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Analysis of molecular diversity and differentiation of photoperiod sensitive and insensitive rice varieties

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Abstract

A study was undertaken to differentiate photoperiod sensitive and photoperiod insensitive rice varieties and to identify putative RAPD marker (s) associated with the trait. The genetic diversity analysis among photoperiod sensitive and photoperiod insensitive rice varieties using different DNA amplicons, grouped 40 varieties into two major clusters and nine sub-clusters. Sixty five per cent of photo sensitive varieties and 60% photo insensitive varieties were grouped in distinct clusters. The result indicated that response to photoperiod had played a major role in the pattern of clustering. The most distant pairs of rice varieties revealed from genetic distance are Bhagya and Ptb 12, Bhagya and Ptb 7, Sagara and Bhagya, and Dhanya and Bhagya. These distant varieties can be utilized in future breeding programmes as parents to get promising recombinants.

Key words: Rice, photoperiod sensitivity, molecular markers, RAPD, Jaccard's similarity index, cluster analysis

Introduction

Rice is one of the agronomically and nutritionally important cereal crops and is the principal staple food in developing countries. Approximately one third of the world's population relies on rice for a significant portion of their food. About 11% of the world's arable land is used for growing rice, and about 29% of total cereal production in 2000 was due to rice. Flowering is a complex phenotype which is the end result of numerous physiological and biochemical processes within a plant. These processes are regulated by the interaction of many genes within an organism, and are also influenced by environmental stimuli [1]. Along with temperature, photoperiod is the most important environmental variable that determines when a plant will flower and set seed. In tropical rice, flowering is associated with the availability of water. In traditional farming systems where precise planting dates and water management are not feasible, it provides a safety mechanism for ensuring that crop reproduction will occur under favorable environmental conditions [2]. In intensive high input farming systems and in environments with high temperature where rice is grown under long days, minimizing the sensitivity to photoperiod has been of critical importance in the development of widely adapted, short season, high yielding varieties. Sensitivity to photoperiod is under genetic control and interacts with other temperature and flowering genes to hasten or delay the flowering response. Though genetic studies are inconclusive as to the number of genes and the type of gene action involved in determining days to flowering, several reports based on the testing of early segregating populations identified genes with major effects to be associated with days to flowering in rice [3]. Based on the development of a series of new isogenic lines, specific genes controlling earliness, lateness, overall heading and their modifiers, inhibitors and enhancers [4] have also been identified, which present evidence for the qualitative action of individual genes. Genetic diversity study is imperative in breeding programmes in order to select distant parents. Morphological traits can be used for assessing genetic diversity, but very often influenced by the environment. Similarly isozyme analysis represents only a part of the genome and subject to environmental variations. The use of molecular markers for the evaluation of the genetic diversity is receiving lot of attention The development of PCR has allowed the introduction of the RAPD (Random Amplified Polymorphic DNA) approach [5] to the molecular analysis of the genome. The major advantage of this approach relies on the fact that it allows the exploration of a much larger genomic portion. The RAPD technique has several advantages such as a relatively unbiased portion of the genome sampled, simplicity of use, lower cost and the use of a small amount of plant material. Identification of a linked marker to a specific trait of interest is imperative in molecular marker aided selection programme and also for gene mapping.

In order to isolate a photo insensitive line from a segregating population, the breeder has to raise the segregating population in all photoperiod seasons, which

is time consuming and labour intensive. An alternative to this procedure would be to screen the population of interest using genetic markers. These markers could be either molecular or morphological. Unfortunately, there are a few morphological markers associated with photoperiod sensitivity; hence development of molecular markers would prove to be ideal. Consequently, upon identification of a suitable molecular marker(s) closely linked to photoperiod sensitive/insensitive gene, one could easily follow the gene in a cross intended to breed photo-insensitive varieties at any time of the year. A study was therefore undertaken to differentiate photoperiod sensitive and photoperiod insensitive rice varieties and to identify RAPD marker (s) associated with photoperiod sensitivity and photoperiod insensitivity.

Materials and methods

Forty rice varieties representing different geographical areas were procured from Kerala and gene bank of National Bank of Plant Genetic Resource, New Delhi. The pedigree and characters of the varieties are given in Table 1. Twenty varieties were photoperiod sensitive type, and 20 were photoperiod insensitive type. Seeds of these rice varieties were germinated and grown under aseptic conditions at about 30°C in green house of NBPGR, New Delhi. Three weeks old seedlings were taken for the isolation of genomic DNA. Genomic DNA was extracted following the protocol of Doyle and Doyle [6]. DNA pooling can be an effective strategy for detecting genetic marker differences among groups of individuals with similar genotypes or phenotypes [7]. DNA bulks were constituted by mixing equal amount of (5ng/micro litre) DNA from 10 different varieties each having the same photoperiod reaction mechanism. Thus, there were two DNA bulks of photoperiod sensitive rice varieties and two DNA bulks of photoperiod insensitive rice varieties. Initially, DNA bulks were used as target DNAs for RAPD analysis. A total number of 120 random decamer primers (from Operon Technology Inc., Alamed, California, USA) (primer kits A to Z) were screened in RAPD assays to identify highly polymorphic well-resolved specific bands for photoperiod sensitivity/insensitivity. The repeatability of the primers, which showed polymorphism among bulks, was tested. Those primers, which showed reproducibility with respect to polymorphic bands in photoperiod sensitive and photoperiod insensitive DNA bulks, were used to screen individual rice varieties, which formed the DNA bulks.

PCR was performed in a 0.2 ml reaction tube. Each 20 micro litre reaction mixture consisted of 10 X assay buffer 2.5 micro litre, 12.5 ng template DNA, and 0.2 mM each deoxy nucleotide triphosphate (dNTP), 5 micro M of 10-mer primer and 1.0 unit *Taq* polymerase. PCR reactions were performed in a Perkin Elmer 9600 Thermal Cycler programmed for 40 cycles of standardized cycling conditions. The reaction involved repeated cycles, each consisting of denaturation 94°C for one minute, primer annealing at 35°C for one minute, primer extension at 72°C for two minutes and final extension at 72°C for 5 minutes. Amplified DNA products were separated on a 1.6% agarose gel, using loading dye, in 1X TAE buffer and stained with ethidium bromide. The amplification products were visualized and photographed under UV light using Polaroid 667 film

The PCR products were scored as present (1) or absent (0) for each primer-genotype combination and used to compute the measures of genetic distance for all pairs of individuals. The data entry was done into a binary data matrix as discrete variables. The statistical analysis was carried out using NTSYS software (version 2.1) [8]. Pair wise comparisons of samples were used to estimate Jaccard's similarity coefficients (GS): a/(n-d), where a = number of positive coincidences, n = total sample size, and d = number of negative coincidences. Genetic distances (GD), between pairs of lines were estimated as GD = 1-GS. Jaccard's similarity coefficients were used to generate dendogram using unweighted Pair Group Method with Arithematic Mean (UPGMA) [9] and relationships between accessions were visualized as dendrogram.

Results and discussion

Level of genetic diversity : A total of 161 polymorphic and reproducible bands were obtained using 12 decamer primers on a set of 40 rice varieties. Number of bands per primer ranged from 7 (OPX 16) to 19 (OPI 20), the average bands per primer being 13.4. The size of the amplicon varied from 0.25Kb to 4. 5Kb. All the 12 primers (100%) revealed polymorphism between varieties. The 161 bands that were scored, 136 (84.5%) were found to be polymorphic. The average number of polymorphic bands per primer was 11.3. The polymorphism revealed by the primer OPK 02 is depicted in Fig. 1.

The genetic diversity of 40 rice varieties, which included photo sensitive and photo-insensitive varieties as revealed by 12 decamer primers is shown in the dendogram (Fig. 2). The first three principal components contributed 74.6%. Similarity indices estimated on the basis of all the decamer primers ranged from 31% to 88% with an average of 59.5%. Maximum similarity was observed between the varieties Ptb 19 and Ptb 20 (90%), which are photo sensitive, and between Ptb 29 and Ptb 30 that are photo insensitive. This indicated that sensitivity to photoperiod played a role in clustering pattern. The most distant pairs of rice varieties revealed from genetic similarity index are Bhagya and Ptb 12 (69%), Bhagya and Ptb 7, Sagara and Bhagya (63%), and Dhanya and Bhagya. (63%). These distant pairs

Table 1. Pedigree and specific characters of rice varieties used for the study

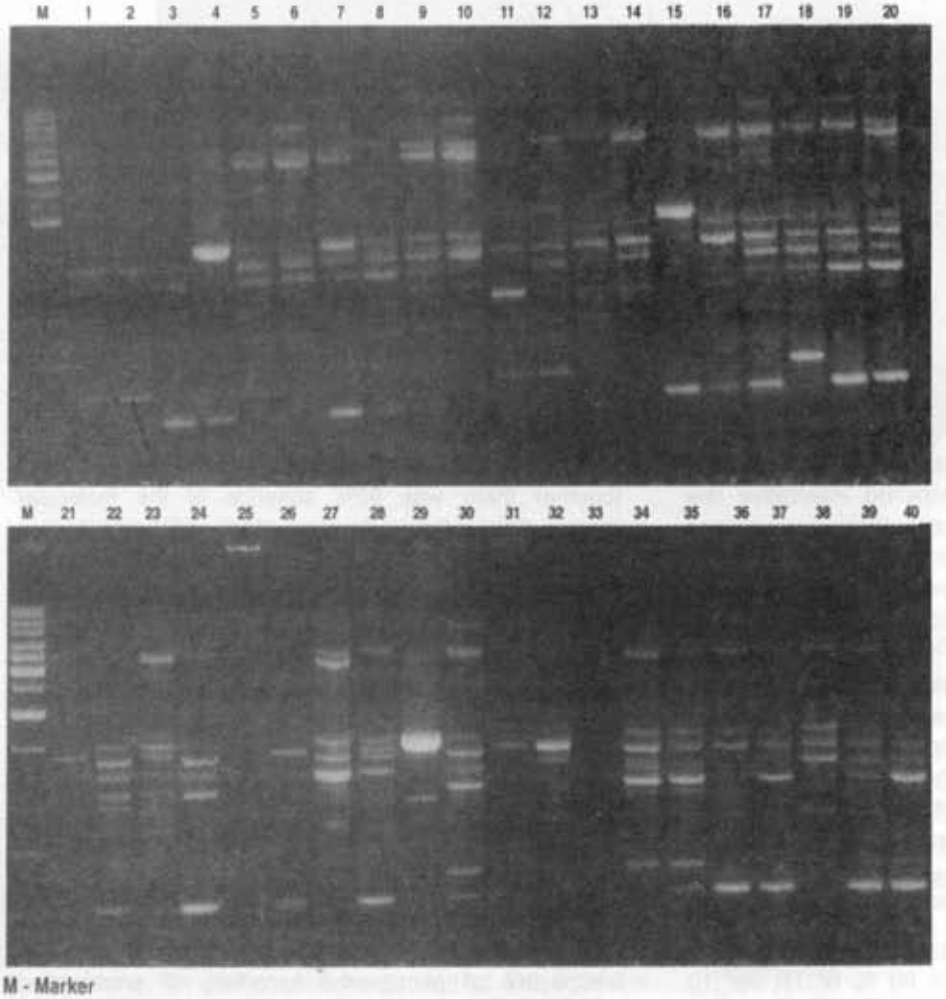
S.No.	Name of varieties	Ogitioeruid sensitive/ photoperiod insensitive	Pedigree	Other specific characters
1	Ptb 1	Photosensitive	Pure line selection from Aryan	Long bold gram, moderately resistant to blast
2	Ptb 2	Photosensitive	Pure line selection from Ponnaryan	Long bold grain
3	Ptb 3	Photosensitive	Pure line selection from Eravapandy	Short bold grain, moderatclv resistant to drought
4	Ptb 4	Photosensitive	Pure line selection from Vellari	Long bold grain, moderately resistant to major pests and diseases
5	Ptb 6	Photosensitive	Pure line selection from Athirikkaya	Long bold grain, moderately resistant to major pests and diseases
6	Ptb 12	Photosensitive	Pure line selection from Chitteni	Short bold grain, moderately resistant to BPH, leaf roller and BLB
7	Ptb 18	Photosensitive	Pure line selection from Eravapandy	Short bold grain, moderately resistant to blast
8	Ptb 19	Photosensitive	Pure line selection from Athikkiraya	Medium bold grain, moderately resistant to sheath blight and gall fly
9	Ptb 20	Photosensitive	Pure line selection from Vadakkan Chitteni	Medium bold grain, resistant to BPH
10	Ptb 21	Photosensitive	Pure line selection from Thekkan	Long bold grain, resistant to three bio-types of BPH, stem borer and GM
11	Ptb 27	Photosensitive	Pure line selection from Kodiyan	Medium bold grain, moderately resistant/tolerant to blast and SB
12	Ptb 33	Photosensitive	Pure line selection from Arikkirai	Short bold grain, resistant to BPH, thrips, leaf roller and SB
13	Reshmi	Photosensitive	Mutant of Oorpandy	Short bold grain, moderately resistant to BPH, GM, stemborer, leaf roller and SB
14	Nila	Photosensitive	Triveni/Vellathilkolappala/CO25	Short bold grain, moderately resistant to BPH, GM and SB but susceptible to blast
15	Lakshmi	Photosensitive	Kottarakkara 1/Poduvi	Long bold grain, moderately resistant to BPH, SN, stem borer, leaf folder and HB
16	Bhagya	Photosensitive	Thadukkan/Jaya	Long bold grain, moderately resistant to BPH, WBPH and SB, tolerant to drought in the early stages
17	Dhanya	Photosensitive	Jaya/Ptb 4	Medium bold grain, moderately resistant to stem borer, leaf roller, SB and blast
18	Sagara	Photosensitive	Pureline selection from Orumundakan local	Short bold grain, moderately resistant to stem borer, SB and blast but susceptible to leaf roller. Highly tolerant to salinity and flood
19	Kumbam	Photosensitive	Mass selection and bulk progeny testing from local Cherady	Tolerant to lodging, short bold grain
20	Makaram	Photosensitive	Mass selection and bulk progeny testing from local Cherady	Short bold grain
21	Ptb 5	Photo insensitive	Pure line selection from Velutharikayama	Long bold grain, moderately resistant to major pests and diseases
22	Ptb 7	Photo insensitive	Pure line selection from Parambuvattan	Medium bold grain, resistant to foot rot, GM, stem borer and withstands drought
23	Ptb 8	Photo insensitive	Pure line selection from Chuvannari Thavalakannan	Medium bold grain, resistant to green leaf hopper
24	Ptb 9	Photo insensitive	Pure line selection from Veluthari Thavalakannan	Short bold grain, moderately resistant to major pests and diseases
25	Ptb 10	Photo insensitive	Pure line selection from Thekkan Cheera	Long bold grain, resistant to GM, BPH and stem borer, high photosynthetic efficiency
26	Ptb 13	Photo insensitive	Pure line selection from Kayama	Short bold grain
27	Ptb 17	Photo insensitive	Pure line selection from Jedduhalliga	Short bold grain, moderately resistant to blast
28	Ptb 22	Photo insensitive	Pure line selection from Veluthavattan	Long bold grain, moderately resistant to blast
29	Ptb 23	Photo insensitive	Pure line selection from Cheriya Aryan	Long bold grain, moderately resistant to blast
30	Ptb 24	Photo insensitive	Pure line selection from Chuvanna Vattan	Medium bold grain, moderately resistant to blast
31	Ptb 25	Photo insensitive	Pure line selection from Thonnuran	Long bold grain, moderately resistant to blast

Table 1. (Contd ....)

32	Ptb 26	Photo insensitive	Pure line selection from Chenkayama	Short bold grain, moderately resistant to blast
33	Ptb 28	Photo insensitive	Pure line selection from Kattamodan	Short bold grain, moderately resistant to blast and drought
34	Ptb 29	Photo insensitive	Pure line selection from Karuthamodan	Long bold grain, moderately resistant to blast and drought
35	Ptb 30	Photo insensitive	Pure line selection from Chuvannamodan	Long bold grain, moderately resistant to blast and drought
36	Ptb 31	Photo insensitive	Pure line selection from Elappappoochampan	Medium bold grain
37	Ptb 32	Photo insensitive	Pure line selection from Aruvakakri	Short bold grain, moderately resistant to SB
38	Ptb 34	Photo insensitive	Pure line selection from Valiya Champan	Short bold grain
39	Onam	Photo insensitive	Kochuvithu/T (N) 1	Long bold grain, moderately resistant to blight, SB, blast and tolerant to drought in the early stages
40	Jyothi	Photo insensitive	Ptb 10/IR 8	Lng bold grain, moderately resistant to BPH, but susceptible to blast and blight

BPH - Brown plant hopper, BLB - Bacterial leaf blight, GM - Gall midge, SB - Sheath blight, HB - Helminthosporium blight

of varieties have similar response to photoperiod except for Bhagya and Ptb 7. These divergent varieties can be used as parents for recombination breeding without disturbing the target trait photoperiod sensitivity/ insensitivity.



M - Marker

Fig. 1. DNA polymorphism detected in a group of photoperiod sensitive rice varieties and photo period insensitive rice varieties as revealed by random primer OPK 02 (See Table 1 for lanes 1 to 40)

At 48% similarity index 40 varieties were grouped in two major clusters which is not similar to photoperiod response. At 71% similarity index, cluster 1 was further sub grouped into 5 sub clusters and three varieties namely Nila, Lakshmi and Ptb 10 were not grouped. Nila is a photo sensitive high yielding variety with short bold grain and moderately resistant to brown plant hopper, gall midge and sheath blight, but susceptible to blast. Lakshmi is a photo sensitive high yielding variety from Kerala with long bold grain and moderately resistant to brown plant hopper, sheath blight, stem borer, leaf folder and Helminthosporium blight. Ptb 10 is a variety which is resistant to the pests brown plant hopper, gall midge and stem borer and it has high photosynthetic efficiency. Out of 20 photo sensitive varieties, 13 varieties (65%) grouped in a single sub cluster (sub cluster 1b). Similarly, all the 12 varieties (60%) in sub

cluster 1c are photo insensitive. This result indicated that reaction to photoperiod response had played a major role in the pattern of clustering to some extent. Maheswaran *et al.*, [10] reported the involvement of 15 QTLs distributed across all the 12 chromosomes and their interaction in controlling days to flowering trait in plants. The presence of these QTLs may be in varying numbers in different varieties and even if all genes are present, their mode of interaction may be different [11]. In the present study, 13 photo sensitive varieties that were grouped in a single cluster along with two photo insensitive varieties might have same number of QTLs but the mode of interaction of genes and interaction of genes with environment may be different. This might be the same reason for the clustering pattern of other 7 photoperiod sensitive varieties in different clusters.

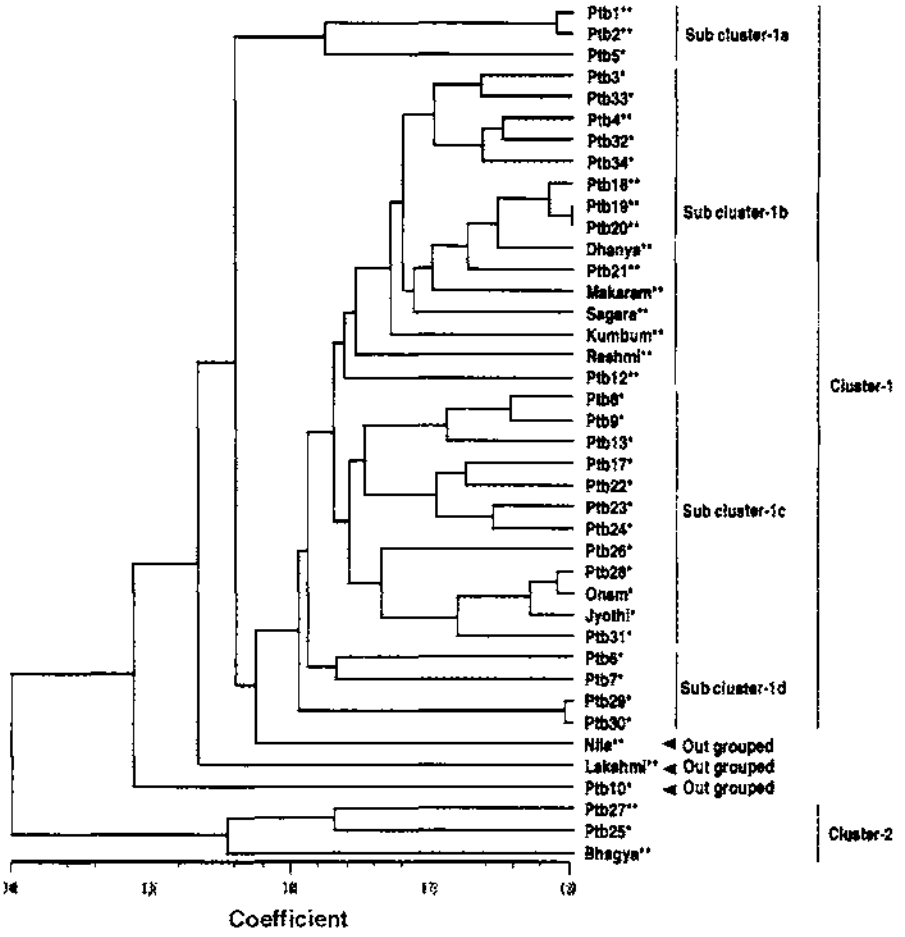


Fig. 2. Dendogram generated for 40 rice genotypes using RAPD markers (*photo insensitive varieties, **photo sensitive varieties)

Differentiation of photoperiod sensitive and photo period insensitive rice varieties : Out of 120 random primers used for screening DNA bulks, 12 RAPD primers consistently differentiated between DNA bulks of photoperiod sensitive and photoperiod insensitive rice varieties. When these polymorphic primers were used to analyze individual varieties of each bulk, the marker OPJ 08_{550}, was present in 80% of 1st set 10 individual photo sensitive varieties, whereas it was absent in 90% of photo insensitive varieties (Fig. 3A). In the 2nd set 10 individual photo sensitive varieties (Fig. 3B), this particular marker (OPJ 08_{550}) was present in 80% of varieties and absent in 60% of photo insensitive varieties. Considering both sets together, the RAPD marker OPJ08 550 was present in 80 % of photo sensitive varieties and absent in 70% of photo insensitive varieties. These results indicated that OPJ 08_{550} may be a putative molecular marker for photoperiod sensitivity in rice.

The DNA profile with the primer OPX 16 showed an amplicon of size 1750 bp in 80 % of 1st set 10 photo insensitive individual varieties, which was absent in 80% of 1st set 10 photo sensitive individual varieties (Fig. 4A). Similar results were observed with the 2nd set, in which 50 % of photo insensitive varieties amplified the marker of same size that was absent in all photo sensitive individuals (Fig. 4B). Considering both sets together there was 90% absence of the molecular marker OPX 16_{750} in photoperiod sensitive varieties and this particular amplicon was present in 55% of photoperiod insensitive rice varieties. These results lead to the inference that OPX 16_{1750} may be a putative molecular marker for photoperiod insensitivity in rice.

Non amplification of the indicated markers for photoperiod sensitivity and photoperiod insensitivity in some of the individual varieties may be due to the fact that the traits might be controlled by more than one gene or allele whose flanking markers can be identified through further screening with more random primers. Maheswaran *et al.*, [10] detected 15 QTLs associated with photo period sensitivity, distributed across all the 12 chromosomes. Presense of molecular markers irrespective of photoperiod sensitivity or photoperiod insensitivity may be due to interaction of quantitative genes/alleles causing to shift in phenotype. Observations

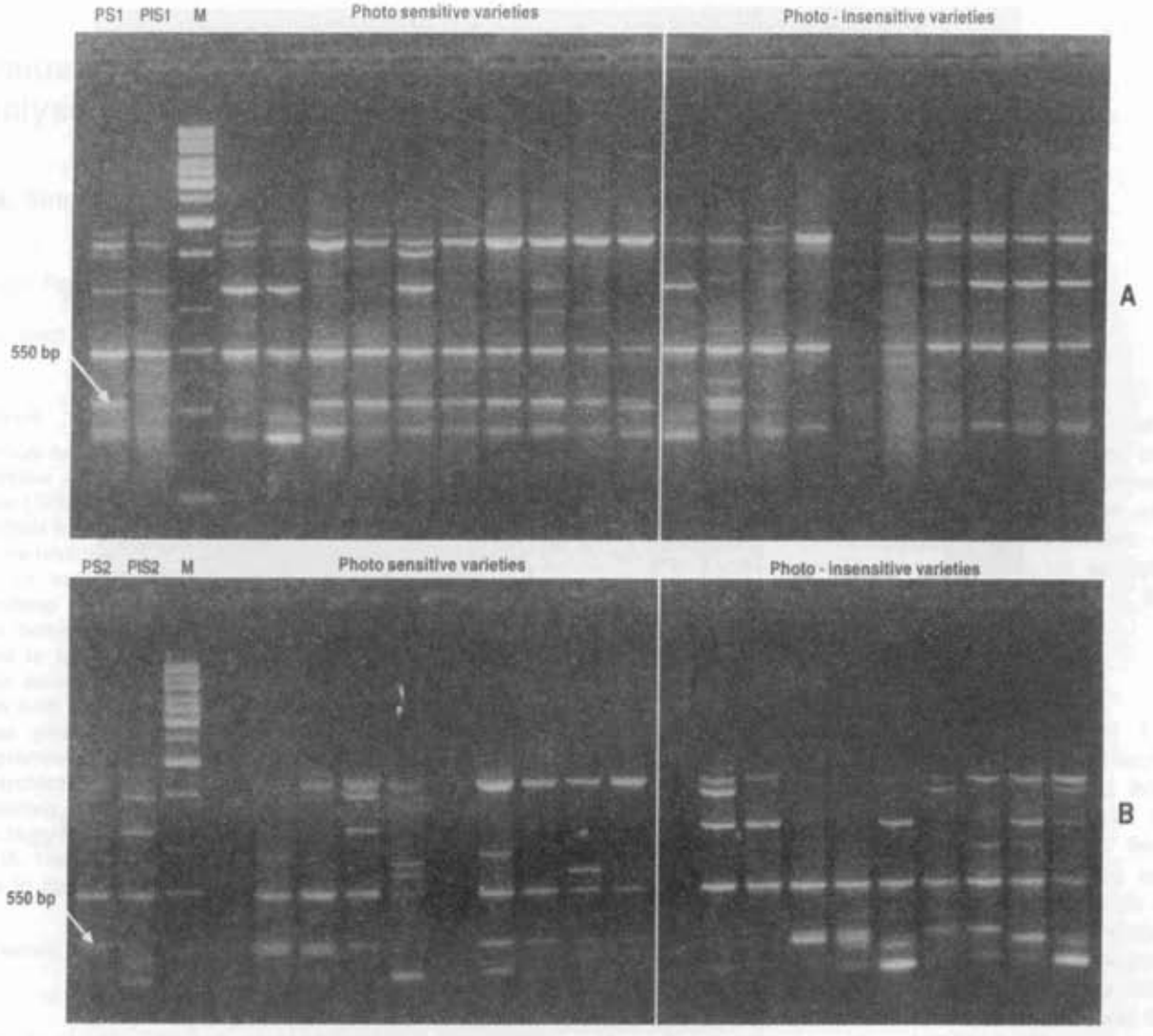


Fig. 3. (A&B) Specific marker for photo period sensitivity revealed by the Decamer primer OPJ08

regarding the interaction of quantitatively inherited flowering genes are not new [11].

The present investigation showed that on the basis of RAPD markers, photoperiod sensitive rice varieties differed from photoperiod insensitive rice varieties. The study also indicated two molecular markers that can be used to differentiate photoperiod sensitive rice varieties from photoperiod insensitive rice varieties and so can be used for transfer of this trait when these varieties are used as donors for photoperiod sensitivity/photoperiod insensitivity in breeding programmes. The indicated polymorphic markers for photoperiod sensitivity and photoperiod insensitivity could be used as starting material to map the gene (s) governing the trait in rice by converting the amplicon to more robust SCAR markers. If it can be proven that these molecular markers are linked to photoperiod sensitivity and photoperiod insensitivity in rice, they could be used as specific markers in breeding programmes to select suitable segregants.

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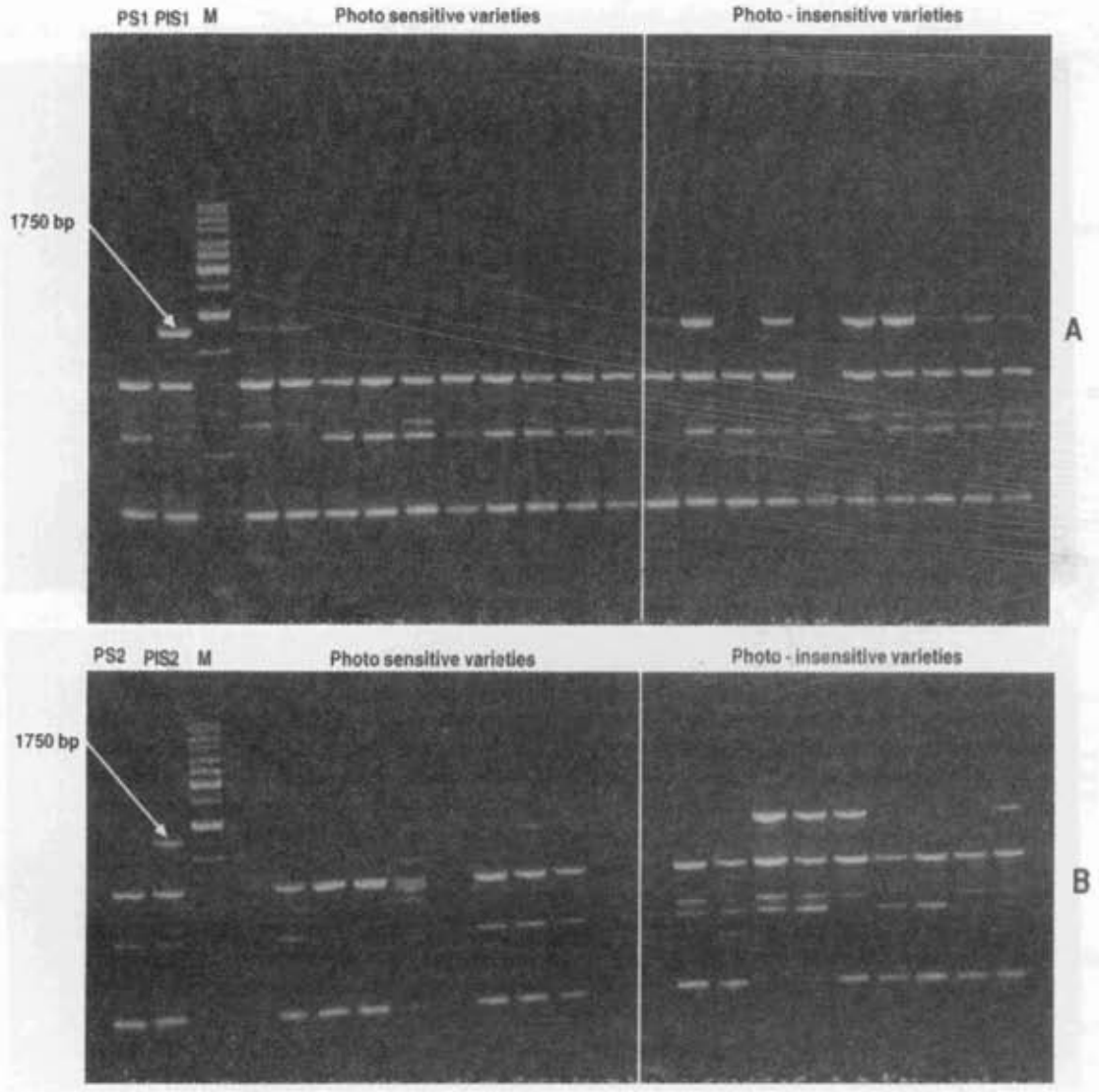

Fig. 4. (A&B) Specific marker for photo period insensitivity revealed by the Decamer primer OPX 16

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