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RFLP relationships of A-genome species in the genus *Oryza*

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RFLPs of 192 accessions of A–genome rice species were analyzed. One plant was used to represent each accession assayed. RFLPs were detected for the combinations of *Dra* I–digested total DNA and twenty–one single–copy genomic clones. A dendrogram was constructed using the UPGMA method from a genetic distance matrix. Classification of the A–genome rice species based on RFLP analysis matched well with the conventional classification. The African annual species, *O. glaberrima* and *O. barthii*, were not clearly differentiated by RFLP loci while the Asian species, *O. sativa*, *O rufipogon* and *O. nivara*, formed a complex wherein none of the species can easily be distinguished from each other. An Asian sub–group that is clearly differentiated from other Asian A–genome germplasm was identified. This consists of some *O. rufipogon* accessions that did not cluster with the cultivated rice but formed loosely knit groups. Some accessions of *O. rufipogon* were closely aligned to Indica rice cultivars while the others, mainly from China, were closely aligned with Japonica cultivars. Most of *O. glumaepatula* accessions from Latin America formed a group which was more closely related to *O. glaberrima* and *O. barthii* than other species. Two accessions of *O. glumaepatula*, however, were aligned with Indica cultivars of Asia. It is possible that the A–genome wild rices in Latin America are of two types. *O. rufipogon* from the Sepik river of Papua New Guinea was the only material which had RFLP fragments found only in *O. meridionalis*.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops and is the staple food for more than half of the world's population. There are more than twenty species in the genus *Oryza*. Two of these species, *O. sativa* and *O. glaberrima* Steud., are cultivated rices. Wild *Oryza* possess genes for resistance to diseases, insects and stress tolerance, but these have not been fully exploited in rice breeding programs.

In order to use useful genes from wild rice species, wide–hybridization techniques have been developed. New rice collections have been added to the already conserved germplasm in recent years (Vaughan, 1994). Molecular markers were identified and used to select plants carrying introgressed useful genes. Information on phylogenetic relationships of wild species also help in efficient gene transfer. If useful genes were found in germplasm collection, a donor accession closely related to target breeding lines can be selected based on the phylogenetic information.

Nuclear restriction fragment length polymorphism (RFLP) is now considered a powerful tool for genetic analysis and plant breeding. RFLP analysis is sensitive to

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genetic changes at the DNA level at both coding and non-coding regions. More importantly, it can be performed easily over many loci. RFLP analysis, therefore, has been widely used to study phylogenetic relationships among populations and species (e.g. Song *et al.*, 1988; Miller and Tanksley, 1990).

The A-genome species (*O. sativa* complex) in the genus *Oryza* were the most widely used in rice breeding. They share the same genome as *O. sativa* and meiotic chromosome pairing of the F_1 hybrids is almost complete (Katayama, 1997), although showing various levels of sterility (Morishima, 1969). Asian wild A-genome species, *O. rufipogon sensu lacto* was considered as wild rice relatives of Asian cultivated rice. It is taxonomically divided into two species, *O. rufipogon* Griff. *sensu stricto* and *O. nivara* Sharma *et* Shastry (Sharma and Shastry, 1965), on the basis of morphological and ecological characteristics. In Africa, annual cultivated rice, *O. glaberrima*, annual wild rice, *O. barthii* A. Chev. and perennial wild rice, *O. longistaminata* Chev. et Roehr., are found. In Latin America, *O. glumaepatula* Steud. is found although they are sometimes included into *O. rufipogon*. Austrarian annual/biennial A-genome wild rice is *called O. meridionalis* Ng.

Nakano *et al.* (1992) studied on RFLP variation of the Asian A-genome species. They identified a group of wild rices which was clearly distinct from *O. sativa* and *O. rufipogon*. The group (cluster 3) did not correspond to any taxonomical classification. Analyzing RFLPs of accessions from broad range of A-genome rice germplasm collections helps to understand the genetic base of the group. It also provides detailed information of phylogenetic relationships of A-genome rice species.

Genetics of A-genome rices had been extensively studied (for review, Oka, 1988). The present RFLP survey is also a continuation of the previous extensive studies (e.g. Morishima, 1969, for morphologocal characters; Second, 1985, for isozymes; Dally and Second, 1990, for chloroplast genomes).

MATERIALS AND METHODS

Plant materials

RFLPs of 67 rice accessions, consisting of *O. sativa*, *O. rufipogon*, *O. nivara*, *O. glaberrima*, *O. barthii*, *O. longistaminata*, *O. glumaepatula* and *O. meridionalis*, were analyzed. Since Nakano's Cluster 3 probably belongs to *O. nivara*, some accessions of *O. nivara* as well as perennial *O. rufipogon* with quite different ecology (OR1–OR15, they are perennial and grew in the area where no cutivated rice was grown) from *O. nivara* were added. The data were combined with those from the previously analyzed materials by Nakano *et al.* (1992). As a result, 192 accessions of A-genome species were subjected to data analysis (Table 1). The seeds or leaves were provided by the National Institute of Agrobiological Resources, Tsukuba, Japan, the National Institute of Genetics (NIG), Mishima, Japan, the International Rice Germplasm Center (IRGC), Internatinal Rice Research Institute, Los Baños, Philippines and Kyushu University, Fukuoka, Japan. Species names used were those given by the source of the seeds. One plant was used to represent each accession assayed.

Table 1. The 192 accessions of A-genome species in the genus *Oryza* analyzed in the study.

Accession number	Variety name/ accession code	Taxa	Origin	Source [1)]	Number of fragments [2)]
Nipponbare	Nipponbare	*O. sativa*	Japan	KY	21
Java14	Java14	*O. sativa*	Indonesia	KY	21
IR24	IR24	*O. sativa*	Philippines	KY	21
CR1		*O. sativa*	South China	NIAR	21
CR2		*O. sativa*	South China	NIAR	21
CR3	Seenaddi	*O. sativa*	Sri Lanka	NIAR	21
CR4		*O. sativa*	Yunnan	NIAR	21
CR5	Gawhtun	*O. sativa*	Myanmar	NIAR	21
CR6	Dange Maruwa	*O. sativa*	Nepal	NIAR	21
CR7	Herosi Bola	*O. sativa*	Assam	NIAR	21
CR8	Muja Shail	*O. sativa*	Bangladesh	NIAR	21
CR9	Ngasein	*O. sativa*	Myanmar	NIAR	21
CR10	Niaw Dam	*O. sativa*	Thailand	NIAR	21
CR11	Mack Kham	*O. sativa*	Laos	NIAR	21
CR12A	Pulat Balachan	*O. sativa*	Malaysia	NIAR	22
CR13	Bongor	*O. sativa*	Malaysia	NIAR	21
CR14	Pulat Beludu	*O. sativa*	Malaysia	NIAR	21
CR15	Nang Dum To	*O. sativa*	Vietnam	NIAR	21
CR16	Ngoc Chum	*O. sativa*	Vietnam	NIAR	21
CR17	Nang Toi	*O. sativa*	Vietnam	NIAR	21
CR18	Pusur	*O. sativa*	India	NIAR	21
CR19B	Juma	*O. sativa*	India	NIAR	21
CR20	Shinriki	*O. sativa*	Japan	NIAR	21
CR21	Kameji	*O. sativa*	Japan	NIAR	21
CR22	Geraldine	*O. sativa*	South America	NIAR	21
CR23	Col/Mk/Palistan/1987/1	*O. sativa*	Pakistan	NIAR	20
CR24B	Khao Eo	*O. sativa*	Laos	NIAR	21
CR25	Dinalaga	*O. sativa*	Philippines	NIAR	21
CR26	Pangkai Kepal	*O. sativa*	Indonesia	NIAR	21
CR27	Masho	*O. sativa*	Myanmar	NIAR	21
CR28	Shinaba	*O. sativa*	Philippines	NIAR	21
CR29	Canabongbong	*O. sativa*	Philippines	NIAR	21
CR30	Menalam	*O. sativa*	Malaysia	NIAR	21
CR31	Siplo	*O. sativa*	Indonesia	NIAR	21
CR32	Ketan Pitik	*O. sativa*	Indonesia	NIAR	21
CR33	Marsi	*O. sativa*	Nepal	NIAR	21
CR34	Dhan	*O. sativa*	Nepal	NIAR	21
CR35	Red Basmati	*O. sativa*	Nepal	NIAR	21
CR36	Bonsaj	*O. sativa*	Bangladesh	NIAR	21
CR41	CPSLO	*O. sativa*	Malaysia	KY	21
CR42	Nekken 2	*O. sativa*	Japan	NIAR	21
CR43	IR26	*O. sativa*	Philippines	NIAR	21
CR44	TKM 6	*O. sativa*	India	NIAR	21
CR45	Norin 8	*O. sativa*	Japan	KY	21
CR101	Shen Shui Lian	*O. sativa*	China	KY	21
CR102	Vear Krochak	*O. sativa*	Cambodia	KY	21
CR103	Co. 24	*O. sativa*	Cambodia	KY	21
CR104	Suon Lory	*O. sativa*	Cambodia	KY	21
CR105	Nang Dum To	*O. sativa*	Vietnam	KY	21
CR106	Nang Quot	*O. sativa*	Vietnam	KY	21

Table 1. (Continued)

Accession number	Variety name/ accession code	Taxa	Origin	Source [1]	Number of fragments [2]
CR107	Bong Sen	*O. sativa*	Vietnam	KY	21
CR108	Chaw Ma-gawk	*O. sativa*	Thailand	KY	21
CR109	Leuang Plah Sew	*O. sativa*	Thailand	KY	21
CR110	Khao Praguad	*O. sativa*	Thailand	KY	21
CR111	Yenanine	*O. sativa*	Myanmar	KY	21
CR112	Taungdi	*O. sativa*	Myanmar	KY	21
CR113	Badobjota	*O. sativa*	Bangladesh	KY	21
CR114	Jhul Digha	*O. sativa*	Bangladesh	KY	21
CR115	Ashani	*O. sativa*	Bangladesh	KY	21
CR116	Bachogadi	*O. sativa*	Bangladesh	KY	21
CR117	Bajramuri	*O. sativa*	Bangladesh	KY	21
CR118	Chakla-59	*O. sativa*	India	KY	21
CR119	Madhukar	*O. sativa*	India	KY	21
CR120	Kalar Harsall	*O. sativa*	India	KY	21
TC65	Taichung 65	*O. sativa*	Taiwan	KY	21
Asominori	Asominori	*O. sativa*	Japan	KY	21
Kasalath	Kasalath	*O. sativa*	India	KY	21
W025(OG)	W025	*O. glaberrima*	West Africa	NIG	21
W106	W106	*O. rufipogon*	India (East Coast)	NIG	21
W120	W120	*O. rufipogon*	India (East Coast)	NIG	21
W137	W137	*O. rufipogon*	India (East Coast)	NIG	22
W168	W168	*O. rufipogon*	Thailand	NIG	21
W558	W558	*O. rufipogon*	Cambodia	NIG	21
W593	W593	*O. rufipogon*	Malaysia	NIG	22
W596	W596	*O. rufipogon.*	Malaysia	NIG	22
W600	W600	*O. rufipogon*	Malaysia	NIG	21
W625	W625	*O. rufipogon*	Myanmar	NIG	19
W629	W629	*O. rufipogon*	Myanmar	NIG	20
W630	W630	*O. rufipogon*	Myanmar	NIG	21
W1244	W1244	*O. rufipogon*	Nepal	NIG	22
W1297	W1297	*O. merdionalis*	Australia	NIG	21
W1299	W1299	*O. merdionalis*	Australia	NIG	21
W1625	W1625	*O. merdionalis*	Australia	NIG	21
W1626	W1626	*O. merdionalis*	Australia	NIG	21
W1627	W1627	*O. merdionalis*	Australia	NIG	19
W1629	W1629	*O. merdionalis*	Australia	NIG	20
W1631	W1631	*O. merdionalis*	Australia	NIG	21
W1633	W1633	*O. merdionalis*	Australia	NIG	20
W1634	W1634	*O. merdionalis*	Australia	NIG	21
W1636	W1636	*O. merdionalis*	Australia	NIG	19
W1637	W1637	*O. merdionalis*	Australia	NIG	21
W1654	W1654	*O. rufipogon*	China	NIG	21
W1680	W1680	*O. rufipogon*	India (East Coast)	NIG	22
W1724	W1724	*O. rufipogon*	China	NIG	22
W1800(s)	W1800(s)	*O. rufipogon*	Cambodia	NIG	21
W1802-1	W1802-1	*O. rufipogon*	Bangladesh	NIG	21
W1802-2	W1802-2	*O. rufipogon*	Bangladesh	NIG	22
W1806-1	W1806-1	*O. rufipogon*	Sri Lanka	NIG	22
W1807	W1807	*O. rufipogon*	Sri Lanka	NIG	22
W1811	W1811	*O. rufipogon*	Sri Lanka	NIG	20

Table 1. (Continued)

Accession number	Variety name/ accession code	Taxa	Origin	Source [1)]	Number of fragments [2)]
W1822	W1822	*O. rufipogon*	Bangladesh	NIG	21
W1863	W1863	*O. rufipogon*	Thailand	NIG	21
W1865	W1865	*O. rufipogon*	Thailand	NIG	21
W1866(s)	W1866(s)	*O. rufipogon*	Thailand	NIG	21
W1944	W1944	*O. rufipogon*	China	NIG	20
W1945	W1945	*O. rufipogon*	China	NIG	21
W1954	W1954	*O. rufipogon*	China	NIG	22
W1956	W1956	*O. rufipogon*	China	NIG	21
W1958–1	W1958–1	*O. rufipogon*	China	NIG	25
W1958–2	W1958–2	*O. rufipogon*	China	NIG	20
W1960	W1960	*O. rufipogon*	China	NIG	21
W1962	W1962	*O. rufipogon*	China	NIG	21
W1964	W1964	*O. rufipogon*	China	NIG	24
W1965(o)	W1965(o)	*O. rufipogon*	China	NIG	21
W1967	W1967	*O. rufipogon*	China	NIG	21
W1970–1	W1970–1	*O. rufipogon*	Indonesia	NIG	22
W1970–2	W1970–2	*O. rufipogon*	Indonesia	NIG	27
W1972–1	W1972–1	*O. rufipogon*	Indonesia	NIG	22
W1972–2	W1972–2	*O. rufipogon*	Indonesia	NIG	22
W1976	W1976	*O. rufipogon*	Indonesia	NIG	21
W1983	W1983	*O. rufipogon*	India (West Coast)	NIG	21
W1987	W1987	*O. rufipogon*	India (West Coast)	NIG	21
W2001	W2001	*O. rufipogon*	India (West Coast)	NIG	30
W2004	W2004	*O. rufipogon*	India (West Coast)	NIG	23
W2036	W2036	*O. rufipogon*	Myanmar	NIG	21
WK1	IRGC 100119	*O. barthii*	Mali	IRRI	21
WK2	IRGC 100223	*O. barthii*	Guinea	IRRI	21
WK5	IRGC 101257	*O. barthii*	Chad	IRRI	21
WK7	IRGC 103910	*O. barthii*	Tanzania	IRRI	21
WK8	IRGC 103912	*O. barthii*	Tanzania	IRRI	21
WK9	IRGC 104081	*O. barthii*	Nigeria	IRRI	20
WK10	IRGC 104103	*O. barthii*	Chad	IRRI	21
WK11	IRGC 104117	*O. barthii*	Chad	IRRI	21
WK12	IRGC 104140	*O. barthii*	Cameroon	IRRI	21
WK13	IRGC 104296	*O. barthii*	Cameroon	IRRI	20
WK14	IRGC 100854	*O. glaberrima*	Congo	IRRI	21
WK15	IRGC 103344	*O. glaberrima*	Senegal	IRRI	21
WK16	IRGC 103474	*O. glaberrima*	Burkina Faso	IRRI	21
WK17	IRGC 103594	*O. glaberrima*	Cameroon	IRRI	21
WK20	IRGC 104034	*O. glaberrima*	Ivory Coast	IRRI	21
WK21	IRGC 104038	*O. glaberrima*	Senegal	IRRI	21
WK22	IRGC 104042	*O. glaberrima*	Chad	IRRI	21
WK23	IRGC 104048	*O. glaberrima*	Cameroon	IRRI	21
WK24	IRGC 100184	*O. glumaepatula*	Cuba	IRRI	22
WK28	IRGC 103810	*O. glumaepatula*	Venezuela	IRRI	21
WK29	IRGC 105465	*O. glumaepatula*	French Guiana	IRRI	19
WK30	IRGC 105662	*O. glumaepatula*	Brazil	IRRI	20
WK31	IRGC 105663	*O. glumaepatula*	Brazil	IRRI	19
WK32	IRGC 105665	*O. glumaepatula*	Brazil	IRRI	19
WK33	IRGC 105666	*O. glumaepatula*	Brazil	IRRI	19

Table 1. (Continued)

Accession number	Variety name/ accession code	Taxa	Origin	Source [1]	Number of fragments [2]
WK34	IRGC 105667	*O. glumaepatula*	Brazil	IRRI	19
WK36	IRGC 105670	*O. glumaepatula*	Brazil	IRRI	19
WK37	IRGC 101200	*O. longistaminata*	Nigeria	IRRI	19
WK38	IRGC 101202	*O. longistaminata*	Nigeria	IRRI	25
WK41	IRGC 101216	*O. longistaminata*	Ivory Coast	IRRI	23
WK42	IRGC 101245	*O. longistaminata*	Mali	IRRI	23
WK43	IRGC 104060	*O. longistaminata*	Nigeria	IRRI	22
WK44	IRGC 104075	*O. longistaminata*	Nigeria	IRRI	29
WK45	IRGC 104977	*O. longistaminata*	Kenya	IRRI	23
WK47	IRGC 105204	*O. longistaminata*	Ethiopia	IRRI	24
WK51	IRGC 105306	*O. meridionalis*	Australia	IRRI	22
WK52	IRGC 105319	*O. nivara*	India	IRRI	21
WK53	IRGC 105428	*O. nivara*	Sri Lanka	IRRI	22
WK54	IRGC 105444	*O. nivara*	Sri Lanka	IRRI	21
WK56	IRGC 105715	*O. nivara*	Cambodia	IRRI	21
WK57	IRGC 106052	*O. nivara*	India	IRRI	21
WK58	IRGC 106111	*O. nivara*	India	IRRI	22
WK59	IRGC 106153	*O. nivara*	Laos	IRRI	21
WK60	IRGC 105709	*O. rufipogon*	India	IRRI	22
WK61	IRGC 105726	*O. rufipogon*	Cambodia	IRRI	21
WK62	IRGC 105889	*O. rufipogon*	Bangladesh	IRRI	23
WK63	IRGC 105898	*O. rufipogon*	Bangladesh	IRRI	25
WK64	IRGC 105910	*O. rufipogon*	Thailand	IRRI	22
WK66	IRGC 106036	*O. rufipogon*	Malaysia	IRRI	22
WK68	IRGC 106128	*O. rufipogon*	India	IRRI	24
WK69	IRGC 106157	*O. rufipogon*	Laos	IRRI	24
WK70	IRGC 106166	*O. rufipogon*	Vietnam	IRRI	25
OR1	VN90-WB5[3]	*O. rufipogon*	Vietnam	IRRI	25
OR2	PNG90-18[3]	*O. rufipogon*	Papua New Guinea	IRRI	20
OR3	PNG91-7[3]	*O. rufipogon*	Papua New Guinea	IRRI	20
OR4	P92-3A[3]	*O. rufipogon*	Philippines	IRRI	21
OR5	P92-3B[3]	*O. rufipogon*	Philippines	IRRI	21
OR6	P92-3C[3]	*O. rufipogon*	Philippines	IRRI	21
OR7	MY90-25[3]	*O. rufipogon*	Myanmar	IRRI	25
OR8	PNG90-27[3]	*O. rufipogon*	Papua New Guinea	IRRI	20
OR9	PNG90-34[3]	*O. rufipogon*	Papua New Guinea	IRRI	22
OR10	PNG90-40[3]	*O. rufipogon*	Papua New Guinea	IRRI	22
OR11	MV89-86[3]	*O. rufipogon*	India	IRRI	27
OR12	PNG90-10[3]	*O. rufipogon*	Papua New Guinea	IRRI	34
OR13	MV89-65[3]	*O. rufipogon*	India	IRRI	28
OR14	PNG90-13[3]	*O. rufipogon*	Papua New Guinea	IRRI	34
OR15	PNG90-14[3]	*O. rufipogon*	Papua New Guinea	IRRI	34

[1] KY=Kyushu University; NIAR=National Institute of Agrobiological Resources; NIG=National Institute of Genetics; IRRI=International Rice Research Institute.

[2] Number of fragments equals the total number of restriction fragments present in each accession across 21 clones.

[3] Only leaves were provided.

Table 2. Probes used in this study.

Probes	Chromosome	Number of unique fragments detected
Npb54	1	3
Npb113	1	4
Npb67	2	5
Npb132	2	3
Npb15	3	11
Npb129	3	8
Npb49	4	4
Npb114	4	4
Npb25	5	7
Npb81	5	3
Npb12	6	5
Npb27	6	2
Npb33	7	3
Npb117	7	2
Npb126	8	10
Npb13	9	7
Npb108	9	5
Npb32	10	7
Npb37	10	6
Npb115	11	7
Npb88	12	5

RFLP probes

RFLPs were detected from the combinations of *Dra* I-digested total DNA and twenty-one single-copy genomic clones (Saito *et al.*, 1991) listed in Table 2.

Data analysis

Each fragment was treated as a unit character, although fragments detected with one clone may be allelic. The character was quantified as 1 and 0 for the presence and absence of the fragment, respectively.

Genetic distances between accessions X and Y were calculated following the formula, $D=-ln\ [2M_{xy}/(M_x+M_y)]$, where M_x and M_y were the number of total fragments in accessions X and Y, respectively, and M_{xy} was the number of common fragments observed between accessions X and Y. This formula is based upon Nei's standard genetic distance (Nei, 1987, p 220 formula 9.24) with the accessions assumed to be homogenous. A dendrogram was then constructed using the UPGMA method (Sokal and Michener, 1958). All data analyses as well as the construction of the dendrogram were done on the Microsoft Excel application program.

Measures of genetic variation within and between groups were done following that of Wang *et al.* (1992). Genetic variation within groups was estimated as the mean genetic distances of all pair-wise comparisons between different accessions from the same group. Genetic variation between groups was estimated as the mean distances from different groups.

The relationship of Asian A-genome species was difficult to understand from the UPGMA dendrogram. One-hundred forty one accessions from Asia, therefore, were analyzed by principal component analysis (PCA). PCA was done using PCA command of MacMul (Thioulouse, 1990) with Centered PCA option.

RESULTS

Polymorphism

As the 21 probes used in this study were single copy clones from *O. sativa*, almost all the *O. sativa* acessions had 21 fragments. The total number of observed fragments per accession ranged from 19 to 34, with an average of 21.6. A total of 111 fragments was observed with all 21 probes.

Variation of A-genome species

The RFLP-derived dendrogram is shown in Fig. 1. Groups of *O. meridionalis*, *O. longistaminata*, and *O. glumaepatula* from Brazil were identified. These RFLP groupings corresponded well with the conventional classification supplied from each source of plant materials. However, the African annual species, *O. glaberrima* and *O. barthii*, were not clearly differentiated. '*O. glaberrima*' RFLP-group, therefore, contained both *O. glaberrima* and *O. barthii*. The Asian species, *O. sativa*, *O. rufipogon* and *O. nivara*, formed a complex wherein none of the species can be clearly separated from each other. In Fig. 1, four labels, 'Indica', 'Japonica', '*O. nivara*' and '*O. rufipogon*' were used for the sake of convenience. Two RFLP-groups, 'Indica' and 'Japonica' mainly contained Indica and Japonica cultivars, respectively. Each of them contained several wild accessions. The group labeled '*O. nivara*' was separated from the other Asian groups and joined *O. glumaepatula* and *O. glaberrima*. Other Asian accessions which clustered with neither cultivated rice nor '*O. nivara*' RFLP-group formed loosely knit sub-groups. '*O. rufipogon*' RFLP-group is a complex of these accessions. The three groups labeled 'Indica', 'Japonica', and '*O. rufipogon*' were the most closely related.

Variation both within and between the eight groups was evaluated. Mean, minimum and maximum genetic distances within and between the groups are shown in Table 3A, 3B and 3C, respectively. *O. longistaminata* showed the largest variation within group and *O. glaberrima* (including *O. barthii*) the smallest. '*O. rufipogon*' group similarly showed relatively large variation.

Based on the mean values of the distance between groups (Table 3A), a dendrogram of all species groups was constructed (Fig. 2). In this dendrogram, '*O. nivara*' RFLP-group was genetically more closely related to the other Asian groups.

PCA was performed to determine further the relationships of Asian species (*O. sativa*, *O. rufipogon*, *O. nivara*), but excluding OR12, 14 and 15 (see discussion) (Fig. 3). In the PCA, the first and second axis corresponded to Indica-Japonica and '*O. nivara*' group-other groups differentiation, respectively. The accessions from 'Japonica' RFLP-group formed a small group while 'Indica' accessions were scattered to a wide area, reflecting large variation. Accessions of '*O. nivara*' group formed a distinct group completely separated from all accessions.

Accession code	Source 1)	Original classification	RFLP classification2)	Origin
WK43	IRRI	O. longistaminata	O. nivara	Nigeria
WK58	IRRI	O. nivara	O. nivara	India
W137	NIG	O. rufipogon	O. nivara	India (east coast)
W593	NIG	O. rufipogon	O. nivara	Malaysia
WK60	IRRI	O. rufipogon	O. nivara	India
W1807	NIG	O. rufipogon	O. nivara	Sri Lanka
WK53	IRRI	O. nivara	O. nivara	Sri Lanka
W598	NIG	O. rufipogon	O. nivara	Malaysia
W1244	NIG	O. rufipogon	O. nivara	Nepal
W1680	NIG	O. rufipogon	O. nivara	India (east coast)
WK54	IRRI	O. nivara	O. nivara	Sri Lanka
WK52	IRRI	O. nivara	O. nivara	India
W2004	NIG	O. rufipogon	O. nivara	India (west coast)
WK31	IRRI	O. glumaepatula	O. glumaepatula	Brazil
WK33	IRRI	O. glumaepatula	O. glumaepatula	Brazil
WK30	IRRI	O. glumaepatula	O. glumaepatula	Brazil
WK32	IRRI	O. glumaepatula	O. glumaepatula	Brazil
WK34	IRRI	O. glumaepatula	O. glumaepatula	Brazil
WK36	IRRI	O. glumaepatula	O. glumaepatula	Brazil
WK29	IRRI	O. glumaepatula	O. glumaepatula	French Guiana
WK8	IRRI	O. barthii	O. glaberrima	Tanzania
WK11	IRRI	O. barthii	O. glaberrima	Chad
WK1	IRRI	O. barthii	O. glaberrima	Mali
WK5	IRRI	O. barthii	O. glaberrima	Chad
WK13	IRRI	O. barthii	O. glaberrima	Cameroon
W025(OG)	NIG	O. glaberrima	O. glaberrima	West Africa
WK2	IRRI	O. barthii	O. glaberrima	Guinea
WK10	IRRI	O. barthii	O. glaberrima	Chad
WK12	IRRI	O. barthii	O. glaberrima	Cameroon
WK14	IRRI	O. glaberrima	O. glaberrima	Congo
WK15	IRRI	O. glaberrima	O. glaberrima	Senegal
WK16	IRRI	O. glaberrima	O. glaberrima	Burkina Faso
WK17	IRRI	O. glaberrima	O. glaberrima	Cameroon
WK20	IRRI	O. glaberrima	O. glaberrima	Ivory Coast
WK22	IRRI	O. glaberrima	O. glaberrima	Chad
WK23	IRRI	O. glaberrima	O. glaberrima	Cameroon
WK21	IRRI	O. glaberrima	O. glaberrima	Senegal
WK7	IRRI	O. barthii	O. glaberrima	Tanzania
WK9	IRRI	O. barthii	O. glaberrima	Nigeria
W1958-2	NIG	O. rufipogon	O. rufipogon	China
W2001	NIG	O. rufipogon	O. rufipogon	India (west coast)
W1944	NIG	O. rufipogon	Indica	China
W2036	NIG	O. rufipogon	Indica	Myanmar
W625	NIG	O. rufipogon	Indica	Myanmar
W829	NIG	O. rufipogon	Indica	Myanmar
W1800(s)	NIG	O. rufipogon	Indica	Cambodia
W1802-1	NIG	O. rufipogon	Indica	Bangladesh
W1865	NIG	O. rufipogon	Indica	Thailand
W1802-2	NIG	O. rufipogon	Indica	Bangladesh
W1806-1	NIG	O. rufipogon	Indica	Sri Lanka
W1954	NIG	O. rufipogon	Indica	China
CR1	NIAR	O. sativa	Indica	South China
W1972-1	NIG	O. rufipogon	Indica	Indonesia
WK86	IRRI	O. rufipogon	Indica	Malaysia
CR3	NIAR	O. sativa	Indica	Sri Lanka
CR5	NIAR	O. sativa	Indica	Myanmar
CR6	NIAR	O. sativa	Indica	Nepal
CR7	NIAR	O. sativa	Indica	Assam
CR9	NIAR	O. sativa	Indica	Myanmar
CR13	NIAR	O. sativa	Indica	Malaysia
CR18	NIAR	O. sativa	Indica	Vietnam
CR17	NIAR	O. sativa	Indica	Vietnam
CR44	NIAR	O. sativa	Indica	India
CR101	KY	O. sativa	Indica	China
CR103	KY	O. sativa	Indica	Cambodia
CR106	KY	O. sativa	Indica	Vietnam
CR107	KY	O. sativa	Indica	Vietnam
CR108	KY	O. sativa	Indica	Thailand
CR110	KY	O. sativa	Indica	Thailand
CR111	KY	O. sativa	Indica	Myanmar
W558	NIG	O. rufipogon	Indica	Cambodia
W1970-1	NIG	O. rufipogon	Indica	Indonesia
W1822	NIG	O. rufipogon	Indica	Bangladesh
CR4	NIAR	O. sativa	Indica	Yunnan
CR114	KY	O. sativa	Indica	Bangladesh
CR109	KY	O. sativa	Indica	Thailand
WK57	IRRI	O. nivara	Indica	India
W1811	NIG	O. rufipogon	Indica	Sri Lanka
W800	NIG	O. rufipogon	Indica	Malaysia
WK56	IRRI	O. nivara	Indica	Cambodia
W630	NIG	O. rufipogon	Indica	Myanmar
WK61	IRRI	O. rufipogon	Indica	Cambodia
W1972-2	NIG	O. rufipogon	Indica	Indonesia
WK70	IRRI	O. rufipogon	Indica	Vietnam
CR41	KY	O. sativa	Indica	Malaysia
CR102	KY	O. sativa	Indica	Cambodia
CR2	NIAR	O. sativa	Indica	South China
CR10	NIAR	O. sativa	Indica	Thailand
CR11	NIAR	O. sativa	Indica	Laos
CR14	NIAR	O. sativa	Indica	Malaysia
CR15	NIAR	O. sativa	Indica	Vietnam
CR31	NIAR	O. sativa	Indica	Indonesia
CR104	KY	O. sativa	Indica	Cambodia
CR105	KY	O. sativa	Indica	Vietnam
CR112	KY	O. sativa	Indica	Myanmar
CR113	KY	O. sativa	Indica	Bangladesh
CR115	KY	O. sativa	Indica	Bangladesh
W168	NIG	O. rufipogon	Indica	Thailand
W1983	NIG	O. rufipogon	Indica	India (west coast)
CR12A	NIAR	O. sativa	Indica	Malaysia
IR24	KY	O. sativa	Indica	Philippines
CR43	NIAR	O. sativa	Indica	Philippines
WK24	IRRI	O. glumaepatula	Indica	Cuba
CR8	NIAR	O. sativa	Indica	Bangladesh
CR33	NIAR	O. sativa	Indica	Nepal
CR120	KY	O. sativa	Indica	India
W1863	NIG	O. rufipogon	Indica	Thailand
Kasalath	KY	O. sativa	Indica	India
W1866(e)	NIG	O. rufipogon	Indica	Thailand
W106	NIG	O. rufipogon	Indica	India (east coast)
WK28	IRRI	O. glumaepatula	Indica	Venezuela
CR16	NIAR	O. sativa	Indica	India
CR19B	NIAR	O. sativa	Indica	India
WK69	IRRI	O. rufipogon	Indica	Laos
W1956	NIG	O. rufipogon	Indica	China
W1958-1	NIG	O. rufipogon	Indica	China
W1654	NIG	O. rufipogon	Indica	China
WK59	IRRI	O. nivara	Indica	Laos
W1965(o)	NIG	O. rufipogon	Japonica	China
W1978	NIG	O. rufipogon	Japonica	Indonesia
Java14	KY	O. sativa	Japonica	Indonesia
CR25	NIAR	O. sativa	Japonica	Philippines
CR26	NIAR	O. sativa	Japonica	Indonesia
CR27	NIAR	O. sativa	Japonica	Myanmar
CR28	NIAR	O. sativa	Japonica	Philippines
CR29	NIAR	O. sativa	Japonica	Philippines
CR32	NIAR	O. sativa	Japonica	Indonesia
CR119	KY	O. sativa	Japonica	India
W1724	NIG	O. rufipogon	Japonica	China
Nipponbare	KY	O. sativa	Japonica	Japan
CR20	NIAR	O. sativa	Japonica	Japan
CR21	NIAR	O. sativa	Japonica	Japan
CR22	NIAR	O. sativa	Japonica	South America
CR24B	NIAR	O. sativa	Japonica	Laos
CR30	NIAR	O. sativa	Japonica	Malaysia
CR42	NIAR	O. sativa	Japonica	Japan
CR45	KY	O. sativa	Japonica	Japan
CR117	KY	O. sativa	Japonica	Bangladesh
TC65	KY	O. sativa	Japonica	Taiwan
Asominori	KY	O. sativa	Japonica	Japan
CR23	NIAR	O. sativa	Japonica	Pakistan
W1967	NIG	O. rufipogon	Japonica	China
W1962	NIG	O. rufipogon	Japonica	China
W1964	NIG	O. rufipogon	Japonica	China
W1960	NIG	O. rufipogon	Japonica	China
CR34	NIAR	O. sativa	Japonica	Nepal
CR35	NIAR	O. sativa	Japonica	Nepal
CR118	KY	O. sativa	Japonica	India
CR116	KY	O. sativa	Japonica	Bangladesh
CR36	NIAR	O. sativa	Japonica	Bangladesh
W1987	NIG	O. rufipogon	Japonica	India (west coast)
W1945	NIG	O. rufipogon	O. rufipogon	China
W1970-2	NIG	O. rufipogon	O. rufipogon	Indonesia
WK82	IRRI	O. rufipogon	O. rufipogon	Bangladesh
W120	NIG	O. rufipogon	O. rufipogon	India (east coast)
WK84	IRRI	O. rufipogon	O. rufipogon	Thailand
WK83	IRRI	O. rufipogon	O. rufipogon	Bangladesh
OR11	IRRI	O. rufipogon	O. rufipogon	India
OR13	IRRI	O. rufipogon	O. rufipogon	India
WK88	IRRI	O. rufipogon	O. rufipogon	India
OR1	IRRI	O. rufipogon	O. rufipogon	Vietnam
OR7	IRRI	O. rufipogon	O. rufipogon	Myanmar
OR3	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR10	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR2	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR8	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR9	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR4	IRRI	O. rufipogon	O. rufipogon	Philippines
OR5	IRRI	O. rufipogon	O. rufipogon	Philippines
OR6	IRRI	O. rufipogon	O. rufipogon	Philippines
OR12	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR14	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
OR15	IRRI	O. rufipogon	O. rufipogon	Papua New Guinea
WK37	IRRI	O. longistaminata	O. longistaminata	Nigeria
WK45	IRRI	O. longistaminata	O. longistaminata	Kenya
WK47	IRRI	O. longistaminata	O. longistaminata	Ethiopia
WK38	IRRI	O. longistaminata	O. longistaminata	Nigeria
WK41	IRRI	O. longistaminata	O. longistaminata	Ivory Coast
WK42	IRRI	O. longistaminata	O. longistaminata	Mali
WK44	IRRI	O. longistaminata	O. longistaminata	Nigeria
W1299	NIG	O. meridionalis	O. meridionalis	Australia
W1625	NIG	O. meridionalis	O. meridionalis	Australia
W1626	NIG	O. meridionalis	O. meridionalis	Australia
W1634	NIG	O. meridionalis	O. meridionalis	Australia
W1297	NIG	O. meridionalis	O. meridionalis	Australia
W1627	NIG	O. meridionalis	O. meridionalis	Australia
W1636	NIG	O. meridionalis	O. meridionalis	Australia
W1629	NIG	O. meridionalis	O. meridionalis	Australia
W1631	NIG	O. meridionalis	O. meridionalis	Australia
W1637	NIG	O. meridionalis	O. meridionalis	Australia
W1633	NIG	O. meridionalis	O. meridionalis	Australia
WK51	IRRI	O. meridionalis	O. meridionalis	Australia

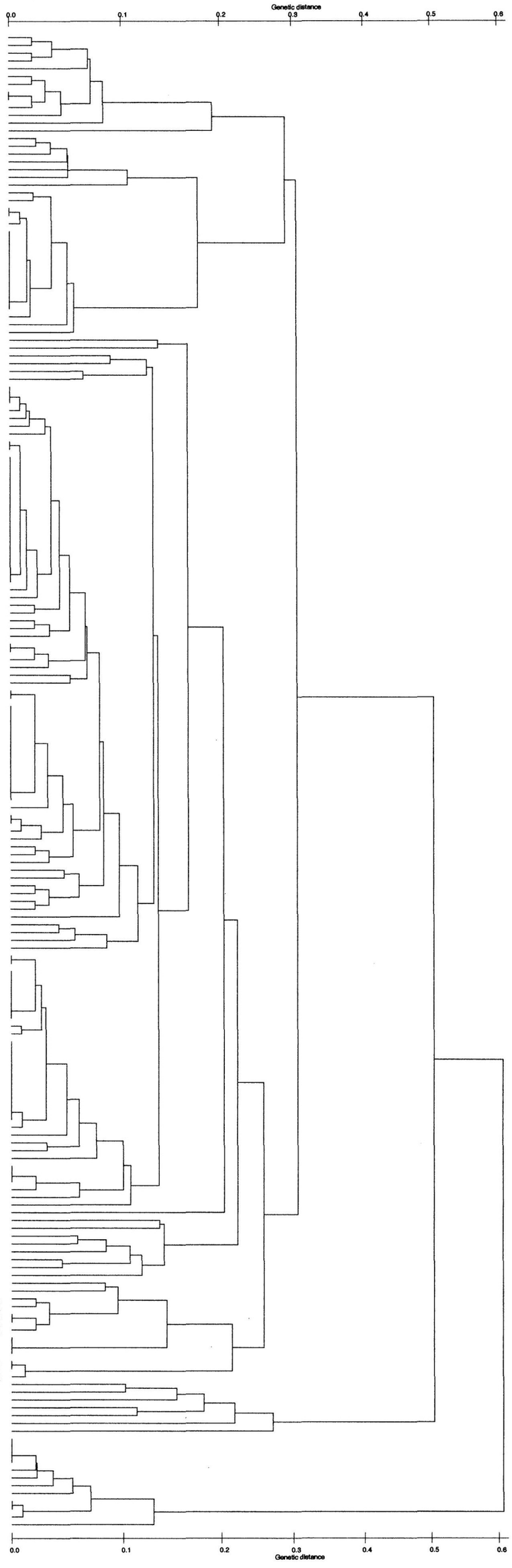


Fig. 1. RFLP-derived dendrogram of all 192 accessions. RFLPs were detected with 21 genomic clones. 1) KY=Kyushu University; NIAR=National Institute of Agrobiological Resources; NIG=National Institute of Genetics; IRRI=International Rice Research Institute. 2) RFLP classification based on the dendrogram.

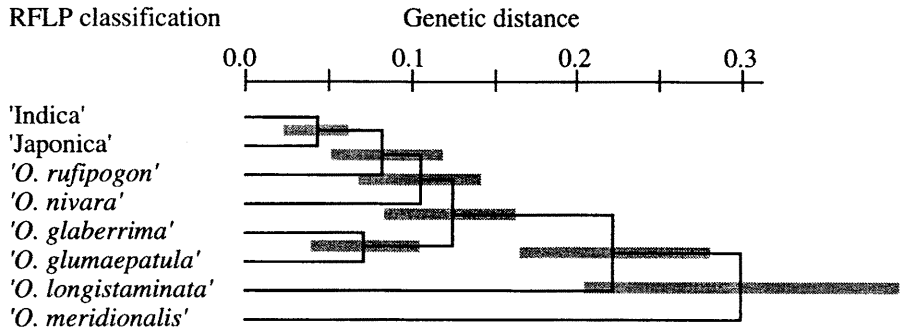


Fig. 2. Dendrogram of A-genome species in the genus *Oryza* based on the recalculated genetic distance among RFLP-groups shown in Table 3A. Gray bars indicate standard deviation of genetic distance at each branch.

DISCUSSION

An average of 5.3 (111/21) unique fragments per probe is between the 3.4 and 11.2 observed in previous studies which included 70 accessions of *O. sativa* (Wang and Tanksley, 1989) and 93 accessions from the whole genus *Oryza* (Wang *et al.*, 1992), respectively. This value is reasonable because the germplasm used in the present study covers A-genome rices.

Nakano *et al.* (1992) identified a unique group of wild rice accessions (Cluster 3). '*O. nivara*' RFLP-group in the present study corresponded to Nakano's cluster 3 (Nakano *et al.*, 1992). It contains 4 out of 7 accessions of *O. nivara* identified at IRRI while only one out of 24 accessions of *O. rufipogon* from IRRI was included. The results of this study, therefore, support the concept of the taxonomic species *O. nivara*. RFLP analysis showed that this group is clearly differentiated from other Asian A-genome germplasm, although we have not yet been able to find any clear key morphological characters to distinguish all members of '*O. nivara*' RFLP-group from *O. rufipogon*. However, since gene flow occurs between A-genome *Oryza* species growing sympatrically in Asia, clear differentiation of these taxonomic species is quite difficult. RFLP markers, as shown here, were useful in identifying specific groups within the species complex.

In this study eight accessions of *O. rufipogon* were in the 'Japonica' group. Six of these accessions came from China. This implies that the genetic base of Japonica is in China. Accessions of *O. rufipogon* from many South and Southeast Asian countries were aligned with Indica varieties. This may reflect the fact that in the lowland tropics of South and Southeast Asia today most varieties are Indica varieties and in many areas, grow sympatrically with wild rice. Recent studies (Wang *et al.*, 1992; Nakano *et al.*, 1992) have shown that some populations of *O. rufipogon* and *O. nivara* complex have a very close relationship to cultivated rice.

Accessions of '*O. rufipogon*' RFLP-group were mainly from Southeast Asia. They appeared to form two relatively large sub-groups and other small groups, although

Table 3. Mean(A), minimum(B) and maximum(C) genetic distance within and between RFLP-identified groups. Classification was based on RFLP-derived dendrogram (see text). The mean distance between all accessions was 0.4323.

A

	Within group	Between groups						
		'Japonica'	'*O. rufipogon*'	'*O. nivara*'	'*O. glaberrima*'	'*O. glumaepatula*'	'*O. longistaminata*'	'*O. meridionalis*'
'Indica'	0.12	0.18	0.33	0.30	0.35	0.44	0.86	1.24
'Japonica'	0.08		0.35	0.38	0.34	0.41	0.96	1.34
'*O. rufipogon*'	0.38			0.52	0.51	0.58	0.99	1.42
'*O. nivara*'	0.16				0.49	0.60	0.92	0.95
'*O. glaberrima*'	0.05					0.29	0.86	1.21
'*O. glumaepatula*'	0.13						0.95	1.50
'*O. longistaminata*'	0.41							1.26
'*O. meridionalis*'	0.13							

B

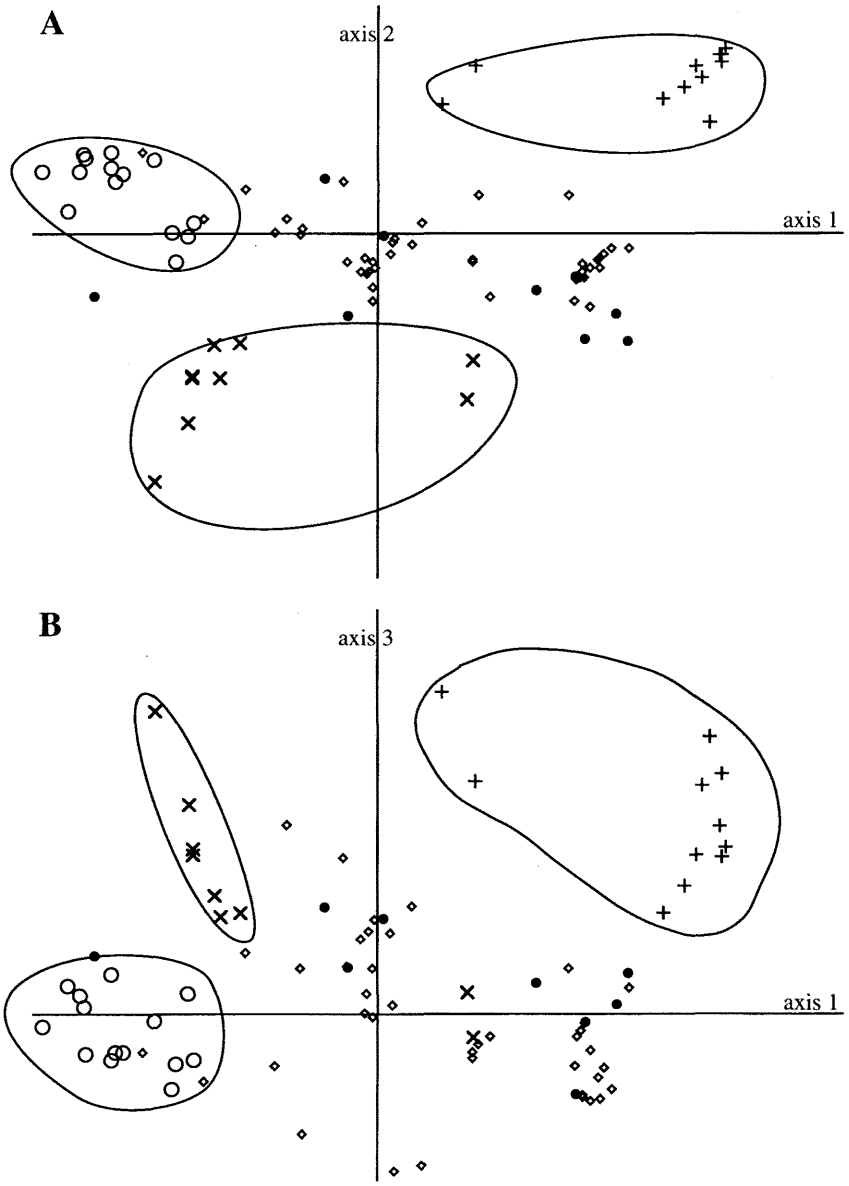
	Within group	Between groups						
		'Japonica'	'*O. rufipogon*'	'*O. nivara*'	'*O. glaberrima*'	'*O. glumaepatula*'	'*O. longistaminata*'	'*O. meridionalis*'
'Indica'	0.00	0.02	0.07	0.10	0.13	0.26	0.65	0.94
'Japonica'	0.00		0.13	0.23	0.21	0.22	0.72	1.07
'*O. rufipogon*'	0.00			0.21	0.31	0.31	0.67	0.44
'*O. nivara*'	0.00				0.32	0.46	0.73	0.74
'*O. glaberrima*'	0.00					0.20	0.61	1.05
'*O. glumaepatula*'	0.05						0.65	1.34
'*O. longistaminata*'	0.21							0.98
'*O. meridionalis*'	0.00							

C

	Within group	Between groups						
		'Japonica'	'*O. rufipogon*'	'*O. nivara*'	'*O. glaberrima*'	'*O. glumaepatula*'	'*O. longistaminata*'	'*O. meridionalis*'
'Indica'	0.41	0.48	0.68	0.61	0.54	0.69	1.43	1.46
'Japonica'	0.28		0.68	0.65	0.54	0.69	1.43	1.66
'*O. rufipogon*'	0.73			0.87	0.72	0.92	1.41	2.33
'*O. nivara*'	0.45				0.65	0.82	1.30	1.28
'*O. glaberrima*'	0.15					0.49	1.14	1.44
'*O. glumaepatula*'	0.26						1.79	1.66
'*O. longistaminata*'	0.62							1.68
'*O. meridionalis*'	0.34							

genetic distance among these groups were too small to consider them as differentiated groups. One relatively large group (which contains W1970-2, WK62, W120, etc.) included accessions mainly from continental Asia that were relatively closer to 'Indica' group based on RFLP-derived dendrogram and PCA analysis (Fig. 3). The other sub-group (which contains OR1, OR7, OR3, etc.) was mainly from the Philippines and Papua New Guinea. Accessions in this sub-group had rather distinctive RFLP variation suggesting that these may have evolved in isolation for a long time compared with other accessions of *O. sativa* and *O. rufipogon*.

O. glumaepatula accessions in Brazil were classified into one group. This species had closer RFLP-relationship with the '*O. glaberrima*' group than with Asian species, although this species has been considered to be a subtype of *O. rufipogon*. This results



○ : 'Japonica' in Fig. 1.
◇ : 'Indica' in Fig. 1.
● ✕ : *'O. rufipogon'* in Fig. 1. (● : continental Asia, ✕: Philippines, Papua New Guinea)
\+ : *'O. nivara'* in Fig. 1.

Fig. 3. Principal component analysis (PCA) of 141 accessions from the *O. sativa*, *O. rufipogon* and *O. nivara*. The first axis extract 19% of total variation, the second, 12% and the third, 11%. Accessions were plotted in the plane defined by axes 1 and 2 of PCA (A), and 1 and 3 (B).

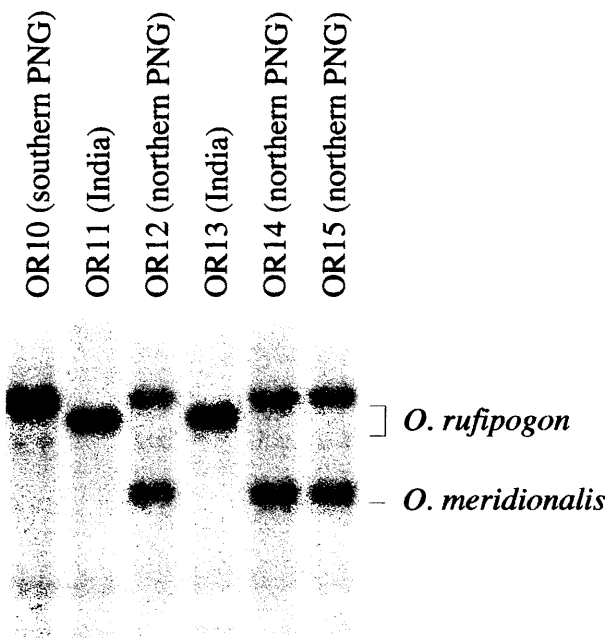


Fig. 4. Autoradiogram of six *O. rufipogon* accessions from India and Papua New Guinea (OR10–OR15) probed with Npb81. OR12, OR14 and OR15 show two bands, one is typical of *O. rufipogon* while the other is typical of *O. meridionalis*.

support previous reports based on ribosomal DNA variation (Sano and Sano, 1989) and RFLP variation (Wang *et al.*, 1992).

WK24 and WK28 were originally classified as *O. glumaepatula*, but in this study, they were classified with Asian species. It is possible that there are actually two types of *O. glumaepatula* in Latin America, an Brazilian race of *O. glumaepatula* which is different from the types found in other parts of Latin America. Second (1985) regarded the American form of *O. perennis* as a subtype of *O. rufipogon*. However, he did not test any Brazilian accession. As the origin of *O. glumaepatula* is not clear (Vaughan, 1989), it is necessary to determine the full range of variation of wild A-genome germplasm from Latin America.

Seven *O. longistaminata* accessions (WK37, 38, 41, 42, 44, 45 and 47) had multiple RFLP fragments with 4 to 8 probes and no signal with two probes, which indicates their high heterozygosity. This species group also showed the largest intra-group variation (Table 3). In this study, however, no information on intra-population variation was obtained as only one plant per accession was tested. Since some useful genes have already been found in *O. longistaminata* (Khush *et al.*, 1990; Maekawa and Tsunoda, 1994), a better understanding of the diversity in this African perennial species is needed.

O. glaberrima and *O. barthii* showed little RFLP variation. *O. glaberrima* has been reported to have little intra-specific variation (Miezan, 1986; Ishii *et al.*, 1988). Results from the present study revealed that *O. barthii* also had little nuclear RFLP variation.

O. rufipogon in Papua New Guinea formed two distinct groups. One group (OR3, 10, 2, 8 and 9) originated from the south of the country. The other group (OR12, 14 and 15) was collected along the Sepik river in the north of Papua New Guinea. The accessions from Northern Papua New Guinea have RFLP fragments which are present in both *O. rufipogon* and *O. meridionalis* (Fig. 4). These accessions (OR12, 14 and 15) are morphologically distinct having large spikelets and sterile lemma and considered floating perennial wild rices (Vaughan, 1990). On the other hand, the accessions from Southern Papua New Guinea have short genetic distance to other Asian accessions. This supports earlier reports that two types of *O. rufipogon* are present on New Guinean island, the Asian and the Oceanian types (Morishima, 1969).

Both annual/biennial *O. meridionalis* and its perennial relatives have not been reported from Papua New Guinea. In this study *O. rufipogon* from the Sepik river of Papua New Guinea have RFLP fragments which were found only in *O. meridionalis* (Fig. 4). Additional survey of germplasm from New Guinean island and Australia, including *O. rufipogon* from Australia not included in this study, therefore, is necessary to clarify the evolution and diversity of A-genome germplasm in this region.

Presently, new information on the A-genome species has been shown. However, to fully understand the diversity in this species complex, integration of data from hybridization, ecological, morphological, isozyme, organelle DNA and nuclear DNA studies is necessary.

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