ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2011) **9**(2); 224–228
doi:10.1017/S1479262111000256

Assessment of genetic diversity among Sri Lankan rice varieties by AFLP markers

Gowri Rajkumar[1], Jagathpriya Weerasena[1]*, Kumudu Fernando[2] and Athula Liyanage[3]

[1]*Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo, Colombo, Sri Lanka,* [2]*Agricultural Biotechnology Centre, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka and* [3]*Plant Genetic Resources Centre, Gannoruwa, Peradeniya, Sri Lanka*

Abstract

Sri Lanka has a valuable repository of germplasm collection due to the availability of a large number of different traditional and improved rice varieties. Molecular techniques can increase the effectiveness of traditional technologies in assessing genetic diversity. Amplified fragment length polymorphism (AFLP) was used to evaluate the genetic diversity among rice varieties available in the germplasm collection of Plant Genetic Resources Centre, Sri Lanka. AFLP analysis of rice varieties using ten different primer combinations yielded a total of 772 polymorphic bands (98.4%). Genetic similarities were estimated using Jaccard's (*J*) similarity coefficient. Unweighted pair group method with arithmetic mean (UPGMA)-based dendrogram was constructed. Genetic similarities varied from 0.073 to 0.565. Cluster analysis by genetic similarity divided the accessions into four main groups. The Cophenetic correlation with $r = 0.781$ indicated high confidence of AFLP data to group the varieties in UPGMA clusters. Principal component analysis further confirmed the patterns obtained by the cluster analysis. The results revealed very high genetic diversity at molecular level among the Sri Lankan rice varieties used in this study.

Keywords: AFLP; dendrogram; genetic diversity; rice; UPGMA

Introduction

Rice varieties grown in Sri Lanka from ancient to middle of the last century are known as traditional varieties. High yielding new improved varieties have been produced from crosses between traditional and exotic genotypes. Almost all rice varieties grown today are new improved varieties. However, traditional genotypes are still conserved *in situ* and/or *ex situ* in Genebanks. As these locally adapted genotypes may contain traits/genes of economic importance, their characterization is essential to use the available genetic diversity. AFLP technique is a robust, reliable and highly informative DNA fingerprinting method used to assess a large number of traits without prior sequence knowledge. Therefore, AFLP is widely accepted as an effective tool for identifying genomic differences (Loh *et al*., 1999). Furthermore, fluorescent labelling in fluorescent AFLP (FAFLP) replaces the radioactive labelling and increases the throughput by enabling automated detection and scoring of fragments generated in AFLP.

The objective of this study was to assess the genetic diversity among different rice accessions available in the Genebank of Sri Lanka by FAFLP markers.

Materials and methods

DNA extraction

Seeds of rice accessions (Table 1) were obtained from the Genebank of the Plant genetic Resources Center,

* Corresponding author. E-mail: jagath@ibmbb.cmb.ac.lk

Table 1. List of traditional rice varieties (TR), cultivated varieties (CV), wild rice species (WS) and hybrid variety (HV) used in this study

Accession no.	Name	Type	Accession no.	Name	Type	Accession no.	Name	Type
5642	Pokkali	TR	6276	Mudukiriel	TR	8919	Bg357	CV
3142	Molaga samba	TR	7799	SuduheenatiIcpy 19	TR	8923	Bg359	CV
3445	Yakada wee	TR	4724	Goda al wee	TR	11630	At306	CV
3470	Hatada wee	TR	2056	Gonabaru	TR	10935	Bg363	CV
3689	Kiriyal	TR	3147	Kaluheenati	TR	6311	Bw267-3	CV
3707	Heenati	TR	3598	Bala mawee	TR	8920	Bg360	CV
3725	Siviru wee	TR	3355	SuduheenatiIcpy 15	TR	10325	At362	CV
3131	Dahanala	TR	6248	Kombili	TR	8682	Bg403	CV
3136	Pachchaiperumal	TR	6233	Kalu kura wee	TR	2835	Bg450	CV
3143	Sulai 27614	TR	4278	Kohumawi B11	TR	10591	Ld356	CV
3145	Podiwi A8	TR	2199	Suwada samba	TR	8540	Bg304	CV
3150	Vellaiperunel	TR	3177	Madael	TR	9103	At353	CV
3151	Vellai Ilankalayan	TR	3724	Goda heenati	TR	9102	At303	CV
3156	Oddavalan	TR	8499	Hondarawala	TR	7182	Bg352	CV
3183	Hathiel	TR	6188	Heen dik wee	TR	10446	Bw361	CV
3484	Kuruluthudu wi	TR	4992	Rathu heenati	TR	5310	Bw351	CV
3133	Murunga 137	TR	3751	Chembavala samba 1	TR	3520	Bg11-11	CV
3444	Dikwee	TR	4614	Chembavala samba 2	TR	2830	Bg379-2	CV
3478	Kahata wee	TR	2079	Murunga	TR	2836	Bg380	CV
4832	Hatapanduru wee	TR	3495	Murungakayan 104	TR	9478	Bg358	CV
4909	Niyan wee	TR	3192	Thahanala	TR	10030	*Oryza rhizomatis*	WS
5630	Peillianel 26081	TR	2714	Gambada samba	TR	5138	*Oryza granulata*	WS
6169	Dewaradderi 26081	TR	2846	Bg745	CV	10199	*Oryza nivara*	WS
6283	Murunga kayan	TR	2840	Bg300	CV	10031	*Oryza eichingeri*	WS
3727	Kottamalli	TR	2837	Bg350	CV	10096	*Oryza rufipogon*	WS
5569	Red leaf variety	TR	4010	Bg90-2	CV	6290	Taichung Native 1	HV
2340	Wedaheenati	TR	4017	Bg34-5	CV		*Hygroryza aristata*	

Sri Lanka. They were planted in pots filled with soil collected from a paddy field and were grown in a green house. Tender leaves were harvested after 2 weeks and stored at −80°C. DNA was extracted from leaves according to the method described by Chen and Ronald (1999). Concentrations of DNA were estimated, and concentrations of all DNA samples were adjusted approximately to 300 ng/μl.

AFLP analysis

AFLP analysis was carried out according to Vos *et al.* (1995) with modifications. DNA was digested with enzymes *Eco*R1 and *Mse*1 at 37°C for 3 h and 30 min. Oligonucleotide adapters were ligated to digested DNA by incubating DNA, and adapters with T4 DNA ligase at 37°C for overnight. Then, digested/ligated DNA was pre-amplified with pre-amplification primers. The pre-amplified products were diluted 20 times with sterile distilled water and used for selective amplification.

Selective amplification reactions were performed using pre-amplified DNA and fluorescently labelled (HEX, TMR or FAM) *Eco*R1 primers and unlabelled *Mse*1 primers. Ten different primer combinations were used for selective amplification. The products were purified by ethanol precipitation followed by washing with 70% ethanol. The dried pellets were re-suspended in 5 μl of water. Finally, 2.5 μl of re-suspended sample was mixed with ET 550-ROX size standard (GE Healthcare Life Sciences, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK) and deionized formamide according to the manufacturer's instructions and denatured at 95°C for 2 min. Fragments were resolved using capillary electrophoresis on MegaBACE 1000 automated DNA sequencer (GE Healthcare Life Sciences). AFLP fragment analysis was performed with Genetic Profiler software 2.2 (GE Healthcare Life Sciences).

Data analysis

The electropherograms in the range of 30–550 bp were analyzed by Genetic Profiler software version 2.2 (GE Healthcare Life Sciences). Each fragment size was treated

https://doi.org/10.1017/S1479262111000256 Published online by Cambridge University Press

as a unique character and converted to binary data (present, 1; absent, 0). Jaccard's (1901) similarity coefficients (*J*) were calculated, and similarity coefficients' matrix was constructed. The matrix was used for cluster analysis by unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973), and the dendrogram was constructed by Multivariate Statistical Package (MSVP 3.1; Kovach, 1998). The confidence

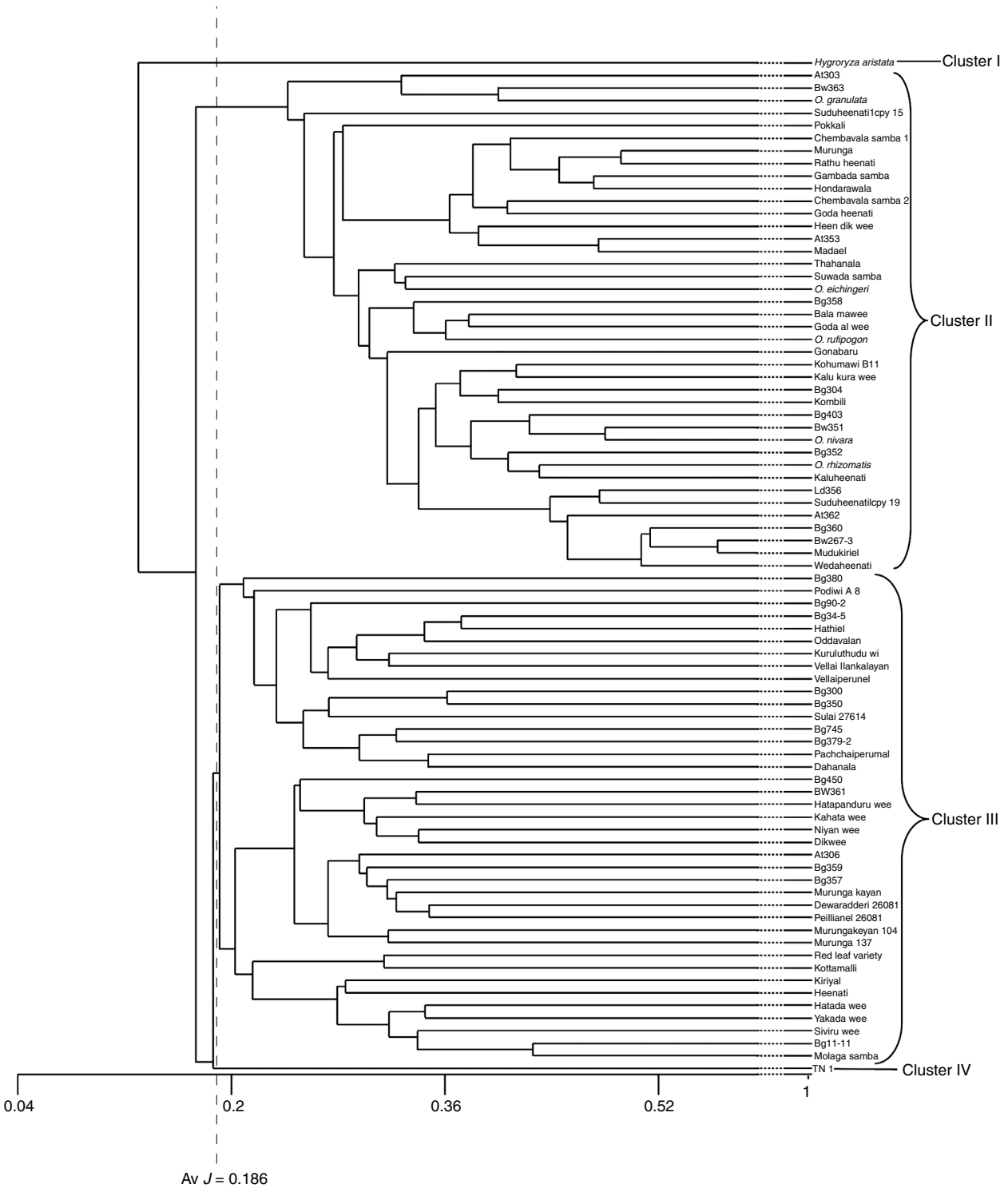

Fig. 1. The UPGMA dendrogram showing genetic diversity among Sri Lankan rice varieties.

https://doi.org/10.1017/S1479262111000256 Published online by Cambridge University Press

of the UPGMA clusters was assessed by Mantel (1967) test to calculate the Cophenetic correlation coefficient (r) as described by Abhijit *et al*. (2004). Cophenetic correlation coefficient is a comparison between the dendrogram and the similarity matrix.

Results and discussion

Ten primer combinations generated 784 fragments. Of which 772 were polymorphic (98.4%) and 12 (1.6%) were monomorphic. The number of amplified products generated by each primer pair ranged from 41 to 171 with an average of 78.4 fragments.

J showed that the genetic similarity varied from 0.083 to 0.565. The traditional rice Dikwee and At353 showed the lowest similarity (0.073), while Mudukiriel and Bw267-3 showed highest genetic similarity (0.565). The UPGMA dendrogram (Fig. 1) separated the accessions into four main clusters at the similarity coefficient of 0.186.

Clusters I and IV contained only one accession, while clusters II and III comprised 37 and 38 accessions, respectively. A modern rice variety (Taichung Native 1 (TN-1)), *Hygroryza aristata* and five wild species were also included in this study to find out the reliability of data and analysis. *H. aristata* previously classified under genus *Oryza* but now separated as a different genus was introduced in the analysis as an outlier. *H. aristata* was found to be the most divergent line (cluster I) by separating from the rest at the similarity coefficient of 0.130. All accessions in other clusters belong to genus *Oryza*. Cluster IV encloses only TN-1, which is the first Indica variety carrying the Dee-geo-woo-gen gene (semi-dwarfing gene), and TN-1 is the most susceptible variety for pest and diseases and photoperiod-insensitive and known to be different from rest of the varieties used. These results confirmed the reliability of data and methods of analyses.

Wild species *Oryza nivara, Oryza rufipogon, Oryza rhizomatis, Oryza granulata* and *Oryza eichingeri* showed clear separation from other accessions representing cluster II. Most of the traditional rice varieties known to be introduced from India (Molaga Samba, Pachchaiperumal, Vellaiperunel, Vellai Illankalayan, Peillianel and Murunga) were in cluster III. These plants had been cultivated in northern part of Sri Lanka during 1930s.

The Cophenetic correlation coefficient (r) from the comparison between the dendrogram and the similarity matrix was 0.781. The high value of Cophenetic correlation coefficient (r) indicates that the UPGMA dendrogram represents similarity data accurately. Principal component analysis of those 81 rice accessions based on the Gower's general similarity coefficient (Gower and Legendre, 1986) also confirmed the distribution of clusters in UPGMA analysis (data not shown). Scatter diagram of first three coordinates (PCo1, PCo2 and PCo3) revealed three well-defined groups. All wild species were found within the same group. Rice varieties with Indian origin were only found in two other groups, while all other verities were scattered among these groups.

The genetic diversity of rice varieties revealed by this study is useful in categorizing the accessions and preventing duplications in core collection in Genebanks. In addition, this information at molecular level can be integrated to devise strategies for *ex situ* and *in situ* genetic conservation, their utilization and exchange of genetic material.

Conclusion

AFLP analysis of the rice varieties revealed significantly high genetic diversity among the Sri Lankan rice germplasm used in this study and degree of genetic resemblance/distance of each one of those varieties. This genetic diversity data will provide more direct and reliable genetic information for selecting suitable parents in rice breeding programmes. Furthermore, the information given here may be useful in the management of *in situ* and *ex situ* preservations of rice germplasm.

Acknowledgements

The authors greatly appreciate the financial support given by the National Research Council of Sri Lanka grant no. 05-61. Technical assistance of Ms Anoma Jayasoma is gratefully acknowledged.

References

Abhijit SD, Kamlesh J, José MG, Vyankatesh JP, Milind SP, Dilip RR and Yogesh SS (2004) Comparison of 16S rRNA gene sequences of genus *Methanobrevibacter*. *BMC Microbiology* 4: 20.

Chen DH and Ronald PC (1999) A rapid DNA mini preparation method suitable for AFLP and other PCR applications. *Plant Molecular Biology Reporter* 17: 53–57.

Gower JC and Legendre P (1986) Metric and Euclidean properties of dissimilarity coefficients. *Journal of Classification* 3: 5–48.

Jaccard P (1901) Étude comparative de la distribuition floraledans une portion des Alpes et des Jura. *Bull Soc Vandoise Sci Nat* 37: 547–579.

Kovach W (1998) *MVSP-A multivariate statistical package. Version 3.1*. Pentraeth, Wales: Kovach Computing Services.

Loh JP, Kiew R, Kee A, Gan LH and Gan YY (1999) Amplified fragment length polymorphism (AFLP) provides molecular markers for the identification of *Caladium bicolor* cultivars. *Annals of Botany* 84: 155–161.

Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*. 27: 209–220.

Sneath PHA and Sokal RR (1973) *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. San Francisco, CA: Freeman.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research* 23: 4407–4414.