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Short Communication

Molecular Characterization of Accessions from a Traditional Rice Cultivar, Suwandel Conserved at Plant Genetic Resources Centre, Sri Lanka

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ABSTRACT: Suwandel is one of the most important traditional rice cultivars in Sri Lanka. The present study was conducted to identify the molecular differences and to determine duplicates among the Suwandel rice accessions conserved at Plant Genetic Resources Centre (PGRC). A total of 20 simple sequence repeat (SSR) markers were used across 11 Suwandel rice accessions. Out of these 20 SSR loci 18 loci showed polymorphism. A total of 47 alleles were detected and the number of alleles per locus ranged from 1-4 with an average of 2 alleles. The frequency of the most common allele at each locus ranged from 45 to 100% with an average of 67%. Genetic diversity among 20 loci ranged from 0.00 to 0.67 with an average of 0.42. Polymorphic information content ranged from 0.00 to 0.62 with an average of 0.35. A phylogenetic tree showed three distinct main clusters with two sub clusters in the cluster three. The highest genetic similarity was obtained between AC 12827 and C 2013-12-778 (91%) which can be identified as closely related Suwandel rice accessions. The lowest similarity was recorded between AC 4197 and AC 4366 (27%). According to the similarity matrix and phylogenetic tree, there were no duplicates among the accessions.

Keywords: Accessions, genetic diversity, polymorphism, SSR markers, Suwandel

INTRODUCTION

Rice has been cultivated in Sri Lanka, since 1000 BC and it is the single most important crop in the country (Samath, 2008). Traditional rice cultivars have paramount importance because those cultivars have evolved thousands of years in the local soil (Rajakumar *et al.,* 2011). Therefore, these cultivars have acquired high adaptability to the environment because of the introgression of desirable traits from the wild relatives and high natural selection pressure of pest and diseases (Rajakumar *et al.,* 2011). Plant breeders keep using this valuable gene pool to produce highly adaptable and agronomically superior commercial varieties.

Sri Lanka has rich genetic diversity of rice and most of the germplasm have been conserved at Plant Genetic Resources Centre (PGRC), Gannoruwa, Peradeniya. It includes traditional varieties, exotic varieties, locally developed old and new improved varieties and wild relatives. Among the traditional rice varieties, *Suwandel* rice is one of the most popular traditional rice cultivars in Sri Lanka due to its exquisite aroma, milky taste and medicinal value (Slow Food Foundation for Biodiversity, 2012). PGRC, Sri Lanka has conserved 11 *Suwandel* rice accessions in their gene bank. Those accessions are differentiated according to

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the information given by farmers based on their observations. But, It is difficult to characterize accessions belonging to the same variety based on physical traits due to morphological similarity of those accessions. Therefore, molecular markers were used to identify the variations among the accessions as they have proven to be powerful tools in assessment of genetic variation and in the elucidation of genetic relationship within and among genetically closer organisms (Miah *et al.,* 2013).Among the molecular markers SSR (Simple Sequence Repeats) are the most widely used to evaluate the genetic variability of rice and those are more informative than other markers (Ravi *et al.,* 2003).

Molecular characterization is important to identify and overcome those duplicates and it helps for efficient gene bank management. Present study was conducted for evaluate the molecular diversity of *Suwandel* rice accessions conserved at PGRC to identify the molecular differences and to determine duplicates among the accessions.

METHODOLOGY

Eleven *Suwandel* rice accessions were obtained from the gene bank of Plant Genetic Resources Centre. Accessions are AC 4197, AC 4366, AC 4471, AC 4595, AC 5420, AC 16640, AC 12827, AC 12844, AC 13136, C 2013-12-788, and AC 4802. DNA was extracted from two weeks old immature leaves using CTAB method described by Murry and Thomson (1980) with minor modification. A total of 20 microsatellite primers were used for genotyping and type of primers are given in Table 1.

The PCR reaction mixture contained 2.5 µl of 15 ng/µl template DNA, 3.0 µl of 5 X buffer, 1.5 µl of 25 mM $MgCl_2$, 0.6 µl of 10 mM forward and reverse primers, 0.08 µl of 5U Taq polymerase and 7.02 µl sterile distilled water in final volume of 15 µl. PCR programme consisted of initial denaturation of the template DNA at 94 ^oC for 5 minute followed by denaturation at 95 ^oC for 1 minute. Primer annealing was done at 55 ^oC for 30 seconds and extension at 72 ^oC for 1 minute. The amplification was terminated by a final extension at 72 ^0C for 7 minutes. PCR amplification was performed by using Applied Biosystem (model # 9902, USA) Thermocycler for 35 cycles. PCR products were resolved in 8 % (Acrylamide: Bis ratio- 29:1) non denaturing polyacrylamide gel electrophoresis in 1 X TBE buffer followed by Ethidium Bromide (0.5 µg/ml) staining. Bands were visualized using gel documentation system (BIO RAD, USA).

Gel images were manually scored by visual observations. Data analysis was done by using POWERMARKER V 3.25 software to estimate the genetic diversity of each accession. Major allele frequency, number of alleles, genetic diversity and polymorphism information content (PIC) for each marker were calculated. Genetic distances were calculated for each pair of populations according to the Nei *et al.,* (1983) formula available in POWERMARKER V 3.25 software. These data was used to construct a phylogenetic tree based on Unweighted Pair Group Method with Arithmetic Averages (UPGMA) algorithm.

RESULT AND DISCUSSION

Allelic diversity

A total of 47 alleles were detected at 20 microsatellite markers across 11 *Suwandel* rice accessions. From 20 SSR markers, only 2 were monomorphic (RM 220 and RM 255) and

other markers were polymorphic and those markers contributed to identify diversity among the 11 *Suwandel* rice accessions. The number of alleles per locus ranged from 1 allele to 4 alleles with an average of 2 alleles across the 20 loci. The highest number of alleles (4.0) per locus was detected in loci RM 217 and RM 207 and lowest number of alleles (1.0) per locus was detected on loci RM 255 and RM 220. The frequency of most common allele at each locus ranged from 45% (RM 217) to 100% (RM 255 and RM 220) with an average of 67%. It was higher than the experimental results of Hossain *et al.,* (2007). Moderate levels of genetic diversity exist among 20 loci studied across 11 *Suwandel* rice accessions, ranging from 0.00 to 0.67 with an average of 0.42 (Table 1).

Table 1. Number of alleles, major allele frequencies, genetic diversity and polymorphic information content (PIC) found among 11 *Suwandel* rice accessions

Marker	Major Allele Frequencies	No. of Alleles	Genetic Diversity	PIC Values
RM 237	0.6364	2	0.4628	0.3557
RM 25	0.5636	3	0.5845	0.5180
RM 259	0.6364	2	0.4628	0.3557
RM 536	0.4727	3	0.6241	0.5481
RM 201	0.6100	2	0.4758	0.3626
RM 219	0.7100	2	0.4118	0.3270
RM 208	0.6091	2	0.4762	0.3628
RM 224	0.5273	3	0.5902	0.5128
RM 241	0.8182	2	0.2975	0.2533
RM 480	0.7273	2	0.3967	0.3180
RM 412	0.8182	2	0.2975	0.2533
RM 220	1.0000	1	0.0000	0.0000
RM 228	0.7000	3	0.4600	0.4102
RM 207	0.6364	4	0.5455	0.5041
RM 216	0.6200	3	0.5432	0.4849
RM 236	0.7273	2	0.3967	0.3180
RM 571	0.5455	2	0.4959	0.3729
RM 255	1.0000	1	0.0000	0.0000
RM 20B	0.7273	2	0.3967	0.3180
RM 217	0.4545	4	0.6777	0.6232
Mean	**0.6770**	**2**	**0.4298**	**0.3599**

PIC value is a measure of polymorphism among varieties for a marker locus used in linkage analysis and it could be evaluated on the basis of allele frequencies (Sajib *et al.,* 2012). It varied from 0.0 (RM 255 and RM 220) to 0.62 with an average 0.35 (Table 1). The Highest PIC value 0.62 was obtained for RM 217 followed by RM 536 (0.54), RM 25 (0.51), and RM 224 (0.51).

Fig. 1 and Fig. 2 shows the 8 % polyacrilamide gel images of amplified fragments produced by primer RM 217, RM 536, RM 25, RM 224, RM 255 and RM 207 across the *Suwandel* rice population. PIC value revealed that the locus RM 217 (0.62) would be the best in screening of 11 *Suwandel* rice accessions followed by RM 536, RM 25 and RM 224.

The PIC values of some of the tested primers indicated that these primers were highly informative and capable of distinguishing the polymorphism among the less diverse genotypes.

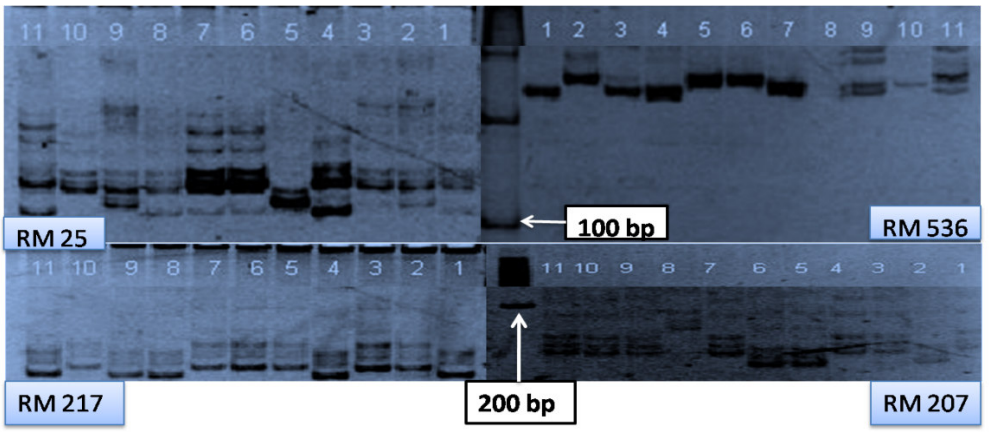

Fig. 1. PCR amplification profiles of RM 25, RM 536, RM 217 and RM 207 primers in 11 *Suwandel* rice accessions in 8% polyacrylamide gel. Sample No. 1-AC4197, 2-AC4366, 3-AC4471, 4-AC4595, 5-AC5420, 6-AC16646, 7-AC12827, 8-AC12844, 9-AC13136, 10-C2013-12-788, 11-AC004802

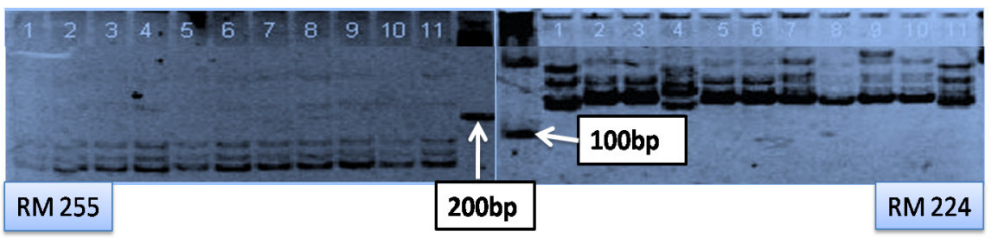

Fig. 2. PCR amplification profiles of RM 255 and RM 224 primers in 11 *Suwandel* rice accessions in 8% polyacrylamide gel. Sample No. 1-AC4197, 2-AC4366, 3-AC4471, 4-AC4595, 5-AC5420, 6-AC16646, 7-AC12827, 8-AC12844, 9-AC13136, 10-C2013-12-788, 11-AC004802

Mean number of alleles per locus and average PIC value in the present study were comparable with the result reported by Sajib *et al.,* (2012) using 12 elite aromatic rice genotypes and Pal *et al.,* (2003) using Basmati and non Basmati rice accessions who reported the mean number of alleles per locus as 3.3 and 3.5, average PIC values as 0.48 and 0.44 respectively. The mean value of allele per locus and PIC value obtained from our study is considerably lower than the genetic study of diverse rice germplasm conducted by Thomson *et al.,* (2006), Hassain *et al.,* (2007) and Pervaiz *et al.,* (2009). One of the reasons for low diversity in this study might be due to consideration of single traditional rice cultivar which was collected from different areas under same name. The diversity existing among the accessions might be due to the genetic drift of the population.

Genetic similarity based analysis

A similarity matrix was used to determine the level of relatedness among the accessions. The Nei *et al.,* (1983) similarity matrix (Table 2) indicated that the highest genetic similarity was between AC 12827 (7) and C 2013-12-788 (10) (91.05% similarity), and followed by the AC 4197 (1) and AC 4595 (4) (90.5 % similarity), AC 4366 (2) and AC 16 646 (6) (84% similarity).The lowest genetic similarity among the *Suwandel* accession was between AC 4197 (1) and AC 4366 (2) (27% similarity), followed by AC 4197 (1) and AC 16646 (6) (33% similarity), AC 5420 (5) and AC 004802 (11) (35.8% similarity).

Table 2. Nei's similarity matrix among 11 *Suwandel* rice accessions using 20 SSR markers

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	1.000										
2	0.270	1.000									
3	0.340	0.805	1.000								
4	**0.905**	0.325	0.405	1.000							
5	0.375	0.745	0.600	0.325	1.000						
6	0.330	**0.840**	0.780	0.370	0.725	1.000					
7	0.465	0.655	0.695	0.505	0.640	0.635	1.000				
8	0.472	0.550	0.572	0.477	0.416	0.555	0.638	1.000			
9	0.545	0.600	0.595	0.570	0.605	0.530	0.735	0.661	1.000		
10	0.447	0.573	0.642	0.484	0.578	0.542	**0.910**	0.676	0.784	1.000	
11	0.747	0.410	0.500	0.815	0.357	0.436	0.513	0.541	0.642	0.483	1.000

Legend: Sample No.1= AC 4197; 2= AC 4366; 3=AC 4471; 4=AC 4595 5=AC 5420; 6=AC 16646; 7=AC 12827; 8=AC 12844; 9=AC 13136; 10= C 2013-12-788; 11= AC 4802

According to these results considerable genetic diversity was observed among *Suwandel* accessions (27% to 91%). Similar results were observed by Pervaiz *et al.,* (2009) in diversity study of Asian rice varieties.

Phylogenetic tree was constructed using Nei *et al.,* (1983) genetic distance data according to the UPGMA algorithm (Fig. 3). Based on the genetic relationship, these accessions were grouped into three clusters in 50% similarity coefficient level. Cluster 1 included AC 4197, AC 4595 and AC 4802 and cluster 2 comprised one accession; AC 12844. Cluster 3 was divided into 2 sub clusters in 60% coefficient similarity threshold level. Sub cluster 1 included AC 4366, AC 16646, AC 4471 and AC 5420. Sub cluster 2 included AC 12827, C 2013-12-788 and AC 13136.

Genetic distance refers to the genetic divergence among population, which is capable of measuring the variety of parameters in relation to the frequency of a particular trait (Sajib *et al.,* 2012). According to the phylogenetic tree, three separate clusters were identified. Within the group some genetic variations could be identified and no 100% similarity was observed. Three pairs of accessions; AC 12827 and C 2013-12-788 (91.05%), AC 4197 and AC 4595 (90.5%) and AC 4366 and AC 16646 (84%) could be identified which have more than 84% similarity level. These results were confirmed by Nei *et al.,* (1983) similarity matrix (Table 2).Sajib *et al.,* (2012) stated that 100% similarity between two accessions can be identified as "duplicates". In the present study, 100% similarity was not observed among these tested accessions.

These accessions had been explored from different locations of the country. Because of the continuous selection by the farmers and cultivation in different locations genetic drift could occur to create genetic variation among populations. Similarity values among the accessions revealed by microsatellite markers provide greater confidence for assessment of genetic diversity and relationship among less diverse populations.

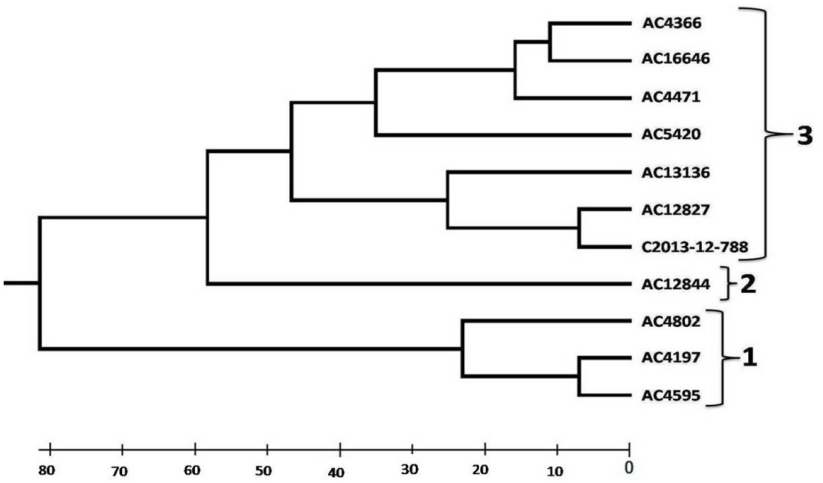

Fig. 3. Phylogenetic tree

CONCLUSIONS

Narrow genetic variation exists among genotypes due to inclusion of accessions from the single rice variety. There were no 100% similar duplicates among the accessions. However, high genetic similarity was observed between AC 12827 and C 2013-12-788 (91.1% similarity), followed by the AC 4197 and AC 4595 (90.5% similarity), AC 4366 and AC 16646 (84% similarity).

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