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Identification of microsatellite markers for finger millet genomics application through cross transferability of rice genomic SSR markers

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Abstract

In the present investigation, 345 rice genomic SSR markers were used for finding the cross transferability in twelve finger millet accessions. Out of which, 202 SSRs (58.6%) showed transferability among the finger millet genotypes and only 26 (13%) were found to be polymorphic. Thirteen markers were polymorphic between two finger millet genotypes, VR708 and GPU48, whereas five markers were between GE86 and PRM801. These markers can be effectively used in mapping populations for construction of linkage maps. Few putative orthologous regions for grain yield and its components like 1000 grain weight, leaf characteristics and root traits between rice and finger millet were detected. Among the biotic stresses, blast and brown plant hopper (BPH) resistance loci were found to be highly conserved. The PIC values of all the polymorphic loci varied from 0.15 to 0.55. Power Marker grouped the finger millet genotypes into two major clusters based on the races. The average gene diversity existing among the genotypes was relatively high (41%) indicating the usefulness of cross transferability in millet

Key words: Finger millet, orthologs, rice genomic SSRs, cross transferability

Introduction

Finger millet is one of the staple foods of the semi-arid tropics of Africa and Asia including India, which constitutes the major portion of all the millets cultivation in terms of area (8%) and production (11%) in the world (Bennetzen et al. 2003). The crop is a rich source of calcium, essential amino acids, and minerals such as manganese, copper and iron, two polyunsaturated fatty acids linoleic acid and α-linolenic acid (Barbeau and Hilu 1993; Birch et al. 2007). It is also considered as functional food which is reported to be beneficial for human health because it contains high amount of dietary fibers, vitamins and antioxidants (Sood et al. 2016). However, in the post-genomic era very little progress is available on application of genomics approaches for the finger millet crop improvement in comparison to other cereals, where there is full genome sequence available for nearly 22 crops including foxtail millet (Hamilton and Buell 2012).

Since there is 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 same family (Moore et al. 1995; Gale and Devos, 1998). The impact of the discovery of conserved synteny is also important in case of minor cereals with reference to the major cereals like rice, maize and wheat. Evidence for similar conserved genome relationships are already well established in rice (Zhao and Kochert 1993), wheat (Roder et al. 1995), legumes (Weeden et al. 1992) and crucifers (Lagercrantz et al. 1996). These results showed that conserved synteny existing among the related species will aid in identification of genes/ QTLs of important agro-morphological traits in minor cereals like finger millet. For the first time Srinivasachary et al. (2008) identified the syntenic regions between rice and finger millet using the genic and genomic finger millet SSR markers. It was found that on an average 85% similarity existed between these two genomes by using 218 markers. Microsatellites or simple sequence repeat (SSR) markers have been useful to molecular breeders and

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geneticists to link phenotypic and genotypic variation and also popular because of their abundance and amenability to high throughput screening. Molecular markers like SSRs are being widely used for cross transferability across species (Shambhavi et al. 2014) and also for identification of syntenic regions among the closely related species (Babu et al. 2014 a, b, c; Kumar et al. 2016). Kumari et al. (2013) demonstrated transferability of 40 foxtail millet eSSR markers in the crops of rice, maize and sorghum, which suggested that the nested chromosome fusion is frequently observed in grass genomes.

Till now very few genomic SSR markers are available in finger millet (Dida et al. 2007). Their number is not enough to be used in any breeding or applied genomics research, since development of SSRs was very costly as it involved high cost of library screening and clone sequencing. The alternate strategy to identify the best suitable markers which can be achieved through transferability of SSRs from the related close species like rice. With this aim, the present study was conducted to identify the best polymorphic genomic SSR markers for their applicability in finger millet genomics. Hence, the present study aimed at identification and validation of rice SSR markers in finger millet for their utility in genetic diversity and syntenic studies.

Materials and methods

Plant materials and DNA extraction

For identification of cross-transferability of SSR markers, initially four finger millet genotypes (GE86, PRM801; VR708, GPU48) were taken to find the transferable and polymorphic markers. Later, a set of 12 finger millet genotypes consisted of 10 landraces and two cultivated genotypes were used for molecular characterization and diversity analysis. The genotypes used in the present study are given in Table 1. The finger millet genomic DNA was isolated as per the earlier reports (Murray and Thomson 1980), and quality and quantity was checked on 0.8% agarose gel electrophoresis (Maniatis et al. 1989).

Details, and amplification of rice microsatellites

For detecting cross-species amplification, a total of 345 rice genomic SSR markers spread throughout all the chromosomes were taken (Table 2). PCR was performed in 15 µL volume containing 10X buffer along with 15 mM MgCl2, 0.2 µM of each forward and reverse primer, 2 mM dNTPs, 1 U of *Taq* DNA polymerase (Invitrogen, USA), and about 25 ng of template DNA. The polymerase chain reaction (PCR) amplification protocol was standardized for rice genomic SSRs used in the study. The primers used for cross transferability were obtained from rice genomics database gramene (www.gramene.org). The reaction conditions were as follows; the initial denaturation at 95^{o}C for 4 min followed by 40 cycles

Table 1. List of finger millet genotypes used in the study

Genotype	Source	Race
Land races		
IE2595	Malawi	Africana
IE3133	India	Spontanea
IE3283	Zimbabwe	Spontanea
IE3468	India	Spontanea
IE3507	Kenya	Spontanea
IE3664	Uganda	Spontanea
IE4225	Zimbabwe	Spontanea
IE4441	Cameroon	Spontanea
IE4443	Cameroon	Spontanea
IE6247	Zimbabwe	Spontanea
Varieties		
VL324	India	Coracana
VL352	India	Coracana

Table 2. The details of the transferability of rice SSR markers in 12 finger millet genotypes

Chromo-some	No. of markers used	No. of amplified markers	Percent of transfer-ability
1	35	24	68.6
2	35	24	68.6
3	33	18	54.5
4	32	17	53.1
5	27	15	55.6
6	32	23	71.9
7	27	13	48.1
8	30	18	60.0
9	27	15	55.6
10	30	17	56.7
11	27	13	48.1
12	10	5	50.0
Total	345	202	58.6

of 94^{o}C denaturation for 30 s, 30 s of annealing temperature at 50^{0}C with an extension of 1.0 min at 72^{o}C, and a final extension at 72^{o}C for 7 min,. The horizontal electrophoresis with 2.5% SFR ((Super fine resolution agarose, Amresco)) agarose was done for 3hr at 110 volts. Gels were visualized using Alpha Imager System (Alpha Innotech, USA).

Data analysis

The scoring of the PCR amplicons was done based on the molecular size of the allele. The data set of polymorphic SSR loci on 12 finger millet accessions 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, 1967 frequency matrix. All the amplified SSR markers were searched in gramene web site (www.gramene.org) to identify the traits to which they linked. Based on the information available in the gramene web site, the syntenic regions were allotted.

Results and discussion

Cross transferability of rice genomic SSRs

Till now most of the works were on the transferability of EST based SSR markers among different species. However, in the present study, a set of 345 rice genomic SSR markers were used initially to amplify four finger millet genotypes *viz*., GE86, PRM801 and VR708, GPU48. The reason behind taking genomic SSRs was that they are more polymorphic and most of the loci were mapped on the rice genome, which made identification of orthologues in finger millet easier. The genotypes, GE86 and PRM801 are being used as contrasting parents for generating mapping populations for calcium content, like wise VR708 and GPU48 are being used as parents for generating mapping population for blast resistance in research farm of VPKAS, Hawalbagh, Almora. A total of 202 (58.6%) markers produced amplicons among all the four genotypes. However, Shambhavi et al. (2014) found 67.3% of transferability of rice genic SSR markers in finger millet which may be due to high synteny between genic regions than genomic regions of closely related species. High transferability was observed in chromosome six followed by one and two (Table 2). The result of the present study helps the finger millet researchers to directly use the reported polymorphic markers directly in their genomic studies of finger millet.

Among the 202 amplified markers, only 26 (13%) were found polymorphic among the genotypes tested. Thirteen markers were found polymorphic between the genotypes, VR708 and GPU48, where as five markers were polymorphic between GE86 and PRM801 (Table 3). These markers can be effectively used in genotyping mapping populations for construction of linkage maps, for generation of comparative maps between rice and finger millet and identification of QTLs. Similar study was conducted by Yadav et al. (2008) where they found 13 primers including both CISPs and EST-SSRs were found to have detectable length polymorphisms in the parental lines of pearl millet mapping populations, which rendered them as useful markers in pearl millet. Though 26 markers found polymorphic but only 14 markers gave clear amplification and banding pattern and hence were used for polymorphic studies among the 12 finger millet genotypes. Among the 202 SSRs, 105 SSRs consisted of di-repeat motifs (52%), whereas 48 SSRs consisted of tri repeat motifs (24%) (Fig. 1). No SSRs were found to have penta-repeat motifs. These results were in close agreement with the earlier reports (Rajput et al. 2014; Reddy et al. 2012), where they found di-repeats, the most frequent repeats in millets. Among the di-repeats, GA repeat motifs (27) are the most frequent followed by TA (14), and AG (13) repeats, which was similar to the earlier reports based on EST based SSRs (Babu et al. 2014d; Reddy et al. 2012).

Table 3. Polymorphic markers between two sets of genotypes

Polymorphic markers between VR708 and GPU48	Polymorphic markers between GE86 and PRM801
RM144	RM144
RM14420	RM451
RM313	RM20930
RM16368	RM403
RM1282	RM121
RM5423	-
RM6887	-
RM5981	-
RM14361	-
RM151	-
RM286	-
RM72	-
RM16296	-

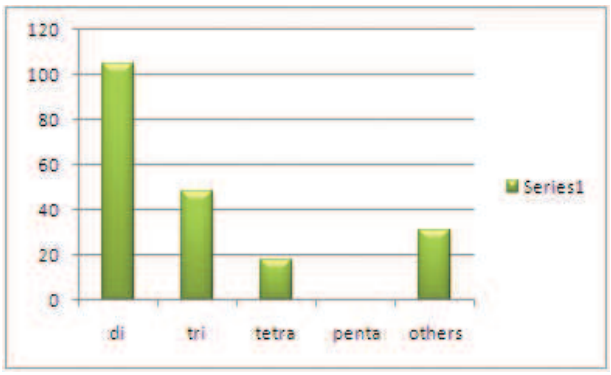

Fig. 1. The graphical representation of the proportion of the frequency and different rice SSR repeat motifs found transferable in finger millet genotypes

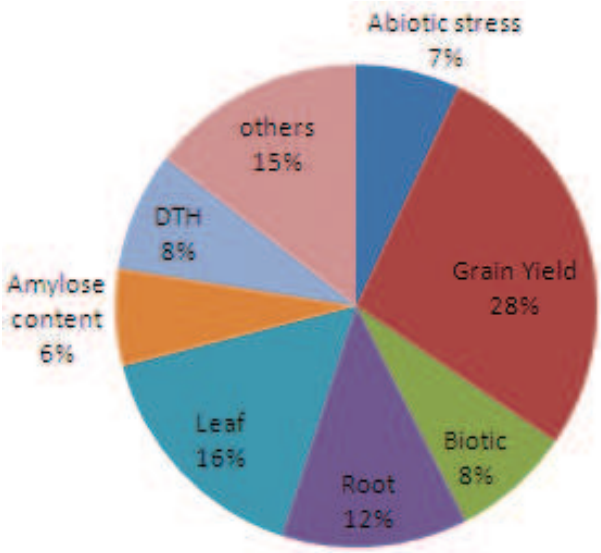

Fig. 2. Representation of syntenic regions observed between rice and finger millet using rice SSR markers

Genetic variation of rice SSR markers on finger millet genotypes

Among the 202 amplified markers, only 26 (13%) markers were found to be polymorphic between the genotypes. This type of low level of polymorphism may be due to that the finger millet crop is highly self pollinated crop or it could be because four genotypes were initially used for polymorphism survey. Similarly very less polymorphism was observed by Shambhavi et al. (2014), in the case of finger millet candidate genes for calcium content. Though 26 markers were found polymorphic, only 14 gave clear amplification and banding pattern and hence were used for polymorphic studies in 12 finger millet genotypes. The polymorphic SSRs yielded 33 scorable alleles with a mean of 2.36 allele per locus. The gel pattern in studied genotypes using the markers RM1282 (a) and RM14361 (b) is given (Fig. 3).

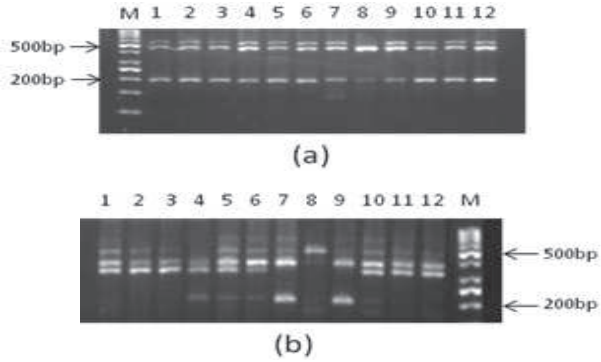

Fig. 3. Polymorphism of finger millet genotypes using rice SSRs RM1282 (a) and RM 14361 (b) (Lane M: 50bp marker, Lane 1-12 finger millet genotypes, IE2595, IE3133, IE3283, IE3468, IE3507, IE3664, IE4225, IE4441, IE4443, IE6247, VL324, and VL352, respectively)

The number of alleles generated ranged from two to where, four SSR marker RM72 was found to contain maximum number of alleles (4), and three markers, RM16296, RM1282 and RM403 had three alleles each (Table 4). The results obtained in the study were similar to our earlier reports, where we used the rice markers of blast resistance in finger millet (Babu et al. 2014a). The polymorphism information content (PIC) values of all the polymorphic loci across the 12 finger millet genotypes varied from 0.15 to 0.55 at an average of 0.33. These results were also in close agreement with our earlier reports (Babu et al. 2014a) where the PIC value ranged from 0.196 to 0.834. This higher PIC may be due to that they used large number of germplasm. This high amount of PIC in the study showed that high amount of polymorphism was existed among the finger millet genotypes and also the identified rice microsatellites can be effectively used to determine the genetic relationships and QTL mapping. The maximum PIC value was observed with the marker RM72 (0.55) followed by RM1282 (0.48) and RM403 (0.41). However, the lowest PIC value was observed for the SSR marker RM20930 (0.15) followed by RM144 and RM208 (0.19 each). Out of the 14 polymorphic SSR loci, nine SSR loci showed highest PIC value of more than the average value (0.33). Among the SSR loci, RM72, RM1282 and RM403 are noteworthy due to their relatively higher level of

polymorphism. The SSR marker RM72 which had more PIC value (0.55) also generated more number of alleles (4). The previous studies showed that rice blast linked genomic SSRs were more polymorphic than EST based SSRs and hence, the polymorphic SSRs obtained in the present study can be used for genomic applications of finger millet (Babu et al. 2014a).

Genetic diversity in finger millet germplasm using rice SSR markers

The polymorphism ability of the SSR markers is generally analyzed using the statistical measures like gene diversity and heterozygosity. The Gene diversity (expected heterozygosity) is termed as the probability that two randomly chosen alleles from the population are different. Gene diversity was in the range of 0.17–0.59 with an average value of 0.41. The expected heterozygosity (*He*) was found to be highest with the SSR marker RM72 (0.59) across the 12 finger millet genotypes, followed by RM1282 (0.57). The expected heterozygosity present among the finger millet genotypes showed that markers used in the present study were more polymorphic. However, the lowest gene diversity was found in the SSR marker RM20930 (0.17) followed by RM144 and RM208 (0.22 each). The heterozygosity, also called as 'observed heterozygosity' (*Ho*) was at an average of 0.53 and ranged from 0.00 to 0.92 which showed a wide range of heterozygosity in the finger millet genotypes. The observed heterozygosity was found to be highest for the SSR marker RM313 and RM1282 (0.92 each) followed by RM5981 (0.83) (Table 4). The observed heterozygosity observed among the selected finger millet genotypes was in congruence with the earlier reports in finger millet (Bharathi 2011). However, the lowest heterozygosity (0.00) was found with the marker RM151. The reason for such high heterozygosity would be allo-tetraploid nature of the finger millet having A and B genomes and may be due to use of landraces. It means the SSR markers had more SSR repeats may be showing high mutation rate. For example in the present study, SSR markers RM313 and RM1282 showed high heterozygous nature and they also had more repeat motifs of (GT)6 (CACG)5-6, (GT)8 and (AG)17 respectively. As a result, many of the markers which displayed heterozygous nature have a large number of SSR units (Dje et al. 2000). Rare and unique alleles were also present in the landraces. Unique alleles were found in SSR markers RM16296 and RM403. The marker RM16296 contained the unique allele size of 150bp, where as RM403 had unique allele of 500bp. Rare alleles were observed by the RM72, RM1282 and RM20930 (two alleles each). The presence of rare alleles in RM72, and RM 20930 may indicate the diversity at a given locus of SSR marker.

Table 4. Details of the rice SSRs like allele number and PIC among 12 finger millet genotypes

Marker	Major allele	Allele number frequency	Gene diversity	Hetero-zygosity	PIC
RM144	0.88	2.00	0.22	0.25	0.19
RM72	0.59	4.00	0.59	0.45	0.55
RM16296	0.83	3.00	0.29	0.33	0.26
RM313	0.54	2.00	0.50	0.92	0.37
RM208	0.88	2.00	0.22	0.25	0.19
RM1282	0.46	3.00	0.57	0.92	0.48
RM6887	0.50	2.00	0.50	0.82	0.38
RM5981	0.58	2.00	0.49	0.83	0.37
RM20930	0.91	2.00	0.17	0.18	0.15
RM14361	0.59	2.00	0.48	0.82	0.37
RM151	0.64	2.00	0.46	0.00	0.36
RM403	0.64	3.00	0.49	0.73	0.41
RM121	0.60	2.00	0.48	0.60	0.36
RM16368	0.83	2.00	0.28	0.33	0.24
Mean	0.68	2.36	0.41	0.53	0.33

Analysis of finger millet genotypes

The genetic diversity analysis among the collection of 12 finger millet genotypes which consisted of land races and cultivated genotypes were done using polymorphic rice genomic SSRs. The dendrogram was generated through UPGMA analysis of Power Marker V3.25 software. These SSR markers grouped the 12 finger millet genotypes into 2 major. Both the clusters comprised of six genotypes each. One cluster was further divided into two sub-groups. The released cultivated and genotypes, VL324 and VL352 clustered along with landraces *viz*., IE3468, IE2595, IE3283 and IE3133. The landraces IE3468 and IE3133 are of Indian origin, and these might be involved in the pedigree of the VL324 and VL352. Similarly, Dida et al. (2008) analyzed a set of 79 finger millet accessions using 45 finger millet SSR markers and were able to differentiate into 2 phylogenetic groups. The second cluster was sub divided into two groups which consisted of two genotypes each, the first sub-cluster comprised IE3664 and IE 4225, and the second consisted of IE4441 and IE 6247. The genotypes under second

cluster belonged to the Spontanea race and all were from exotic source. The genotype IE4443 found close with the genotypes in second sub cluster except IE6247, which is from Zimbabwe. The average gene diversity existing among all the genotypes were relatively high (41%), indicating existence of high levels of polymorphisms among the finger millet.

Identification of putative orthologous regions in finger millet

All the amplified SSR markers were searched in gramene web site (www.gramene.org) to identify the traits to which they linked. Based on the information available in the gramene web site, the syntenic regions were allotted. Though the methodology may not give full details, but we attempted initially to explore the possible syntenic regions common between rice and finger millet. 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 identification of few putative syntenic regions between rice and finger millet. It was found that high synteny was observed for grain yield characters like 1000 grain weight, yield, and grain weight, leaf characteristics followed by root traits (Fig. 2). The leaf senescence was found to be conserved highly among the leaf characteristics. Synteny was also observed for root characteristics (10%), amylase content (6%), days to heading (8%), plant height, seed dormancy, spikelet fertility and tiller number. The inadequate genomic information available in finger millet lead to a major hurdle for the finger millet crop improvement. In the present investigation, 345 rice genomic SSR markers were explored for the cross transferability and orthologous regions in finger millet. Nearly 59% of rice SSRs were amplified in finger millet. Twenty six (13%) markers were found to be polymorphic which can be effectively used in genotyping mapping populations. It was found that high synteny of rice markers was observed for grain yield characters like 1000 grain weight, yield, and grain weight, leaf characteristics followed by root traits.

Authors' contributions

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

Conflict of interest

The authors declare that there is no conflict of interest.

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