Theor Appl Genet (2003) 107:203–209
DOI 10.1007/s00122-003-1252-x

K. C. Park · N. H. Kim · Y. S. Cho · K. H. Kang · J. K. Lee · N.-S. Kim

Genetic variations of AA genome *Oryza* species measured by MITE-AFLP

Received: 18 October 2002 / Accepted: 27 January 2003 / Published online: 20 March 2003
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Abstract MITEs (miniature inverted-repeat transposable elements) are the major transposable elements in *Oryza* species. We have applied the MITE-AFLP technique to study the genetic variation and species relationship in the AA-genome *Oryza* species. High polymorphism was detected within and between species. The genetic variation in the cultivated species, *Oryza sativa* and *Oryza glaberrima*, was comparatively lower than in their ancestral wild species. In comparison between geographical lineages of the AA genome species, African taxa, *O. glaberrima* and *Oryza barthii*, showed lower variation than the Asian taxa, *O. sativa, Oryza rufipogon*, and *Oryza nivara*, and Australian taxon *Oryza meridionalis*. However, another African taxon, *Oryza longistaminata*, showed high genetic variation. Species relationships were analyzed by the pattern of presence or absence of homologous fragments, because nucleotide sequences of the detected MITE-AFLP fragments revealed that the same fragments in different species shared very high sequence homology. The clustering pattern of the AA-genome species matched well with the geographical origins (Asian, African and Australian), and with the Australian taxon being distant to the others. Therefore, this study demonstrated that the MITE-AFLP technique is amenable for studying the genetic variation and species relationship in rice.

Keywords *Oryza* species · MITE-AFLP · Genetic variation · Species relationship

Communicated by D.J. Mackill

K. C. Park · N. H. Kim · J. K. Lee · N.-S. Kim (✉)
Division of Biotechnology, Kangwon National University, Chunchon, Korea, 200-701
e-mail: kimnamsu@kangwon.ac.kr

Y. S. Cho · K. H. Kang
Rice Breeding Division, Crop Research Station, RDA, Suwon, Korea

Introduction

The *Oryza* genus contains approximately 24 species of diploids and tetraploids with the basic chromosome number of 12. Because of their economic importance, considerable effort has been devoted to studying the genome relationships and genetic diversity in the rice genome and species. With the studies of 42 morphological characters by numerical taxonomic methods, Morishima and Oka (1970) have grouped the *Oryza* species into three complexes, *Oryza sativa, Oryza officinalis*, and *Oryza ridleyi*, depending on their genome constitution. While the *O. sativa* complex constitutes mainly the diploid AA-genome species, the *O. officinalis* complex consists of BB, CC, EE diploids and BBCC, CCDD tetraploids, and the *O. ridleyi* complex consists of HHJJ genomes (Ge et al. 2001).

The *O. sativa* complex contains seven or eight diploid species including two cultivated species *O. sativa* and *Oryza glamberrima* depending on the classification (Vaughan 1994). *O. sativa* is grown worldwide, but mostly in South and Southeast Asia. It has two subspecies, *O. sativa indica* and *O. sativa japonica*, which have been separated by a sterility barrier in F_1 plants (Chu et al. 1969). *Oryza rufipogon* and *Oryza nivara* are believed to be the direct wild relatives of *O. sativa* (Khush 1997). Since these two Asian AA wild taxa are interfertile and variation between the two types is continuous, they can be classified as a single species *O. rufipogon* (Tateoka 1964; Oka 1988). Another cultivated AA genome rice is the *O. glaberrima* grown in West Africa. *Oryza longistaminata* and *Oryza barthii* are thought to be the progenitors of *O. glaberrima*. *Oryza glumaepatula* and *Oryza meridionalis* are also AA-genome diploid species found in Latin America and Australia, respectively.

Several lines of evidence indicate that the transposable elements (TEs) have been the main driving force to create genomic diversity (Kumar and Bennetzen 1999; Fedoroff 2000; Zhang et al. 2000). Differential amplification of TEs was demonstrated to be responsible for the C-value differences in plant species (Bennetzen 2000). There are

two kinds of TEs, class 1 and class 2, depending on their transposition mechanism. While class-1 TEs transpose via RNA intermediates and are present in very high copy numbers in the host genome, class-2 TEs are DNA transposons and are usually present in less than 100 copies per genome with the exception of MITEs (miniature inverted-repeat transposable elements) which were discovered relatively recently in various eukaryotic species (see review by Wessler et al. 2001). Unlike the other class-2 elements, MITEs are present in very high copy numbers in the genome (Bureau and Wessler 1992, 1994a, b).

Although rice has the smallest genome among the cereal species, all classes of TEs are present in the *Oryza* species. A 340-kb contig around the *Adh1* gene in rice contains 28.5% repetitive DNA (Tarchini et al. 2000). Retrotransposons, DNA elements and MITEs constitute 14.4%, 8.7% and 5.3% in that contig DNA, respectively. The MITEs consisted of 78 elements, or one MITE per 4.4 kb. Therefore, the MITE sequences should be one of the major components of repetitive DNA families in the rice genome. Since MITEs have very high sequence identity within a family and high polymorphism in their insertion sites, Wessler et al. (2001) argued that the MITEs are the major source of allelic diversity in rice. From the presence or absence of MITE elements in specific loci, Kanazawa et al. (2000) demonstrated the species relationship among the AA-genome wild rice species.

MITE-transposon display, which is a modification of the conventional AFLP (amplified fragment length polymorphism) with the consensus MITE sequences in each family, demonstrated high allelic diversity in a segregating mapping population of maize (Casa et al. 2000). Wessler et al. (2001) showed very high allelic diversity in rice by a MITE-transposon display (MITE-TD) using a MITE-family *Olo* element. We isolated a conserved MITE-family, *Pangrangja*, which means a vagabond in Korean, from grass species (Park et al. 2002). Moreover, this new MITE element was found to be more abundant in AA-genome *Oryza* species. In this article, genomic variation and species relationships among AA diploid *Oryza* species were explored using MITE-TD with *Pangrangja*.

Materials and methods

Plant materials

The seeds of the *Oryza* species were obtained from by Dr. M.T. Jackson, Genetic Resources Center, IRRI, Las Baňos, The Philippines. The accession numbers and their species names are shown in the cluster dendrogram (see Fig. 3). The rice genomic DNA was isolated from young leaves with the protocol of Dellaporta et al. (1983).

MITE-AFLP

The protocol of MITE-AFLP used was that of Casa et al. (2000) with minor modifications. Completely digested genomic DNA (100 ng) with *Mse*I was ligated with appropriate adaptors in a volume of 20 µl. The nucleotide sequences for adaptors were 5'-GACGATGAGTCCTGAA-3' and 5'-TACTCAGGACTCAT-3'. The genomic DNA was amplified with a complementary primer to the adaptor and PangMAP primer in a volume of 50 µl which contained 5 µl of the digested and adaptor ligated genomic DNA, 0.5 µM of each primer, 0.2 mM of dNTP, 1.5 mM of $MgCl_2$ and 1.5 units of *Taq* DNA polymerase (Biotools, Spain). Primer sequences are shown in Table 1. The polymerase chain reaction was performed using one cycle of 72 °C for 2 min and 94 °C for 3 min; 25 cycles of 94 °C for 30 s, 53 °C for 30 s, 72 °C for 1 min; and a final extension at 72 °C for 5 min before completion. The pre-amplified products were diluted 50 fold after the final amplification cycle. Five microliters of the diluted products were amplified with 0.5 µM of the *Mse*I anchor primer with selective bases, 0.5 µM of PangMAP primer, 0.2 mM of dNTP, 1.5 mM of $MgCl_2$ and 1 unit of *Taq* Polymerase (Biotools, Spain) in a 30-µl volume. The PCR condition was 94 °C for 5 min, ten “touchdown” cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 1 min with the decrease of annealing temperature to 1 °C in each cycle, 26 cycles of 94 °C for 30 s, 53 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 5 min to terminate the reaction.

Five microliters of the final reaction was mixed with 10 µl of electrophoresis loading-buffer (98% formamide, 0.02% bromophenol blue, 0.02% Xylene C and 5 mM of NaOH). The samples were denatured at 95 °C for 5 min and cooled immediately on ice before electrophoresis. Two microliters of the sample were loaded in 6% denaturing (7.5 M urea) acrylamide-bisacrylamide gel (19:1) in 1 × TBE (0.89 M of Tris, 0.89 M of borate and 20 mM of EDTA, pH 8.0) and electrophoresed at 1800 V and 60 W for 130 min. After electrophoresis, the separated fragments were visualized with a silver-staining kit (Promega, USA).

PCR fragments were isolated from the PAGE gel, with the same primer combination and cloned into the pGEM-easy T vector. Nucleotide sequences were determined with an automatic DNA sequencer (ABI377, Applied Biosystems, USA). DNA sequences of the fragments in this study were deposited in GenBank with accession numbers from AF527490 to AF527510.

Table 1 Adaptor and anchor primer sequences of the MITE-AFLP analysis

Primer name	Sequence
Adaptors	
KRMA-1	GACGATGAGTCCTGAG
KRMA-1	TACTCAGGACTCAT
*Mse*I anchors	
KRMP-0	GACGATGAGTCCTGAGTAA
KRMP-1	GACGATGAGTCCTGAGTAAAA
KRMP-2	GACGATGAGTCCTGAGTAAAC
KRMP-3	GACGATGAGTCCTGAGTAAAG
KRMP-4	GACGATGAGTCCTGAGTAAAT
KRMP-5	GACGATGAGTCCTGAGTAACA
KRMP-7	GACGATGAGTCCTGAGTAACG-
KRMP-8	GACGATGAGTCCTGAGTAACT
KRMP-11	GACGATGAGTCCTGAGTAAGG
KRMP-13	GACGATGAGTCCTGAGTAATA
KRMP-14	GACGATGAGTCCTGAGTAATC
KRMP-15	GACGATGAGTCCTGAGTAATG
KRMP-16	GACGATGAGTCCTGAGTAATT
Pangrangja	
PangMAP	AAR*CAGTTTGACTTTGATC

*R = A, G

Data analysis

The presence or absence of specific bands of the MITE-AFLP were recorded as a binary code, 1 or 0, in each accession. A genetic

dendrogram was constructed on the basis of Nei and Li's algorithm (1979), $S_{xy} = 2N_{xy}/(N_x + N_y)$, where N_{xy} refers to the number of bands in common between plant x and y. N_x and N_y denote the total number of bands in each plant x and y, respectively. The calculations were done using an arithmetic average option in the NTSYS-pc program (Roholf 1992) and bootstrapping was done using 'WINBOOT' developed at IRRI (Yap and Nelson 1996). Two-thousand bootstrap replications were carried out to obtain a robust consensus tree. Genetic variation within species and genetic distances between species were calculated with the Microsoft Exel PC-program from the NTSYS data set.

Results

Polymorphism by MITE-AFLP

The *Pangrangja* MITE-AFLP produced highly polymorphic fragments with each primer combination. The number of polymorphic fragments varied from 22 to 50 depending on the primer combination among the AA species (Table 2). Analysis of 66 accessions of AA species with 12 MITE-AFLP primer combinations detected 423 fragments, of which 416 fragments were polymorphic at the inter- or intra-species level. At the species level, the average number of fragments per primer also varied from 11.8 in *O. glaberrima* to 15.3 in *O. longistaminata*. The Asian taxa, *O. sativa, O. rufipogon*, and *O. nivara*, showed comparatively higher polymorphism than the African taxa, *O. glaberrima* and *O. barthii* and the Australian taxon, *O. meridionalis*. However, *O. longistaminata*, which has an African origin, showed high polymorphism with 13.5 polymorphic fragments per primer combination. *O. glaberrima* showed the least polymorphism with 4.08 polymorphic fragments per primer on average. For example, the primer combination of M-AA produced all monomorphic fragments among the 11 accessions of *O. glaberrima* (Table 2). The profiles of amplified fragments of *O. barthii* were very similar to those of *O. glaberrima* in most of the primer combinations (Fig. 1).

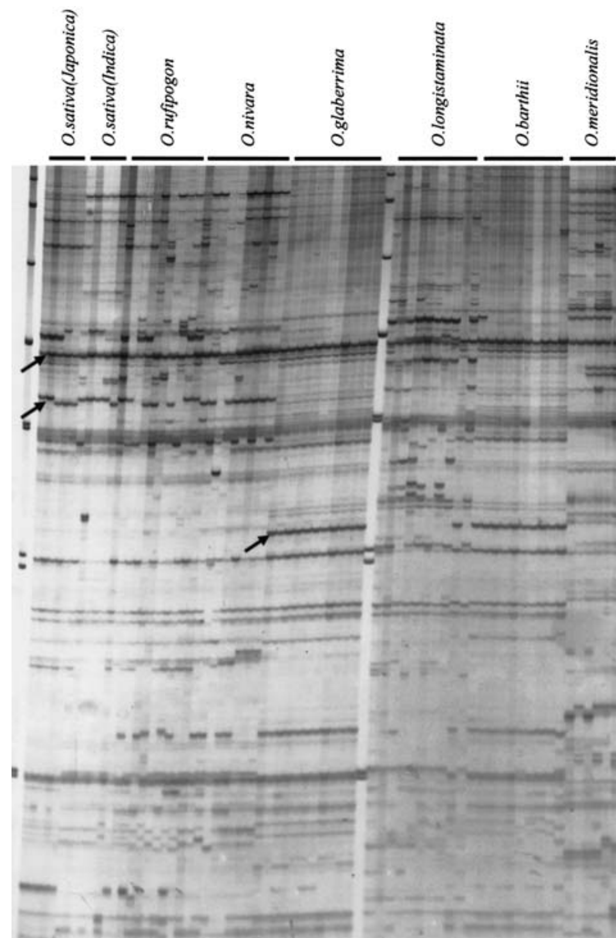

Fig. 1 MITE-AFLP profile of the AA diploid *Oryza* species using the KRMP-3 *Mse*I anchor primer in amplification. The fragments designated by arrows were cloned and sequenced to identify the sequence homology between species. The top arrow indicates the universal fragment in all AA species. Arrows in the middle and lower part indicate the fragments either specific to all Asian taxa or African taxa, *O. glaberrima* and *O. barhii*

Table 2 Distribution of MITE-AFLP markers detected with different primers in AA *Oryza* species

Primers used	Polymorphic/total	Number of markers in each *Oryza* species (polymorphic/total)						
		O. sat (10)*	*O. ruf.* (9)	*O. niv.* (10)	*O. gla.* (11)	*O. lon.* (10)	*O. bar.* (10)	*O. mer.* (6)
-AA	37/37	6/12	12/13	12/14	0/13	19/21	4/14	8/13
-AC	37/37	10/10	12/12	12/12	5/10	11/11	6/10	9/12
-AG	40/42	9/17	10/17	11/17	3/16	12/19	4/16	11/21
-AT	48/50	16/25	17/25	19/24	8/17	15/17	10/17	10/19
-CA	35/35	14/21	18/21	17/20	3/7	8/10	2/7	1/7
-CG	40/41	16/20	17/21	18/20	5/14	16/18	7/15	6/12
-CT	25/26	9/14	11/13	10/13	7/13	14/16	6/13	5/10
-GG	22/22	6/7	8/8	7/7	1/5	7/7	3/5	6/9
-TA	34/34	5/13	10/14	12/14	4/13	15/16	8/13	5/9
-TC	45/45	11/14	13/16	14/15	7/16	21/23	10/16	11/20
-TG	23/23	5/9	8/9	8/9	2/7	10/10	4/7	5/10
-TT	30/31	6/11	9/13	11/14	4/10	14/16	4/10	4/9
Average	34.7/35.3	9.42/14.4	12.0/15.2	12.6/14.9	4.08/11.8	13.5/15.3	5.67/11.9	6.75/12.6
Total	416/423	113/173	145/182	151/179	49/141	162/184	68/143	81/151

*: number of accessions

Table 3 Genetic distance matrix showing within- and between- species for AA *Oryza* species

Species	Within species	Between species					
		O. ruf.	*O. niv.*	*O. gla.*	*O. lon.*	*O. bar*	*O. mer.*
O. sativa	0.119	0.148	0.167	0.338	0.365	0.331	0.485
O. rufipogon	0.15	–	0.155	0.319	0.352	0.317	0.47
O. nivara	0.145	–	–	0.308	0.341	0.304	0.458
O. glaberrima	0.049	–	–	–	0.284	0.07	0.449
O. longistaminata	0.131	–	–	–	–	0.285	0.398
O. barthii	0.067	–	–	–	–	–	0.448
O. meridionalis	0.098	–	–	–	–	–	–

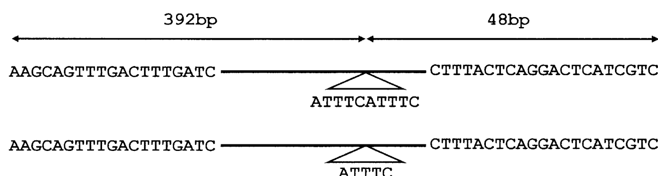

Fig. 2 Differences between large and small fragments of the Asian taxa-specific fragment shown in Fig. 1. The duplication of the penta-nucleotide, ATTTC, accounts for the size difference between them. The rest of the sequences were identical except for a few insertions or deletions

Table 3 shows the genetic variation in each species. In Asian taxa, genetic variation in the wild species, 0.15 in *O. rufipogon* and 0. 14 in *O. nivara*, was higher than that of the cultivated species *O. sativa*, 0.12. In African taxa, while *O. glaberrima* and *O. barthii* showed a similar level of genetic variation at 0.049 and 0.067, respectively, *O. longistaminata* had a genetic variation of 0.131.

Three fragments (designated by arrows in Fig. 1) were present either in all AA-genome species, in only Asian taxa or in only African taxa. Since the fragments were either species-specific or specific to the geographic lineages, nucleotide sequences in these fragments were determined to check their identity. The nucleotide sequences of the fragment present in all AA species revealed that they were homologous in all of them. The Asian taxon-specific fragments were also all homologous. The presence of a duplicate 5-bp sequence, ATTTC, resulted in the fragments being slightly longer than those with a single-copy of ATTTC (Fig. 2). We did not observe plants having both fragments. The *O. glaberrima*- and the *O. barthii*-specific fragments designated in Fig. 1 showed perfect sequence homology between fragments from the two species.

Cluster analysis and genetic affinities among the A-genome *Oryza* species

The AA-genome *Oryza* species clustered into three groups at a similarity level of 0.471 (Fig. 3). The first group consisted of the three Asian taxa, *O. sativa, O. rufipogon* and *O. nivara*. The second group consisted of the three African taxa, *O. glaberrima, O. barthii* and *O. longistaminata*. The Australian species, *O. meridionalis*, did not cluster with the others. Although bootstrap values were low within a geographical group, high bootstrap values on the nodes between geographical clusters also support the phylogenetic inferences of the AA-genome species according to their geographical origins.

In Asian taxa, there were three subclusters at a similarity level of 0.73. Interestingly, *O. sativa japonica* and *O. sativa indica* types were not grouped in the same subcluster. Of the eight accessions in the first subcluster, five *O. sativa japonica*, two of *O. rufipogon*, and one of *O. nivara*, were clustered together at a similarity level of 0.75. There were 18 accessions in the second subcluster. When the accessions in the second subcluster were further classified at a similarity level of 0.73 two groups were formed. One group contained five *O. sativa indica* accessions, six *O. rufipogon* accessions and one *O. nivara* accession. Another group contained only six *O. nivara* accessions. The third subcluster of Asian taxa contained two accessions of *O. rufipogon* and one accession of *O. nivara*. In African taxa, two subclusters existed at a similarity level of 0.58. In the first subcluster, 11 *O. glaberrima* accessions, ten *O. barthii*, and one accession of *O. longistaminata* (acc. no. 100930) were clustered. (This *O. longistaminata* accession turned out to be a mislabeled *O. barthii* accession since its morphology was similar to *O. barthii*.) The second subcluster contained only the nine accessions of *O. longistaminata*. All accessions of the Australian taxon, *O. meridinalis*, were clustered separately from others at a similarity level of 0.76.

Genetic variations and genetic distances within and between species among the AA-genome *Oryza* species were associated with their geographic origins. The genetic variation among the Asian taxa (0.137) was higher than that of the African (0.08) and Australian taxa (0.098). The genetic distance between Asian AA-genome species and African AA-genome species (0.33) was closer than the distances between Asian AA-genome species and Australian AA-genome species (0.471), and African AA-genome species and Australian AA-genome species (0.427).

Discussion

This study demonstrated the successful application of MITE-AFLP for studying the genetic variation and species relationships in AA-genome diploid *Oryza* spe-

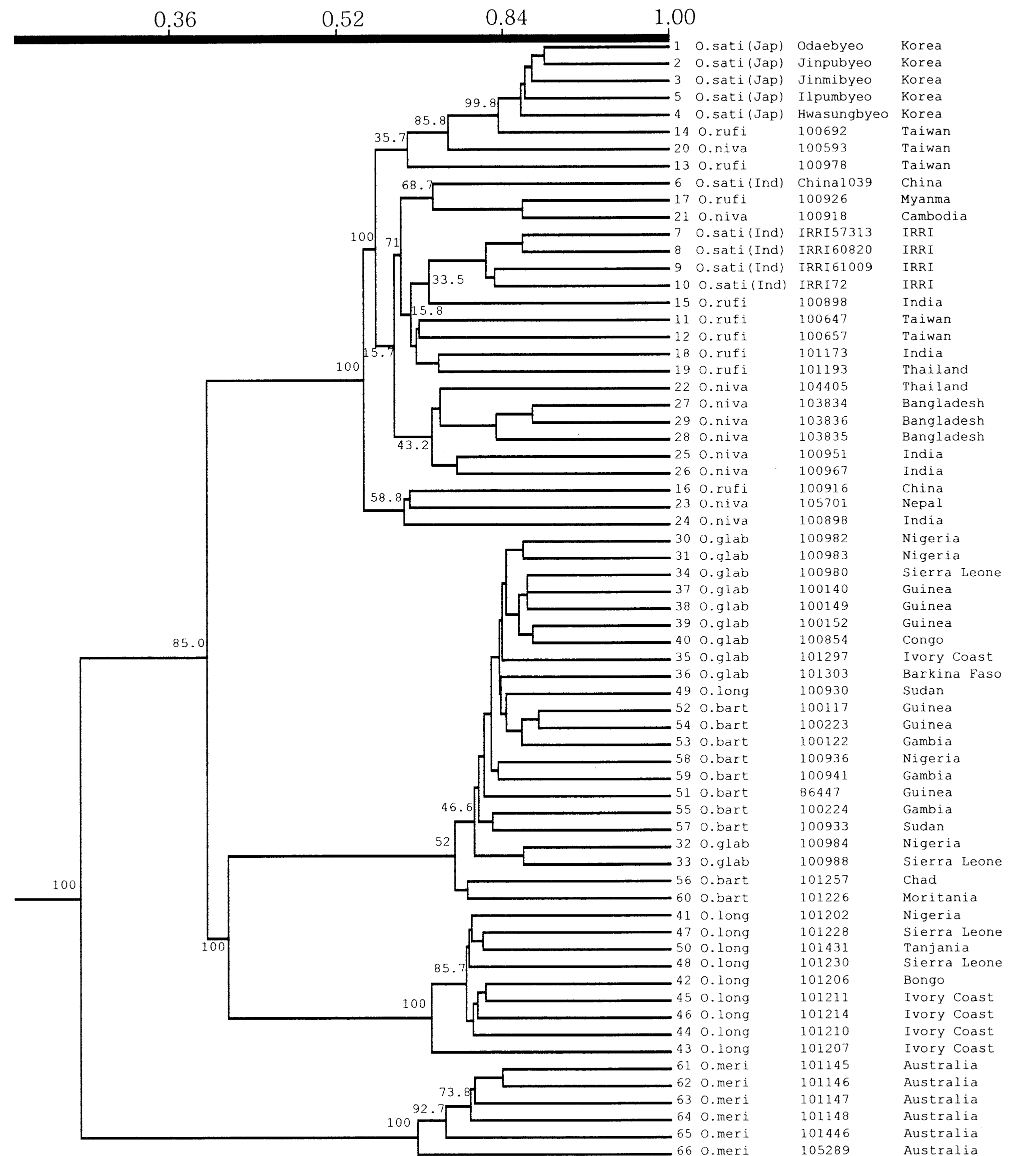

Fig. 3 Cluster dendrogram of the AA-genome *Oryza* species based on MITE-AFLP. Numbers shown at different nodes represent bootstrap values in each node. The scale shown above is the measure of genetic similarity

cies. The conventional AFLP technique successfully allowed the species relationship among AA-genome rices to be determined (Aggarwal et al. 1999). The number of detectable markers in MITE-AFLP analysis is similar to that of conventional AFLP markers in *Oryza* species (Wessler et al. 2001). Moreover, high polymorphism in our results demonstrated that the genetic resolution by MITE-AFLP is amenable for studying genetic variation and species relationships among AA-genome diploid *Oryza* species. Kanazawa et al. (2000) showed that the pattern of presence or absence of MITE elements in the *Stowaway* family was highly associated with speciation among AA-genome *Oryza* species. Compared to the three discrete genetic loci in their analysis, our study is more extensive considering more than 400 loci. Analysis of *Stowaway* MITE elements also revealed that gene flow between different species has been highly restricted among the AA-genome wild rices (Kanazawa et al. 2000). If gaining a MITE at a specific locus occurred before species divergence, presence of the MITE at this locus could be maintained within the species derived from that lineage, which was shown by the sequence analysis in our study. Kanazawa et al. (2000) also noted that the frequency of transposition of MITEs is low, indicating that the loci containing MITEs are free from selection pressure. Insertion of the MITE in a common fragment in all AA-genome species, therefore, must have occurred before the species divergence among the AA-genome *Oryza* species. With the same logic, the Asian taxon-specific fragment gained the MITE sequence at this locus before divergence of *O. sativa, O. rufipogon* and *O. nivara*, but after divergence from other AA-genome taxa in South and Southeast Asia. The same logic can also be applied to the African or Australian species-specific fragments.

High genetic variation in wild progenitors has been consistently observed by various analyses (Aggarwal et al. 1999; Joshi et al. 2000; Ishii et al. 2001; Sun et al.

2001). From the analysis of 122 accessions of *O. rufipogon* and 75 accessions of *O. sativa* by 44 single-copy RFLP markers, Sun et al. (2001) proposed that many alleles were lost through natural and human selection, leading to the lower genetic diversity of the cultigens during the course of evolution from wild rice to cultivated rice. In this MITE-AFLP analysis, *O. rufipogon* and *O. nivara* accessions showed higher polymorphism than *O. sativa* accessions. In the lineage of African taxa, *O. longistaminata* accessions also showed higher polymorphism than *O. glaberrima*. However, *O. barthii*, which is believed to be the direct wild progenitor of *O. glaberrima*, showed similar levels of polymorphism as *O. glaberrima*. In microsatellite analysis, Ishii et al. (2001) noted that *O. barthii* and *O. glaberrima* showed similar levels of polymorphism and shared the same alleles. In our analysis, the high variation by MITE-AFLP among the Asian taxa might be attributed to the wide geographical distribution as compared to the African taxa. Similarly, microsatellite variation in Asian taxa were comparably higher than in African taxa (Ishii et al. 2001).

The clustering pattern among the AA-genome diploid species by the present MITE-AFLP analysis matched with their geographic forms. This supports the conclusion of Morishima (1969) who demonstrated that the Asian, African and Australian taxa evolved distinctively and independently. The parallel evolution of ancestral lineages of two cultivated rices, *O. sativa* and *O. glaberrima*, was well established with many criteria such as isozymes, chromosome pairing, breeding data and geographical distribution (see review by Oka 1988). Several other molecular studies are also congruent with the geographical separation of the AA-genome diploid species (Aggarwal et al. 1999; Joshi et al. 2000; Buso et al. 2001).

In Asian taxa, differentiation of *indica-japonica* types of *O. sativa* from wild progenitors is an interesting feature in rice evolution since there have been two different schools of thought on the evolution of these two plant types, monophyletic evolution (Oka 1988; Wang et al. 1992; Joshi et al. 2000) and diphyletic evolution (Oka and Morishima 1982; Second 1982; Glaszmann 1987). Joshi et al. (2000) reported that the *indica*/*japonica* cluster joined together and then joined with *O. nivara* before joining to the cluster of *O. rufipogon*, leading to their interpretation of monophyletic evolution. On the other hand, the hypothesis of diphyletic evolution is based on the theory that wild rice can potentially evolve into *indica* as well as *japonica* types since these two types consistently differ from each other and show closer affinity with different wild accessions (Second 1982). In our analysis, the clustering pattern of the Asian taxa supports diphyletic evolution since the independent clustering of *japonica* and *indica* accessions showed closer affinity to different wild accessions of *O. rufipogon* and *O. nivara*. However, the equivocal distinction between several accessions of *O. rufipogon* (acc. no 100692, 100978, 100926 and 100916) and *O. nivara* (acc. no 100593 and 100918) is notable in our analysis since these two species separated distinctively in AFLP analysis by Aggarwal et al. (1999). In the analysis of *Stowaway* MITEs, Kanazawa et al. (2000) reported that the pattern of presence or absence in discrete loci was correlated with annual (*nivara*) or perennial (*rufipogon*) types of *O. rufipogon*. Therefore, studies with more accessions of *O. rufipogon* and *O. nivara* using MITE-AFLP may provide solutions for these discrepancies.

Compared to the diphyletic origin of *O. sativa*, another cultigen *O. glaberrima* was monophyletic in our analysis. Because *O. glaberrima* and *O. barthii* showed very similar patterns by MITE-AFLP and they belong to the same subcluster in the African cluster, *O. barthii* can be deduced to be the immediate progenitor of *O. glaberrima*. This conclusion is supported by many other criteria of species relationships (Oka 1988). *O. barthii* is distributed mostly in the savanna areas of West Africa where *O. glaberrima* is cultivated (Vaughan 1994). Hybrid swarms between these two species have been often found in the *O. glaberrima* growing areas, and these hybrids show a continuous series of variation between wild and cultivated forms (Morishima and Oka 1970). *O. longistaminata*, another African wild species that is rhizomatous, seemed to be more distantly related with *O. glaberrima* and *O. barthii*. *O. longistaminata* is distributed throughout the savanna areas of West to East Africa. Many of the habitats of *O. barthii* and *O. longistaminata* were found near fields where *O. glaberrima* is grown (Oka 1988).

O. meridionalis is only found in northern Australia. The remote clustering of *O. meridionalis* compared to other taxa in our analysis agreed with other reports (Morishima 2001). Our observation of the genetic affinity of *O. meridionalis* to other species is slightly different from the report of Vaughan (1994) who indicated that *O. meridionalis* belongs to the *O. sativa* complex and is related to *O. rufipogon* and *O. nivara*. In our analysis, the average genetic distance between *O. meridionalis* and the *O. glaberrima* complex was less than that between *O. meridionalis* and the *O. sativa* complex. The difference between this study and the report of Vaughan (1994) might be resolved by using more accessions of the AA species.

In summary, this study demonstrated a successful application of MITE-AFLP for studying genetic variation and species relationships in *Oryza*. The MITE-AFLP can detect many discrete loci as efficiently as the conventional AFLP technique. Since many different MITE families are known to be present with abundant copies in the genomes of *Oryza* species (Turcotte et al. 2001), the number of detectable MITE-AFLP markers can be very high, which can be utilized in genetic mapping and tagging genes for specific traits.

Acknowledgements We thank the Ministry of Science and Technology, Republic of Korea, for funding this research through the Crop Functional Genomics Center (Project Number CG3122). Thanks are also extended to Dr. K.K. Jena for critical reading of the manuscript.

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