MITE-transposon Display Efficiently Detects Polymorphisms among the *Oryza* AA-genome Species

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Miniature inverted repeat transposable elements (MITEs) are a major component of interspersed repetitive sequences in the rice genome. These elements serve as excellent tools for fine genomic analysis throughout the genome. Using the rice genome database, we evaluated MITEs present in a 200-kb region surrounding the rice *waxy* locus and selected four MITE subfamilies for MITE-transposon display (MITE-TD) analyses. MITE-TD, which is an AFLP-related technique based on MITE sequences, was applied to detect polymorphisms among the AA-genome *Oryza* species using the four selected MITEs. The MITE-TD used here enabled the most efficient detection of polymorphisms of all the molecular marker techniques applied to date in the *Oryza* species. Of the four MITEs, Mashu, a new MITE family, was found to be the best system for detecting the polymorphisms, with a detection frequency 3-1.5 times higher than that of the other three MITEs. The MITE-TDs also revealed information about genetic variations within the AA-genome species, and the complexity of the genetic relationships between *O. sativa* and *O. rufipogon*.

Key Words: *Oryza* species, MITE, transposon display, phylogenetic tree.

Introduction

The grass species vary in genome size (Bennetzen 2000). The larger-genome species of grasses such as maize and barley arose via a massive amplification of class 1 type elements, long terminal repeat (LTR) retrotransposons (SanMiguel *et al.* 1996, Suoniemi *et al.* 1996). Inevitably, the amplification of LTR retrotransposons resulted in expansion of the spacer regions in those grass species (Bennetzen *et al.* 1998, Kumar and Bennetzen 1999). On the other hand, the small genome species such as rice do not successfully amplify LTR retrotransposons, and thus they contain small spacers (Tarchini *et al.* 2000, Dubcovsky *et al.* 2001). However, abundant miniature inverted repeat transposable elements (MITEs) have been found in the spacer regions of the rice

genome (Bureau and Wessler 1994a, 1994b, Lisch *et al.* 2001, Nagano *et al.* 2002). MITE is a collective name for small transposon-like elements with terminal inverted repeats (TIRs) at the ends (Wessler *et al.* 1995). MITE families have been found in plants and animals, and most seem to be inactive (Feschotte *et al.* 2002). In contrast with active class 2 type elements, MITEs are characterized by uniformly small sizes (< 500 bp) and by target site preferences (Feschotte *et al.* 2002). MITEs also display a very high copy number and an even distribution throughout the chromosomes (Casa *et al.* 2000). They are known to be major elements contributing to the repetition in the rice genome architecture (Bureau *et al.* 1996, Turcotte *et al.* 2001, Nagano *et al.* 2002). However, little is known about the relative distribution and dynamism of MITEs among various rice species. If MITEs can be inserted into species-specific unique sites, they would provide a useful tool for characterizing genetic relationships at the whole-genome level.

In this study, we used the transposon-display (TD) method for MITEs-mediated phylogenetic analysis among rice-related species (Van den Broeck *et al.* 1998, Casa *et al.* 2000). We characterized repetitive sequences in a 200-kb region around the rice *waxy* locus, and identified 55 transposable element (TE)-like sequences (Nagano *et al.* 2002) therein. Fifty of the 55 TE-like sequences consisted of MITEs or MITE-like sequences, most of which are categorized into three representative MITE families, Stowaway, Tourist and Mu-like families (the Mu-like family is usually distinguishable from MITEs, since this family normally consists of copies larger than 500 bp). Here we studied major subfamilies with an abundant copy number predicted in the rice genome database from each of the three representative MITE families. In addition, one element from a newly identified MITE family around the rice *waxy* locus was also studied. The MITEs examined here were expected to become useful molecular markers within the AA-genome *Oryza* species from now on. Among the four MITE subfamilies selected for this study, Mashu, the new MITE subfamily, was found to have a markedly conserved copy organization, and to create polymorphisms in the AA-genome species at high frequency, suggesting that it has been active until recently. The phylogenetic study via MITE-TD showed that *O. rufipogon* possesses a wide range of genetic variation, suggesting its association with the cultivated rice species.

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Materials and Methods

Plant materials and genomic DNA extraction

Twenty-one lines from six *Oryza* species with the AA-genome and *O. officinalis* with the CC-genome were used in these experiments (Table 1). They were maintained as inbred lines by several selfings, except for *O. officinalis*. Total genomic DNAs were isolated as described by Nagano *et al.* (1999).

MITE-TD

The TD method used here was basically similar to the method described by Casa *et al.* (2000). We targeted four MITE elements: Stowaway OS-1 in the Stowaway/Tnr1 family, Tabito II in the Tourist family, Onaga I in the Mu-like family and Mashu in the Mashu family (Nagano *et al.* 2002). All the primers used here are listed in Table 2. The *TaqI* adaptor was prepared by annealing the two adaptor primers at room temperature after boiling. The *TaqI* adaptor

(50 µM) was ligated to the *TaqI*-digested genomic DNA (200 ng) by adding a mix containing 10 X ligation buffer, 0.5 µM ATP and 1 Weiss unit of T4 DNA ligase and incubated at 16°C overnight. Each of the primary primer sets was designed from the adaptor sequence and the terminal sequences of each MITE, and the first PCR reaction was performed in 20 µl of reaction mixture containing 1 X PCR buffer, 0.2 mM dNTPs (Takara Shuzo Co Ltd., Osaka), 15 ng of the adaptor-ligated DNA, 10 pmol of each primary primer and 1 unit of EX Taq polymerase (Takara Shuzo Co Ltd., Osaka). The first PCR reaction was carried out as follows: 72°C/2 min; 94°C/3 min; 24 cycles of 94°C/30 sec, 54°C/30 sec and 72°C/1 min, and a final cycle of 72°C/5 min. The PCR product was then diluted 20-fold with sterilized water.

Nested primers were used for the secondary PCR: 5' end of the secondary MITE primers were labeled with FITC, and the 3' end of the secondary adaptor primers were overhung by three selective nucleotides (in this study, we ran-

Table 1. A list of the *Oryza* strains used in this study

Genome	<i>Oryza</i> species	Type	Strain	Origin
AA	<i>O. sativa</i>	Japonica	A58	Japan
			Koshihikari	Japan
			T65	Taiwan
		Javanica	221	Philippines
			627	Celebes Is.
		Indica	IR36	Philippines
			Kasalath	India
			414	India
			868	Taiwan
	<i>O. rufipogon</i>	annual	W107	India
			W2002	India
			W630	Myanmar
			W1551	Thailand
		perennial	W120	India
			W149	India
			W1718	China
			W025	Guinea
			W1579b-2	Cameroon
CC	<i>O. glaberrima</i>		W1185	Surinam
	<i>O. barthii</i>		W1625	Australia
	<i>O. glumaepatula</i>		W0002	Unknown
	<i>O. meridionalis</i>			
	<i>O. officinalis</i>			

Table 2. Oligonucleotide sequences used for adaptors, adaptor primers and MITE primers

Type	Name	Sequence (5'→3')
Adaptor		GTGCTTGATGCTTGAAAC+CGGTTTCAAGCATCAA
Adaptor primer	adaptor 1st	GTGCTTGATGCTTGAAACCGA
	adaptor 2nd	GTGCTTGATGCTTGAAACCGANN
MITE primer	Stowaway Os-1 1st	GAATGACTTACATTGTGAAACGGAG
	Stowaway Os-1 2nd	GACTTACATTGTGAAACGGAGGGAG
	Tabito II 1st	GATGGAAAAAGTTGRAAGTTTGAAGAAAAAG
	Tabito II 2nd	TTGAAGAAAAAGTTWGGAACTAAACWCGGC
	Onaga I 1st	AATCGGATTTCGTTGACAAGTTAAGG
	Onaga I 2nd	CGTTGACAAGTTAAGGGACCTYAGAT
	Mashu 1st	ATAGATAGCACCTTACTTTACCATTG
	Mashu 2nd	CCTTACTTTACCATTGYGGGTGCTC

domly selected three kinds of secondary adaptor primers for each MITE-TD). The second PCR reaction was performed with the same reaction mixture as the first PCR plus 2 μ l of 50% DMSO. For the temperature cycling, we used a modification of the "touchdown method": 94°C/5 min, then 10 cycles beginning with 94°C/30 sec, 68°C/30 sec, 72°C/1 min, followed by a reduction of the annealing temperature of the first cycle (68°C) in 1°C increments per cycle, and then 27 cycles at an annealing temperature of 58°C, and a final incubation at 72°C/5 min.

The reaction products were electrophoresed on a 3.5% denaturing polyacrylamide gel containing 50 ml of a mixture of 3.5 ml of Long Ranger (equivalent to 50% polyacrylamide:bisacrylamide solution=19:1) and 7.0 M urea. After the gel was pre-run using 1 X TBE as running buffer, the samples were electrophoresed at a constant 1700 V for 80 min. The bands were visualized by scanning the fluorescent signals in the gel plate with a Typhoon 8600 (Molecular Dynamics Inc. Sunnyvale, California).

Data analysis

After the scan, the visualized gel profiles were saved as an ImageQuant image file (Molecular Dynamics Inc. Sunnyvale, California), and the sizes of the bands on the image were characterized by Fragment Analysis (Molecular Dynamics Inc. Sunnyvale, California). Each informative band was scored independently as 1 for "presence" and 0 for "absence". To estimate the level of polymorphisms between a pair of lines, the similarity coefficient (SC) (Dice 1945) between a pair of entries was calculated as

$$SC = N/(N_i + N_j - N)$$

where N is the number of shared fragments and N_i and N_j are the total numbers of fragments for entries i and j (Lynch 1990, Zhou and Gustafson 1995). The genetic distance coefficient (GDC) was calculated as

$$GDC = 1 - 2N/(N_i + N_j)$$

for constructing the phylogenetic trees. We constructed a neighbor-joining (NJ) tree based on these estimates (Saitou and Nei 1987). The tree was drawn using PAUP*4.0 (Swofford 1998).

Results and Discussion

Selection of MITEs for genome survey

A high copy number, conserved structure and unique insertion sites are the essential features of MITEs for use as efficient DNA markers. We searched for such MITEs by selecting elements with a high copy number residing in a 200-kb region around the *wx* locus in the *Oryza sativa* genome (Nagano *et al.* 1999, 2000). MITEs are major components of the repetitive sequences in the rice genome, particularly of the type of class II elements in the Tourist, Stowaway/Tnr1 and Mu-like families, which are frequently found in the rice genome. Each of these three families comprises several subfamilies (Nagano *et al.* 2002). The standard sequence of each subfamily was determined as the consensus of at least

10 independent sequences with over 95% homology. We performed a BLAST search (version 2.0, <http://www.ncbi.nlm.nih.gov/blast/>) against the rice genome sequences available as of July 2002. Using 50 bp of the 5' end region of each element as the query, we counted the number of rice genomic segments containing sequences resulting in a score of more than 50 bits (the maximum score is 100 bits) (Table 3). These outcomes indicate the numbers of hit segments deposited in the rice genome database, not the number of sequences present in the rice genome database. Therefore, these numbers express the degree of frequency of the elements in the rice genome. The numbers of the hit segments (> 50 bits) varied among the subfamily elements. We selected subfamilies with the highest or second-highest score among each of the three families: Tabito II (Tourist family), Stowaway OS-1 (Stowaway/Tnr1 family) and Onaga I (Mu-like family) obtained 398, 551 and 216 hit numbers, respectively. Moreover, a similar search was performed using five new MITEs. Among the five new elements, Mashu was found to be the most abundant (171 hit numbers).

Characteristics of the selected MITEs

We next investigated the characteristics of the selected MITE subfamilies, focusing on the degree of genetic divergence within these families. The segments listed in Table 3 are mostly large genomic fragments (> 100 kb), which were generally analyzed for the rice genome project, so that many segments contained several sequences homologous to the queries. For each segment, we counted the total number of sequences that had > 50 bits against the 50 bp of the 5' ends of the four standard MITEs. The sequences that scored more than 50 bits for the query of Stowaway Os-1, Tabito II, Onaga I and Mashu hit 1454, 764, 419 and 251 sequences, respectively. Since Stowaway OS-1 and Onaga I have long TIRs, 70 bp and 297 bp, respectively, the 50 bp from their 5' ends include TIRs. By replacing the sequence number by the copy number, we could roughly estimate the copy number per segment in each MITE as 1.3 (Stowaway Os-1), 1.0 (Onaga I), 1.9 (Tabito II) and 1.5 (Mashu). These results suggest that Tabito II shows a preference for localizing near another Tabito II element, as Jiang and Wessler (2001) reported, while the other elements show a relatively weaker insertion preference.

Fig. 1 shows the distributions of the bit scores and the percentages of the sequences hit by each MITE query in the BLAST analysis. A score of 100 bits indicates 100% homology with the 50 bp of the 5' ends of the standard sequences (but note that 50 bits is not equal to 50% homology). The majority of the copies belonging to Stowaway OS-1 and Tabito II were distributed in the low score range, and the ratio was reduced as the score increased. This tendency reflects a diverged structural organization of the two MITE subfamilies. Onaga I had a maximum range of 70-61 bits, which indicates a rather small degree of divergence among the copies. For Mashu, as many as 60% of the total number of sequences were in the range of 100-81 bits, indicating that

Table 3. The MITE subfamilies found around the rice *waxy* locus and the number of segments in the rice genome database obtained by BLAST search

Tourist	No. segments ¹⁾	Stowaway/TnrI	No. segments	Mu-like	No. segments	New MITEs	No. segments
TouristI	161	TnrI	445	RMu-A23	15	Akan	27
Castaway	105	StowawayOs1 ²⁾	551	Mermite	41	Mashu ²⁾	171
Ditto/TypeF ³⁾	454	StowawayOs4	12	OnagaI ²⁾	216	Saroma	37
Gaijin	192	TypeA	35	OnagaII	125	Shikotsu	53
TypeC	4	TypeB	198			Toya	13
TypeE	191	KiseruI	213				
TabitoI	31	KiseruII	163				
TabitoII ²⁾	398	KiseruIII	16				
TabitoIII	17	KiseruIV	345				
TabitoIV	133						
TabitoV	104						
TabitoVI	172						

All the subfamilies are categorized into the three representative MITE families and the new families.

These subfamilies are taken from Nagano *et al.* (2002).

¹⁾ No. segments means the number of segments with over 50 bits hit by BLAST analysis (<http://www.ncbi.nlm.nih.gov/blast/>).

²⁾ The subfamilies with double asterisks were used for further analysis.

³⁾ Ditto/TypeF had the largest number of segments hit with BLAST analysis, but we failed to design an appropriate primer for it for TD.

these were well-conserved elements among the four MITEs. The different patterns of divergence of the four MITEs lead us to suppose that the evolutionary times when they had activities in the *Oryza* genome differed in order of: Tabito II, Stowaway OS-1, Onaga I and Mashu (early to late). Tabito II is thought to have become inactive long before the other MITEs, because there are no sequences matching with 100–91 bits. On the other hand, more than 15% copies of Mashu correspond to 100–91 bits. Thus, it is possible that Mashu is an active element in the rice genome if its autonomous element is present.

MITE-TD analysis of the *Oryza* AA-genome species

We performed MITE-TD with the four selected subfamilies against 21 lines of *Oryza*. Appropriate primers were designed for each element and from the adaptors carrying three additional nucleotides as described in Materials and Methods. We carried out three displays on each of the MITE subfamilies with different adaptor primers. The plant materials subjected to MITE-TD have been maintained by selfing for several generations; thus, most loci are considered to have become homozygous. Table 4 summarizes the average numbers of bands detected with a single set of adaptor primers estimated from the total band numbers of MITE-TDs with the three adaptor primers. The same MITE primer resulted in nearly the same number of bands in 20 lines of the AA-genome species (Table 4). The consistent number of bands for each MITE within the AA-genome species implies that these MITEs were in the ancestral genome of the AA-genome species, and that these MITEs have not been amplified in a species-specific manner. Compared with the AA-genome species, the CC-genome species, *O. officinalis*, showed markedly fewer detectable bands in Stowaway OS-1, Tabito II and Mashu. In the AA-genome, the fact that four MITEs had saturated copy numbers might indicate that the

elements are in an inactive state.

MITE-TD readily revealed polymorphisms among the 20 lines of *Oryza* AA-species. The data extracted from 9 lines of *O. sativa* are summarized in Table 5. Hitherto, amplified fragment length polymorphism (AFLP) analysis showed the best performance for producing polymorphic bands in the *Oryza* species (Maheswaran *et al.* 1997, Zhu *et al.* 1998, Aggarwal *et al.* 1999). For instance, Maheswaran *et al.* (1997) reported that AFLP detected an average of 47.3 bands using a single primer pair and 10.4 bands (21.8% of total number of bands) as polymorphism between IR64 (Indica) and Azucena (Japonica). The results of the MITE-TD analyses performed here revealed that each of the four trials was superior to AFLP analysis in terms of both total band numbers and frequency of occurrence of the polymorphisms between Indica and Japonica. In particular, Mashu generated polymorphisms at frequencies of 3–1.5 times higher than the other elements in most of the plots (Table 5). The copies of Mashu tend to reside in unique sites, suggesting that Mashu became active recently.

Phylogenetic trees produced with MITE-TD

Based on the polymorphisms detected by MITE-TD, we constructed phylogenetic trees for the 21 *Oryza* strains (Fig. 2). The four trees made for individual MITE-TDs were constructed by the NJ method. The four dendrograms show basically similar topologies for the 21 lines from the seven *Oryza* species, but each tree revealed differences in the relative times at which the four MITEs had been active (Fig. 2). The trees reveal that the nine strains of *O. sativa* are divided into two clusters: one includes Japonica and Javanica strains and the other includes Indica strains. *O. rufipogon* contributes to both of the clusters as the wild progenitor. A perennial strain of *O. rufipogon* from China (W1718) seems to be included in the Japonica and Javanica cluster, whereas the

Fig. 1. Distributions according to their scores of MITE sequences as estimated by BLAST analysis against the rice genome.

Each 50 bp from the 5' end of the four MITEs was subjected to BLAST analysis (<http://www.ncbi.nlm.nih.gov/blast/>). The outcome sequences with more than 50 bits obtained by the BLAST search were sorted out every 10 bits. In the case of 100% matching with the 50-bp query, the score corresponds to 100 bits. The number of the outcome sequences obtained by the BLAST search is indicated above the histograms.

Table 4. Number of bands detected by the four MITE-TDs in the 21 *Oryza* strains ^{1,2)}

MITE	AA																			CC	
	A58	Koshi-hikari	T65	221	627	IR36	Kasa-lath	414	868	W107	W2002	W630	W1551	W120	W149	W1718	W025	W1579b-2	W1185	W1625	W0002
Stowaway Os-1	88.0	87.3	87.7	94.0	89.0	87.3	95.3	89.7	83.0	96.7	87.0	87.0	92.0	90.3	92.3	94.0	84.0	82.0	79.3	81.3	59.3
Tabito II	68.7	69.0	71.0	73.0	71.3	69.0	68.3	71.7	70.0	73.0	60.0	66.3	67.7	75.3	73.3	68.7	62.3	64.0	58.0	60.3	3.3
Onaga I	52.0	52.7	54.7	52.3	53.3	54.3	52.7	55.7	55.7	50.7	46.3	47.3	50.3	48.3	62.0	53.3	47.0	50.7	44.7	42.7	46.0
Mashu	64.7	68.3	64.3	64.7	62.3	67.3	71.0	64.0	66.0	72.7	60.0	64.7	70.3	69.3	74.0	63.3	58.3	55.7	55.3	35.3	13.3

¹⁾ The numbers are the averages of three trials with different adaptor primers.

²⁾ 21 *Oryza* strains are listed in Table 1.

other *O. rufipogon* strains from India or South-East Asian countries are included in the Indica cluster. In addition, an African cultivated rice species, *O. glaberrima*, made a distinct cluster together with its wild progenitor, *O. barthii*. Regarding the annual-perennial differentiation in the wild progenitors of Asian cultivated rices, Aggarwal *et al.* (1999) reported two distinct clusters corresponding to annuals or perennials based on AFLP analyses; however, the present study based on MITE-TDs showed that the W107 (annual) and W120 (perennial) from India are closely related to each other. These results support the idea that the differentiation in the longevity of the Asian wild rices generated responding

to ecological properties in wild rice, but not a consequence of speciation (Morishima *et al.* 1984). To resolve the questions noted above, it will be necessary to examine a large number of strains of wild and cultivated rice species.

MITE-TD analysis has the potential of locating markers throughout *Oryza* genomes, and of producing detailed phylogenetic trees. This method is also applicable for the isolation of genes tagged with a specific MITE and exploring molecular markers linked with MITEs. Our MITE-TD system generated polymorphic bands between Japonica and Indica at more than twice the frequency of AFLP analysis. All four MITEs used here were very useful for examining

Table 5. The frequency indexes of polymorphisms between two lines of the nine *O.sativa* strains detected by the four MITE-TDs¹⁾

Stowaway Os-1	Japonica			Javanica		Indica			
	A58	Koshihikari	T65	221	627	IR36	Kasalath	414	868
A58		0.07	0.09	0.24	0.26	0.50	0.52	0.49	0.51
Koshihikari			0.04	0.21	0.24	0.48	0.50	0.48	0.50
T65				0.20	0.24	0.48	0.50	0.48	0.50
221					0.18	0.50	0.47	0.46	0.50
627						0.48	0.47	0.45	0.49
IR36							0.37	0.29	0.34
Kasalath								0.36	0.43
414									0.27
868									

Tabito II	Japonica			Javanica		Indica			
	A58	Koshihikari	T65	221	627	IR36	Kasalath	414	868
A58		0.08	0.09	0.20	0.30	0.54	0.55	0.55	0.56
Koshihikari			0.05	0.21	0.29	0.54	0.55	0.53	0.55
T65				0.19	0.28	0.53	0.56	0.54	0.54
221					0.22	0.49	0.53	0.50	0.49
627						0.51	0.53	0.50	0.51
IR36							0.43	0.32	0.32
Kasalath								0.45	0.44
414									0.24
868									

Onaga I	Japonica			Javanica		Indica			
	A58	Koshihikari	T65	221	627	IR36	Kasalath	414	868
A58		0.04	0.06	0.21	0.22	0.48	0.50	0.52	0.46
Koshihikari			0.04	0.21	0.21	0.48	0.49	0.53	0.46
T65				0.24	0.24	0.49	0.50	0.53	0.47
221					0.18	0.44	0.43	0.46	0.39
627						0.43	0.41	0.44	0.41
IR36							0.43	0.28	0.25
Kasalath								0.36	0.36
414									0.27
868									

Mashu	Japonica			Javanica		Indica			
	A58	Koshihikari	T65	221	627	IR36	Kasalath	414	868
A58		0.14	0.11	0.40	0.41	0.70	0.68	0.72	0.72
Koshihikari			0.15	0.43	0.43	0.71	0.70	0.73	0.73
T65				0.42	0.40	0.71	0.69	0.72	0.72
221					0.47	0.70	0.67	0.73	0.72
627						0.64	0.65	0.65	0.64
IR36							0.56	0.42	0.46
Kasalath								0.56	0.60
414									0.35
868									

¹⁾ The frequency index was calculated as 1-SC (SC is described in Materials and Methods).

the genetic diversity among closely related species such as the AA-genome species. For distantly related species such as the AA- and CC-genomes, however, this method is not suitable because the distribution of MITEs differs in them. Among the MITEs examined here, Mashu could most efficiently detect polymorphisms for the AA-genome species. We could not conclude whether Mashu has stabilized or still remains active. Even if Mashu is inactive, it is a powerful MITE system for providing molecular markers throughout

the rice genome for TD analysis.

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Stowaway Os -1 **Tabito II** **Onaga I** **Mashu**

Fig. 2. Phylogenetic trees of the 21 strains of *Oryza* species constructed with individual MITE-TDs.

Dendrograms were drawn with the NJ methods based on polymorphisms obtained by MITE-TDs. The root length, which reflects the genetic distance, is equivalent for all the trees. The abbreviations for species names and types are as follows; sat: *sativa*, ruf: *rufipogon*, gla: *glaberrima*, bar: *barthii*, glu: *glumaepatula*, mer: *meridionalis*, off: *officinalis*, jp: Japonica, jv: Javanica, in: Indica, an: annual, pe: perennial.

Literature Cited

- 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.
- Bennetzen, J.L. (2000) Transposable element contributions to plant gene and genome evolution. *Plant Mol. Biol.* 42: 251-269.
- Bennetzen, J.L., P.SanMiguel, M.S.Chen, A.Tikhonov, M.Francki and Z.Avrarova (1998) Grass genomes. *Proc. Natl. Acad. Sci. USA* 95: 1975-1978.
- Bureau, T.E. and S.R.Wessler (1994a) *Stowaway*: a new family of inverted repeat elements associated with the genes of both monocotyledonous and dicotyledonous plants. *Plant Cell* 6: 907-916.
- Bureau, T.E. and S.R.Wessler (1994b) Mobile inverted-repeat elements of the *Tourist* family are associated with the genes of many cereal grasses. *Proc. Natl. Acad. Sci. USA* 91: 1411-1415.
- Bureau, T.E., P.C.Ronald and S.R.Wessler (1996) A computer-based

- systematic survey reveals the predominance of small inverted-repeat elements in wild-type rice genes. *Proc. Natl. Acad. Sci. USA* 93: 8524-8529.
- Casa, A.M., C.Brouwer, A.Nagel, L.Wang, Q.Zhang, S.Kresovich and S.R.Wessler (2000) The MITE family *heartbreaker (Hbr)*: molecular markers in maize. *Proc. Natl. Acad. Sci. USA* 97: 10083-10089.
- Dice, L.R. (1945) Measures of the amount of ecological association between species. *Ecology* 26: 297-302.
- Dubcovsky, J., W.Ramakrishna, P.J.SanMiguel, C.S.Busso, L.L.Yan, B.A.Shiloff and J.L.Bennetzen (2001) Comparative sequence analysis of colinear barley and rice bacterial artificial chromosomes. *Plant Physiol.* 125: 1342-1353.
- Feschotte, C., N.Jiang and S.R.Wessler (2002) Plant transposable elements: where genetics meets genomics. *Nat. Rev. Genet.* 3: 329-341.
- Jiang, N. and S.R.Wessler (2001) Insertion preference of maize and rice miniature inverted repeat transposable elements as revealed by the analysis of nested elements. *Plant Cell* 13: 2553-2564.
- Kumar, A. and J.L.Bennetzen (1999) Plant retrotransposons. *Annu. Rev. Genet.* 33: 479-532.
- Lisch, D.R., M.Freeling, R.J.Langham and M.Y.Choy (2001) *Mutator* transposase is widespread in the grasses. *Plant Physiol.* 125: 1293-1303.
- Lynch, M. (1990) The similarity index and DNA fingerprinting. *Mol. Biol. Evol.* 7: 478-484.
- Maheswaran, M., P.K.Subudhi, S.Nandi, J.C.Xu, A.Parco, D.C.Yang and N.Huang (1997) Polymorphism, distribution, and segregation of AFLP markers in a doubled haploid rice population. *Theor. Appl. Genet.* 94: 39-45.
- Morishima, H., Y.Sano and H.I.Oka (1984) Differentiation of perennial and annual types due to habitat conditions in the wild rice *Oryza perennis*. *Plant Syst. Evol.* 144: 119-135.
- Nagano, H., S.Kawasaki, Y.Kishima and Y.Sano (2000) Structural differences in the vicinity of the *waxy* locus among the *Oryza* species with the AA-genome: identification of variable regions. *Theor. Appl. Genet.* 100: 376-383.
- Nagano, H., M.Kunii, T.Azuma, Y.Kishima and Y.Sano (2002) Characterization of the repetitive sequences in a 200-kb region around the rice *waxy* locus: diversity of transposable elements and presence of veiled repetitive sequences. *Genes Genet. Syst.* 77: 69-79.
- Nagano, H., L.Wu, S.Kawasaki, Y.Kishima and Y.Sano (1999) Genomic organization of the 260 kb surrounding the *waxy* locus in a Japonica rice. *Genome* 42: 1121-1126.
- Saitou, N. and M.Nei (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.
- SanMiguel, P., A.Tikhonov, Y.K.Jin, N.Motchoulskaia, D.Zakharov, A.MelakeBerhan, P.S.Springer, K.J.Edwards, M.Lee, Z.Avramova and J.L.Bennetzen (1996) Nested retrotransposons in the intergenic regions of the maize genome. *Science* 274: 765-768.
- Suoniemi, A., K.AnanthawatJonsson, T.Arna and A.H.Schulman (1996) Retrotransposon BARE-1 is a major, dispersed component of the barley (*Hordeum vulgare* L.) genome. *Plant Mol. Biol.* 30: 1321-1329.
- Swofford, D.L. (1998) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Method), Version 4. Sinauer Association, Sunderland, Massachusetts.
- Tarchini, R., P.Biddle, R.Wineland, S.Tingey and A.Rafalski (2000) The complete sequence of 340 kb of DNA around the rice *Adh1-adh2* region reveals interrupted colinearity with maize chromosome 4. *Plant Cell* 12: 381-391.
- Turcotte, K., S.Srinivasan and T.Bureau (2001) Survey of transposable elements from rice genomic sequences. *Plant J.* 25: 169-179.
- Van den Broeck, D., T.Maes, M.Sauer, J.Zethof, P.De Keukeleire, M.D'Hauw, M.VanMontagu and T.Gerats (1998) *Transposon display* identifies individual transposable elements in high copy number lines. *Plant J.* 13: 121-129.
- Wessler, S.R., T.E.Bureau and S.E.White (1995) LTR-retrotransposons and MITEs: important players in the evolution of plant genomes. *Curr. Opin. Genet. Dev.* 5: 814-821.
- Zhou, Z. and J.P.Gustafson (1995) Genetic-variation detected by DNA-fingerprinting with a rice minisatellite probe in *Oryza sativa* L.. *Theor. Appl. Genet.* 91: 481-488.
- Zhu, J., M.D.Gale, S.Quarrie, M.T.Jackson and G.J.Bryan (1998) AFLP markers for the study of rice biodiversity. *Theor. Appl. Genet.* 96: 602-611.