Indian J. Genet., **53** (2): 118–126 (1993)

RFLP FINGERPRINTING OF *ORYZA AUSTRALIENSIS* GENOTYPES USING CULTIVATED RICE GENOMIC PROBES

K. K. JENA AND G. KOCHERT

Department of Botany, University of Georgia, Athens, GA 30602, U.S.A.

(Received: December 12, 1991; accepted: June 15, 1992)

ABSTRACT

Four accessions of *O. australiensis* with EE genome were examined for restriction fragment length polymorphism (RFLPs). Fifteen RFLP clones of *O. sativa*, with known chromosomal positions showed RFLPs among the accessions. However, 54.6% monomorphic banding patterns indicated conserved nature of DNA of *O. australiensis* genome. The differences in electrophoretic banding patterns were parsimoniously interpreted as the result of 108 mutations. Three RFLP clones (RG118, RG190, RG207) revealed a number of RFLPs among the accessions which could fingerprint the four genotypes. The genetic relationship between the accessions were determined by the positions of the accessions in the phylogenetic tree and by computation of mutation distances between the accessions. We found that the accession 100882 is closer to the accession 101397 in terms of genetic distance than the accessions 103318 and 101410.

Key words: RFLP, *Oryza*, *O. australiensis*, DNA fingerprint, phylogeny, genotype.

The genus *Oryza* L. belongs to the subfamily *Oryzoideae*, in the family *Poaceae* (Gramineae) and has two cultivated species and about 20 wild species [1, 2]. These species grow in a wide range of geographical locations and are found in tropical and temperate regions worldwide [3]. The genus *Oryza* is divided into four distinct species complexes and two distinct species such as *O. schlechteri* and *O. brachyantha*. Cytogenetic observations of chromosome pairing have so far identified five genomes: AA, BB, CC, BBCC, CCDD, EE and FF [4, 5]. The cultivated species *O. sativa* and *O. glaberrima* share the common genome AA with their closely related wild species. The diploid species with DD genome is not yet identified and may be extinct. *O. australiensis* Domin. is diploid with EE genome and belongs to *O. officinalis* complex [2] or *O. latifolia* complex [6, 7].

The species in the genus *Oryza* have a number of desirable traits for rice improvement [8]. They have genes for resistance to biotic and abiotic stresses and it has been possible to introgress disease and insect resistance genes into the genome of cultivated rice across crossability and recombination barriers [9, 10]. *O. australiensis* grows in the northern tropical

zone of Queensland, Northern Territory and Western Australia. A few available accessions of *O. australiensis* have been screened and they show high level of resistance to several insects [11] and tolerance to drought (K. Ingram, personal communication). The accessions of this species are morphologically similar. Nevertheless, isozyme studies have detected genetic variability between the accessions of this species [6, 12]. However, apparent genetic variability of the accessions detected by morphological and isozyme analysis may be caused by differential gene expression in response to the environment or to the stages of plant development. Restriction fragment length polymorphism (RFLP) analysis of nuclear DNA provides an additional tool for studying genetic variation and phylogenetic relationships among populations and species of crop plants [13–17]. Since RFLP analysis is done directly at the DNA level, it reflects heritable changes in the nucleotide sequence both in the coding and noncoding regions. As a result, RFLP studies are more sensitive to genetic changes than isozymes which reflect only those changes resulting in aminoacid substitutions.

In this study, we have used RFLP analysis by rice genomic nuclear DNA probes to identify genetic variation among the four genotypes of *O. australiensis* and to fingerprint the respective genotypes. Based on RFLP variability, we have determined phylogenetic relationships among the accessions which could be useful for selecting genotypes to introgress useful genes for rice improvement.

MATERIALS AND METHODS

PLANT MATERIAL

Four accessions of *O australiensis*, which represented four independent collections from Australia, were grown in the green house from seeds provided by the International Rice Germplasm Center (IRGC) at the International Rice Research Institute (IRRI), Los Baños, Philippines (Table 1).

Table 1. Accessions of *O. australiensis* used in RFLP analysis

Identifier	Species	Genome	Accession No.	Origin or source
A1	*O. australiensis*	EE	100882	Japan*
A2	"	EE	101397	Australia
A3	"	EE	103318	Australia
A4	"	EE	101410	Australia

*Origin unknown but the seed kept at IRRI was obtained from NIG, Japan.

RFLP PROBING

Genomic DNA extraction, restriction endonuclease digestion, electrophoresis, Southern blotting, hybridization of labelled probes were generally similar to the methods described by McCouch et al. [18]. Genomic DNA of four *O. australiensis* accessions was cut with eight different restriction enzymes (EcoRI, EcoRV, HindIII, BamHI, PstI, DraI, XbaI, ScaI) according to the supplier's instructions (Bethesda Research Laboratories and New England Biolabs), electrophoresed in 0.8% agarose gels and Southern-blotted to GeneScreen Plus hybridizatioon membrane (DuPont) by the method of Southern [19]. Bacteriophage lambda cut with Hind III were included as molecular size standards on each gel. Fifteen genomic RFLP clones previously mapped to AA genome rice chromosomes [18], were kindly supplied by Dr. Steven D. Tanksley, Cornell University, Ithaca, New York (Table 2). Inserts were isolated from plasmids according to Maniatis et al. [20], random hexamer-labelled with ^{32}P-dCTP to high specific activity [21] and probed to the Southern-blots. Following overnight hybridization at 65°C, the membranes were washed with 2XSSC + 0.1% SDS (once) and 1XSSC + 0.1% SDS (twice) at 65°C for 20–30 minutes. Washed membranes were exposed to Kodak X-OMat film at –80°C for autoradiography.

Table 2. RFLP probes used

Probe	Copy	Mol. size (kb)	Chromosomal location*
RG108	Single	2.1	1
RG140	"	1.5	1
RG345	"	1.2	1
RG144	"	0.8	2
RG139	"	1.2	2
RG143	"	1.2	4
RG169	"	1.1	4
RG214	"	1.4	4
RG182	"	3.4	5
RG207	"	1.7	5
RG172	Multiple	1.8	6
RG213	"	1.3	6
RG118	Single	2.0	11
RG190	"	1.4	12
RG341	"	1.6	12

*Chromosome numbering is according to the International Rice Genetics Cooperative agreement, May, 1991.

DATA ANALYSIS

The bands revealed by autoradiography were assigned molecular weights based on their positions relative to the lambda molecular weight standards and each fragment was treated as a unit character. For each probe/enzyme combination, restriction fragments across all accessions were assigned numbers (1, 2, 3, ..., n) in order of their decreasing molecular weight. Common fragments among the four accessions were assumed to reflect shared restriction sites and to be indicative of homologies in DNA sequences. The data were organized into a 1–0 matrix and analyzed with the computer program "Phylogenetic Analysis Using Parsimony" (PAUP version 2.4) developed by D. L. Swofford (Illinois

Natural History Survey, Champaign, IL). The branch and bound method using addition= stepwise and mulpar functions was used to generate phylogenetic trees. In addition to phylogenetic analysis using parsimony (PAUP), we compared accessions in a pairwise fashion [22] using the total number of polymorphic restriction fragments between pairs of accessions.

RESULTS AND DISCUSSION

RFLP VARIABILITY

Of the 238 DNA fragments identified by 72 probe/enzyme combinations, 45 (18.9%) were unique fragments and 130 (54.6%) fragments were common to four accessions indicating extensive DNA sequence homology among the genotypes of *O. australiensis*. All the accessions showed similar banding patterns at eight restriction sites with the probes RG108 and 144 (Fig. 1A). However, 63 fragments (26.5%) were polymorphic among the accessions and separated the genotypes from one another (Fig. 1B). Genomic or cDNA clones have previously been used to identify genomes or genotypes in several crop species like potato, rice, soybean, tomato and beets [13, 15, 23–25]. In this study, we could differentiate the genotypes of *O. australiensis* with the selected random genomic probes of cultivated rice with known

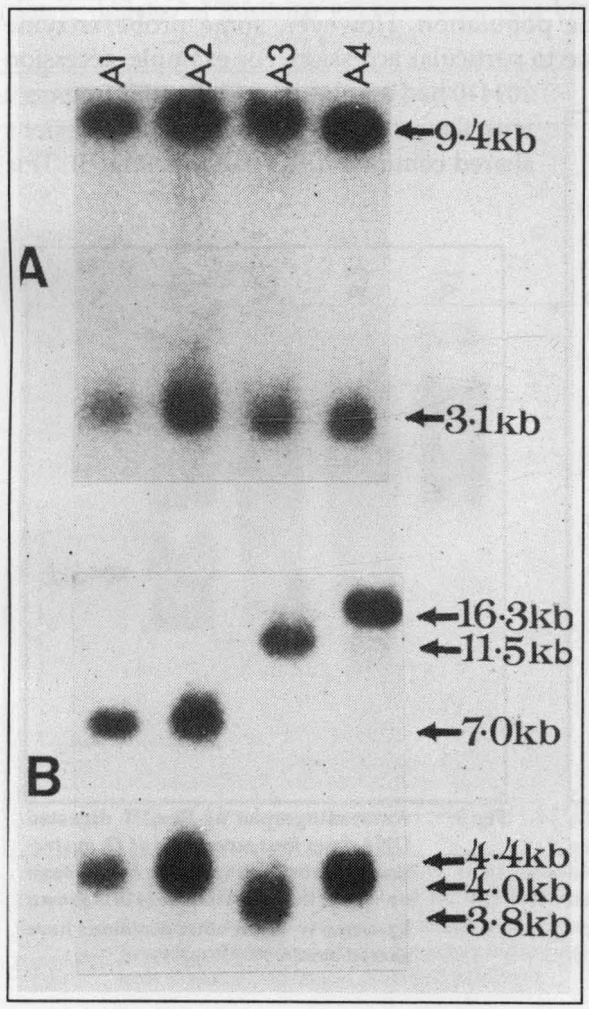

Fig. 1. **Autoradiographs of EcoRI digested DNA from four accessions of *O. australiensis*. A) Southern-blot was probed with RG 139. Note that two bands (9.4 kb and 3.1 kb) are common for all accessions. B) Southern-blot was probed with RG214. Note RFLP variations among the accessions. The sizes of banding patterns are in kilobases (kb) and pointed out by arrows (←).**

chromosomal positions. Interestingly, three single copy probes (RG118, RG190 and RG207) showed multiple banding patterns for the accessions at three different restriction sites (EcoRI, DraI and EcoRV) and showed RFLP differences among the accessions. For example, the probe RG190 showed 23 kb and 6kb DraI fragments specific to the accession 100882, 5 kb and 2.5 kb fragments for the accession 101410 and the accessions 101397 and103318 shared a common 3.5 kb fragment (Fig. 2). Thus the three probes (Rg118, RG190 and RG207) may be used to fingerprint EE genome species at the genotype level and this type of banding patterns resembled with the patterns detected in apple genotypes by M13 fingerprint probes [26]. Identification of the genotypes of *O. australiensis* by random genomic probes is also supported by previous findings at the chloroplast DNA level [27].

The population genetics of wild rice species is not well known and our data do not include multiple plants from the same population. However, some probe/enzyme combinations produced fragments unique to particular accession. For example, accession 101410 had a unique 6.0 kb BamHI fragment with the probe RG190 while other accessions shared common RFLP patterns (Fig. 3). This

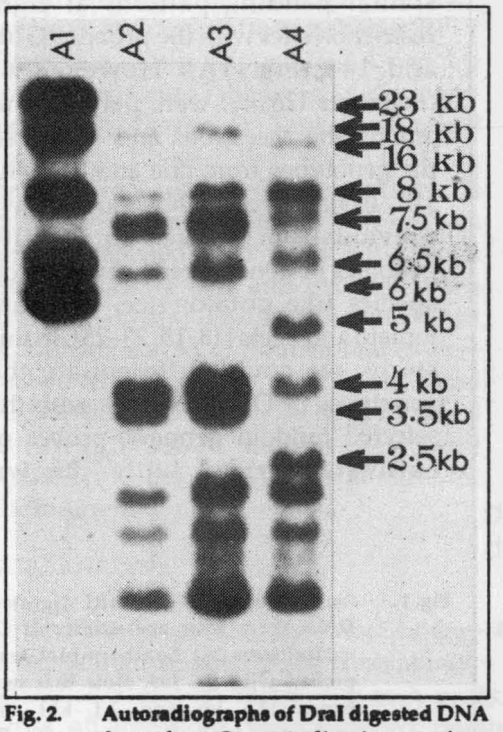

Fig. 2. **Autoradiographs of DraI digested DNA from four *O. australiensis* accessions probed with RG190. Note multiple banding patterns in all four accessions. Differentiating band sizes are given in kilobases (kb) and marked by arrows (←).**

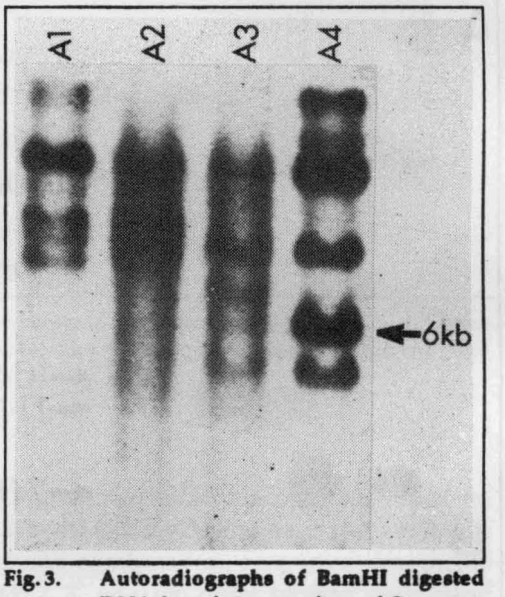

Fig. 3. **Autoradiographs of BamHI digested DNA from four accessions of *O. australiensis* probed with RG190. A 6 kb band unique to the accessions 101410 is shown by arrow (←) and other accesions have shared bands.**

may be a population specific marker and we are now studying such variation among the plants of a single genotype and their inheritance in an interaccession F_2 population.

PHYLOGENETIC RELATIONSHIP

The total number of 108 dissimilar RFLPs among the accessions were obtained by totaling the number of polymorphic restriction fragments between pairs of accessions for 72 probe/enzyme combinations. A comparison of these dissimilar RFLPs reflects the relationship between the genotypes of *O. australiensis* and the numbers in Table 3 represent minimum mutation distances between the genotypes. By parsimony analysis, one shortest tree of length 114 and over all consistency index of 0.93 were found. Of the four accessions of *O. australiensis*, the accessions 103318 and 101410 were clustered into one group, whereas the other two accessions (100882 and 101397) form two distinct groups in the phylogenetic tree (Fig. 4). The phylogenetic analysis of the accessions revealed that the accessions 103318 and 101410 might have originated from one geographical location with high genetic diversity (mutation distance 62) whereas the accessions 100882 and 101397 might have originated from two independent locations in Australia and have close genetic relationships (mutation distance 19). However, the accession 100882 is genetically diverged from the accessions 103318 and 101410. Minimum mutation distances for the individual branches in the phylogenetic tree correlated with the values in Table 3 and reflects the degree of genetic variability among the accessions. Similar studies have been employed in the classification of the genotypes at species and subspecies level in cultivated rices [17] and for identification of *Brassica* germplasm [28].

Table 3. Number of dissimilar RFLPs between pairs of *O. australiensis* accessions

Accessions	100882	101397	103318	101410
100882		19	67	77
101397			54	80
103318				62
101410				

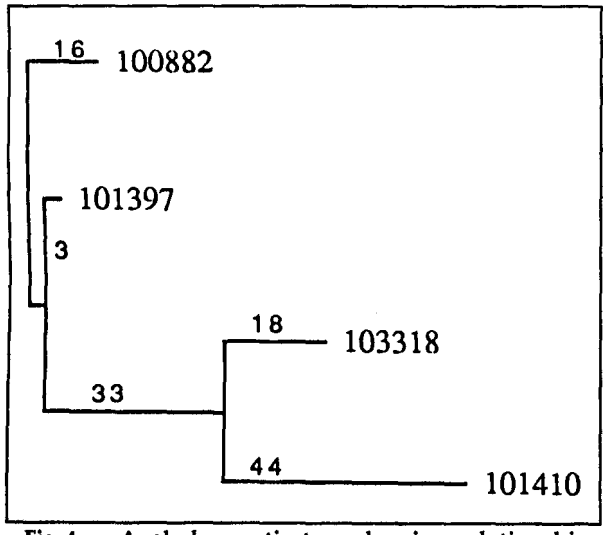

Fig. 4. A phylogenetic tree showing relationships between the accessions of *O. australiensis*. Accession numbers are given at the end of each branch and numbers on the branches indicate the unit distance (minimum mutation distance).

ACKNOWLEDGEMENTS

We are grateful to the Rockefeller Foundation International Programme on Rice Biotechnology for the financial support of this project.

REFERENCES

1. T. Tateoka. 1962. Taxonomic studies of *Oryza*. II. Several species complexes. Bot. Mag. Tokyo, **75**: 455–461.

2. D. A. Vaughan. 1989. The genus *Oryza* L. Current status and taxonomy. IRRI Research Paper Series, No. 138: 1–21.

3. T. T. Chang and D. A. Vaughan. 1989. Conservation and potentials of rice genetic resources. *In*: Biotechnology in Agriculture and Forestry (eds. Y. P. S. Bajaj). Springer–Verlag, Berlin (in press).

4. T. Katayama. 1982. Cytogenetical studies on the genus *Oryza*. XIII. Relationship between the genomes E and D. Japan J. Genet., **57**: 613–621.

5. N. M. Nayar. 1973. Origin and cytogenetics of rice. Adv. Genet., **17**: 153–292.

6. G. Second. 1985. A new insight into the genome differentiation in *Oryza* L. through isozyme studies. *In*: Advances in Chromosome and Cell Genetics (eds. A. K. Sharma and A. Sharma). Oxford and IBM Co., New Delhi: 311–323.

7. T. Tateoka. 1962. Taxonomic studies of *Oryza*. I. *O. latifolia* complex. Bot. Mag. Tokyo, **75**: 418–427.

8. M. S. Swaminathan. 1987. Plant research and world agriculture. Plant. Mol. Biol. Rep., **4**: 1–17.

9. K. K. Jena and G. S. Khush. 1990. Introgression of genes from *O. officinalis* wall ex Watt. to cultivated rice, *O. sativa* L. Theor. Appl. Genet., **80**(6): 737–745.

10. G. S. Khush, K. C. Ling, R. C. Aquino and V. M. Aguiero. 1977. Breeding for resistance to grassy stunt in rice. Proc. 3rd Intern. Cong. SABRAO. Canberra, Australia. Plant Breeding Papers, vols. **1–4**(6): 3–9.

11. E. A. Heinrichs, F. G. Medrano, H. R. Rapusas. 1985. Genetic Evaluation for Insect Resistance in Rice. International Rice Research Institute, Manila, Philippines.

12. B. B. Sahi, H. Morishima, H. I. Oka. 1969. A survey of variation in peroxidase, acid phosphatase and esterase isozymes of wild and cultivated *Oryza* species. Japan J. Genet., **44**: 303–319.

13. K. K. Jena and G. Kochert. 1991. Restriction fragment length polymorphism analysis of CCDD genome species of the genus *Oryza* L. Plant Mol. Biol., **16**: 831–839.

14. G. Kochert, T. Halward, W. D. Branch and C. E. Simpson. 1991. RFLP variability in peanut (*Arachis hypogaea*) cultivars and wild species. Theor. Appl. Genet., **81**: 565–570.

15. J. C. Miller and S. D. Tanksley. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor. Appl. Genet., **80**: 437–448.

16. K. Song, T. C. Osborn and P. H. Williams. 1990. *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLP). 3. Genome relationships in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). Theor. Appl. Genet., **79**: 497–506.

17. Z. Y. Wang and S. D. Tanksley. 1989. Restriction fragment length polymorphism in *Oryza sativa* L. Genome, **32**: 1113–1118.

18. S. R. McCouch, G. Kochert, Z. H. Yu, Z. Y. Wang, G. S. Khush, W. R. Coffman and S. D. Tanksley. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet., **76**: 815–829.

19. E. M. Southern. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol., **98**: 503–517.

20. R. Maniatis, E. F. Fritsch and J. Sambrook. 1982. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y.

21. A. P. Feinberg and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction fragments to a high specific activity. Anal. Biochem., **132**: 6–13.

22. W. M. Fitch and E. Margoliash. 1967. Construction of phylogenetic trees. Science, **155**: 279–284.

23. T. Debner, F. Salamini and C. Gebhardt. 1990. Phylogeny of wild and cultivated *Solanum* species based on nuclear restriction fragment length polymorphisms (RFLPs). Theor. Appl. Genet., **79**: 360–368.

24. P. Keim, R. C. Shoemaker and R. G. Palmer. 1989. Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet., **77**: 786–792.

25. T. Nagamine, G. A. Todd, K. P. McCann, H. J. Newburry and B. V. Ford-Lloyd. 1989. Use of restriction fragment length polymorphism to fingerprint beets at the genotype and species levels. Theor. Appl. Genet., **78**: 847–851.

26. H. Nybom and B. A. Schaal. 1990. DNA "fingerprints" applied to paternity analysis in apples (*Malus domestica*). Theor. Appl. Genet., **79**: 763–768.

27. A. M. Dally and G. Second. 1990. Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza*, section Oryza). Cladistic-mutation and genetic-distance analysis. Theor. Appl. Genet., **80**: 209–222.

28. K. Song, T. C. Osborn and P. H. Williams. 1988. *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLP). 2. Preliminary analysis of subspecies within *B. rapa* (syn. *campestris*) and *B. oleracea*. Theor. Appl. Genet., **76**: 573–600.