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GENETIC DIVERSITY OF WEST BENGAL INDICA RICE LANDRACES USING ISOZYME AND SITE SPECIFIC PUTATIVE TRANSPOSON SEQUENCE AMPLIFICATIONS

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A study on genetic variability in indica rice landraces at both the protein and DNA levels was made using isozyme and Site Specific Putative Transposon Sequence Amplification (SSPTSA) analyses. The results showed that the level of polymorphism between the two methods varied widely, ranging from only 12.38% (isozymes) to 46.66% (SSPTSA). Genetic dissimilarity matrix between the two methods, tested by the Spearman Rank Correlation analysis, indicated a moderate correlation between isozymes and SSPTSA (rs = 0.36). It was concluded that the DNA based markers (SSPTSA) will be more useful than isozyme markers in detecting genetic diversity among the indica landraces of West Bengal.

Key words : Rice landraces, isozymes, plant transposon, genotyping, genetic diversity

Landraces are generally considered to be a rich source of genetic variation for cultivar development. Because of the ancient origin and wide spread distribution of rice, enormous numbers of landraces have been accumulated (Chang, 1976), and portions of these landraces have been collected and maintained in the form of gene banks in several national and International germplasm programs. However, there are very few reported studies designed to assess the relative richness of genetic variability in these landraces in comparison to modern cultivars.

Isozyme analysis is a valuable tool for studying genetic variation in natural population, identification of species and hybrids and to delineate clones (reviewed in Nevo, 1988a, Rajora, 1990, Linhart and Grant, 1996), providing many advantages over morphological methods. However, allozyme studies are limited by the number of enzymes and loci that can be resolved, and reveal only genetic changes in coding regions of the genome that have resulted in changed amino-acid sequences. On the other hand, transposable elements are integral components of most, if not all, genome. The majority of middle or interspersed repetitive DNA may be composed of transposons (Flavell, 1986). Few dispersed mobile DNA families were identified and found to be associated with genes in several plant species including rice (Bureau *et al.*, 1996). If the conserved domains of mobile genetic elements are amplified, they would reveal polymorphism and may be used for genotyping.

Studies comparing marker techniques in detecting genetic diversity are recently appearing (Russel *et al.*, 1997; Sun *et al.*, 1999). However, no studies on applying Site Specific Putative Transposon Sequence Amplification (SSPTSA) and

isozyme markers to the same set of individuals have been reported. Such a study would provide experimental data to verify the relative merits of the different methods and would indicate whether genetic diversity obtained by one method is similar to that obtained by other method. The objectives of our study were (i) to evaluate levels of genetic variability in indica rice landraces at both the protein and DNA levels with isozyme and SSPTSA markers; (ii) to compare the results obtained from these two methods with each other; and (iii) to determine a suitable strategy to characterize indica rice germplasm.

MATERIALS AND METHODS

Plant material

Seeds of 11 landraces and one cultivar (IR8) (listed in Table 1), procured from the Rice Research Station (CRRS), Chinsurah, West Bengal.

Table 1. Details of the rice cultivar/landraces

Code	Cultivar	Accessions
1	CB1	Chinsurah Boro-1
2	CB2	Chinsurah Boro-2
3	Bhasamanik	Chinsurah-3
4	Patnai23	Chinsurah-7
5	Rupsail	Chinsurah-13
6	Kumargore	Chinsurah-19
7	Latisail	Chinsurah-25
8	Tilakachari	Chinsurah-31
9	SR26B	Chinsurah-45
10	Matla	Chinsura
11	Jaldhi I	Chinsurah
12	IR8	Chinsurah

Extraction of protein and DNA

Crude enzymes from approximately 200 mg of 4 to 6 days old, etiolated seedlings were extracted in 400 µml of 100 mM Tris-HCl buffer, pH 6.8 and centrifuged at 15,000 rpm for 20 minutes at 4°C. The supernatant containing crude enzymes was taken for isozyme analysis. DNA was extracted from 5 to 10 days old etiolated seedlings raised in a growth chamber using a modified protocol of Walbot (1988).

Polyacrylamide gel electrophoresis and staining for isozymes

To obtain a good separation of proteins, slab gel electrophoresis was performed with 7.5 per cent polyacrylamide (Davis, 1964) in Tris-glycine buffer, pH 8.3. Gels were run at 30 mA constant current for 4 hours at 4°C. After electrophoresis, the gels were stained as per standard procedures for Esterase, Peroxidase, Superoxide Dismutase (SOD) and Alcohol Dehydrogenase (ADH) (Kephart, 1990). When the bands were visible, the gels were fixed in a solution of methanol/water/ glacial acetic acid in a ratio of 4:5:1 and photographed by placing the gels on frosted glass and providing fluorescent lights from below. Line drawings of the visible bands were made manually and the gels scanned in a Pharmacia LKB densitometric scanner (model 2210) at 632.8 nm wave length provided from a Helium Neon Laser.

Sequencing of RG104 clone

The RFLP marker RG104 (a gift from Prof. S.D. Tanksley of Cornell University, USA) was cloned from the supplied pUC8 into an expression vector (pBluescript II), at the *Pstl* site and was sequenced using the (-29) universal primer by dideoxynucleotide chain termination method (Sanger *et al.*, 1977). The sequence was reported in the EMBL data bank as OSY07612.

PCR primers

From the repeat motifs of the RG104, a total of twelve primers, each 12 nucleotides long were designed. The primers along with their positions on the RG104 sequence are described elsewhere (Prasad *et al.*, 2000).

Polymerase chain reaction

DNA amplification was carried out in 50-µl reaction mixtures, each containing 100 ng template DNA, 200 ng primer, 200 µM each of the dNTPS, 2.5 mM $MgCl_2$, 1X PCR buffer and 1U *Taq* DNA polymerase using the following PCR profile in a Perkin Elmer DNA thermal cycler 2400. Initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C for 1 min, 37°C for 1 min, 72° C for 2 min, and final extension at 72°C for 5 min. The amplification products were resolved on 1.5 per cent agarose gels.

Data analysis

For the purpose of assessing genetic diversity leading to the preparation of a dendrogram, gels (Allozyme profiles and Amplification profiles) were scored in binary format, with the presence of a band scored as unity and its absence scored as zero. Nei's dissimilarity indices [1- (2 × No. of shared bands/Total no. of bands)] were calculated (Nei and Li, 1979) and clustering was done using unweighted pair group with arithmetic mean (UPGMA) method (Sneath and Sokal, 1973). Correlation between assays were calculated using Spearman Rank Correlation (SRC).

RESULTS AND DISCUSSION

Fingerprinting

Four enzyme systems (Esterase, Peroxidase, Alcohol dehydrogenase and Superoxide dismutase) were used in this study due to their activity, high resolution and polymorphism. A schematic representation of allozyme profiles of one enzyme (Esterase) is given in Fig. 1. A total of 226 isozyme bands was recorded over the four enzyme systems (Table 2). Twenty-nine (12.83%) bands showed polymorphism across the tested samples. Clear polymorphism in banding pattern was observed in each zymograms, though estarase and peroxidase activities are more polymorphic than that of other two enzymes. Data on the number

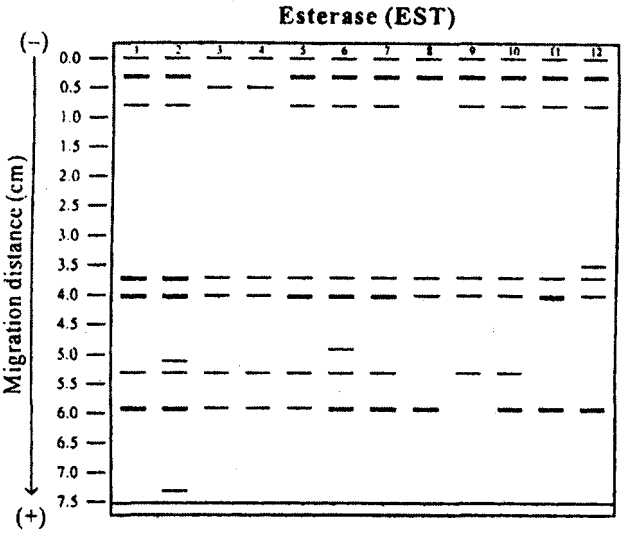

Fig. 1. Diagrammatic representation of the Esterase isozyme profiles in the twelve rice landraces/cultivars. Lane 1, Tilakachari; lane 2, IR8; lane 3, Bhasamanik; lane 4, CB1; lane 5, Kumargore; lane 6, Latisail; lane 7, Rupsail; lane 8, CB2; lane 9, Jaladhi1; lane 10, Patnai23; lane, 11, Matla; lane 12, SR26B

of allozymes and number of polymorphic allozymes for four enzymes is given Table 2. From the zymograms, it is apparent that in several cases though the banding patterns are similar but the band intensity are different, thereby indicating polymorphism.

Table 2. Name of enzyme, number and number of polymorphic allozymes in indigenous rice germplasm

Enzyme	Total bands	Dissimilar bands	% polymorphism
Esterase	69	9	13.04
Peroxidase	56	10	17.85
SOD	50	7	14.00
ADH	51	3	05.88

The unusual high frequency of short repeats and the presence of a transposon-like element in the RG104 sequence led to the thought that this region in the rice genome may be hypervariable amongst different cultivars (Chaudhuri *et al.*, 1996). Based on this rationale, from the repeat motif of the RG104, a total of twelve primers,

each 12 nucleotides long were designed (named as RCP5-RCP16). The primers along with their positions on the RG104 sequence are described elsewhere (Prasad *et al.*, 2000). Reproducible DNA amplification profiles of the rice cultivars were obtained using the "hot start" method in which the *Taq* DNA polymerase was added to the reaction tubes at 72°C 9the third step in the second cycle). A total of 105 bands was scored, resulting in an average of 4.08 fragments per primer with a range of one to seven fragments per primer. Primer RCP8 generated the highest number of polymorphic fragments among the 12 primers used (Fig. 2). The level of polymorphism

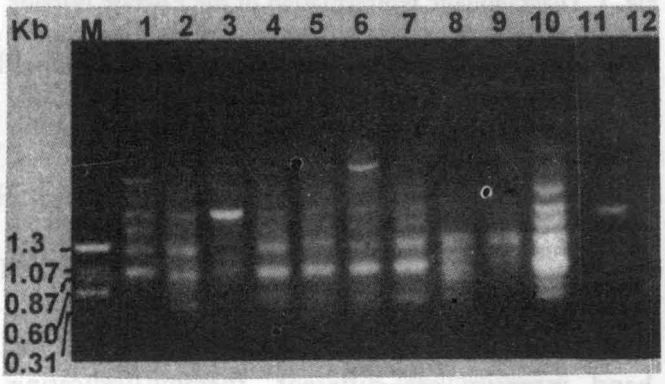

Fig. 2. Amplification profiles of eleven landraces and one cultivar (IR8) obtained using primer RCP8. Lane M, φX174/HaeIII molecular weight (Kb) marker; lane 1, SR26B; lane 2, Matla; lane 3, Rupsail; lane 4, Latisail; lane 5, Bhasamanik; lane 6, Patnai23; lane 7, Tilakachari; lane 8, Kumargore; lane 9, CB1; lane 10, CB2; lane 11, IR8; lane 12, Jaladhi I.

between the two methods varied widely, ranging from only 12.83% (isozymes) to 46.66%) (SSPTSA). The intrinsic differences between the two entire genome of both coding and non-coding regions, in case of SSPTSA, whereas, isozymes reflect polymorphism only in the coding regions that codes for certain enzymes. Moreover, considerations of the genetic code and of electrical properties of amino acids suggest that only one third of all amino acid replacements are detectable by gel electrophoresis which consequently underestimates the variation actually present. Higher degree of variability for RAPD over isozymes has also been reported on other species e.g. *Tylophora indica* (Parani and Parida, 1997) and *Elymus caninus* (Sun *et al.*, 1999).

Assessment of genetic diversity

The diversity in rice germplasm based on PCR analysis has also been initiated (Parsons *et al.*, 1997; Nadarajan *et al.*, 1999) for exploitation of this technology in rice improvement. Pairwise dissimilar values for the different combinations of cultivars were calculated from the compiled zymogram of four enzymes and also from the compiled amplification profile of twelve primers. The coefficient values ranged from 17.54 (CB1 and CB2) to 45.61 (Rupsail and IR8) for the isozymes and from 6.25 (Bhasamanik and Patnai23) to 61.65 (SR26B and IR8) for SSPTSA. Dendrograms based on dissimilarity values from

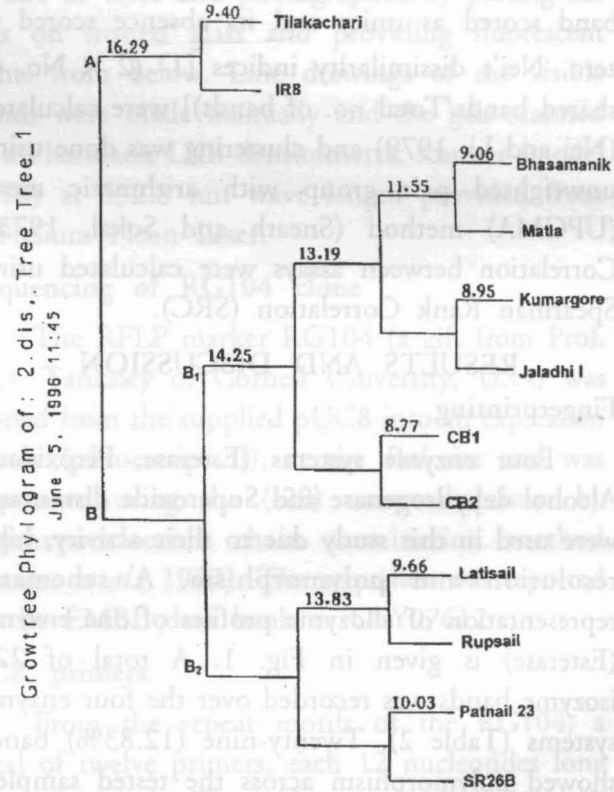

Fig. 3. Dendrogram based on culster analysis fo isozyme data showing the genetic relatedness between cultivars. Accessions are indicated at the end of each branch and numbers on the branches indicate the unit distance.

isozyme and SSPTSA data were constructed to reveal similarities between accessions. Two independent dendrograms for the two types of markers were generated (Figs. 3, 4) and several clusters were recognised from the two dendrograms. The grouping of accessions based on isozyme and SSPTSA data differed from each other. However, some of them occupied in the same relative position in both the dendrograms. Similar discrepancies was also reported by Parani and Parida (1997) in *Tylophora indica* and Sun *et al.*, (1999) in *Elymus caninus*. A possible explanation for the differences found among the two dendrograms might be based on the kind of information provided by each type of marker. Isozyme variation reflects only differences in protein-coding genes and is environment sensitive. Coding sequences are under a greater selection pressure to maintain functional sequences. On the other hand, SSPTSA can detect variation in both coding and non-coding regions and environment independent.

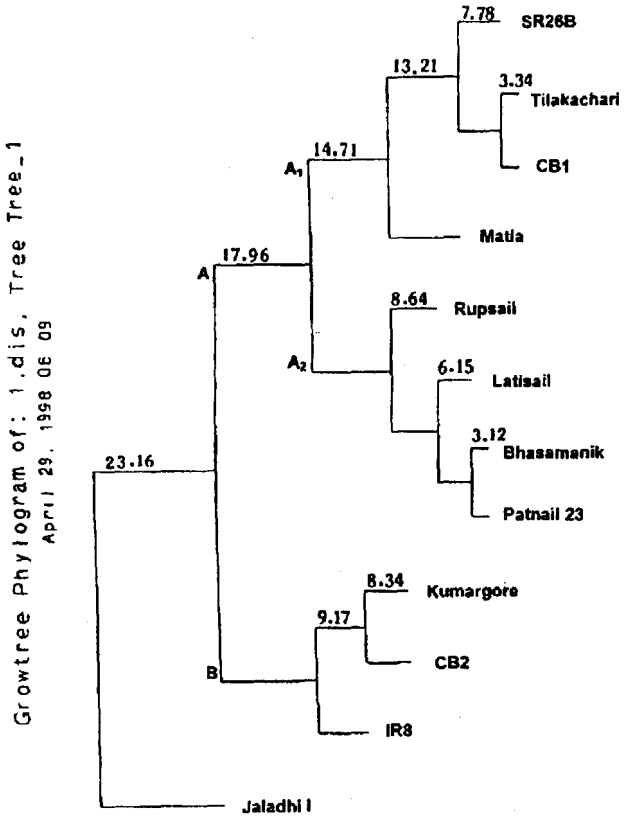

Fig. 4. Dendrogrambased on cluster analysis of DNA amplification profile data showing the genetic relatedness between cultivars. Accessions are indicated at the end of each branch and numbers on the branches indicate the unit distance

The correlation between the two genetic dissimilarity matrices was tested using the Spearman Rank Correlation (SRC). The results for SRC (which compares how each system ranks pairwise similarities) gave moderate correlation between isozymes and SSPTSA (rs = 0.36) and over 40 per cent of the genotypes ranking in the same order. In several recent studies, fingerprints based on different markers were compared using genotypes from different species. Lanner-Herrera *et al.*, (1996) also recorded a moderate Spearman's Rank Correlation (r = 0.38) between RAPD and isozyme distances in *Brassica oleracea*. Similar results were also reported by Beer *et al.*, (1993) and Heun *et al.*, (1994) in *Avena sterlis* L., and by Sun *et al.*, (1999) in *Elymus caninus*.

The results of the present study on isozyme and SSPTSA patterns, in general, provided an insight into the interrelationships among the studied varieties and have thereby helped to formulate phylogenetic hierarchies. Further, to the best of our knowledge, this has been the first report of analysis of evolutionary relationship s among the landraces of West Bengal, some of which have been extensively used in variety development programmes nationally and internationally, using biochemial (isozyme) and DNA markers. Overall, it could be concluded that the DNA based markers (SSPTSA) will be more useful than isozyme markers in detecting the genetic diversity among the landraces of West Bengal.

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