Genetic diversity in cultivars and landraces of *Oryza sativa* subsp. *indica* as revealed by AFLP markers

S.R. Prashanth, M. Parani, B.P. Mohanty, V. Talame, R. Tuberosa, and A. Parida

Abstract: Genetic diversity among 49 Indian accessions of rice (*Oryza sativa* subsp. *indica*), including 29 landraces from Jeypore, 12 modern cultivars, and 8 traditional cultivars from Tamil Nadu, was investigated using AFLP markers. In total, nine primer combinations revealed 664 AFLPs, 408 of which were found to be polymorphic. The percentage of polymorphic AFLPs was approximately the same within the cultivars and landraces. Similar results were obtained when genetic diversity values were estimated using the Shannon–Weiner index of diversity. Genetic diversity was slightly higher in the modern cultivars than in the traditional cultivars from Tamil Nadu. Among the landraces from Jeypore, the lowland landraces showed the highest diversity. The present study showed that the process of breeding modern cultivars did not appear to cause significant genetic erosion in rice. Cluster analysis and the first component of principle component analysis (PCA) both showed a clear demarcation between the cultivars and landraces as separate groups, although the genetic distance between them was narrow. The modern cultivars were positioned between the landraces from Jeypore and the traditional cultivars from Tamil Nadu. The second component of PCA further separated medium and upland landraces from lowland landraces, with the lowland landraces found closest to the traditional and modern cultivars.

Key words: rice, *Oryza sativa*, AFLP, landraces, genetic diversity.

Résumé : La diversité génétique parmi 49 accessions de riz (*Oryza sativa* ssp. *indica*), dont 29 variétés de pays du Jeypore et 8 variétés traditionnelles du Tamil Nadu, a été examinée à l'aide de marqueurs AFLP. Au total, neuf combinaisons d'amorces ont permis de révéler 664 AFLP dont 408 étaient polymorphes. La proportion d'AFLP polymorphes était semblable au sein des cultivars et des variétés de pays. Des résultats semblables ont été obtenus lorsque les valeurs de diversité génétique ont été estimées en utilisant l'indice Shannon–Weiner de diversité. La diversité génétique était légèrement supérieure au sein des cultivars modernes que parmi les variétés de pays du Tamil Nadu. Parmi les variétés de pays du Jeypore, les variétés cultivées en faible altitude ont montré le plus de diversité. La présente étude montre que les méthodes modernes d'amélioration génétique n'auraient pas causé d'érosion génétique significative chez le riz. Une analyse de groupement et la première composante au sein d'une analyse en composantes principales (PCA) ont toutes deux montré que les cultivars et les variétés de pays formaient deux groupes distincts, bien que la distance génétique entre eux était faible. Les cultivars modernes logeaient entre les variétés de pays du Jeypore et les cultivars traditionnels du Tamil Nadu. La seconde composante de l'analyse en composantes principales a permis de séparer davantage les variétés cultivées en altitudes moyenne ou élevée des variétés cultivées en faible altitude, ces dernières étant les plus proches des cultivars traditionnels et modernes.

Mots clés : riz, *Oryza sativa*, AFLP, variétés de pays, diversité génétique.

[Traduit par la Rédaction]

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops grown worldwide and is the staple food for half of the world population (Sasaki and Burr 2000). The varieties of *O. sativa* have been traditionally classified as *O. sativa* subsp. *indica*, subsp. *japonica*, and subsp. *javanica*. *O. sativa* subsp. *indica* is grown in tropical countries, such as India, where traditionally only native landraces or their derivatives have been cultivated. These varieties have shown excellent adaptation to local conditions but their productivity is very low. Increasing the productivity through high-input agriculture has not been possible because native landraces are not responsive to fertilizers. In 1964, direct introduction

Received 30 July 2001. Accepted 18 January 2002. Published on the NRC Research Press Web site at http://genome.nrc.ca on 21 March 2002.

Corresponding Editor: R.S. Singh.

S.R. Prashanth, M. Parani, B.P. Mohanty, and A. Parida.[1] M.S. Swaminathan Research Foundation, III Cross Street, Institutional Area, Taramani, Chennai 600 113, India.
V. Talame and R. Tuberosa. University of Bologna, Department of Agronomy, Fillippo Re, 6, 40126, Bologna, Italy.

[1]Corresponding author (e-mail: pmb@mssrf.res.in).

 DOI: 10.1139/G02-003

of a semi-dwarf, fertilizer-responsive, high-yielding exotic variety, TN-1, derived from 'Deo-Geo-Woo-Gen' × 'Tsai-Yuan-Chung' from Taiwan failed because of its susceptibility to bacterial blight. Two years later, in 1966, the introduction of IR-8, derived from 'Deo-Geo-Woo-Gen' × 'Peta' (International Rice Research Institute (IRRI)), became a great success, but it was not well adapted to all of the rice ecosystems in India. Therefore, efforts were made to incorporate the desirable traits of 'Deo-Geo-Woo-Gen' into local varieties and landraces. As a result, two high-yielding varieties, derived from the cross between TN-1 and a locally adapted line, T-141, were released in 1968. Since then, more than 500 high-yielding modern and locally adapted varieties containing the dwarfing gene from 'Deo-Geo-Woo-Gen' have been developed from landraces and traditional cultivars (Anonymous 1992).

Diversity and polymorphism using molecular markers have been studied in more detail in *O. sativa* subsp. *japonica* than in subsp. *indica* or subsp. *javanica* (Mackill 1995; Redona and Mackill 1996; Ashikawa et al. 1999). However, information on *O. sativa* subsp. *indica* can be obtained from the comparative studies on *O. sativa* subsp. *japonica* and *O. sativa* subsp. *indica* (Zhang et al. 1992; Yang et al. 1994; Liu et al. 1996; Mackill et al. 1996; Zhu et al. 1998). There are only two studies that have specifically investigated genetic diversity in accessions of *O. sativa* subsp. *indica* using molecular markers (Singh et al. 1998; Singh et al. 1999). Given the large amount of unexplored genetic diversity in cultivated *O. sativa* subsp. *indica* varieties, particlarly among accessions of its sexually compatible wild relatives, it becomes important to better characterize the extent of such diversity.

Different types of molecular markers have been used to investigate the biodiversity of rice (Glaszmann 1987; Wang and Tanksley 1989; Wu and Tanksley 1993; Zeitkiewicz et al. 1994; Virk et al. 1995; Zhou and Gustafson 1995; Ashikawa et al. 1999; Dong et al. 2000). Among the different types of molecular markers, AFLP (amplified fragment length polymorphism; Vos et al. 1995) combines the ease of random amplified polymorphic DNA markers (RAPDs; Williams et al. 1990) with the robustness of restriction fragment length polymorphism markers (RFLPs; Botstein et al. 1980). AFLP analysis is sensitive, does not require prior sequence information, and has already been used for investigating genetic distances among accessions of crop species (Maughan et al. 1996; Ellis et al. 1997; Cho et al. 1999; Lotti et al. 2000). AFLPs generate complex banding patterns with up to 100 DNA fragments in a single reaction (Mackill et al. 1996; Zhu et al. 1998) and offer wider genome coverage and informativeness than other molecular marker systems (Zhu et al. 1998; Singh et al. 1999).

The objective of our study was to use AFLPs to analyse the genetic diversity present in 49 accessions of *O. sativa* subsp. *indica* from India cultivated in the three major rice-growing environments (lowland, midland (medium land), and upland) that represent both traditional and modern breeding pools.

Materials and methods

Plant material

The 49 accessions used in this study included 8 traditional and 12 modern cultivars obtained from Ambasamudram Rice Research Station (ARRS) and Madurai Agricultural College (MAC), Tamil Nadu, India, and 29 landraces collected from the Jeypore tract of Orissa, India (Table 1).

Extraction of genomic DNA

DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). For each accession, about 5 g of bulked leaf tissue collected from 20 plants was ground to a fine powder using liquid nitrogen and then suspended in 20 mL of extraction buffer (20 mM EDTA (pH 8.0), 100 mM Tris-HCl (pH 8.0), 1.5 M NaCl, 2% CTAB, and 1% β-mercaptoethanol). The suspension was mixed well, incubated at 60°C for 45 min, followed by chloroform – isoamyl alcohol (24:1) extraction and precipitation with 2/3 of the volume of isopropanol at –20°C for 1 h. The DNA was pelleted down by centrifugation at 12 000 rpm for 10 min and suspended in TE buffer (10 mM Tris-HCl – 1 mM EDTA (pH 8.0)). The DNA was purified from RNA and proteins by standard procedures (Sambrook et al. 1989) and DNA concentration was estimated by agarose-gel electrophoresis and staining with ethidium bromide.

AFLP analysis

Preselective amplification

Genomic DNA (250 ng) was digested to completion with 5 U each *Mse*I and *Eco*RI restriction enzymes (Boehringer-Manheim, Germany). The double-digested DNA fragments were ligated to 5 pmol *Eco*RI and 25 pmol *Mse*I adaptors (Vos et al. 1995). The adaptor-ligated DNA was diluted 10 times with TE buffer and subjected to pre-selective amplification with *Mse*I adaptor+A and *Eco*RI adaptor+C primers. A 25-μL reaction mixture containing 2.5 μL diluted DNA, 75 ng of each primer, 1× reaction buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$), 1 U *Taq* DNA polymerase (Bangalore Genei, India), and 250 μM of dNTPs was prepared and subjected to 20 cycles of 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s in a thermal cycler (Perkin-Elmer 9700; Perkin-Elmer, Foster City, Calif.). The concentration of the amplified DNA was checked in 1.5% agarose gel and diluted 25 times in TE buffer.

Selective amplification

The *Eco*RI+3 and *Eco*RI+2 primers (30 ng) were end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Life Technologies) in a 50-μL reaction as recommended by the manufacturer. Selective amplification mixture (20 μL) was prepared with 0.25 μL labelled *Eco*RI primer, 0.3 μM *Mse*I+3 primer, 5 μL diluted preselective reaction mixture, 1× buffer (20 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, and 50 mM KCl), 125 μM dNTPs, and 1.0 U *Taq* DNA polymerase. Selective amplification was carried out for one cycle at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s, and the annealing temperature was then repeatedly lowered by 1°C for each of the next nine cycles, followed by 23 cycles at 94°C for 15 s, 56°C for 30 s and 72°C for 30 s. Amplified products were mixed with equal volume of formamide loading buffer (98% formamide, 10mM EDTA, 0.1% bromophenol blue, 0.1% Xylene cyanol), denatured at 90°C for 3 min, and resolved on 6% denaturing polyacrylamide gels (acrylamide–bis-acrylamide (20:1), 7.5 M urea – 1× TBE buffer) at 60 W

Table 1. List of cultivars and landraces of *Oryza sativa* subsp. *indica* used in the present study.

Accession	Description*	Source
Traditional Cultivars:		
ASD-1	Pureline selection from Kar Samba Red (115)	ARRS
ASD-2	Pureline selection from Kar Samba White (110)	ARRS
ASD-3	Pureline selection from Veethi Vidangan (135)	ARRS
ASD-4	Pureline selection from Kuruva Kalayan (135)	ARRS
ASD-7	Pureline selection from Kar-Samba Red-Early (105)	ARRS
ASD-8	Pureline selection from Thuyamalli (85)	ARRS
ASD-9	Pureline selection from Avasara Samba (90)	ARRS
GEB-24	Konamani mutant, photo-insensitive (150)	ARRS
Modern Cultivars:		
ASD-11	Natural cross of GEB-24/PJB-15 (150)	ARRS
ASD-12	Natural cross of GEB-24/PJB-15 (165)	ARRS
ASD-14	Derived from TN1/ ASD1 (115)	ARRS
ASD-15	Derived from IR26/IR22 (120)	ARRS
ASD-16	Derived from ADT-31/CO-39 (110)	ARRS
ASD-17	Derived from ADT-31/Ratna/ ASD-8/IR-8 (100)	ARRS
ASD-19	Derived from Lainakanda/IR30 (130)	ARRS
ASD-18	Derived from ADT-31/IR-50 (105)	ARRS
ASD-20	Derived from IR-18348/IR-25863 /IR-58 (110)	ARRS
TN-1	Dwarf gene, dee-geo-woo-gen donor (110)	ARRS
MDU-3	Derived form IR8/W 1263 (120–125)	MACRI
MDU-4	Derived form AC 2836/Jagannath (120–125)	MACRI
Landraces[†]:		
Dhobkhuji	Lowland (150)	Chendenga, Malkangiri
Rajamuan	Lowland (180)	Kundri, Koraput
Machackanta	Lowland (150)	Badakumuli, Nabrangapur
Kalamali	Lowland (150)	Malkangiri, Malkangiri
Patadhan	Lowland (135)	Chedenga, Malkangiri
Biaganmanjidhan	Lowland (170)	Malkangiri, Malkangiri
Gadakhuta	Lowland (135)	Masigaon, Koraput
Kalajira	Lowland (160)	Masigaon, Koraput
Sindhikohli	Lowland (140)	Lima, Koraput
Baunasaganthi	Lowland (140–150)	Mahulli, Koraput
Basubhoga	Lowland (150)	Kotpad, Koraput
Tikichudi	Lowland (140–150)	Masigaon, Koraput
Paiken	Upland (90)	Kaniguma, Kalahandi
Dular	Upland (95)	Chendenga, Malkangiri
Mora	Upland (100)	Jeypore, Koraput
Kakmaranga	Upland (90)	Dadaki, Kandhamal
Dangardhan	Upland (85–95)	Masigaon, Khurda
Bhataguda	Upland (90)	Jeypore, Koraput
Matidhan	Upland (70)	Haladikund, Koraput
Pandakagura	Upland (90)	Jeypore, Koraput
Osagathiali	Medium land (120–130)	Jeypore, Koraput
Khutuli	Upland (90)	Kaniguma, Kalahandi
Bhatakubudi	Medium land (130)	Mentri, Koraput
Mer	Medium land (110–120)	Kotpad, Koraput
Baringa	Medium land (120)	Badakumuli, Nabrangpur
Bhatachudi	Medium land (130)	Jeypore, Jeypore
Sapuri	Medium land (130)	Masigaon, Khurda
Limbachudi	Medium land (110)	Kotpad, Koraput
Bodikaburi	Medium land (110)	Mohuli, Koraput

Note: ARRS, Ambasamudram Rice Research Station; MACRI, Madurai Agricultural College and Research Institute.
*Figure given in parentheses indicates duration of the cultivar or landrace.
[†]Source for the landraces is given as the village and the district of the site of collection.

Table 2. Total number of AFLP bands and the percentage of polymorphic bands in different groups of *indica* landraces and cultivars.

	Lowland landraces		Midland landraces		Upland landraces		All landraces		Modern cultivars		Traditional cultivars		All cultivars		All accessions	
Primer combination	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
E-AAC–M-CTA	44	41	45	27	45	29	46	59	46	43	45	38	45	44	47	65
E-AGC–M-CAT	53	32	51	31	53	38	51	47	53	40	51	29	52	42	53	64
E-AGG–M-CAC	67	60	72	17	70	41	72	67	73	62	73	48	72	63	73	75
E-AGC–M-CAC	58	36	58	29	56	39	57	53	50	56	55	42	56	62	58	74
E-AGG–M-CAA	105	48	103	18	104	31	111	48	111	42	108	29	110	50	112	61
E-ACC–M-CTT	94	17	92	14	92	16	96	31	91	37	91	15	95	45	97	50
E-AAC–M-CTT	66	39	56	21	58	25	64	48	64	31	64	29	65	37	66	62
E-AG–MCTT	86	37	87	18	87	20	90	41	90	38	86	30	90	40	91	48
E-AG–M-CTA	63	40	60	27	64	22	66	56	65	45	65	35	65	48	67	64
Total and mean	636	39±11.6	624	22±7.8	629	29±8.9	653	50±10.4	643	44±9.6	638	33±9.45	650	48±9.1	664	62±9.1

constant power. The gels were dried and autoradiographed overnight. Nine primer combinations used for selective amplifications are reported in Table 2.

Data analysis

Each informative AFLP band was scored independently as 1 for presence and 0 for absence. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Total diversity was calculated using the Shannon–Weiner (SW) index (Shannon and Weaver 1949) as

$$H_W = -\sum f_i \times \ln(f_i)$$

where f_i is the frequency of an AFLP band across all the samples. SW index within a subset of data was calculated as

$$H_S = -\sum f_i \times \ln(f_i)$$

where f_i is the frequency of an AFLP band within a subset. The average diversity between different groups was calculated as

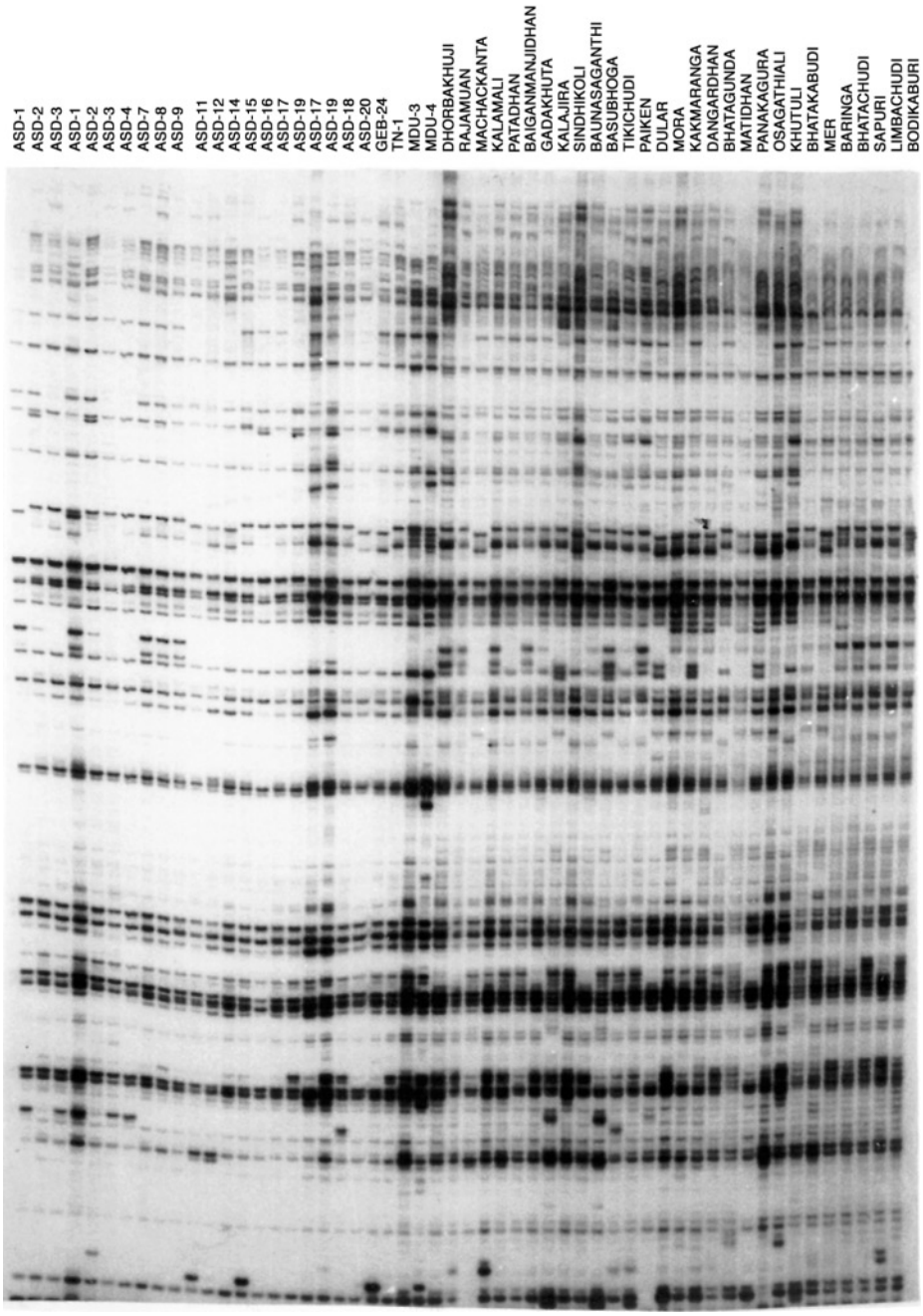
$$H_A = 1/nH_S$$

where H_A is the average group diversity over n groups. The intra- and inter-diversity components (H_A/H_W and $(H_W - H_A)/H_W$) were also compared (Maughan et al. 1996). Genetic similarities among rice accessions were calculated with the simple matching coefficient (Sokal and Michener 1958) using NTSYS-pc version 2.0 software (Rohlf 1992). The resulting similarity matrix was first subjected to cluster analysis by the unweighted pair-group method with the arithmetic averages (UPGMA) and then to principal component analysis (PCA).

Results and discussion

India is the second-largest rice-producing country in the world, where about 60 000 landraces were being grown in the 1950s (Anonymous 1992). Until 1968, the varieties developed by agricultural research stations in India were largely by direct pureline selection from landraces and occasionally derived by selection after hybridization between purelines. Such varieties are referred to as traditional cultivars in this paper. Later on, modern cultivars were developed by hybridization of the local landraces and traditional cultivars with exotic germplasm. The present study included 29 landraces collected from Jeypore that includes eight districts of Orissa in southeastern India. These landraces represent lowland (12 accessions), midland (8 accessions), and upland (9 accessions) rice ecosystems. The 8 traditional cultivars (including GEB-24) were from southern India, and the 12 modern cultivars were developed for the same region by hybridization of locally adapted landraces and traditional cultivars with exotic varieties like TN-1 and IR-8 to incorporate agronomically desirable traits. The number of AFLP bands per primer combination observed across all 49 accessions varied between 41 and 112 over the nine primer combinations used. The number of polymorphic AFLP per primer combination varied from 29 to 68 with an average of 45. In total, 408 fragments out of 664 (62%) were found polymorphic (Table 2). Each primer combination could differentiate all of the accessions used. The AFLP pro-

Fig. 1. AFLP profile of 49 accessions of rice cultivars and landraces generated by selective amplification with *Eco*RI adaptor+AGC and *Mse*I adaptor+CAT primer combination resolved in 6% denaturing polyacrylamide gel. AFLP profiles from ASD-1, ASD-2, ASD-3, ASD-17, and ASD-19 are replicated once.



file of the 49 accessions obtained with the primer combination E-AGC–M-CAT is shown in Fig. 1.

The percentage of polymorphic AFLPs was almost the same within the cultivars (traditional and modern together) and the landraces (48 and 50%, respectively) (Table 2). The level of genetic diversity within rice cultivars and landraces was found to be similar, as reported earlier based on variation in rDNA intergenic spacer regions (Liu et al. 1996) and microsatellite markers (Yang et al. 1994). Furthermore, it has been reported that the most frequent alleles at the majority of the loci in landraces were also maintained as the most common alleles at their respective loci in cultivars (Yang et al. 1994). In the present study a higher level of polymorphism was found in modern cultivars in comparison with traditional cultivars (44 and 33%, respectively) when the modern and traditional cultivars were considered as separate groups. Within the landraces, lowland landraces were more polymorphic than midland and upland landraces (39, 22, and 29%, respectively). However, across the cultivars and the landraces 62% of the AFLPs were polymorphic. Therefore, a certain portion of variation is unique to each group. This indicated a moderate level of genetic divergence between the cultivars (traditional and modern together) and the landraces studied. Furthermore, the midland and upland landraces

Table 3. Shannon–Weiner diversity index values for different groups of *Oryza sativa* subsp. *indica* landraces and cultivars.

Primer combination	Lowland landraces	Medium land landraces	Upland landraces	All landraces	Modern cultivars	Traditional cultivars	All cultivars	All accessions
E-AAC–M-CTA	4.26	1.95	3.34	3.91	5.30	5.41	5.65	4.98
E-AGC–M-CAT	4.60	3.21	4.56	6.43	4.90	3.68	4.74	5.61
E-AGG–M-CAC	8.86	1.72	5.74	7.41	10.20	8.85	10.43	9.26
E-AGC–M-CAC	4.79	4.58	4.96	5.17	6.27	5.83	6.71	6.78
E-AGG–M-CAA	10.40	4.01	7.68	10.85	11.23	9.04	11.81	12.87
E-ACC–M-CTT	5.79	4.60	5.66	6.76	7.54	3.50	8.04	20.48
E-AAC–M-CTT	7.76	3.94	5.61	6.89	6.27	9.30	8.26	10.59
E-AG–M-CTT	4.98	3.90	4.26	6.14	5.48	5.09	6.37	6.41
E-AG–M-CTA	6.71	3.58	4.34	8.9	6.66	5.46	6.85	8.08
Total	58.15	31.50	46.1	62.4	63.97	56.42	68.8	85.06
Mean	6.46±2.14	3.5±1.03	5.1±1.24	6.94±1.88	7.1±2.16	6.26±2.20	7.65±2.26	9.45±4.84

were more divergent than the lowland landraces from the cultivars.

In the present study, the frequency of individual polymorphic AFLP fragments ranged from 0.02 (present in only one accession) to 0.98 (present in all but one accession), which is in accordance with previous results reported in rice (Zhu et al. 1998). When measuring the level of polymorphism in terms of percentage of polymorphic AFLPs, a fragment with a frequency of 0.02 as well as 0.98 are given the same weight. Consequently, this approach does not indicate to what extent rare alleles or common alleles contribute to the observed level of polymorphism. Therefore, to measure the genetic content of the accessions, we used the SW index, which is essentially a sum of frequency of occurrence of events (the AFLP bands in this case). The average SW index and its standard deviation was 6.94 ± 1.88, 6.26 ± 2.20, 7.1 ± 2.16, and 9.45 ± 4.84 for landraces, traditional cultivars, modern cultivars, and the pooled data from all the accessions, respectively, (Table 3). These results indicate that the choice of the primer combination influences the measure of genetic diversity, particularly when the sample size is large and diverse in origin, such as in the case of the pooled data from all accessions.

The results obtained with the SW index can be analyzed with the four following comparisons:

(*i*) Traditional cultivars compared with modern cultivars from Tamil Nadu. Diversity value for modern cultivars was slightly higher than that for traditional cultivars (63.9 and 56.4, respectively). These modern cultivars were developed for the same region where the traditional cultivars were grown. In fact, some of these traditional cultivars were used as parents to develop the modern cultivars (see Table 1). Therefore, hybridization of exotic germplasm with local germplasm during the course of breeding programs might have contributed to the increased diversity among the modern cultivars compared with the traditional cultivars.

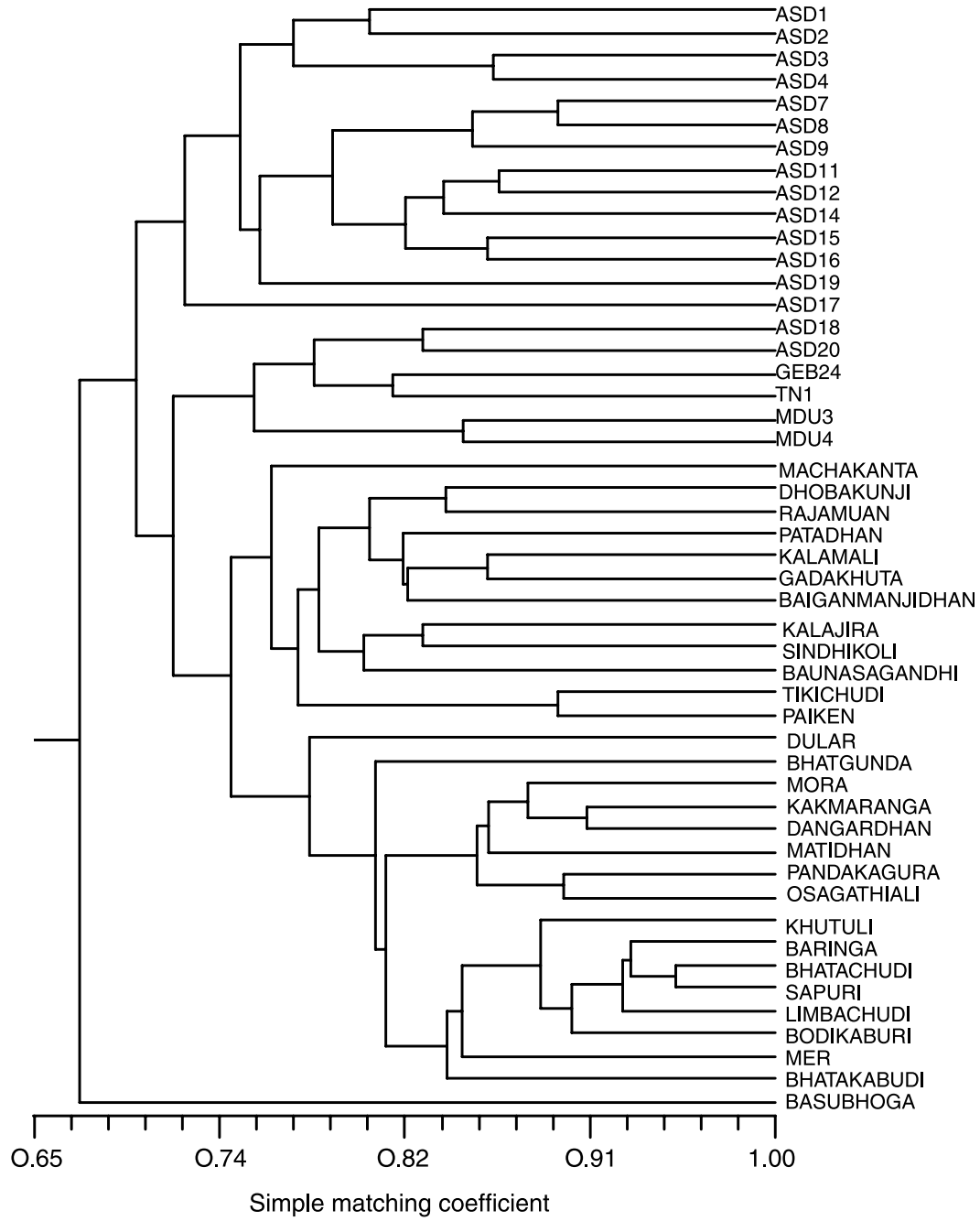
(*ii*) Landraces from Jeypore compared with those from Tamil Nadu. The eight traditional cultivars analyzed in the present study are essentially the landraces from Tamil Nadu (Ambasamudram; Table 1) that were obtained by direct pureline selection. Therefore, a comparison between these cultivars and the landraces from Jeypore could provide a comparative estimate of diversity present in the landraces from these two regions. The diversity value for landraces from Jeypore was higher than that for landraces from Tamil Nadu (62.4 and 56.4, respectively). This could be explained by the fact that Jeypore is considered a secondary centre of origin for rice (Ramaiah 1953; Ramaiah and Ghose 1951) and a hot spot for genetic diversity in this species.

(*iii*) Comparison of lowland, midland, and upland landraces. Lowland landraces showed remarkably higher genetic diversity than the midland and upland landraces (58.2, 31.5, and 46.1, respectively). Analysis of the data after excluding the accession Basubhoga, which was found to be an outlier (Fig. 2), did not alter the SW index for diversity very much and it remained the highest for lowland landraces (56.9) in comparison with upland and midland landraces. This result was interpreted after taking into consideration that the lowland environment, as compared with the midland and upland environments, is more complex, unpredictable, and subjected to several biotic and abiotic stresses. This, in turn, may have required greater genetic variability to reach and sustain adequate yield levels.

(*iv*) Comparison across all accessions. We compared the intra- and inter-group diversity components of AFLP variation (H_a/H_w and $(H_w - H_a)/H_w$, respectively) considering the lowland, midland, and upland landraces and the modern and traditional cultivars as five separate groups. It was found that the observed variation in AFLP profiles was almost equally divided between the intra- and interdiversity components.

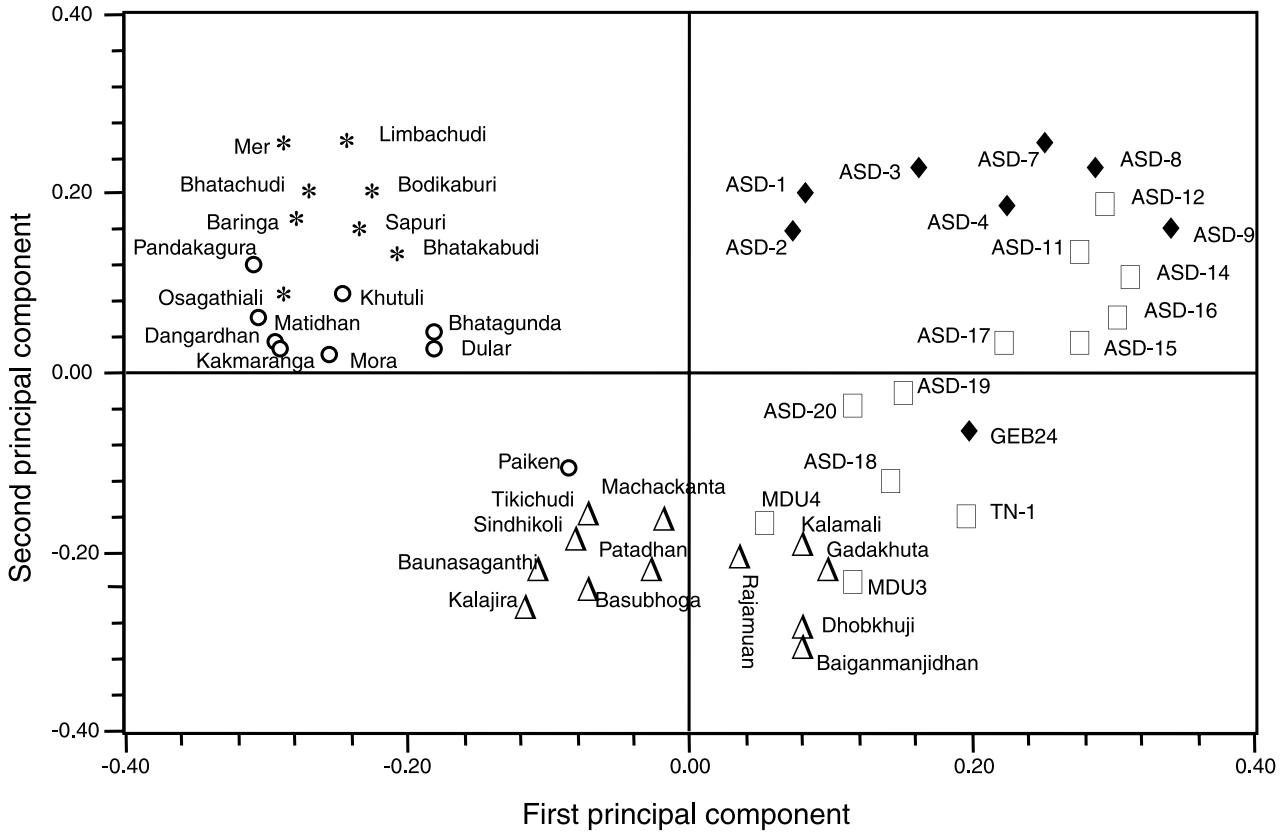
The dendrogram based on the analysis with the simple matching coefficient (Fig. 2) showed two major clusters with about 70% similarity. One of the two major clusters included a sub-cluster all of the landraces, except Basubhoga, in a group with approximately 75% similarity, and a second subcluster with six accessions (GEB-24, TN-1, MDU-3, MDU-4, ASD-18, and ASD-20). The other major cluster included all of the other cultivars. Among the landraces, lowland landraces were closer to the modern cultivars even though the latter were not especially derived from lowland landraces or developed for the lowland ecosystem. Basubhoga, a lowland landrace with white and aromatic kernel was found to be an outlying accession among all other accessions studied.

Fig. 2. Dendrogram showing relationships among the 49 accessions of rice cultivars and landraces based on NTSYS simple matching coefficient and UPGMA.



To better understand the relationships among rice accessions, PCA was also carried out using the same genetic similarities data set (Fig. 3). On the basis of the first principal component, which accounted for 16.7% of the total variation, the landraces adapted to midland and upland cultivation were clearly separated from lowland landraces and cultivars. The latter were more widely spread than the landraces across the second principal component, which explained 9.0% of the total variation. The lowland landraces were positioned between the upland and midland landraces and the cultivars, although they were closer to the latter. The tight cluster including the midland and upland landraces was better resolved by the third principal component, which accounted for 5.5% of the total variation and, with the exception of Paiken, allowed for the separation between midland and upland landraces. The modern cultivars were positioned between the traditional cutivars from Tamil Nadu and the landraces from Jeypore. Modern cultivars are primarily nonsegregating lines selected at advanced generations raised after hybridization between locally adapted purelines from Tamil Nadu and exotic varieties. Modern cultivar breeding selection was more towards the phenotype of the respective pureline, except for a few selected traits like semi-dwarfism or erect leaves that were acquired from the

Fig. 3. Cluster diagram showing the relationships among the 49 accessions of rice cultivars and landraces based on principal component analysis performed. ◆, traditional cultivars; □, modern cultivars; Δ, landraces for lowland cultivation; ○, landraces for upland cultivation; and asterisks indicates landraces for midland cultivation.



exotic cultivars. Indeed, the modern cultivars show more affinity towards the traditional cultivars from Tamil Nadu rather than forming a separate cluster distant from landraces and traditional cultivars.

Modern cultivars showed more diversity than the landraces from the same region, and yet clustered closer to the traditional cultivars. Therefore, these results indicate that the development of modern cultivars does not appear to have caused genetic erosion. In fact, after hybridization of the landraces and their derivatives with the exotic germplasm, genetic diversity has increased in terms of available polymorphisms at single loci. However, in terms of cultivated genotypes, total genetic diversity erosion is probable, because only a small number of the 500 modern cultivars (Anonymous 1992) are currently being cultivated in India, and they occupy more than 95% of the area under rice cultivation. Therefore, it remains essential to conserve the biodiversity present in the rice landraces and use it to broaden the genetic base of cultivated rice varieties. Molecular markers, such as AFLPs, will play an essential role in characterizing biodiversity for its exploitation in modern breeding programmes.

Acknowledgements

The farmers from the Jeypore tract of Orissa who maintain, cultivate, and contributed the landraces for this study are gratefully acknowledged. This research was supported by the Department of Biotechnology, Government of India, and by the Ministry of Foreign Affairs, India and Italy.

References

Anonymous. 1992. Miracle rice varieties of India. Central Rice Research Institute, Cuttack, Orissa, India.

Ashikawa, I., Fukuta, Y., Tamura, K., and Yagi, T.A.D. 1999. Application of AFLP technique that uses non-radioactive fluorescent primers for the detection of genetic diversity in Japanese rice cultivars and cloning of DNA sequences derived from an *indica* genome. Breed. Sci. **49**: 225–231.

Botstein, D., White, R.L., Skolnik, M., and Davis, R.W. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. **32**: 314–331.

Cho, Y.C., Shin, Y.S., Ahn, S.N., Kang, K.H., and Moon, H.P. 1999. DNA fingerprinting of rice cultivars using AFLP and RAPD markers. Korean J. Crop Sci. **44**: 26–31.

Dong, N.V., Subudhi, P.K., Luong, P.N., Quang, V.D., Quy, T.D., Zheng, H,G., Wang, B., and Nguyen, H.T. 2000. Molecular mapping of a rice gene conditioning thermosensitive genic male sterility using AFLP, RFLP and SSR techniques. Theor. Appl. Genet. **100**: 727–734.

Ellis, R.P., McNicol, J.W., Baird, E., Booth, A., Lawrence, P., Thomas, B., and Powell, W. 1997. Use of AFLPs to examine the genetic relatedness of barley. Mol. Breed. **3**: 359–369.

Glaszmann, J.C. 1987. Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. **74**: 21–30.

Liu, K.D., Yang, G.P., Zhu, S.H., Zhang, Q., Wang, X.M., and

Saghai-Maroof, M.A. 1996. Extraordinarily polymorphic ribosomal DNA in wild and cultivated rice. Genome, **39**: 1109–1116.

Lotti, C., Salvi, S., Pasqualone, A., Tuberosa, R., and Blanco, A. 2000. Integration of AFLP markers into an RFLP-based map of durum wheat. Plant Breed. **119**: 1–10.

Mackill, D.J. 1995. Classifying *japonica* rice cultivars using RAPD markers. Crop Sci. **35**: 889–894.

Mackill, D.J., Zhang, Z., Redona, E., and Colowit, P.M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome, **39**: 969–977.

Maughan, P.J., Saghai-Maroof, M.A., Buss, G.R., and Heustis, G.M. 1996. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance and near isogenic line analysis. Theor. Appl. Genet. **93**: 392–401.

Ramaiah, K. 1953. Rice breeding and genetics. Indian Counc. Agricul. Res. Monogr. **19**: 360–362.

Ramaiah, K., and Ghose, R.L.M. 1951. Origin and distribution of cultivated plants of South Asian rice. Indian J. Genet. Plant Breed. **11**: 7–13.

Redona, E.D., and Mackill, D.J. 1996. Molecular mapping of quantitative trait loci in *japonica* rice. Genome, **39**: 395–403.

Rohlf, F.J. 1992. NTSYS-pc: numerical taxonomy and multivariate analysis system. Exeter software, New York.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. 1984. Ribosomal spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. U.S.A. **83**: 1757–1761.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sasaki, T., and Burr, B. 2000. International rice genome sequencing project: the effort to completely sequence the rice genome. Curr. Opin. Plant Biol. **3**: 138–141.

Shannon, C.E., and Weaver, W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, Ill.

Singh, S., Sidhu, J.S., Uberoi, S.K., Sodhi, M., Vikal, Y., and Dhaliwal, H.S. 1998. Genetic diversity analysis of *Oryza* using amplified fragment length polymorphism. Crop Improv. **25**: 15–20.

Singh, K.N., Nandi, R., Shanmugasundaram, P., Sadasivam, S., Ning, H., Brar, D.S., Khush, G.S., and Huang, N. 1999. High resolution DNA fingerprinting of Indian rice (*Oryza sativa* L.) varieties by amplified fragment length polymorphism. Genet. Resour. Crop Evol. **46**: 427–433.

Sokal, R., and Michener, C. 1958. A statistical method for evaluating systematic relationships. Univ. Kans. Sci. Bull. **38**: 1409–1438.

Virk, P.S., Ford-Lloyd, B.V., Jackson, M.T., and Newbury, H.J. 1995. Use of RAPD for the study of diversity within the plant germplasm collection. Heredity, **74**: 170–179.

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

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

Williams, J.G.K., Kubelik, A.R., Livak, J., Rafalski, A., and Tingey, S.V. 1990. DNA primers identified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535.

Wu, K.S., and Tanksley, S.D. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. **241**: 225–235.

Yang, G.P., Saghai-Maroof, M.A., Xu, C.G., Zhang, Q.F., and Biyashev, R.M. 1994. Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Mol. Gen. Genet. **245**: 187–194.

Zeitkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, **20**: 176–183.

Zhang, Q., Saghai-Maroof, M.A., Lu, T.Y., and Shen, B.Z. 1992. Genetic diversity and differentiation of *indica* and *japonica* rice detected by RFLP analysis. Theor. Appl. Genet. **83**: 495–499.

Zhou, Z., and Gustafson, J.P. 1995. Genetic variation detected by DNA fingerprinting with a rice minisatellite probe in *Oryza sativa* L. Theor. Appl. Genet. **91**: 481–488.

Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T., and Bryan, G.J. 1998. AFLP markers for the study of rice biodiversity. Theor. Appl. Genet. **96**: 602–611.