Indian J. Pl. Genet. Resources 12(1): 77-80, 1999

RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS OF PHOTOPERIOD SENSITIVE ACCESSIONS OF RICE (*Oryza sativa* L)

S. K. MISHRA, Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

RAPD patterns were studied using twenty photoperiod sensitive accessions of rice for assessing genetic diversity among them. Out of 60 decamer random primers used, six primers (OPE 9, OPG 14, OPJ 14, OPK 12, OPN 18 and OPO 15) revealed polymorphic DNA banding pattern. All the genotypes were uniquely distinguished and were clustered into six distinct clusters based upon similarity index represented by the ratio of common bands and total number of bands for each pair of all the accessions. Maximum number of genotypes were found to occur in clusters IV and V each having five genotypes followed by the cluster I which comprised four genotypes. The cluster II was monogenotypic.

Key words : Rice, RAPD, photoperiod sensitivity, diversity.

Rice (*Oryza sativa* L.) is one of the most important food crop grown over variable agro-climatic conditions ranging from rainfed upland to deep water conditions. Possibly this resulted in accumulation of greater diversity in this food crop. Genetic markers are of great value in crop improvement and inheritance studies (Weining and Langridge, 1991). Traditionally, markers based on morphological characters have been used. Different biochemical and molecular markers have also been employed for germplasm characterisation because of their added advantage over morphological markers. Variation in protein profiles and isozyme pattern have frequently been used as biochemical markers (Smith, 1986) but were unable to maintain specificity in most cases. The development of new techniques involving polymorphism in naturally occurring DNA sequences have represented a significant improvement in this regard as they offer greater diversity (Waugh and Powell, 1991). The techniques like restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) have added a new dimension to the development of genetic markers to construct genetic maps and tagging of agronomically and physiologically important trait (Brown *et al.*, 1991, Mohapatra *et al.*, 1992 and Wang *et al.*, 1994) including rice crop (Second, 1991). However, the technical complexity commonly associated with the use of radioactive isotope has restricted the applied aspects of RFLP specially in crop improvement programmes (Waugh and Powell, 1991). Contrary to this, polymerase chain reaction based technique, RAPD, primarily based on primers of arbitary sequences, has been preferred in a variety of applications over RFLP's due to its simplicity (Mullis and Floona, 1987).

Taking above consideration in view, RAPD analysis was conducted to assess the genetic diversity in twenty accessions of photoperiod sensitive rice genotypes from Germplasm Centre of International Rice Research Institute, Manila, Philippines.

MATERIALS AND METHODS

A. Plant material : 20 accessions of photoperiod sensitive rice *viz.*, 4131, 4132, 4135, 4137, 4138, 4144, 4145, 4147, 4151, 4152, 4153, 4154, 4155, 4156, 4158, 4165, 4167, 4168, 4172 and R 9678 were obtained from Germplasm Centre of IRRI, Manila, Philippines. These accessions were grown in glasshouse conditions at IRRI.

B. *DNA extraction* : Total DNA was extracted from young leaves as per method developed by Dellaporta, *et al.* (1983) with suitable modifications as per requirement.

C. *Primer for amplification* : A set of sixty decamer (10-mer) oligonucleotides (Primers) of arbitrary sequences from Operon Technologies, California, USA were used for screening polymorphism.

D. *RAPD analysis* : The starting mixture of PCR was made in 25 µl volume containing 5 µl DNA, 1.25 µl primer, 2.5 µl PCR buffer, 2.5 µl dNTP, 2.5 µl Mg Cl_2, 2.0 µl Taq polymerase and 9.25 µl water. DNA amplification was performed in techne Thermalcycler programmed for 45 cycles of 1.0 min at 92°C, 1.0 min at 34°C and 2.0 min at 70°C.

The DNA fragments, thus generated by PCR, were electrophoressed on 2 per cent agarose gel containing 1 per cent agarose and 1 per cent nusieve. The ethidium bromide (1 µg/ml) stained gels were photographed under UV-Transilluminator with Polaroid camera.

E. *Data analysis* : Data obtained from RAPD patterns were used to calculate the similarity index. Diversity and clustering analysis were done as per standard statistical procedure of Nei and Li (1979) and Rao (1952).

RESULTS AND DISCUSSION

A total of sixty primers of arbitary sequences were surveyed to generate amplification products (RAPD). It was observed that most of the amplification products, thus generated, were monomorphic or in few cases proper amplification could not occur. In case of only six primers *viz.*, POE 9, OPG 14, OPJ 14, OPK 12, OPN 18 and OPO 15, the polymorphic bands among the genotypes were observed. A few typical example of amplification pattern has been presented in Fig. 1.

All the 20 genotypes under study were grouped into six clusters based on the similarity index values. The cluster composition (Table 1)

Table 1. Cluster composition of 20 photoperiod sensitive accessions of rice based on RAPD analysis

Cluster	Number of genotypes	Accession number
I	4	4137, 4151, 4153, 4154
II	1	4145
III	2	4131, 4158
IV	5	4132, 4135, 4144, 4152, 4168
V	5	4155, 4156, 4165, 4167, 4172
VI	3	4147, 4148, R 9678

indicated that clusters IV and V were consisting of 50 per cent of the genotypes having five genotypes in each cluster. The number of genotypes in other cluster were monogenotypic (cluster II), two (cluster III), three (cluster VI) and four (clusterI). These observations clearly indicated that sufficient genetic variability is present at molecular level in these genotypes although they belong to only one group i.e. photoperiod sensitive based on morphological assessment. In addition, these genotypes also have other morphological features more or less identical e.g., plant height, foliage color and maturity

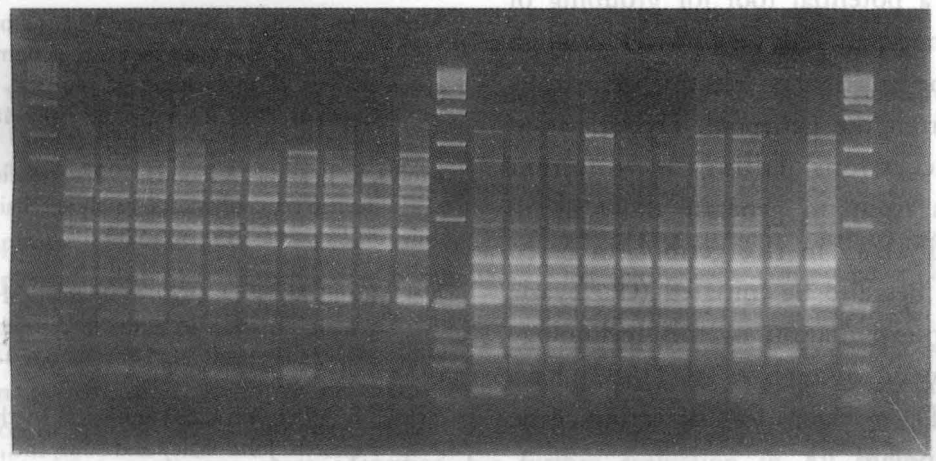
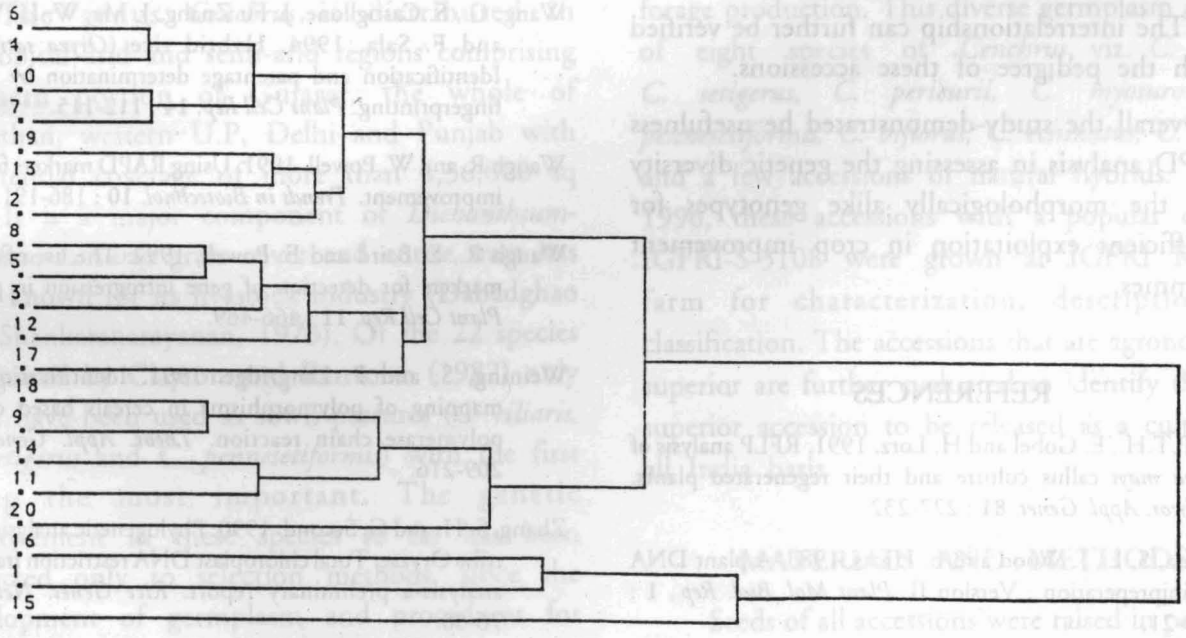


Fig. 1. Amplification pattern of 20 photoperiod sensitive accessions with the primer OPN-18 (Lane 1-DNA marker, Lane 2-Acc. 4148, Lane 3-Acc 4131, Lane 4-Acc 4135, Lane 5-Acc 4151, Lane 6-Acc 4152, Lane 7-Acc 4137, Lane 8-Acc 4154, Lane 9-Acc 4144, Lane 10-Acc 4156, Lane 11-Acc 4145, Lane 12-Acc 4165, Lane 13-Acc 4168, Lane 14-Acc 4158, Lane 15-Acc 4172, Lane 16-Acc R-9678, lane 17-Acc 4147, Lane 18-Acc 4132, Lane 19-Acc 4155, Laner 20-Acc 4153, Lane 21-Acc. 4153, Lane 22-24-DNA markers



DENDROGRAM OF PHOTOPERIOD SENSITIVE ACCESSIONS BASED ON RAPD ANALYSIS

Fig. 2. 1. Acc-4148, 2. Acc-4131, 3. Acc-4135, 4. Acc-4151, 5. Acc-4152, 6. Acc-4137, 7. Acc-4154, 8. Acc-4144, 9. Acc-4156, 10. Acc-4145, 11. Acc-4165, 12. Acc-4167, 13. Acc-4168, 14. Acc-4158, 15. Acc-4172, 16. Acc-9678, 17. Acc-4153, 18. Acc-4155, 19. Acc-4147, 20. Acc-4132

duration. It is practically not possible to distinguish these genotypes based on their morphological features. In this context, the present investigation offers RAPD as a potential tool for grouping of closely related genotypes. This may provide enough opportunity to the breeders in selecting genetically diverse parents from the genepool. Hybridization between the accessions falling in different groups/clusters is likely to generate more useful segregates. The diversity in rice germplasm based on PCR analysis has also be initiated (Garzeyazie *et al.*, 1995) for exploitation of this technology in rice improvement. Waugh *et al.* (1992) have also used RAPD markers for detecting gene introgression in potato.

The average linkage dendrogram (Fig. 2) based on genetic distances was also prepared to show the inter-relationship among these genotypes as per Zhang and Second, 1990. The pattern confirmed the finding that enough genetic diversity is available in these 20 accessions used under study. The interrelationship can further be verified through the pedigree of these accessions.

Overall the study-demonstrated he usefulness of RAPD analysis in assessing the genetic diversity among the morphologically alike genotypes for their efficient exploitation in crop improvement programmes.

REFERENCES

Brown, P.T.H., E. Gobel and H. Lorz. 1991. RFLP analysis of *Zea mays* callus culture and their regenerated plants. *Theor. Appl. Genet.* **81** : 227-232.

Dellaporta, S. L., J. Wood and B. Hicks. 1983. A plant DNA minipreperation : Version II. *Plant Mol. Biol. Rep.*, **1** : 19-21.

Gharzeyazie, B., N. Huang, G. Second, J. Bennet and G. S. Khush. 1995. Classification of rice germplasm. I. Analysis using ALP and PCR based RFLP. *Theor. Appl. Genet.* **91**: 218-227.

Mohapatra T., R. P. Sharma and V. L. Chopra. 1992. Cloning and use of low copy sequence genomic DNA for RFLP analysis of somaclones of mustard (*Brassica juncea* L. Czern & Coss). *Curr. Sci.* **62** : 482-486.

Mullis, K. B. and F. A Floona. 1987. Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. *Methods in Enzymol.* **155** : 335-350.

Nei, M. and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.*, USA **76**: 5269-5273.

Rao C. R. 1952. Advanced Statistical Methods in Biometrical Research. John Wiley and Sons, Inc., New York.

Second, G. 1991. Cytoplasmic DNA markers, phylogeny, and systematics in Oryzae. Rice Genetics II, IRRI, Manila, Philippines : 335-350.

Smith, D.B. 1986. Variation in the electrophoretic pattern of tuber proteins from somaclones of potato *J. Agric. Sci.* **106** : 427-428.

Wang, G., S. Castigllone, J. Fu Znahg, J. Ma, W. Li, Y. Sun, and F. Sala. 1994. Hybrid rice (*Oryza sativa* L): Identification and parentage determination by RAPD fingerprinting. *Plant Cell Rep.* **14** : 112-115.

Waugh R. and W. Powell. 1991. Using RAPD markers for crop improvement. *Trends in Biotechnol.* **10** : 186-191.

Waugh R., E. Baird and E. Powell. 1992. The use of RAPD markers for detection of gene introgression in potato. *Plant Cell Rep.* **11** : 466-469.

Weining, S. and P. Langridge. 1991. Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. *Theor. Appl. Genet.* **82**: 209-216.

Zhang, S. H. and G. Second. 1990. Phylogenetic analysis of the tribe Oryzae. Total chloroplast DNA restriction fragment analysis-a preliminary report. *Rice Genet. Newsl.* **6**: 76-80.