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Transferability of Rice SSR Markers to Sorghum

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Abstract

In the present investigation, five genotypes each from rice and sorghum were evaluated for marker transferability. Upon PCR amplification, twenty markers distributed evenly on rice chromosomes, were separated on Agarose Gel. It was observed that nine rice primers amplified in sorghum. This showed that rate of transferability (45.0 %) of rice primers among sorghum genotypes. Hence, screening existing markers through transferability test from closely related species or family is resource efficient.

Keywords: Genome, molecular markers*, oryzasativa*, sorghum, transferability

Sorghum and rice belongs to family Poaceae. *Sorghum bicolor* is a widely grown cereal crop, particularly in Africa, ranking 5th in global cereal production. Sorghum's genome is relatively small (~730 M) and simple (10 chromosomes, diploid) compared to other C_4 crops in the Poaceae subfamily, such as maize and sugarcane (Dillon *et al*., 2007 and Luo *et al*., 2016).

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world. Rice, wheat, and maize together account for about half of the world's food production and rice itself is the principal food of half of the world's population (Joshi *et al*., 2010). Rice is the obvious choice for the first whole genome sequencing of a cereal crop. The rice genome is well mapped and well characterized, and it is the smallest of the major cereal crop genomes at an estimated 400 to 430 Mb. The next largest genome of an important cereal crop isthat of sorghum at 750 to 770 Mb and the wheat genome is nearly 37 times the size of the rice genome at close to16,000 Mb (Chapman *et al*., 2015).

It has been estimated that rice diverged from the common ancestor of sorghum and maize approximately 50 million years ago (Paterson, 2004). Sorghum-rice alignments based on the completely sequenced *S. bicolor* and *O. sativa* genomes, demonstrate high levels of DNA conservation between the two species. In addition, the number and sizes of sorghum gene families are similar to those of Arabidopsis and rice. It has been observed that 39.9 per cent of rice sorghum aligned sequences are conserved at the 70 per cent / 100 bp level and 77.5 per cent of the length of sorghum exon sequences overlap with those of rice (Yu *et al*., 2002; Matsumoto *et al*., 2005; Paterson and Bowers, 2009). However, the number of rice SSR markers that have been transferred into sorghum is still limited, and there is a lack of systemic surveys from different rice chromosomes.

Due to their ubiquitous distribution in genomes, Simple Sequence Repeats (SSR) markers showed a high transferability among common cereal species. The transferability of SSR markers across species orgenera has been reported in several cereal cropssuch as rice, wheat, barley, sorghum, maize and bitter gourd (Pandian *et al.,* 2000; Cordeiro *et al.,* 2001; Gupta *et al.,* 2003; Thiel *et al*., 2003; Wang *et al*., 2005; Zhang *et al*., 2005; Tang *et al*., 2008; Singh *et al*., 2013 and Saxena *et al*., 2015). The transferability of Rice SSR to Bamboo genera has been established by Chen *et al.,* 2010. Thus, developed SSR markers from the model crops could also be transferred to other cropsystem for sustaining beneficial agronomical traits.Therefore, transferable SSR markers from the complete sequenced rice genome would be useful for genetic analyses in sorghum.

Several studies have been conducted on the transferability of SSR markers across species or genera of several cereal crops which belong to grass family such as rice and wheat (Varshney *et al.,* 2005);

rye and triticale (Kuleung *et al*., 2004); barley (Thiel *et al*., 2003); sugar cane (Cordeiro *et al*., 2001; Banumathi *et al*., 2010), sorghum (Savadi *et al*., 2012) major cereal to minor grass (Wang *et al*., 2005) and pearl millet (Yadav *et al*., 2008). There are also reports about transferability of SSRs from cereals such as rice and sugar cane to bamboo species (Sharma *et al*., 2008). Sharma *et al.* (2009) also conducted an experiment on identification and amplification of EST-SSR markers in different bamboo species. However, there has been no report on transferability of rice SSR markers to sorghum. The objective of this study was to perform the transferability test of SSR markers from rice to sorghum and to employ the transferable markers in the genetic diversity analysis of selected sorghum genotypes.

Material and Methods

Plant materials and experimental conditions : The present experiment was conducted during *Kharif*-2015. The experimental material for this investigation consists of five genotypes each from riceand sorghum (Table I). They were grown at field of aerobic rice laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru in Randomized Complete Block Design (RCBD) with three replications. Total genomic DNA was isolated from young leaves of 21 days old plants using cetyltrimethylammonium bromide (CTAB) procedure, as described by Doyle & Doyle (1990).

Table I

List of rice sorghum varieties used in present study

Genotype	
Rice	Sorghum
AM 65	CSH-14
AM 72	DHANVI
ARB 6	MLHT
MOROBEREKAN	ROAGRO
MTU1001	SJH-1

Selection of rice SSR markers : Selection of rice SSR markers information on rice SSR markers was derived from McCouch *et al*. (2002) and the Gramene website (http://www.gramene.org). Total of twenty SSR markers were used to screen for transferability. The details of SSR primers used are presented in Table II.

PCR amplification : Primers developed for rice SSR by McCouch *et al*. (1997) were used for the PCR reactions with the DNA of the five sorghum species. To standardize the PCR conditions, the annealing temperature of each rice SSR marker used with sorghum DNA was the same as that originally used with rice DNA (McCouch *et al*., 2002).

PCR amplification reactions were done in 10 µl reaction mixtures, containing 2 µl of template DNA (50ng/µl), 0.5 µl of each forward and reverse primer (5pmol), 4 µl of PCR master mix and 3 µl ddH_2O. PCR cycling consisted of initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing (according to different SSR primer pairs) and primer elongation at 72 °C for 1 min followed by afinal extension at 72 °C for 10 min. Amplified products were stored at -20 °C until further use. PCR amplification of the markers was carried out in Mastercycler® Nexus Gradient, Eppendorf. Agarose electrophoresis was done with 3 per cent gels to visualize the amplicons and then photographed using Alpha Innotech gel documentation instrument.

Among the markers SSRs are widely used in genetic diversity and parental analysis owing to their co-dominant, high reproducibility, abundance in the genome and transferability across species or genera. The development of these markers for a species might be costly and time consuming. Hence, screening existing markers through transferability test from closely related species or family is resource conscious.

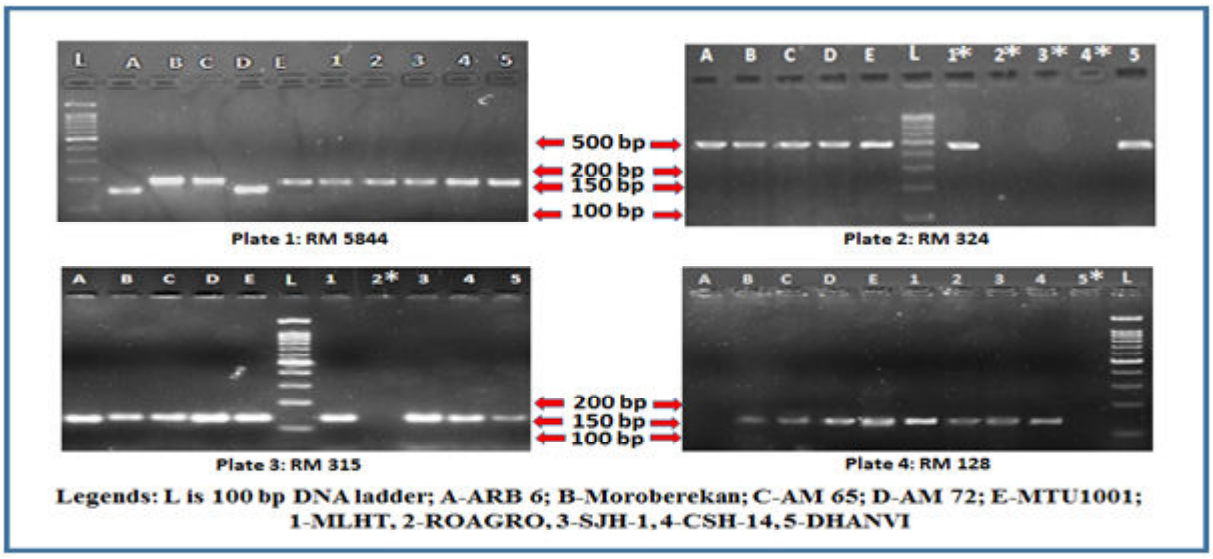
Results and Discussion

Among the twenty markers used in present study, as expected, most of them showed amplicons in all the genotypes of rice. However, only nine primers *viz.,* RM166, RM234, RM315, RM324, RM318, RM128, RM5844, RM140 and RM348 showed amplification in sorghum.

TABLE II

List of selected rice SSR markers tested for their transferability to sorghum

Primer	Chromosome No.	Forward (5' --3')	Reverse (5' --3')	Optimized Annealing Temperature (°C)	Expected Product size (bp)	Repeat motif
RM157	1	CCTCCTCCTCACGAATCCCGCC	GGGCTTCTTCTCCGCCGGCTTC	60	106	(CT)11(TC)10
RM315	1	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	56.6	133	(AT)4(GT)10
RM128	1	AGCTTGGGTGATTTCTTGGAAGCG	ACGACGAGGAGTCGCCGTGCAG	63.9	148	(GAA) 9
RM140	1	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	64.6	261	(CT)12
RM543	1	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCCCC	55	98	(GCG)10
RM211	2	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	60	161	(TC)3A(TC)18
RM166	2	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	60.3	321	(T)12
RM324	2	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	60	175	(CAT)21
RM318	2	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC	60.3	140	(GT)15
RM563	3	CGACCCTAGGGTTTCTCC	CTCGACGTCGTGGAAAGC	60	185	(CCT)6
RM148	3	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG	59.9	129	(TG)12
RM185	4	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC	64.6	197	(AGG)9
RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	68.4	216	(CT)19
RM518	4	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	60	171	(TC)15
RM1153	4	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC	60.3	114	(AG)13
RM348	4	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC	50.4	136	(CAG)7
RM146	5	CTATTATTCCCTAACCCCCATACCCTCC	AGAGCCACTGCCTGCAAGGCCC	60	345	(CT)11(CT)7
RM5844	5	TGACTAACGTGGCATCCATG	GCTAGGAGCCATTGTCGAAG	60	195	(ATA)22
RM234	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	60.3	156	(CT)25
RM195	8	AGAAAGAGAGGCCGTCGGCGGC	GGGCTCACCCCCAAACCTGCAG	63.9	311	(GA)9(CT)8



* Not amplified

Of the nine amplified SSR markers, two were amplified in all sorghum genotypes (RM348 and RM5844) (Plate 1), whereas, RM324 amplified only in two sorghum varieties (Plate 2) and two were amplified (RM315 and RM128) in four sorghum varieties (Plate 3 and 4). This indicates that the rice SSR primers are operating efficiently in sorghum. Other eleven markers were not amplified in sorghum even after replicating the experiment thrice. Hence, absence of bands is true absence of that particular DNA sequence in the genomic DNA of the respective crop and not due to any kinds of technical error and indicates null allele.

TABLE III

Transferability of rice SSR markers in sorghum genotypes

Sorghum Genotypes	Amplified markers (x)	Transferability* (%)
CSH-14	2	10.0
DHANVI	2	10.0
MLHT	2	10.0
ROAGRO	1	5.0
SJH-1	2	10.0
Total	9	45.0

* Transferability, the ratio of amplified markers (x) / tested markers (n)

Among the selected 20 rice SSR markers, nine SSR markers were successfully amplified in sorghum, which is a transferability of 45.0 per cent (Table III). However, transferability rate differed among sorghum genotypes. The transferability rate obtained in this study was higher than the one reported for pearl millet using sorghum, rice and other cereals (Yadav *et al*., 2008). However, the rate is slightly lower than the rice SSR transferability tobamboo that was reported to be 68 per cent (Chen *et al*., 2010). It is possible to improve the transferability rate of markers by using markersthat were developed from expressed sequences (Kuleung *et al*., 2004).

The transferability (45.0%) in this study was lower than that reported for the transfer of apple SSR markers cross-amplified inpear (100%; Yamamoto *et al*., 2001) and for maize SSR markers to Miscanthus (74.5%; Hernandez *et al*., 2001). However, it was higher than that reported for rice SSR markers amplified in Indian bamboo species (44.9%; Sharma *et al*., 2008) and the intrageneric amplification of SSR markers in the grass family (Kuleung *et al*., 2004; Saha *et al*., 2006) and intergeneric amplification of barley (Thiel *et al*., 2003). The transferability of rice SSR markers into sorghum in our study was lower, possibly due to the lack of sequence similarity in sorghum, which are the primer binding sites for SSRs in rice.

The results of study determines that the rice SSR markers can be a valuable marker source for those

plant species for where little molecular marker information is available. This study also suggests that more rice SSR markers derived from those rice chromosome regions showing a very high transferability should be tested in the future with the aim of obtaining more markers in the sorghum genotypes. Trait specific markers from rice when transferred to sorghum could be used directly for MAS.

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