Transferability and Comparative Mapping in Millet and Non-Millet Species. PLoS ONE, 8(6): e67742. doi: 10.1371/ journal.pone.0067742.
- Liu K. and Muse M. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics, **21**: 2128-2129.
- Maniatis T., Sambrook J. and Fritsch E. F. 1989. Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Moore G., Devos K. M., Wang Z. and Gale M. D. 1995. Grasses, line up and form a circle. Curr. Biol., **5**: 17-23.
- Murray M. G. and Thompson W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucl. Acids Res., 8: 4321-4326
- Muthamilarasan M., Venkata Suresh, Pandey B., Kumari G., Parida S. K. and Prasad M. 2014. Development of 5123 Intron-Length Polymorphic markers for largescale genotyping applications in Foxtail millet. DNA Res., 21: 41-52.
- Nirgude M., Kalyana Babu B., Shambhavi Y., Singh U. M., Upadhyaya H. D. and Anil Kumar. 2014. Development and molecular characterization of genic molecular markers for grain protein and calcium content in finger millet [*Eleusine coracana* (L.) Gaertn.]. Mol. Biol. Rep., **41**(3): 1189-1200.
- Nozawa S., Nakai H. and Sato Y. I. 2004. Characterization of microsatellite and ISSR polymorphisms among *Echinochloa* (L.) Beauv. spp. Jap. Breed. Res., **6**: 87-93.
- Pandey G., Mishra G., Kumari K., Gupta S., Parida S. K., Chattopadhyay D. and Prasad. 2013. Genome-wide development and use of microsatellite markers for large-scale genotyping applications in foxtail millet *Setaria italica* (L.). DNA Res., 20: 197-207.

- Pritchard J. K. and Wen W. 2003. Documentation for the structure software, version 2. Department of Human Genetics, University of Chicago, Chicago. http:// pritch.bsd.uchicago.edu/software.
- Rajput S. G., Tammy P. H. and Dipak K. S. 2014. Development and Characterization of SSR Markers in Proso Millet Based on Switchgrass Genomics. Amer. J. Plant Sci., **5**: 175-186.
- Roder M. S., Plaschke J., Konig S. U., Borner A., Sorrells M. E., Tanksley S. D. and Ganal M. W. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Genet. Genom., 246: 327-333.
- Shambhavi Y., Vikram Singh G., Jaiswal J. P. and Anil Kumar. 2014. Simple sequence repeat (SSR) analysis in relation to calcium transport and signaling genes reveals transferability among grasses and a conserved behavior within finger millet genotypes. Plant Syst. Evol., **300**: 1561-1568.
- Sood S., Khulbe R. K., Gupta A., Agrawal P. K., Upadhaya H. D. and J. C. Bhatt. 2015a. Barnyard millet- A Potential Food and Feed Crop of Future. Plant Breed., **134**: 135-147. doi:10.1111/pbr.12243.
- Sood S., Khulbe R. K., Kumar A., Agrawal P. K. and Upadhaya H. D. 2015b. Barnyard millet global core collection evaluation in the submontane Himalayan region of India using multivariate analysis. The Crop J., **3**: 517-525.
- Udupa S. M. and Baum M. 2001. High mutation rate and mutational bias at (TAA)n microsatellite loci of chickpea (*Cicer arietinum* L.). Mol. Genet. Genom., 265: 1097-1103.

- Ugare R., Chimmad B., Naik R., Bharati P. and S. Itagi. 2014. Glycemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. J. Food Sci. Tech., **51**: 392-395.
- Upadhyaya H. D., Gowda C. L. L. and Sastry D. V. S. S. R. 2008. Plant genetic resources management: collection, characterization, conservation and utilization. J. SAT Agri. Res., **6**: 1-15.
- Varshney R. K., Kumar A., Balyan H. S., Roy J. K., Prasad M. and Gupta P. K. 2001. Characterization of microsatellites and development of chromosome specific STMS markers in bread wheat. Plant Mol. Biol. Rep., 18: 5-16.
- Veena S., Bharati V. C., Rama K. N. and Shanthakumar G. 2005. Physico-chemical and nutritional studies in barnyard millet. Karnataka J. Agri. Sci., 18: 101-105.
- Wallace J. G., Upadhyaya H. D., Vetriventhan M., Buckler E. S., Tom Hash and Ramu P. 2015. The genetic makeup of a global barnyard millet germplasm collection. Plant Genome, 8. http://dx.doi.org/ 10.3835/plantgenome 2014.10.0067.
- Yabuno T. 1987. Japanese Barnyard Millet (*Echinochloa utilis*, Poaceae) in Japan. Econ. Bot., **41**(4): 484-493.
- Yadav O. P., Mitchell S. E., Fulton T. M. and Kresovich S. 2008. Transferring molecular markers from sorghum, rice and other cereals to pearl millet and identifying polymorphic markers. J. SAT Agri. Res., **6**.
- Zhao X. and Kochert G. 1993. Phylogenetic distribution and genetic mapping of a (GGC) n microsatellite from rice (*Oryza sativa* L.). Plant Mol. Biol., **21**: 607-614.

Errata

Inadvertently an error in printing of the second cover page displaying the Executive Council (EC), Editorial Board (EB), membership fee and subscription rates for the year 2016-2017 occurred in Volume 78(1) of Indian Journal of Genetics and Plant Breeding. The error was rectified in the next issue of the Journal, Volume 78(2) with newly elected EC, EB, membership fee and the rates of subscription for the year 2018-2019. The error is regretted.

Editor