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ORIGINAL PAPER

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A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers

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Abstract In order to estimate genetic relationships of the AA-genome *Oryza* species, RAPD and SSR analyses were performed with 45 accessions, including 13 cultivated varieties (eight *Oryza sativa* and five *Oryza glaberrima*) and 32 wild accessions (nine *Oryza rufipogon*, seven *Oryza nivara*, three *Oryza glumaepatula*, four *Oryza longistaminata*, six *Oryza barthii*, and three *Oryza meridionalis*). A total of 181 clear and repeatable bands were amplified from 27 selected RAPD primers, and 101 alleles were detected from 29 SSR primer pairs. The dendrogram constructed using UPGMA from a genetic-similarity matrix based on the RAPD data supported the clustering of distinct five groups with a few exceptions: *O. rufipogon*/*O. nivara*/*O. meridionalis*, *O. barthii*/*O. glaberrima*, *O. glumaepatula*, *O. sativa* and *O. longistaminata.* The dendrogram based on the SSR analysis showed a more-complicated genetic variation pattern, but the *O. longistaminata* and *O. barthii*/*O. glaberrima* accessions were consistently separated from all other accessions, indicating significant differentiation of the African AA-genome *Oryza* species. For accessions in the *O. rufipogon*/*O. nivara*/*O. sativa* complex, it is apparent that geographical isolation has played an important role in differentiation of the Asian AA-genome *Oryza* taxa. It is also demonstrated from this study that both RAPD and SSR analyses are powerful methods for detecting polymorphisms among the different AA-genome *Oryza* accessions. However, the RAPD analysis provides a more-informative result in terms of the overall genetic relationships at the species level compared to the SSR analysis. The SSR analysis effectively reveals diminutive variation among accessions or individuals within the same species, given approximately the same number of primers or primer-pairs used in the studies.

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Introduction

The genus *Oryza* L. is an economically valuable plant group in the grass family (Poaceae), because it includes the world's single most-important food crop, rice, that is a primary food source for more than one-third of the global population (Khush 1997). There are two cultivated rice species and over 20 wild species widely distributed in the pan-tropics and subtropics (Lu 1998). The Asian cultivated rice *Oryza sativa* originated in South and Southeast Asia (Chang 1985) and is now grown worldwide, whereas the African cultivated rice, *Oryza glaberrima*, domesticated in western Africa, is only cultivated in local agricultural ecosystems in West Africa. All wild species in *Oryza* are important genetic resources and have played significant roles in rice breeding by contributing genes valuable for resistance to disease and insect pests, and tolerance to abiotic stress (Chang et al. 1975; Sitch et al. 1989; Khush 1997).

Species in the genus are highly diverse in morphology (Clayton and Renvoize 1986; Vaughan 1994), and are represented genetically by ten different genome types, i.e. the AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH and JJKK genomes, providing opportunities to explore variations for rice breeding programs. Of the wild relatives, those containing the AA-genome are the most accessible genetic resources, because the cultivated *O. sativa* also shares the same AA-genome, and transfer of alien genes from those wild relatives can be easily achieved through sexual hybridization. As a matter of fact, only a small portion of the valuable genes has been utilized through introgression into cultivated rice at present (Jena and

Khush 1990). The fuller exploitation of the wild-rice gene pool essentially relies on the better understanding of genetic diversity and relationships of the *Oryza* species, which will pose a significant impact on rice production (Sharma 1983). Therefore, a thorough assessment of genetic diversity and relationships of rice and its wild relatives, particularly those with the AA-genome, has become increasingly important not only for their efficient utilization, but also for their effective conservation (Sun et al. 2001; Lu et al. 2002).

For the assessment of genetic relationships of *Oryza* species, several types of protein and molecular markers have been applied, including isozyme (Second 1982), RAPD (Ishii et al. 1996; Martin et al. 1997), RFLP (Sun et al. 2001; Lu et al. 2002), AFLP (Aggarwal et al. 1999) and inter simple-sequence repeat (ISSR) markers (Joshi et al. 2000). Advantages as well as disadvantages are observed with these methods. The RAPD technique has several distinct advantages over isozyme and other DNA fingerprinting technologies in the speed of data-acquisition possible, the low cost of reactions, the small amounts of plant material required and the ability to perform analyses without the need for prior sequencing of the genome. (Huff et al. 1993). Because of these advantages, RAPD analysis has recently become a popular method for estimating genetic diversity and relationships among plant populations, involving the characterization of cultivars and germplasm accessions (Yu and Nguyen 1994; Virk et al.1995; Ishii et al.1996; Martin et al.1997; Buso et al.1998; Qian et al. 2001).

Microsatellites, also referred to as simple sequence repeats (SSR), are a useful marker for genome analysis, because of the significant level of allelic diversity that may be revealed (Ishii et al. 2001). Polymorphisms in the microsatellite region are considered to result from misreplication of repeated sequences (Richards and Sutherland 1994), and the polymorphisms can be readily detected by PCR, using pairs of primers specific to the sequences flanking the microsatellite repeats. The microsatellite markers are supposed to be particularly suitable for evaluating genetic diversity and relationships among closely related plant accessions or individuals, such as different rice cultivars (Akagi et al. 1997).

The objectives of this study were to estimate genetic relationships of the closely related *Oryza* species sharing the AA-genome using RAPD and SSR marker technologies, and to assess the levels of polymorphisms detected by the two different methods.

Materials and methods

Plant materials

Both cultivated and wild *Oryza* species with the AA-genome used in this study were donated by the International Rice Gene Bank, at the International Rice Research Institute (IRRI), Los Baños, Philippines. A total of 45 accessions representing diverse origins were analyzed, including 13 cultivated accessions (eight *Oryza sativa* and five *Oryza glaberrima*) and 32 wild accessions (nine *Oryza rufipogon* and seven *Oryza nivara* accessions from Asia, three *Oryza glumaepatula* accessions from the Latin America, four *Oryza longistaminata* and six *Oryza barthii* accessions from Africa, and three *Oryza meridionalis* accessions from Australia, see Table 1). One seedling each from germinated seed was used to represent the individual accessions analyzed.

Total genomic DNA extraction

A simple DNA extraction procedure was performed using a modified CTAB (cetyltrimethyl ammonium bromide) method described by Murray and Thompson (1980). All DNA samples were extracted from young leaves generated from germinated seeds.

RAPD PCR amplification

A total of 27 arbitrary primers from Shanghai Sangon Biological Engineering Technology and Service Co. Ltd were used for RAPD analysis. DNA amplification was performed in a PTC-100 (Programmable Thermal Controller), programmed for an initial 5 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 38°C, and 1.5 min at 72°C and 8 min at 72°C for the final extension. Reactions were carried out in a total volume of 25 μl containing 10 mM of Tris·HCl (pH 8.8), 25 mM of KCl, 1.5 mM of $MgCl_2$, 0.2 μM each of dATP, dCTP, dGTP and dTTP, 0.2 μM of random primer, 100 ng of genomic DNA and 1 unit of *Taq* polymerase. The amplified RAPD fragments were separated electrophoretically in 1.5% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light using a Gel Doc 2000 (Bio-RAD), and saved into a computer. Molecular weights were estimated using a PCR marker (DL2000 Takara).

SSR PCR amplification

A total of 29 nuclear microsatellite (SSR) markers were examined. At least two primer pairs were selected from each of the linkage groups corresponding to the 12 chromosomes of the cultivated rice. PCR was performed in a total volume of a 25 μl reaction-solution containing 100 ng of template DNA,10 mM of Tris·HCl (pH 8.8), 50 mM of KCl, 0.001% Gelatin, 2.5 mM of $MgCl_2$, 0.2 μM each of dATP, dCTP, dGTP and dTTP, 0.2 μM of SSR primer pairs, and one unit of *Taq* polymerase. DNA amplification was performed in a PTC-100 (Programmable Thermal Controller), programmed for an initial 5 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 55°C, and 1.5 min at 72°C and 8 min at 72°C, for the final extension. The amplified SSR products were separated electrophoretically in 4% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light using a Gel Doc 2000 (Bio-RAD), and saved into a computer. Molecular weights were estimated using a PCR marker (DL2000 Takara).

Data encoding and analysis

Polymorphic fragments from each primer or primer-pair detected among the 45 accessions were used to make comparisons between accessions. The dominant RAPD markers were scored as present (1) or absent (0) for each DNA sample, and the co-dominant SSR markers were scored as homozygous alleles (e.g. AA or BB) and the heterozygous allele (AB) for each DNA sample. The generated data matrixes were subject to a statistical analysis using an NTSYS-pc analytical software (Rohlf 1994). For the RAPD markers, the Jaccard coefficient was calculated as a relative measure of similarity among the sampled individuals. For the SSR alleles, the ratios of shared DNA fragments and similarity coefficients between accessions were quantified according to Nei (1978). Dendrograms showing genetic relationships of the 45 accessions was constructed using the unweighted pair-group method with the

Table 1 The AA-genome *Oryza* species used in this study for the RAPD and SSR analyses with their code, accession number and origins

No.	Species	Code	Accession no.	Origin
1	*O. barthii*	BA-Cam	101255	Yagoua, Cameroon
2	*O. barthii*	BA-Sen	101959	Senegal
3	*O. barthii*	BA-Cha	103580	N'Djamena-att, Yao, Chad
4	*O. barthii*	BA-Nig-1	104078	Sakoto, Nigeria
5	*O. barthii*	BA-Nig-2	104081	Arugungu, Nigeria
6	*O. barthii*	BA-Gui	106194	Boro, Guinea
7	*O. glaberrima*	GA-Sen	101791	Senegal
8	*O. glaberrima*	GA-Bfa	101855	Burkina Faso
9	*O. glaberrima*	GA-Mal	102452	Mali
10	*O. glaberrima*	GA-Nig	104540	Nigeria
11	*O. glaberrima*	GA-Lib	102641	Liberia
12	*O. glumaepatula*	GL-Sur	100968	Paramaribo, Suriname
13	*O. glumaepatula*	GL-Col	105561	Meta, Colombia
14	*O. glumaepatula*	GL-Bra	105661	Pracwba, Amap, Brazil
15	*O. longistaminata*	LO-Ico	101213	Korhogo, Ferkesseougou, Ivory Coast
16	*O. longistaminata*	LO-Cha	104127	Bousso, N'Djamena, Chad
17	*O. longistaminata*	LO-Cam	104147	Maroua, Maroua, Cameroon
18	*O. longistaminata*	LO-Eth	105204	Gambela, Gonder/Debretabor, Ethiopia
19	*O. meridionalis*	ME-Aus-1	101411	Northern Territory, Darwin, Australia
20	*O. meridionalis*	ME-Aus-2	105283	Northern Territory, Darwin, Australia
21	*O. meridionalis*	ME-Aus-3	105303	Weipa-Hurukum, Queensland, Australia
22	*O. nivara*	NI-Ban	103836	Tarash, Pabna, Bangladesh
23	*O. nivara*	NI-Ind-1	101971	Madhaya Pradesh, Jeypore, India
24	*O. nivara*	NI-Ind-2	104705	Saligaou, Maharashtra, India
25	*O. nivara*	NI-Sla	105419	Nochchivagama, Anuradhapura, Sri Lanka
26	*O. nivara*	NI-Nep	105704	Koholpul Panchayat, Banke, Nepal
27	*O. nivara*	NI-Cab-1	105736	Makara, Takeo, Cambodia
28	*O. nivara*	NI-Cab-2	106309	Koksai, Phnom Penh, Cambodia
29	*O. rufipogon*	RU-Chi-1	D-01	Dongxiang, Jiangxi, China
30	*O. rufipogon*	RU-Chi-2	D-02	Dongxiang, Jiangxi, China
31	*O. rufipogon*	RU-Chi-3	G-01	Guangxi, China
32	*O. rufipogon*	RU-Tha	100219	Chumpae, Konkhen, Thailand
33	*O. rufipogon*	RU-Ban	105887	Fakirhat, Bagerhat, Bangladesh
34	*O. rufipogon*	RU-Mal	106036	Kota Bharu, Kelangtan, Malaysia
35	*O. rufipogon*	RU-Ind	106083	Puruliya, West Bengal, India
36	*O. rufipogon*	RU-Lao	106158	Sikhotabong, Vientiane, Laos
37	*O. rufipogon*	RU-Cab	106321	Klatran, Kandal, Cambodia
38	*O. sativa* (*indica*)	SA-Ina1-i	Peta	Indonesia
39	*O. sativa* (*indica*)	SA-Kor-i	My23	Korea
40	*O. sativa* (*indica*)	SA-Chi1-i	Teqing	China
41	*O. sativa* (*indica*)	SA-Chi2-i	MBP98	China
42	*O. sativa* (*japonica*)	SA-US-j	New Bonnet	USA
43	*O. sativa* (*japonica*)	SA-Ita-j	Balilla	Italy
44	*O. sativa* (*japonica*)	SA-Jap-j	Akihikar	Japan
45	*O. sativa* (*javanica*)	SA-Ina2-jav	CPSL017	Indonesia

arithmetic mean (UPGMA) and SHAN (the sequential hierarchical agglomerative and nested clustering). The allelic diversity of the SSR was calculated according to the polymorphism information-content (PIC) value described by Botstein et al. (1980) and modified by Anderson et al. (1993) for self-pollinated species as follows: $PIC_i=1-\sum p_{ij}^2$, where p_{ij} is the frequency of the *j*th pattern for marker *i* and summation extends over *n* patterns.

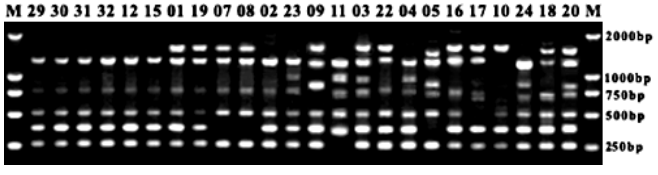

Fig. 1 RAPD fragment variation patterns of the AA-genome *Oryza* accessions generated by the primer S415. The identity of accessions (no.) refers to Table 1, *M* = molecular ladder

Results

Genetic variation patterns of the 45 accessions of the AA-genome rice species were examined using the 27 selected RAPD primers, and all the primers produced clear and repeatable bands as shown in Fig. 1. The portion of polymorphic bands generated by the 27 primers was considerably high ranging from 50.0 to 90.9% among the individual primers (Table 2). A total of 181 bands, with molecular weight of 200–2,200 bp, were identified in the *Oryza* accessions, corresponding to an average of 6.70 bands per primer used. Patterns of genetic variation within the 45 *Oryza* accessions were also determined by the 29 selected pairs of SSR primers designed based on the nucleotide sequences of *O. sativa* cv IR36. Two to six

Table 2 Primers used for RAPD analysis

Primer	Sequence	No. of bands amplified	Polymorphic bands/total no. of bands
S246	*ACCTTTGCGG*	7	85.7%
S259	*GTCAGTGCGG*	7	85.7%
S274	*CTGCTGAGCA*	7	85.7%
S278	*TTCAGGGCAC*	7	85.7%
S415	*GACCTACCAC*	7	85.7%
S435	*CAGCGACTGT*	6	83.3%
S444	*AAGTCCGCTC*	8	87.5%
S448	*CCTCCAGTGT*	6	83.3%
S482	*TCTGTCGGTC*	6	83.3%
S494	*GGACGCTTCA*	2	50.0%
S1009	*AGAACCGAGG*	4	75.0%
S1084	*ACAACGGTCC*	7	85.7%
S1097	*CCGCAAGTGT*	5	80.0%
S1099	*TCTGCCAGTG*	8	87.5%
S258	*GAGGTCCACA*	10	90.0%
S270	*TCGCATCCCT*	7	85.7%
S276	*CAGCCTACCA*	5	80.0%
S290	*CAAACGTGGG*	6	83.3%
S420	*AGGTCTTGGG*	6	83.3%
S441	*GGCACGTAAG*	11	90.9%
S447	*CAGCACTGAC*	10	90.0%
S460	*ACACACGCTG*	6	83.3%
S483	*GGTCACCTCA*	5	80.0%
S1008	*TTCCCGTGCC*	8	87.5%
S1083	*CCCACCCTTG*	8	87.5%
S1088	*GTCGCCCTCA*	5	80.0%
S1098	*GGAGTGGACT*	7	85.7%

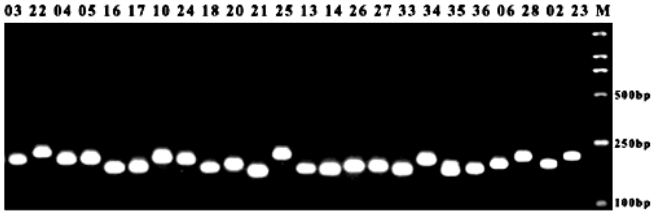

Fig. 2 SSR fragment variation patterns of the AA-genome *Oryza* accessions generated by the primer pair RM 551. The identity of accessions (no.) refers to Table 1, *M* = molecular ladder

alleles per primer pair were observed from the cultivated and wild *Oryza* accessions, using the 29 SSR primer pairs. A total of 101 polymorphic alleles, with molecular weight ranging from 100 to 500 bp, were detected from all the *Oryza* materials studied (Fig. 2 and Table 3). Noticeably, a considerable portion (about 9.5%) of heterozygous alleles was detected in the rice accessions, indicating high frequencies of introgression in some samples. The polymorphism information content (PIC) for each SSR primer pair varied to a large extent between 0.129 and 0.712 (Table 3). It is also worthwhile to mention that a very low frequency of null alleles (>3%) was also detected in some of the primer pairs with the *Oryza* accessions.

The two molecular-marker systems revealed considerable genetic diversity of the AA-genome *Oryza* species from different locations, with similarity coefficient levels ranging from 0.59 to 0.92 for the RAPD, and from 0.36 to 0.96 for the SSR, analysis. Genetic-diversity levels of the

Table 3 Primer pairs used in SSR analysis

Locus	Chromosome no.	Motifs	No. of alleles	PIC
RM405	5	(AC)14	5	0.526
RM423	2	(TTC)9	4	0.273
RM434	9	(TC)12	3	0.522
RM462	1	(GA)12	4	0.375
RM479	11	(TC)9	4	0.200
RM496	10	(TC)14	6	0.588
RM512	12	(TTTA)5	2	0.231
RM519	12	(AAG)8	2	0.129
RM521	2	(TC)14	4	0.543
RM551	4	(AG)18	4	0.712
RM558B	11	(ATTG)5	3	0.275
RM560	7	(CT)12	3	0.263
RM573	2	(GA)11	4	0.444
RM578	1	(GA)19	2	0.486
RM587	6	(CTT)18	2	0.543
RM413	5	(AG)11	4	0.645
RM432	7	(CATC)9	4	0.636
RM458	8	(TAG)8	3	0.426
RM466	1	(AG)17	3	0.546
RM484	10	(AT)9	3	0.085
RM509	5	(TC)11	3	0.555
RM515	8	(GA)11	6	0.539
RM520	3	(AG)10	2	0.300
RM546	3	(CCT)7	2	0.166
RM553	9	(CT)10	3	0.434
RM559	4	(AACA)6	3	0.350
RM567	4	(GA)21	3	0.239
RM577	1	(TA)9(CA)8	6	0.586
RM585	6	(TC)45	4	0.261

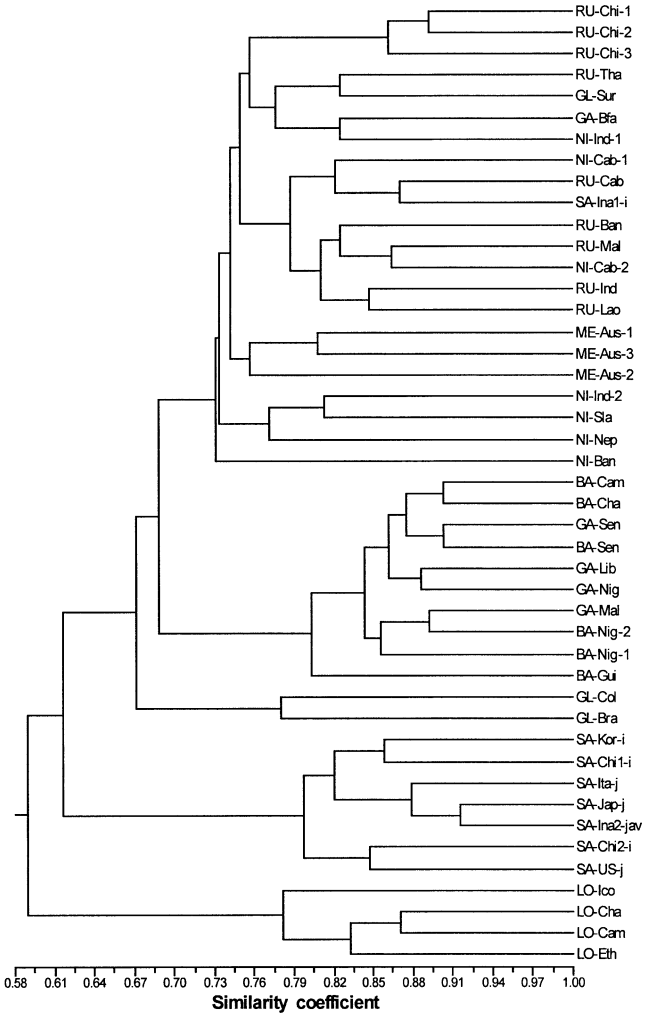

Fig. 3 A dendrogram illustrating genetic relationships of the 45 AA-genome *Oryza* accessions generated by the UPGMA cluster analysis of 181 RAPD fragments produced by 27 RAPD primers

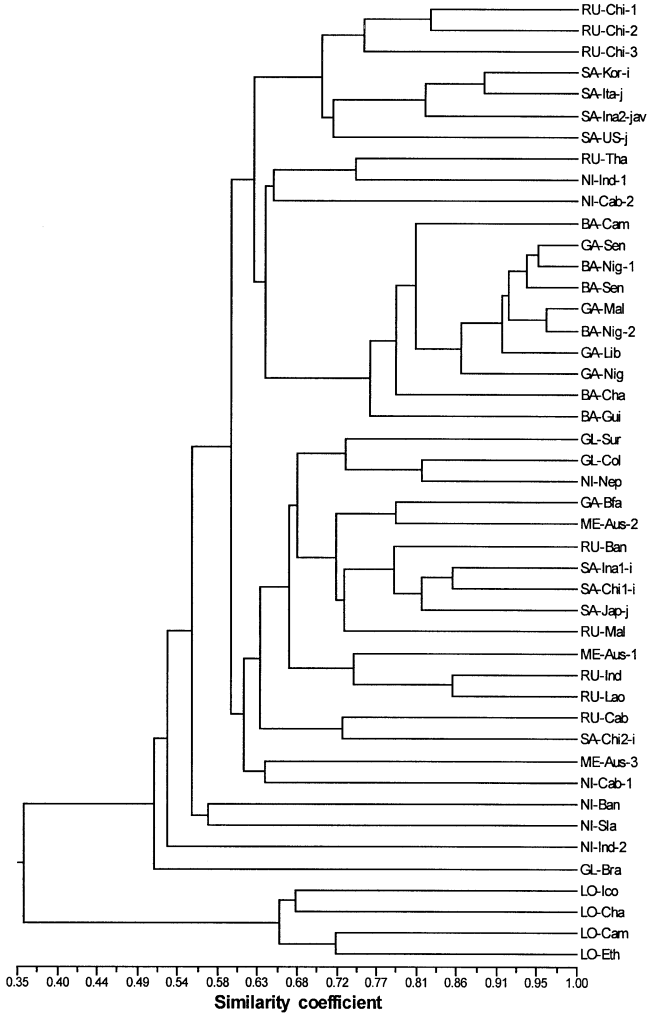

Fig. 4 A dendrogram illustrating genetic relationships of the 45 AA-genome *Oryza* accessions generated by the UPGMA cluster analysis of 101 SSR alleles produced by 29 SSR primer pairs

Oryza accessions detected by the two systems are comparable. Two dendrograms were constructed based on the similarity coefficient calculated from the 181 RAPD and 101 SSR markers generated from the 45 *Oryza* accessions (Figs. 3 and 4). The dendrograms constructed by the two different analyses illustrated a clear, but relatively different genetic variation pattern among the examined *Oryza* accessions.

The dendrogram generated by the RAPD analysis showed five distinct groups at the similarity coefficient level of 0.69 (Fig. 3). The first large group (uppermost) included all the Asian and Australian wild-rice species, *O. rufipogon*, *O. nivara* and *O. meridionalis*, only with the exception of one accession each of *O. sativa* (SA-Ina-j), *O. glumaepatula* (GL-Sur) and *O. glaberrima* (GA-Bfa). Considerable variation was evident in this subgroup, in which the Australian *O. meridionalis* formed a distinct subgroup, and *O. rufipogon* and *O. nivara* accessions were separated by their geographic distribution. In other words, the Chinese *O. rufipogon* accessions tended to be clustered together, and the accessions of *O. nivara* from South Asian countries, and *O. nivara*/*O. rufipogon* from Southeast Asian countries, were divided into separate subgroups, although with a few exceptions. The second group included only the *O. barthii* and *O. glaberrima* accessions from Africa, where the cultivated and wild-Africa rice species were randomly mixed with each other with relatively low variation. The third group only contained two *O. glumaepatula* accessions from Latin America, and was closely linked with the African *O. barthii*/*O. glaberrima* group. The fourth group only encompassed accessions of the cultivated *O. sativa*, with low levels of variation among the different accessions. The African *O. longistaminata* accessions were clustered into an independent group, and were placed far apart from other groups, with a little within-group variation.

The dendrogram generated by the SSR analysis showed a more-complicated genetic variation pattern (Fig. 4), where only one species, *O. longistaminata*, formed a distinct group that was separated from all other

Oryza species included. The African *O. barthii*/*O. glaberrima* complex formed an apparent group among the other *Oryza* accessions, although one *O. glaberrima* accession (GA-Bfa) was excluded from this group. Noticeably, accessions of the Africa species demonstrated their close relationships. The Asian *O. rufipogon*/*O. nivara*/*O. sativa* complex, the Latin American *O. glumaepatula* and the Australian *O. meridionalis*, did not exhibit a well-resolved pattern of variation, although there were weak associations between accessions with their similar geographic distributions. The two dendrograms showed relatively different variation patterns of the 45 AA-genome *Oryza* accessions, but there was a certain feature in common for the two dendrograms. For example, all *O. longistaminata* accessions consistently included a distinct group, and the *O. barthii* and *O. glaberrima* accessions were constantly clustered in the same group, with one *O. glaberrima* accession (GA-Bfa) included in the *O. rufipogon*/*O. nivara* complex.

Discussion

There have been a number of studies that report the assessment of genetic or phylogenetic relationships of *Oryza* species, either including all members in the genus (Aggarwal et al. 1999; Joshi et al. 2000) or only including taxa with the AA-genome (Ishii et al. 1996; Doi et al. 2000; Lu et al. 2002). Molecular fingerprinting techniques, such as RAPD, RFLP, SSR, and AFLP, were applied to facilitate the assessment. The results from these studies, in principle, supported the traditional classification of the *Oryza* species based mainly on morphological characterization, although with a certain discrepancy in terms of taxonomic treatments and genetic relationships of some species. Questions regarding relationships of *O. glumaepatula O. longistaminata* and *O. meridionalis* in the AA-genome *Oryza* group still remain unresolved.

The genetic relationships of the AA-genome *Oryza* group revealed by the RAPD data in this study support its traditional classification at species level and some conclusions drawn from other studies based on RAPD (Ishii et al. 1996), ISSR (Joshi et al. 2000) and RFLP studies (Doi et al. 2000). However, differences on the grouping of certain species were observed in this study. Our data clearly showed that the largest group includes all *O. rufipogon*, *O. nivara* and *O. meridionalis* accessions, in addition to a few accessions of other species. It is not surprising that *O. rufipogon* and *O. nivara* cluster together due to their close genetic relationship supported by several other authors (e.g., Oka 1988; Vaughan 1994; Lu et al. 2002). Interestingly, many Japanese scientists already treat the two as the "perennial" or "annual" types of *O. rufipogon* (Morishima et al. 1992). In fact, any attempt to use molecular-markers to identify samples designated as *O. rufipogon* or *O. nivara* is usually an extremely difficult task because of their close genetic relationships. The best solution might be to treat *O. nivara* and *O. rufipogon* (including *O. sativa*) as a large species-complex, as suggested by Lu et al. (2000, 2002).

However, there are two points worthy of mention regarding this grouping. First, in this analysis, the Australian *O. meridionalis* was supported in this group as a more-or-less distinct subgroup, which is different from all other previous studies. It is known that the Australian *O. meridionalis* occurs only in the northern part of Australia and the southern tip of Irian Jaya of Indonesia (Vaughan 1994; Lu and Silitonga 1999), and considerably overlapped with *O. rufipogon* in its distribution areas. The Asian and Australian AA-genome *Oryza* species also share considerable morphological similarities, and have an appreciable sexual compatibility (Naredo et al. 1997) and full chromosome-pairing in meiosis of their interspecific hybrids (Lu et al. 1997). In fact, *O. rufipogon* was the only recognized AA-genome *Oryza* species in many Australian floras, and *O. meridionalis* is taxonomically lumped with the perennial *O. rufipogon*. This information may provide confirmation of the close genetic relationship of the Asian and Australian AA-genome wild-rice species. This finding provides a sound basis for the effective utilization of *O. meridionalis* as the most accessible genetic resource for use in rice breeding, given that the Asian common wild-rice species are the putative ancestors of the cultivated rice. Second, there is a clear tendency that geographically closely distributed *O. rufipogon*/*O. nivara* accessions, such as samples from China/Southeast Asia versus South Asia, demonstrate a relatively close genetic relationship to one another. This result not only supports our earlier findings (Lu et al. 2002) where the genetic differentiation pattern of *O. rufipogon*/*O. nivara*/weedy rice complex is associated with their geographical isolation, but also suggests that a more-complete geographical allocation should be taken into consideration when *ex situ* conservation of these wild-rice genetic resources is planned. Almost all *O. sativa* accessions were clustered into an independent group, with the exception of one *O. sativa* accession that was included in the *O. rufipogon*/*O. nivara* complex. This demonstrates significant genetic differentiation of the cultivated rice from its ancestors.

It is generally recognized that the African cultivated rice *O. glaberrima* and its direct ancestor *O. barthii* are genetically closely related species. This statement is strongly supported in this study by their inclusion into the same group with relatively low genetic variation. It is also evident that the two taxa were not clearly distinguished by RAPD analysis, similar to the results reported by Doi et al. (2000) using RFLP data. This demonstrates convincingly the limited genetic differentiation between the African cultivated rice from its ancestor (Chu et al. 1969), due to the relatively short time of domestication of *O. glaberrima* and spontaneous genetic introgressions with *O. barthii*. The only *O. glaberrima* accession (GA-Bfa), which fell into the *O. rufipogon*/*O. nivara* group, might be interpreted as the result of introgression between the African and Asian cultivated rices. Similarly, the inclusion of one *O. glumaepatula* accession into the *O.*

rufipogon/*O. nivara* group can also be explained as the result of introgression in some *O. glumaepatula* accessions with the Asian cultivated rice introduced to Latin America. The frequent introgressions of *O. glumaepatula* with its Asian counterpart have been well documented (Lu et al. 1997; Naredo et al. 1997; Juliano et al. 1998). Furthermore, the relatedness of the Latin American *O. glumaepatula* with the *O. barthii*/*O. glaberrima* complex observed in this study has also been documented in other studies (Doi et al. 2000).

The *O. longistaminata* accessions consistently showed significant differentiation from all other AA-genome *Oryza* species as shown in the two dendrograms generated by RAPD and SSR analyses. In fact, *O. longistaminata* is morphologically very distinct from all other AA-genome species in *Oryza*, and it possesses several unique features that cannot be found in other AA-genome species. For example, it is self-incompatible, rhizomatous, and has unique ligular characteristics that make this species especially different in this AA-genome group. This analysis indicates that the differences of *O. longistaminata* from other A-genome *Oryza* species, both in reproductive biology and the above-mentioned morphological features, are mirrored in its considerable genetic differentiation as revealed by the molecular markers.

The dendrogram generated by the SSR analysis showed a more-complicated genetic-variation pattern that does not clearly indicate the interspecific genetic relationships of the AA-genome *Oryza* species. While the *O. longistaminata* and *O. barthii*/*O. glaberrima* accessions clustered in two distinct groups, demonstrating a similar within-group genetic variation pattern, compared to the RAPD analysis, all other accessions did not show a significant variation. The *O. rufipogon*/*O. nivara*/*O. sativa* and *O. meridionalis* accessions were randomly clustered in different groups, and *O. glumaepatula* accessions were divided into two groups, with one accession (GL-Bra) separated from all other species and the other two included among *O. rufipogon*, *O. nivara* and *O. sativa* accessions. Similarly, one *O. glaberrima* accession (GA-Bfa) also showed a close association with the Asian AA-genome *Oryza* species in the SSR variation pattern, suggesting introgression with the Asian species.

Although RAPDs are dominant markers in comparison with the co-dominant SSRs, the two types of markers used in this study are proven to be effective as fingerprinting systems for detecting polymorphisms among the AA-genome *Oryza* accessions. Polymorphisms detected by the RAPD analysis are somehow more informative than those obtained through SSR analysis for resolving the overall genetic relationships among these accessions. Theoretically, polymorphisms detected by the RAPD markers are mainly due to nucleotide sequence differences resulting in base substitutions or insertions/deletions, whereas those detected by the microsatellite (SSR) markers are due to variation in the number of repeat units. The two types of molecular markers used in this study are all effective for detecting polymorphisms in *Oryza* accessions with relatively high resolution. However, since the expansion/contraction of simple-sequence repeats is a more-frequent event than base substitution or insertion/deletion, SSR technology could be very effective for distinguishing higher levels of polymorphisms; particularly in cases where diminutive variation has occurred in a relatively short evolutionary span among the closely related individuals/varieties. The mutation rate of simple sequence repeats might be affected by the number of nucleotides in the repeat motif and the number of perfect repeat units (Chakraborty et al. 1997; Cho et al. 2000). Undoubtedly, SSRs are very useful markers for detecting allelic variation among species at population level or accessions, if an appropriate number of SSR primer pairs are used in analyses. However, Doyle et al. (1998) point out some problems that might be encountered when only a limited number of markers are used for phylogenetic and evolutionary studies, causing homoplasy (where amplified bands of the same molecular weight contain different internal mutations). Therefore, we encourage using an adequate number of SSR primer pairs in analyzing genetic relationships of related species to achieve a better resolution.

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References

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor Appl Genet 98:1320–1328

Akagi H, Yokozeki Y, Inagaki A, Fujimura T (1997) Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. Theor Appl Genet 94:61–67

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36:181–186

Botstein D, While RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Buso GSC, Rangel PH, Ferreira ME (1998) Analysis of genetic variability of South American wild-rice populations (*Oryza glumaepatula*) with isozymes and RAPD markers. Mol Ecol 7:107–117

Chakraborty R, Kimmel M, Stivers DN, Davison LJ, Deka R (1997) Relative mutation rates at di-,tri-,and tetra-nucleotide microsatellite loci. Proc Natl Acad Sci USA 94:1041–1046

Chang TT (1985) Crop history and genetic conservation: rice—a case study. Iowa State J Res 59:425–455

Chang TT, Qu Sh, Pathak MD, Ling KC, Kauffman HE (1975) The search for disease and insect resistance in rice germplasm. In: Frankel OH, Hawkes JG (eds) Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, England, pp 183–200

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayres N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:713–722

Chu YE, Morishima H, Oka HI (1969) Reproductive barriers distributed in cultivated rice species and their wild relatives. Japan J Genet 44:207–223

Clayton WD, Renvoize SA (1986) Genera *Graminum*. Grasses of the world. Her Majesty's Stationary Office, London, Kew Bulletin Series XIII

Doi K, Nonomura MN, Yoshimura A, Iwata N, Vaughan DA (2000) RFLP relationships of A-genome species in the genus *Oryza*. J Faculty Agric Kyushu Univ 45:83–98

Doyle JJ, Morgante M, Tingey SV, Powell W (1998) Size homoplasy in chloroplast microsatellites of wild perennial relatives of soybean (*Glycine* subgenus *Glycine*). Mol Biol Evol 15:215–218

Huff DR, Peakall R, Smouse PE (1993) RAPD variation within and among natural populations of outcrossing buffalo-grass [*Buchloe dactyloides* (Nutt.) Engelm]. Theor Appl Genet 86:927–934

Ishii T, Nakano T, Maeda H, Kamijima O (1996) Phylogenetic relationships in A-genome species of rice as revealed by RAPD analysis. Genes Genet Syst 71:195–201

Ishii T, Xu Y. McCouch SR (2001) Nuclear- and chloroplast-microsatellite variation in A-genome species of rice. Genome 44:658–666

Jena KK, Khush GS (1990) Introgression of genes from *Oryza officinalis* Well ex. Watt to cultivated rice, *O. sativa* L. Theor Appl Genet 80:737–745

Joshi SP, VS Gupta, RK Aggarwal, PK Ranjekar, DS Brar (2000) Genetic diversity and phylogenetic relationship as revealed by inter simple-sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor Appl Genet 100:1311–1320

Juliano AB, Naredo MBE, Jackson MT (1998) Taxonomic status of *Oryza glumaepatula* Steud. I. Comparative morphological studies of New World diploids and Asian AA-genome species. Genetic Resour Crop Evol 45:197–203

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35:25–34

Lu BR (1998) Diversity of rice genetic resources and its utilization and conservation. Biodiversity Sci 6:63–72

Lu BR, TS Silitonga (1999) Wild-rice *Oryza meridionalis* was first found in Indonesia. Int Rice Res Notes 24:28

Lu BR, Naredo MBE, Juliao A, Jackson MT (1997) Hybridization of AA-genome rice species from Asia and Australia. II. Meiotic analysis of *Oryza meridionalis* and its hybrids. Genetic Res Crop Evol 44:25–31

Lu BR, Naredo MBE, Juliano AB, Jackson MT (2000) Preliminary studies on taxonomy and biosystematics of the AA-genome *Oryza* species (Poaceae). In: Jacobs SWL, Everett J (eds) Grasses: systematics and evolution, pp 51–58

Lu BR, Zheng KL, Qian HR, Zhuang JY (2002) Genetic differentiation of wild relatives of rice as referred by the RFLP analysis. Theor Appl Genet 106:101–106

Martin C, Juliano A, Newbury HJ, Lu B-R, Jackson MT, Ford-Lloyd BV (1997) The use of RAPD markers to facilitate the identification of *Oryza* species within a germplasm collection. Genetic Res Crop Evol 44:175–183

Morishima H, Sano Y, Oka HI (1992) Evolutionary studies in cultivated rice and its wild relatives. Oxford Surveys Evol Biol 8:135–184

Murray MG, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Naredo ME, A Juliao, Lu BR, Jackson MT (1997) Hybridization of AA-genome rice species from Asia and Australia. I. Crosses and development of hybrids. Genetic Res Crop Evol 44:17–24

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Oka HI (1988) Origin of cultivated rice. Japan Scientific Societies Press, Elsevier, Tokyo

Qian W, Ge S, Hong DY (2001) Genetic variation within and among populations of a wild-rice *Oryza granulata* from China detected by RAPD and ISSR markers. Theor Appl Genet 102:440–449

Rohlf FJ (1994) NTSYS-pc, numerical taxonomy and multivariate analysis system, vol 1.8. Exeter Software, New York

Richards R, Sutherland GR (1994) Simple repeat DNA is not replicated simply. Nature Genet 6:114–116

Second G (1982) Origin of the genetic diversity of cultivated rice (*Oryza* spp.): study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57:25–57

Sharma SD (1983) Conservation of wild species of *Oryza*. In: Rice germplasm conservation workshop. The International Rice Research Institute and the International Board for Plant Genetic Resources, pp 21–25

Sitch LA, Dalmacio RD, Romero GO (1989) Crossability of wild *Oryza* species and their potential use for improvement of cultivated rice. Rice Genet Newslett 6:58–60

Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison of the genetic diversity of common wild-rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theor Appl Genet 102:157–162

Vaughan DA (1994) The wild relatives of rice: a genetic resources guide book. International Rice Research Institute, Los Banos, The Philippines

Virk PS, Newbury HJ, Jackson MT, Ford-lloyd V (1995) The identification of duplicate accessions with a rice germplasm collection using RAPD analysis. Theor Appl Genet 90:1049-1055

Yu LX, Nguyen HT (1994) Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor Appl Genet 87:668–672