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= Research Article

Differential distribution of miniature inverted-repeat transposable elements in wild *Oryza* species

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Summary: Miniature inverted-repeat transposable elements (MITEs) are short DNA transposons. To understand the distribution of MITEs in genus *Oryza*, the presence or absence of seven types of MITE was investigated in 44 accessions of 19 *Oryza* species covering nine genome constitutions. PCR and sequencing analyses showed that AA genome species harbored all of the seven types of MITE, whereas the other genome species harbored not all but zero to three types, respectively. *Ditto-Os1* was found to be the most widely distributed MITE: it was detected in 15 *Oryza* species. Phylogenic tree of genus *Oryza* showed that *Ditto-Os1* is the eldest among the seven MITEs. This study also suggests that the insertion of MITEs is one of the major sources making genetic variations in natural populations of genus *Oryza*.

Key words: Genus *Oryza*, transposable elements, miniature inverted-repeat transposable element (MITE), genetic variation, genome

Introduction

In eukaryotic organisms, most of the genome is composed of transposable elements (TEs). TEs are important genetic factors that promote chromosomal rearrangements, and alterations of gene structures and functions through their transposition. Thus TEs have played major roles in genome evolution, diversification, and expansions (Hurst and Werren 2001, Bergero *et al.* 2008). Among the numerous types of TEs, miniature inverted-repeat transposable elements (MITEs) are a subset of non-autonomous DNA transposons. They are characterized by their small sizes (less than 600 bp) with short (10-30 bp) terminal inverted repeats (TIRs) at both ends flanked by direct repeats of 2-8 bp known as the target site duplication (TSD), and are present in high copy numbers in a genome (Feschotte *et al.* 2002, Takagi *et al.* 2003). Moreover, MITEs reach several thousands in copy numbers and are frequently inserted in or near genes (Feschotte and Pritham 2007).

In rice (*Oryza sativa* L.), MITEs are the numerically prominent type of TEs in the genome (Jiang *et al.* 2004). In fact, MITEs account for nearly 50% of the entire repetitive DNA of chromosome 4 (Feng *et al.* 2002). Moreover, MITEs contain coding sequence, poly(A) sites, transcription start sites, and splicing sites of more than 300 protein-coding genes (Oki *et al.* 2008). Hence, MITEs are likely to significantly contribute to gene regulation and host genome evolution. However, very little is known about their evolution, transposition mechanisms, and specific biological functions because their distributions among species in different geographical regions are not well understood.

The genus *Oryza* contains 22 wild and two cultivated species, each of which has either 24 (diploid) or 48 (amphidiploid) chromosomes, and has been divided into ten genome types, viz., AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK (Aggarwal *et al.* 1997, 1999). The phylogeny and evolutionary history of the genus *Oryza* are well analyzed and understood (Guo and Ge 2005). Therefore, the genus *Oryza* is an ideal experimental material to investigate the origin and roles of MITEs in the host genome evolution and diversification.

In the present study, the presence and distributions of seven types of rice specific MITEs were investigated in 19 species of genus *Oryza* to trace their origin during the evolutionary process of *Oryza* species.

Materials and methods

Plant materials

Forty-two accessions of 18 wild *Oryza* species, which were provided by the National Institute of Genetics, Japan, were used. In addition, two *O. sativa* cultivars, Nipponbare (*O. sativa* ssp. *japonica*) and IR36 (*O. sativa* ssp. *indica*), were used. The accession number, genomic constitution, species name, and habitat country of each wild *Oryza* accession are shown in Table 1.

DNA extraction

Genomic DNA was extracted from young leaves, either by the modified CTAB method of Murray and Thompson (1980) or by DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) according to the

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Table 1 List of the plant materials used in the present study

S.N.	Genome	Acc. no.	Species	Country
1	AA	Nipponbare	*O. sativa (japonica)*	Japan
2	AA	IR36	*O. sativa (indica)*	Philippines
3	AA	W0106	*O. rufipogon*	India
4	AA	W0120	*O. rufipogon*	India
5	AA	W1294	*O. rufipogon*	Philippines
6	AA	W1866	*O. rufipogon*	Thailand
7	AA	W1921	*O. rufipogon*	Thailand
8	AA	W2003	*O. rufipogon*	India
9	AA	W1625	*O. meridionalis*	Australia
10	AA	W1635	*O. meridionalis*	Australia
11	AA	W0652	*O. barthii*	Sierra Leone
12	AA	W1588	*O. barthii*	Cameroon
13	AA	W1169=W1165	*O. glumaepatula*	Cuba
14	AA	W2145	*O. glumaepatula*	Brazil
15	AA	W2199	*O. glumaepatula*	Brazil
16	AA	W1413	*O. longistaminata*	Sierra Leone
17	AA	W1508	*O. longistaminata*	Madagascar
18	BB	W1514	*O. punctata*	Kenya
19	BBCC	W1024	*O. punctata*	Ghana
20	BBCC	W1213	*O. minuta*	Philippines
21	BBCC	W1331	*O. minuta*	Philippines
22	CC	W1527	*O. eichingeri*	Uganda
23	CC	W1805	*O. eichingeri*	Sri Lanka
24	CC	W0002	*O. officinalis*	Thailand
25	CC	W1361	*O. officinalis*	Malaysia
26	CC	W1830	*O. officinalis*	Unknown
27	CCDD	W0017	*O. alta*	Surinam
28	CCDD	W1182	*O. alta or O. latifolia*	British Guinea
29	CCDD	W0613	*O. grandiglumis*	Brazil
30	CCDD	W1194	*O. grandiglumis*	Brazil
31	CCDD	W2220	*O. grandiglumis*	Brazil
32	CCDD	W1166	*O. latifolia*	Mexico
33	CCDD	W1197	*O. latifolia*	Colombia
34	CCDD	W2200	*O. latifolia*	Brazil
35	EE	W0008	*O. australiensis*	Australia
36	EE	W1628	*O. australiensis*	Australia
37	FF	W1401	*O. brachyantha*	Sierra Leone
38	FF	W1711	*O. brachyantha*	Cameroon
39	GG	W0003	*O. granulata*	India
40	GG	W0067(B)	*O. granulata*	Thailand
41	GG	W1356	*O. meyeriana*	Malaysia
42	HHJJ	W1220	*O. longiglumis*	Dutch New Guinea
43	HHJJ	W0001	*O. ridleyi*	Thailand
44	HHJJ	W0604	*O. ridleyi*	Malaya

manufacturer's protocol.

Selection of MITEs

A total of seven rice (*O. sativa*) specific MITEs, *Castaway-Os1*, *Ditto-Os1*, *Wanderer* (these are referred in Bureau *et al.* 1996), *Kiddo* (Yang *et al.* 2001), *Mashu* (Takagi *et al.* 2003), *Stowaway2-Os*-like (Kanazawa *et al.* 2000) and *Stowaway17-Os*-like (Bureau and Wessler 1994) were selected from the database (Table 2).

PCR amplification

To investigate the presence or absence of the above MITEs, PCR was carried out using element specific primers (Table 2). The genomic DNA of each accession was used as the template for PCR using *Ex* Taq DNA polymerase (TaKaRa, Shiga, Japan). The PCR conditions were as follows: pre-denaturation for 3 min at 94 ℃ followed by 30 cycles of polymerization reaction, each consisting of a denaturation step for 10 s at 98 ℃, an annealing step for 1 min at 60 ℃ and an extension step for 1 min at 72 ℃, with a final extension step for 7 min at 72 ℃. PCR products were visualized under UV light following electrophoresis on a 1.4% agarose gel in 0.5x TBE buffer.

Cloning, sequencing, and data analysis

For each MITE, five to 10 PCR amplicons were cloned. Cloning was done using TOPO TA Cloning Kit Version R (Invitrogen, Nihonbashi, Japan) following its instruction manual. Plasmid DNA consisting of PCR product was transformed into *Escherichia coli* DH5α strain. The extraction of plasmid DNA was done using the Plasmid DNA Purification Kit (MACHEREY-NAGEL, Duren, Germany). The cloned fragments were sequenced using CEQ 8000 Genetic Analysis System (BECKMAN COULTER, Tokyo, Japan). The sequence

Table 2 Name of the MITEs analyzed in this study and the PCR primers used to amplify them

Name of MITEs	NCBI accession no.	Forward primers (5' to 3')	Reverse primers (5' to 3')	Size (bp)
Castaway-Os1	AC021891	GTCCCTTTGAATCATAGGGTTG	GCGCCATTTGAATGAAATGA	360
Ditto-Os1	AF488413	GAGCAAGTTTAATAGTATAGCCA	GAGCAGGTACAATAGCATGCTA	244
Kiddo	AF484680	GGGGCTGTTTGGTTCCCAGCCA	TTTGGTTGCAAGCTACACTTTG	269
Mashu	AB077839	AATGGTAAAGTAAGGTGCTCTC	GGGCACCCRCAATGGTTATCTA	263
Stowaway2-Os-like	AF488413	TCCATATTTTAATATATAACGC	CTCCCTCCGTATTTTAATGTATG	235
Stowaway17-Os-like	AB092509	CTCCCTCCATACTCATAAAGGA	CTCCGTACTTATAAATGAAATCG	259
Wanderer	AC134517	TCTCGTTTTCCGCGCGCATGC	GTCTGAGGAGAAGGGGATTG	208

data were analyzed by nucleotide-nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and CLUSTALW (http://align.genome.jp/) to confirm the presence of each MITE.

Results

Differential distribution of seven MITEs in *Oryza* species

PCR with specific primers for *Castaway-Os1* was conducted; consequently, only AA genome species except *O. meridionalis* yielded PCR products of appropriate size (ca. 360 bp) (Fig. 1). Subsequent sequence analysis revealed that PCR products were identical with each other. Contrary, the other species yielded larger or no products. Likewise, *Mashu*, *Stowaway17-Os*-like, and *Wanderer* were found to be present in all the AA genome species.

Kiddo and *Stowaway2-Os*-like MITEs were found to be more widely distributed in genus *Oryza* than the other MITEs: *Kiddo* was present in all the AA, BB, BBCC, and CC genome species, and *Stowaway2-Os*-like was present in *O. punctata* (BB, BBCC), *O. minuta* (BBCC) in addition to all the AA genome species. Out of the seven MITEs examined, *Ditto-Os1* was most widely distributed: it was present in 15 species with AA, BB, BBCC, CC, CCDD, EE, or FF genome (Fig. 2). These results are summarized in Table 3.

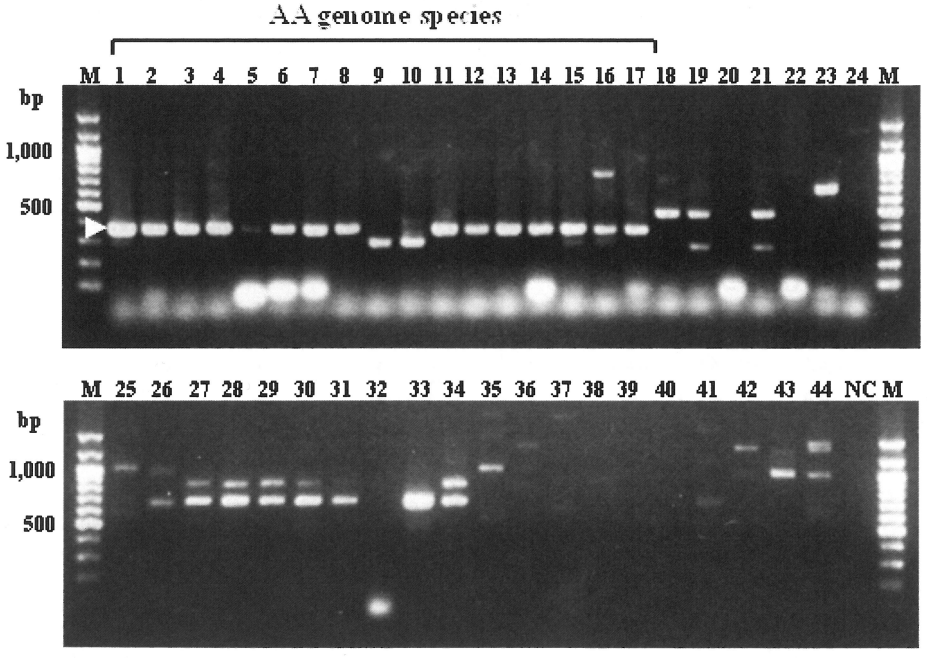

Fig. 1 Investigation of presence or absence of *Castaway-Os1* MITE in genus *Oryza*. M: DNA size marker (100 bp ladder, Nacalai, Japan); Lanes are- 1,2: *Oryza sativa*; 3-8: *O. rufipogon*; 9,10: *O. meridionalis*; 11,12: *O. barthii*; 13-15: *O. glumaepatula*; 16,17: *O. longistaminata*; 18,19: *O. punctata*; 20,21: *O. minuta*; 22,23: *O. eichingeri*; 24-26: *O. officinalis*; 27,28: *O. alta*; 29-31: *O. grandiglumis*; 32-34: *O. latifolia*; 35,36: *O. australiensis*; 37,38: *O. brachyantha*; 39,40: *O. granulata*; 41: *O. meyeriana*; 42: *O. longiglumis*; 43,44: *O. ridleyi*; and NC: negative control (distilled water). Lane numbers represent the serial numbers of the accessions in Table 1. Arrowhead shows the perspective band (ca. 360 bp). This MITE is present only in the AA genome species.

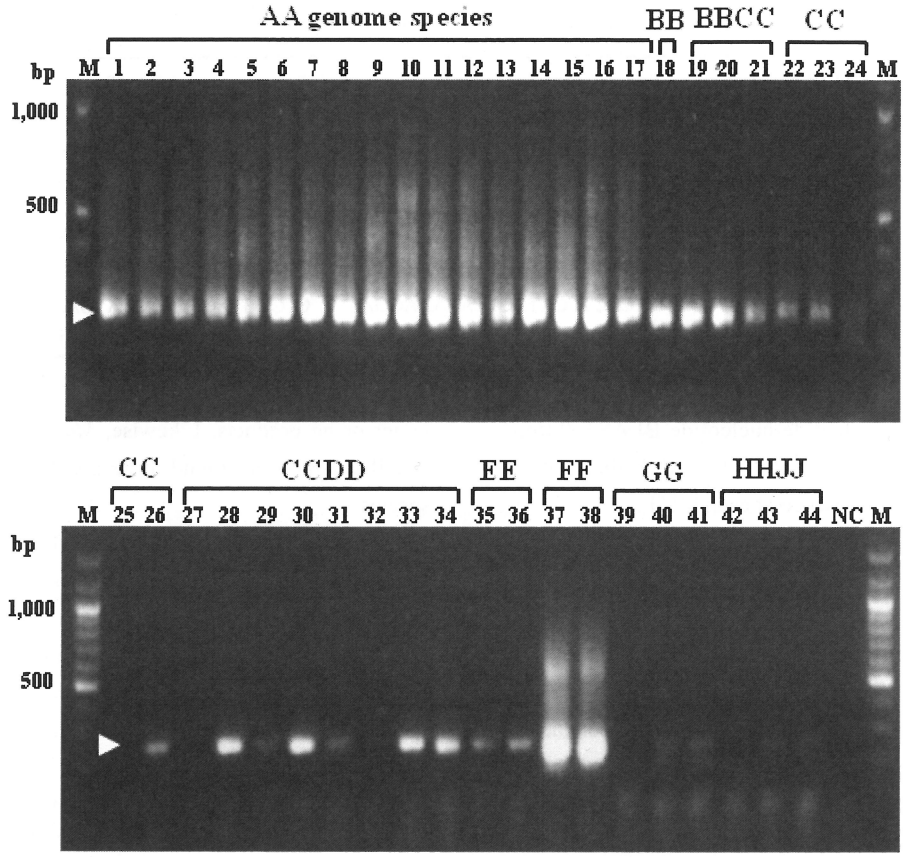

Fig. 2 Distribution of *Ditto-Os1* in genus *Oryza*. Lane numbers are the same as in Fig. 1 and represent the serial number of the accessions in Table 1. Lane M: DNA size marker (100 bp ladder, Nacalai, Japan). Arrowheads represent the band of expected size (ca. 244 bp).

Table 3 Distribution of MITEs in genus *Oryza*

Species	Genome	*Kiddo*	*Ditto -Os1*	*Stowaway17 -Os*-like	*Castaway -Os1*	*Mashu*	*Stowaway2 -Os*-like	*Wanderer*
Oryza sativa	AA	+	+	+	+	+	+	+
O. rufipogon	AA	+	+	+	+	+	+	+
O. barthii	AA	+	+	+	+	+	+	+
O. glumaepatula	AA	+	+	+	+	+	+	+
O. longistaminata	AA	+	+	+	+	+	+	+
O. meridionalis	AA	+	+	+		+	+	+
O. punctata	BB	+	+				+	
O. minuta	BBCC	+	+				+	
O. eichingeri	CC	+	+					
O. officinalis	CC	+	+					
O. alta	CCDD		+					
O. grandiglumis	CCDD		+					
O. latifolia	CCDD		+					
O. australiensis	EE		+					
O. brachyantha	FF		+					
O. granulata	GG							
O. meyeriana	GG							
O. longiglumis	HHJJ							
O. ridleyi	HHJJ							

Relationship between the presence of MITEs and speciation in genus *Oryza*

To verify the evolutionary history of each MITE, the relationships of distribution pattern of MITE and phylogenic tree of genus *Oryza* (Zhu and Ge, 2005) were investigated. All of the seven *Oryza sativa* specific MITEs were present in AA genome species, the latest divergent out of the 10 genome species of genus *Oryza* (Fig. 3). *Ditto-Os1* was present even in ancient species such as *O. brachyantha* (FF genome). As described above, *Castaway-Os1* was present in all the AA genome species excluding *O. meridionalis*, the earliest divergent lineage in the AA genome (Zhu and Ge 2005). Thus, it was considered that *Castaway-Os1* was inserted into the AA genome species after *O. meridionalis* separated from the other AA genome species, or it was eliminated from *O. meridionalis* after the specification. Similar results were obtained in earlier studies in which three of the MITE insertions were detected in all the AA genome species except for *O. meridionalis* (Zhu and Ge 2005). These results suggest that the specific MITEs came into existence at different times during the divergence and evolutionary process of genus *Oryza*.

Discussion

Mashu, *Castaway-Os1*, *mPing*, *Stowaway17-Os*-like, and *Wanderer* were present only in AA genome species, whereas *Kiddo*, *Ditto-Os1* and *Stowaway2-Os*-like were present not only AA genome species but also in other genome species. This suggests that the first five MITEs came into existence later than the second three. The presence of *mPing* is known to be limited to two of six AA genome species, *O. sativa* and *O. rufipogon* (Hu *et al.* 2006). This suggests that among the AA genome specific MITEs, *mPing* came into existence latest during the evolutionary process of genus *Oryza*. In other words, *mPing* is the youngest MITE in genus *Oryza*. On the other hand, *Kiddo* and *Stowaway2-Os*-like were present not only in AA genome species, but also in BB, CC, and BBCC genome species. Likewise, *Ditto-Os1* was present even in CCDD, EE, and FF genome species in addition to AA, BB, BBCC, and CC genomes. This may indicate that *Ditto-Os1* is the eldest MITE, and contributed to the differentiation of genus *Oryza* into several genome types and species. It is known that a *Tourist C* element, which is one of the MITE in rice, is inserted into a functional domain in 5' region of *CatA* gene coding a rice catalase protein, and this insertion is observed in all the

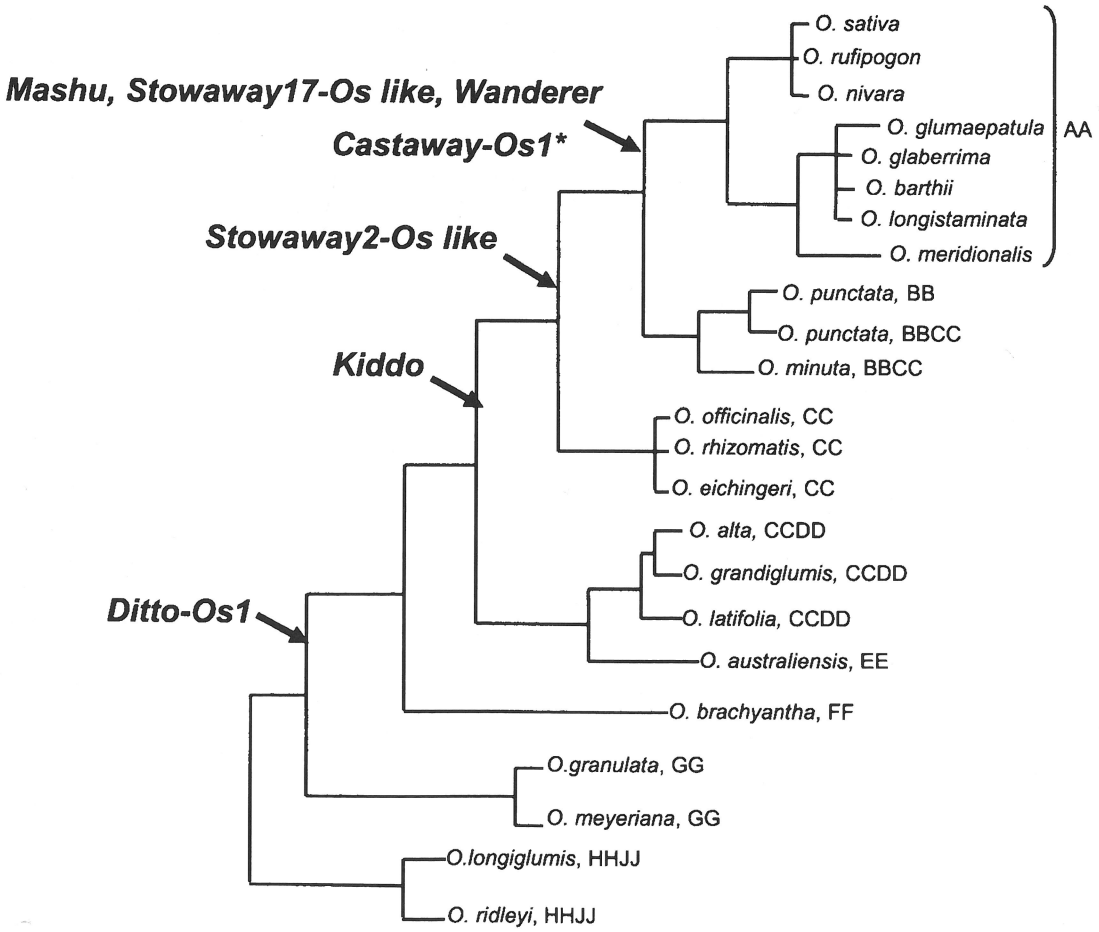

Fig. 3 An evolutionary model for seven MITEs analyzed. Phylogenetic tree of *Oryza* species is based on Zhu and Ge (2005). *: Not present in *O. meridionalis*.

four *Oryza* species complexes (Iwamoto *et al.* 1999). This suggests a possibility that the insertion of *Ditto-Os1* into important genes has been conserved up to now. Such insertion might have favorable effects on genus *Oryza* rather than neutral effects.

The transposition of TEs causes alteration of DNA sequences, such as addition and deletion in the host genome, thereby giving rise to various sequence polymorphisms (Morgante *et al.* 2005). Such indels (insertion and deletion) cause inter- and intra-specific variations in DNA sequence, which serve as useful markers to infer phylogenic relationships. Likewise, the presence of MITEs is one of the major causes of genetic diversity among natural populations of *Oryza* species. Kanazawa *et al.* (2000) found that the pattern of the presence or absence of MITEs is highly associated with speciation in AA genome species of wild rice. Moreover, *Pangrangja* elements are found to be present in AA, BB, CC, BBCC, CCDD, and EE genome species, and proved to be useful to study the genetic variations and species relationships in *Oryza* (Park *et al.* 2003). The present study also showed that differential distribution of MITEs in wild *Oryza* species consists with the evolutionary history of genus *Oryza*. Thus, genome- and/or species-specific MITEs might have contributed significantly to the adaptation, differentiation, speciation, and diversification processes of the genus *Oryza*.

References

Aggarwal, R. K., D.S. Brar and G.S. Khush (1997) Two new genomes in the *Oryza* complex identified on the basis of molecular divergence analysis using total genomic DNA hybridization. Mol. Gen. Genet. 254 : 1 - 12.

Aggarwal, R.K., D.S. Brar, S. Nandi, N. Huang and G.S. Khush (1999) Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor. Appl. Genet. 98 : 1320 - 1328.

Bergero, R., A. Forrest and D. Charlesworth (2008) Active miniature transposons from a plant genome and its nonrecombining Y chromosome. Genetics 178 : 1085 - 1092.

Bureau, T. E., P. C. Ronald and S. R. Wessler (1996) A computer-based systematic survey reveals the predominace of small inverted-repeat elements in wild-type rice genes. Proc. Natl. Acad. Sci. USA 93 : 8524 - 8529.

Bureau, T. and S. R. Wessler (1994) *Stowaway*: A new family of inverted repeat elements associated with the genes of both monocotyledonous and dicotyledonous plants. Plant Cell 6 : 907 - 916.

Feng, Q., Y. Zhang, P. Hao, *et al.* (2002) Sequence and analysis of rice chromosome 4. Nature 420 : 316 - 320.

Feschotte, C., N. Jiang and S. R. Wessler (2002) Plant transposable elements: where genetics meets genomics. Nat. Rev. Genet. 3: 329 - 341.

Feschotte, C. and E. J. Pritham (2007) DNA transposons and the evolution of eukaryotic genomes. Annu. Rev. Genet. 41 : 331 - 368.

Guo, Y. L. and S. Ge (2005) Molecular phylogeny of Oryzeae (Poaceae) based on DNA sequences from chloroplast, mitochondrial, and nuclear genomes. American Journal of Botany 92 : 1548 - 1558.

Hu, H., J. Mu, H. J. Zhang, Y. Z. Tao and B. Han (2006) Differentiation of a miniature inverted transposable element (MITE) system in Asian rice cultivars and its inference for a diphyletic origin of two subspecies of Asian cultivated rice. J. Integr. Plant Biol. 48 : 260 - 267.

Hurst, G. D. and J. H. Werren (2001) The role of selfish genetic elements in eukaryotic evolution. Nat. Rev. Genet. 2 : 597 - 606.

Iwamoto, M., H. Nagashima, T. Nagamine, H. Higo and K. Higo (1999) A *Tourist* element in the 5'-flanking region of the catalase gene *Cat*A reveals evolutionary relationships among *Oryza* species with various genome types. Mol. Genet. Genomics 262 : 493 - 500.

Jiang, N., Z. Bao, X. Zhang, H. Hirochika, S. R. Eddy, S. R. McCouch and S. R. Wessler (2003) An active DNA transposon family in rice. Nature 421 : 163 - 167.

Jiang, N., C. Feschotte, X. Zhang and S. R. Wessler (2004) Using rice to understand the origin and amplification of MITEs. Curr. Opin. Plant Biol. 7 : 115 - 119.

Kanazawa, A., M. Akimoto, H. Morishima and Y. Shimamoto (2000) Inter- and intra-specific distribution of *Stowaway* transposable elements in AA-genome species in rice. Theor. Appl. Genet. 101 : 327 - 335.

Kikuchi, K., K. Terauchi, M. Wada and H. Y. Hirano (2003) The plant MITE *mPing* is mobilized in anther culture. Nature 421 : 167 - 170.

Morgante, M., S. Brunner, G. Pea, K. Fengler, A. Zuccolo and A. Rafalski (2005) Gene duplication and exon shuffling by *Helitron*-like transposons generate intraspecies diversity in maize. Nat. Genet. 37 : 997 - 1002.

Murray, M. and W. F. Thompson (1980) Rapid isolation of high-molecular weight plant DNA. Nucleic Acids Res. 8 : 4321 - 4325.

Nakazaki, T., Y. Okumoto, A. Horibata, S. Yamahira, M. Teraishi, H. Nishida, H. Inoue and T. Tanisaka (2003) Mobilization of a transposon in the rice genome. Nature 421 : 170 - 172.

Oki, N., K. Yano, Y. Okumoto, T. Tsukiyama, M. Teraishi and T. Tanisaka (2008) A genome-wide view of miniature inverted-repeat transposable elements (MITEs) in rice, Oryza sativa ssp. japonica. Genes Genet. Syst. 83 : 321 - 329.

Park, K. C., J. K. Lee, N. H. Kim, Y. B. Shin, J. H. Lee and N. S. Kim (2003) Genetic variation in *Oryza* species detected by

MITE-AFLP. Genes Genet. Syst. 78 : 235 - 243.

Takagi, K., H. Nagano, Y. Kishima and Y. Sano (2003) MITE-transposon display efficiently detects polymorphisms among the *Oryza* AA-genome species. Breed. Sci. 53 : 125 - 132.

Yang, G., J. Dong, M. B. Chandrasekharan and T. C. Hall (2001) *Kiddo*, a new transposable element family closely associated with rice genes. Mol. Genet. Genomics 266 : 417 - 424.

Zhu, Q.H. and S. Ge (2005) Phylogenetic relationships among A-genome species of the genus *Oryza* revealed by intron sequences of four nuclear genes. New Phytol. 167 : 249 - 265.