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DNA FINGERPRINTING OF TRADITIONAL RICE (*Oryza sativa* L.) ACCESSIONS FOR GENETIC DIVERSITY ANALYSIS

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ABSTRACT

Assessment of level and pattern of genetic diversity and identification of duplicates are important prerequisites in systematic germplasm management, conservation and utilization. Present study was conducted to assess the genetic diversity and relationships of twenty one rice germplasm (fifteen traditional accessions including ten different *Al-wee* accessions, four exotic accessions and two improved accessions), which are conserved at the seed bank of Plant Genetic Resources Centre (PGRC), Sri Lanka, using 8 polymorphic SSR markers. Data were analyzed using PowerMarker® Ver. 3.25. A significant genetic diversity was found among the accessions. Allele richness ranged from 2-5 per locus. The mean Polymorphic Information Content (PIC) value was 0.62 and the mean gene diversity was 0.67. The results reflect the high level of diversity in these accessions and the usefulness of selected SSR loci as informative markers. The cluster analysis based on SSR data identified eight clusters. The *Al-wee* accessions were clustered into different groups. The accessions *Mahagoda-al* and *Al-wee-4* showed the minimum genetic distance (0.13) and these two can be identified as genetically similar accessions.

KEYWORDS: Traditional rice, DNA fingerprinting, Cultivar identification, Genetic diversity

INTRODUCTION

Plant genetic resources are a non-renewable natural resource indispensable for the sustenance of human life on the earth. The genetic diversity present in the plant genetic resources are highly essential for crop improvement. Since the beginning of agriculture (more than 10,000 years ago), during the process of domestication and cultivation of crop plants, a wealth of genetic diversity has been utilized and partly preserved. However, with the extensive use of modern agriculture, many traditional crop varieties have been replaced worldwide by a few improved high yielding varieties narrowing down the crop diversity thus leading to serious genetic erosion. As a precautionary measure, substantial crop diversity is being conserved at present in the gene banks around the world.

The Plant Genetic Resources Center (PGRC) in Sri Lanka is the focal point for germplasm conservation and evaluation in agricultural crops. At present, over 12,000 accessions from a variety of agricultural crops have been conserved in the seed gene bank. Out of these, more than 4000 are accessions that include wild relatives, traditional varieties, advanced breeding lines, locally developed varieties

and exotic germplasm. In systematic conservation, presence of duplicates is one of the main constraints faced by gene banks worldwide. Normally, in the gene banks, germplasm are conserved under the common names used by farmers and therefore, repetitions of the same accession under different names and/or different accessions conserved under the same name could occur. According to the plant genetic resource data base at the PGRC in Sri Lanka, around 200 accessions of the traditional rice (*Oryza sativa* L.) variety *Al-wee* have been explored from different parts of the country and conserved at the seed gene bank. However, to-date, no records are available to identify the diversity among these accessions. Analysis of the levels and pattern of genetic diversity in these conserved germplasm accessions would be invaluable as it opens up the possibilities for systematic management, conservation, introgression of desirable genes from diverse genetic sources into the elite material, and derivation of novel and useful germplasm. Proper and appropriate characterization of germplasm is fundamental for efficient and systematic germplasm management and utilization, which leads to accurate identification of accessions avoiding duplicates. Further, with the international development of various aspects of intellectual property rights, special attention has to be paid to develop legal framework for cultivar identification and protect plant genetic resources in a country.

Morphological characterization has been traditionally used for cultivar identification and for studying the genetic diversity among accessions. However, phenotypic markers are not an ideal technique for diversity analysis due to limited abundance, low degree of polymorphism, environmental influences, and low throughput. In some instances, the morphological diversity has been found to be misleading the cultivar identification. Perales *et al.* (2005) studied about the maize diversity in Mexico and reported that though the accessions are genetically very close to each other, there can be significant phenological, morphological and yield differences within landrace accessions cultivated at a specific region due to the specific environmental adaptation and continuous selection done by farmers.

Molecular markers can support detailed, reliable and unambiguous characterization of genetic resources. A vast potential lies in their ability to identify the structure of genetic diversity within and among accessions. Microsatellite or Simple Sequence Repeat (SSR) markers are highly preferred by plant molecular breeders, as these markers are mapped, genetically co-dominant, highly abundant, highly polymorphic, robust, reproducible and amenable to automation (Powell *et al.*, 1996), and hence been widely used in crops including rice.

The present study was conducted to study the genetic diversity of selected set of rice germplasm accessions conserved at the seed gene bank of the PGRC in

Sri Lanka, and to investigate their genetic relationships, genotypic similarities among those rice accessions.

MATERIALS AND METHODS

A total of 21 rice accessions comprising of traditional (15), introductions (4) and improved (2) rice varieties were selected from the seed gene bank of the Plant Genetic Resources Centre (PGRC), Gannoruwa, Sri Lanka for this study (Table 1). Experiment was conducted at the Biotechnology Division at the PGRC.

Table 1. List of rice accessions used for the study

Serial No.	*Accession No.*	*Accession Name*	*Origin*	*Type*
1	003136	Pachchaiperumal	Sri Lanka	Traditional
2	003578	Al- wee-1	Sri Lanka	Traditional/Al-wee
3	004013	Pokkali	Sri Lanka	Traditional
4	004023	Al- wee-2	Sri Lanka	Traditional/Al-wee
5	004171	Rathal	Sri Lanka	Traditional/Al-wee
6	004724	Goda -al -wee	Sri Lanka	Traditional/Al-wee
7	004802	Suwada- al	Sri Lanka	Traditional/Al-wee
8	004905	Ma goda -al	Sri Lanka	Traditional/Al-wee
9	004909	Niyan -wee	Sri Lanka	Traditional
10	004919	Al-wee-3	Sri Lanka	Traditional/Al-wee
11	004930	Hal- al	Sri Lanka	Traditional/Al-wee
12	004948	Maha-goda-al	Sri Lanka	Traditional/Al-wee
13	005387	Blackgora	Philippine	Exotics
14	005393	UPIR 15	Philippine	Exotics
15	005395	Al- wee-4	Sri Lanka	Traditional/Al-wee
16	006338	Sudu-wee	Sri Lanka	Traditional
17	006454	Kolanathi- wee	Sri Lanka	Traditional
18	006897	Moraberekan	Philippine	Exotics
19	006924	Kinandang-patong	Philippine	Exotics
20	008540	Bg 304	Sri Lanka	Improved
21	008922	Bg 2225 – 1	Sri Lanka	Improved

The DNA was extracted from each accessions based on the 'population DNA bulk strategy' (Rebourg *et al.*, 2001). Each bulk was prepared by pooling an equal amount of leaf material that was taken from 20 individuals of each accession. The bulk strategy allows the estimation of within-population allele frequencies accurately. The DNA isolation was carried out after the pooling step, using a modified CTAB (Murray and Thompson, 1980).

Eight SSR primer pairs representing the rice genome were selected (Temnykh *et al.*, 2000) and used to analyze the genetic variation among the selected accessions. Details of the selected primers are summarized in Table 2.

Table 2. Details of selected primers

Marker name	*Sequence*	*Repeat motif*	*Fragment sizes**
RM201	F- ctcgtttattacctacagtacc R- ctacctcctttctagaccgata	(CT)17	158
RM273	F-gaagccgtcgtgaagttacc R- gtttcctacctgatcgcgac	(GA)11	207
RM332	F- gcgaaggcgaaggtgaag R- catgagtgatctcactcaccc	(CTT)5-12-(CTT)14	183
RM341	F- caagaaacctcaatccgagc R- ctcctcccgatcccaatc	(CTT)20	172
RM284	F- atctctgatactccatccatcc R- cctgtacgttgatccgaagc	(GA)8	141
RM286	F- ggcttcatctttggcgac R- ccggattcacgagataaactc	(GA)16	110
RM287	F- ttccctgttaagagagaaatc R- gtgtatttggtgaaagcaac	(GA)21	118
RM339	F- gtaatcgatgctgtgggaag R- gagtcatgtgatagccgatatg	(CTT)8 CTT(CTT)5	148

* Expected fragment sizes

Amplification of SSR was done using a protocol optimized at the Biotechnology Division of PGRC [30 ng template DNA, 1XPCR buffer (Promega flexy buffer), 0.2 mM dNTP mixture, 1.5 mM $MgCl_2$, 0.5U Taq DNA polymerase, 0.5µM of each primer]. The PCR amplification was performed using a Eppendorf thermo-cycler, according to the cycle profile of initial denaturation step at 94 °C at 4 min followed by a process of 35 cycles of denaturation at 95 °C for 1 min, annealing for 1 min in which the specific temperature for each primer was maintained and an extension step at 72 °C for 2 min. At the end of the final cycle, final extension was operated at a temperature of 72 °C for 7 min, with subsequent holding temperature at 4 °C. The PCR amplified products were resolved in 4 % Super Fine Resolution (SFR) agarose. A 50 bp ladder was used as a reference. The banding pattern of the SFR gel was manually scored by visual observation. Summary statistics including the allele richness (number of alleles per locus), Polymorphism Information Content (PIC) and the gene diversity were estimated using the software PowerMarker® Version 3.25 (Liu and Muse, 2005). The genetic distances were computed using Nei's genetic distance (Nei, 1972), which is based

on the infinite allele model (Kimura and Crow, 1964). To elucidate the relationship among selected rice accessions the similarities/dissimilarities among the selected rice accessions were calculated and phylogenetic tree was constructed using Nei's genetic distance data and Neighbour Joining algorithm.

RESULTS AND DISCUSSION

SSR allelic diversity

All the SSR used in the present study were polymorphic among the tested accessions. The diversity parameters including the number of alleles detected per locus, Polymorphic Information Content (PIC), and gene diversity (GD) analysis are presented in Table 3. The loci RM286 showed the maximum number of alleles per locus as six. All tested loci showed more than three alleles per locus.

Table 3. Details of SSR primers used for the study and summary statistics of SSR diversity

Marker name	*Allele No.*	*Gene Diversity*	*PIC**
RM201	3	0.6058	0.5380
RM273	3	0.6363	0.5645
RM332	3	0.5850	0.5129
RM341	4	0.6850	0.6270
RM284	3	0.6607	0.5867
RM286	6	0.7750	0.7402
RM287	5	0.7650	0.7286
RM339	4	0.6778	0.6241
Mean	3.8750	0.6738	0.6152

*Polymorphic Information Content

The PIC value, which measures the allelic diversity at a locus and reflects the allelic diversity and allelic frequency among the cultivars, ranged from 0.51 (RM332) to 0.74 (RM286) with the average of 0.62. Higher PIC value of a locus, indicate the higher the number of alleles detected. The high PIC values found in these tested loci indicated the informativeness of these SSR markers. Similar PIC values were observed in a study done using traditional rice germplsam in India (Seetharam *et al.*, 2009).

The gene diversity is a composite measure that summarizes the genetic variation among the tested accessions at the allele level. In this study, the gene diversity of tested loci ranged from 0.59 (RM332) to 0.78 (RM286) with an average of 0.67. The loci RM286, RM287, RM341 and RM339 were highly diverse among the tested populations. The genetic diversity depends on the proportion of polymorphic loci, the number of alleles per polymorphic locus and the evenness of

allele frequency within population (Berg and Hamrick, 1997). The accessions used in the study showed high genetic diversity at the tested SSR loci.

Genetic relationship among rice accessions

The maximum genetic distance was observed between Bg 304 and UPLR15, *Kinandang-patong* and *Pachchaiperumal*, *Pachchaiperumal* and UPLR15 was 1.0, while the least genetic distance was observed between *Al-wee-4* and *Mahagoda-al* (0.13). The accession *Mahagoda-al* and *Niyan-wee* also showed a considerably low genetic distance (0.25). The phylogenetic tree, which was developed using the distance data based on Neighbour Joining algorithm, showed that the tested populations could be grouped into eight clusters (Figure 1). However, a specific pattern of grouping according to the accession name or origin of accessions was not observed. *Pokkali* and *Pachchaiperumal* were clustered with Bg 304.

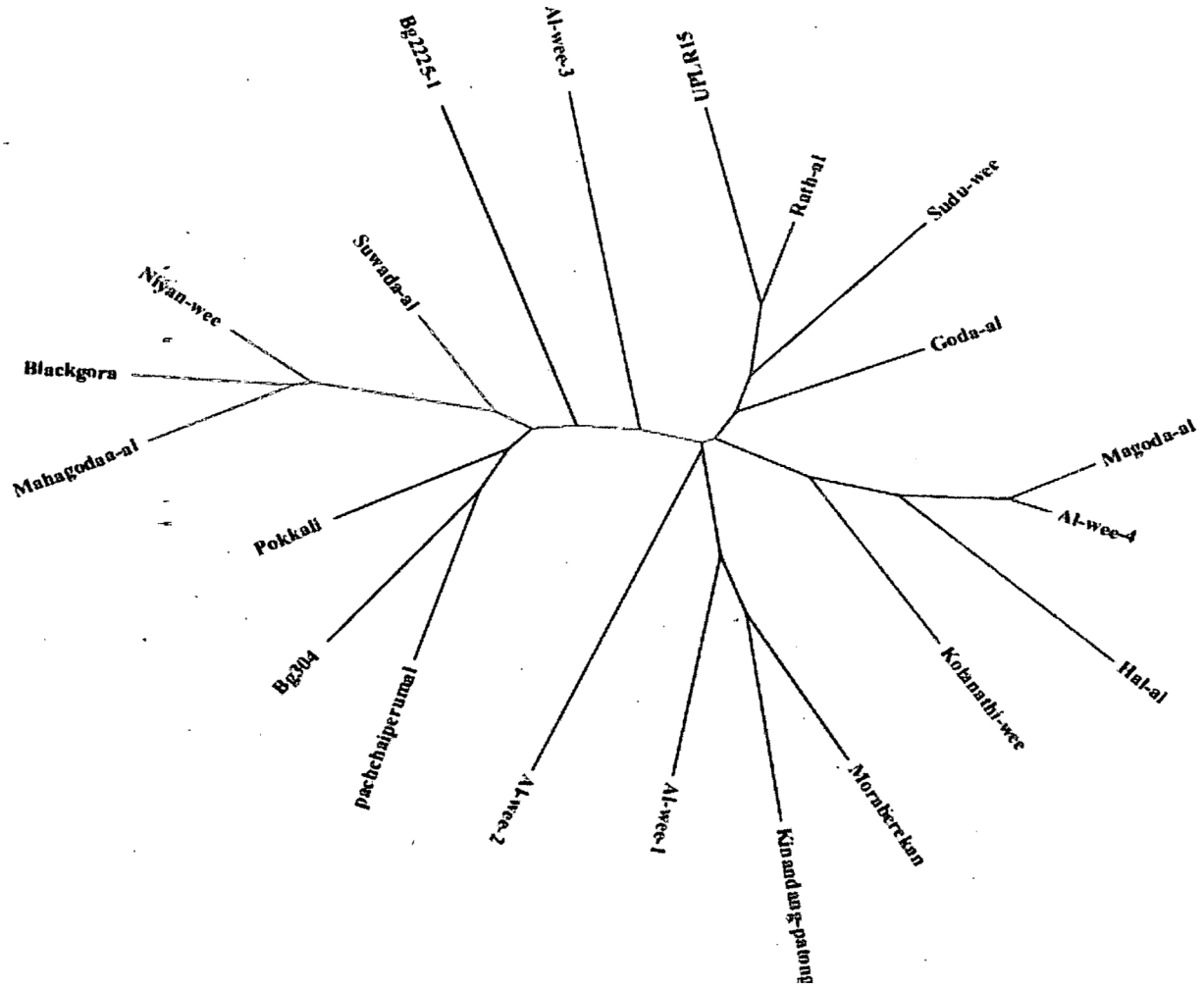

Figure 1. Genetic relationships among the 21 accessions of rice , based on analysis of SSR dataset using Nei's genetic distance (Nei, 1972) and Neighbour Joining algorithm.

The two exotic accessions (*Moraberekan* and *Kinandang-patong*) were clustered with *El wee-1*. *Suwada-al*, *Niyan-wee* and *Mahagoda-al*, and the exotic variety *Blackgora*. The accessions *Goda-al*, *Rath-al*, *Sudu-wee* and UPLR15 were clustered together. *Kolanethi-wee*, *Hal-al*, *Al-wee-4* and *Mahagoda-al* formed a different cluster. The clustering pattern of accessions gave an idea on the similarity among accessions within a particular cluster. Such type of information is important to study the desirable traits. Two exotic rice accessions (*Moraberekan* and *Kinandang-patong*) are known to be drought tolerant (Kamoshita *et al.,* 2008). Further, *Pokkali* and *Pachchaiperumal* are well known salinity-tolerant accessions.

An accurate assessment of the level and patterns of genetic diversity is important for different applications, including opening up of possibilities for introgression of desirable genes from diverse germplasm into the available genetic base, identification of subsets of core collections with utility for specific breeding purposes, systematic germplasm conservation management, and identification of duplicates (Mohammadi and Prasanna, 2003). It is important to make core collections to retain the maximum genetic diversity with the minimum number of accessions for systematic germplasm conservation (Frankel *et al.*, 1995). A considerable number of rice accessions are available with same common names (PGRC, 1999). For example, the availability of more than 185 different *Al-wee* accessions at the gene bank of the PGRC in Sri Lanka. The morphological and genetic similarities among these accessions have not been studied extensively. The genetic distance data generated from fingerprinting studies could be used efficiently and effectively to identify duplicates present in the gene banks.

In the present study, the ten different *Al-wee* accessions (Table 1) used for molecular characterization did not cluster together. These accessions were dispersed in six different clusters. The *Al-wee-2* and *Al-wee-3* that did not cluster with any other accession and hence, these two could be considered as two genetically distinct accessions. The least minimum genetic distance was observed between *Mahagoda-al* and *Al-wee-4* (0.13), and thus, these two accessions are genetically close. No similarity was observed between *Al-wee-1* and other different *Al-wee* accessions.

This present study showed the possibility of utilization of DNA fingerprint data for identification of duplicates and presence of similarity/ dissimilarity among rice accessions. Moreover, the fingerprint data could be utilized to identify molecular markers that are linked to desirable traits. The present global scenario in crop improvement focuses on the expansion of genetic diversity of crop species by exploiting desirable traits in traditional cultivars and wild relatives. Although such candidates generally perceived to be poor in yield, they are rich sources for some favorable genes and QTLs that could be a valuable resource for crop improvement. These genes and QTLs can be identified through the association analysis of molecular and phenotypic data. Prior to such applications, study of genetic diversity of the available germplasm is essential. Several studies have been conducted to identify the presence of genetic diversity among rice accessions originated from different geographical regions (Ni and Markill, 2002) and to identify the presence of molecular diversity within biotic traits (Garris *et al.*, 2003; Garland *et al.*, 2000; Jain and McCouch, 2004; Nagaraju *et al.*, 2002). Further, with the international development of various aspects of property rights, special attention should be paid to develop a legal framework to protect the plant genetic resources in the country.

This study highlighted the feasibility of utilization of molecular fingerprint data for diversity analysis and genetic similarities among rice accessions. Only eight polymorphic SSR markers were utilized for the characterization of accessions in this study. The precision of this characterization could be enhanced by utilizing more number of SSR markers to represent the whole rice genome and utilize high resolution methods such as capillary electrophoresis, which will give more informative fingerprint data.

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