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Insertional Mutation of the Rice Blast Resistance Gene, *Pi-b*, by Long Terminal Repeat of a Retrotransposon

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The *Pi-b* is the rice gene conferring race specific resistance to the blast fungus *Magnaporthe grisea* race having a corresponding avirulence gene, *AVR-Pi-b*. All resistant cultivars have two copies of the *Pi-b* gene, but susceptible cultivars have a single copy of the gene. About 1 Kbp insertion sequence was detected in the open reading frame of the *Pi-b* gene from the susceptible cv. Nipponbare. The nature of insertion sequence was identified as a solo long terminal repeat (LTR) of new rice *Ty1-copia*-like retrotransposon. LTR was widely distributed in the rice genome. Various types of different patterns of restriction fragment length polymorphism of LTR were detected in *indica* cultivars, whereas a single type was detected from *japonica* cultivars. The insertion of LTR sequence in the *Pi-b* gene in the susceptible cultivar suggested that retrotransposon-mediated insertional mutation might played an important role in the resistance breakdown as well as evolution of resistance genes in rice.

Keywords : LTR, *Pi-b*, resistance, retrotransposon, RFLP.

Rice blast, caused by *Magnaporthe grisea*, is one of the most devastating diseases throughout rice-growing regions in the world. Breeding for resistant varieties has been a major tool for controlling this disease (Bonman, 1992). However, frequent breakdown of resistance has been reported relatively short after the resistant cultivars released in the field (Kiyosawa, 1982). Much effort has been conducted to understand the mechanisms responsible for breakdown of resistance. The phenomenon of resistance-breakdown to date has been mostly explained by genetical changes in the fungal pathogens through heterokaryosis (Suzuki, 1965), parasexual recombination (Genovesi and Magill, 1976), and aneuploid (Kameswar Row et al., 1985). More recently, unstability of avirulence (*AVR*) genes of the fungal pathogen such as *AVR-Pita*, *AVR1-TSUY* and *PWL2* was suggested as an important factor in virulence changes at the molecular level (Valent and Chumley, 1994). It was also reported that *M. grisea* race containing *AVR-Pita* gained virulence through mutations including deletions, point mutation and insertion of *Pot3* in the *AVR* gene (Valent and Chumley, 1994). However, the precise mechanism of resistance-breakdown in rice against fungal infections remains to be elucidated. Although several potential mechanisms for new race appearance have been proposed, little information is available on the mechanisms of resistance-breakdown in the plant.

During the last decade, rapid technical advances in plant molecular genetics made it possible to study on the mechanism of resistance-breakdown in rice. Several resistance genes in rice were identified, mapped and characterized at the molecular level. Furthermore, accumulation of genome sequencing data helps to understand the structure and distribution of these genes in the rice genome. The *Pi-b* is the resistance gene conferring resistance to rice blast fungus *M. grisea* race having an avirulence gene, *AVR-Pi-b*. This gene was originally found in *indica* cultivars and introgressed into *japonica* background on the telomere region of chromosome 2 (Yokoo et al., 1978). The *Pi-b* had been mapped with high resolution and cloned by positional cloning strategy (Miyamoto et al., 1996; Monna et al., 1997; Wang et al., 1999b).

Since McClintock (1948) developed the concept of transposable elements as mobile genetic entities, numerous transposable elements from various plants were identified and characterized. Transposons alter their chromosomal locations and consequently often induce mutations by integrating into other genes and destroying their structural integrity (Gierl and Saedler, 1992; Hirochika, 1993; Hirochika et al., 1996). Several transposons such as a family of *Tos*, *copia*-like retrotransposon, p-SINE1, *RIRE1*, *RIRE2* have been identified in *Oryza sativa* and wild rice species (Hirochika et al., 1992; Wang et al., 1999a; Mochizuki et al., 1992; Noma et al., 1997; Ohtsubo et al., 1999). It has been suggested that some of the ancient transposable elements may have played an important role in the evolution of plant genomes by altering structures of coding regions, pattern of splicing, and regulation of gene expression

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(Finnegan, 1989; Weil et al., 1990; Wessler et al., 1995). The higher plant genome undergoes transposon-mediated structural changes during plant development as well as over evolutionary time period (Finnegan, 1989). Induction and insertion of retrotransposon *Tos*17 were also activated in rice during tissue culture (Hirochika, 1997). In the process of genetic rearrangement by transposons, mutations could occur in resistance genes or flanking regions. *Tourist* and *Drone* transposons were to be involved in loss of resistance in maize against *Cochliobolus carbonum* by insertional mutation at *hm*1 locus (Bureau and Wessler, 1992; Mutani et al., 1998).

However, there has been no report on the retrotransposon-mediated breakdown of resistance in rice. In the process of sequencing analysis of contig clones covering the *Pi-b* gene from both resistant and susceptible cultivars, insertion of retrotransposon-like sequence was detected in the open reading frame (ORF) of *Pi-b* gene from the susceptible cultivars. Here, we report the possible role of insertion of retrotransposon in the resistant gene on the breakdown of resistance.

Materials and Methods

Rice cultivars. Plant materials used in these experiments are listed in Table 1.

Cloning of long terminal repeat (LTR) and sequencing analysis. Part of the 4th and 5th exons of *Pi-b* was amplified by polymerase chain reaction (PCR) from both resistant cv. BL-1 and susceptible cv. Nipponbare (Fig. 2). The pair of the primer [sense: 5'-GGTAGAAAATAGAGTCAGCAGTAAG-3'(Exn-4), antisense: 5'-TATGTACCTCTGATGTCTAGCA-3', (Exn-5)] were used. Two 5' end of *Pi-b* primers [sense: 5'-ATCAACTCTGCCACAAAATCC-3', antisense: 5'-CCCATATCACCACTTGTTCCCC-3' (designated as PIB5-F, PIB5-R)] were designed based on *Pi-b* cDNA sequence data (Wang et al., 1999b) and listed in Fig. 2. For the specific amplification of LTR fragment, a primer pair [sense: 5'-AGGCCAAGTTCGGAGTTTCTGACGCCGG-3', (LTR-5), antisense: 5'-CGCAGTTCATTAGGATAGCTGGCACTGGTT-3', (LTR-3)] was prepared according to the sequence of 1Kb LTR sequence identified in susceptible *Pi-b* region (Fig. 3). Thirty cycles of amplification was performed under the following condition. Template DNA was denatured 1 min at 94°C, annealed at 55°C for 1 min, denatured at 94°C for 1 min and DNA was synthesized at 72°C for 3 min. PCR products were cloned in TOPO TA cloning kit according to the manufacturer's instruction (Invitrogen, Carlsbad, CA, USA). Sequencing reaction was carried out on DNA Amp PCR reaction system 9600 (Perkin Elmer, Foster City, CA, USA) using BigDye cycle sequencing kit with AmpliTag DNA polymerase FS (Perkin Elmer) and sequenced by PE ABI prism 377 DNA sequencer (Perkin Elmer) according to the manufacturers instruction. All the sequencing data were analyzed using Genetyx software (Software development, Tokyo, Japan). Homology search of nucleotide and amino acid sequences was conducted with a BLAST against the sequences in the GenBank and EMBL databases.

Genomic Southern blot hybridization. Rice genomic DNA was extracted according to the method of Rogers and Bendich (1985). Genomic DNAs of susceptible cultivars, resistant near isogenic lines (NILs) and *indica* cultivars were digested with *Eco* RV, fractionated on 0.7% agarose gel and blotted onto Hybond-N^+ membrane (Amersham Pharmacia biotech, Buckinghamshire, UK). Probe DNA fragments were amplified with PCR reactions with two sets of primers LTR-5/LTR-3 and PIB5-F/PIB5-R. Southern hybridization was performed normally for 16 hr at 65 °C. The membranes were washed with (0.2% SDS, 2X SSC), (0.2% SDS, 1X SSC) and (0.2% SDS, 0.2X SSC) at 65 °C for 10 min each time. Washed membranes were exposed onto medical blue X-Ray film (Agfa-Gevaert, Germany).

Identification of a retrotransposon with LTR. Genomic DNA from cv. Nipponbare was partially digested with *Hind* III and used to construct genomic library. Digested DNA was ligated into pBI121 vector and transformed into *Escherichia coli* DH10B. Colonies on LB agar with antibiotics were blotted onto Hybond N^+ nylon membrane. Positive clones were identified by colony hybridization using a PCR product amplified with two primers (LTR-5 and LTR-3) as a probe. Four clones were selected and characterized by sequencing analysis to obtain a full length of a retrotransposon.

Table 1. Rice cultivars used in this study

Species	Origin	Varietal group	Cultivar	Source[a]
Oryza sativa	Japan	*japonica*	Nipponbare	SNU
	Japan	*japonica*	Shimokita	SNU
	Japan	*japonica*	BL-1	NIAR
	Japan	*japonica*	BL-3	NIAR
	Japan	*japonica*	BL-5	NIAR
	Japan	*japonica*	BL-7	NIAR
	Japan	*japonica*	Touhoku IL9	MPFAES
	Indonesia	*indica*	Tjina	SNU
	Malaysia	*indica*	Milek Kuning	SNU
	Philippine	*indica*	Taducan	SNU
	India	*indica*	Kasalath	SNU

[a]Gifts from Prof. Hee Jong Koh of Seoul National University (SNU). Gene bank of National Institute of Agrobiological Resources (NIAR, Tsukuba, Japan). Gifts from K. Matunaga and T. Sasaki of Miyagi Prefectural Furukawa Agriculural Experiment Station (MPFAES, Furukawa, Japan)

Results

Distribution of the *Pi-b* gene in rice genome. As the first step to understand the molecular nature of the *Pi-b* gene in rice, sequencing analysis of the *Pi-b* regions was conducted with contig clones. Sequencing data revealed that an additional non-functional *Pi-b* homologue existed near to a functional *Pi-b* gene in a resistant cultivar BL-1. To understand the relationship between the copy number of the *Pi-b*

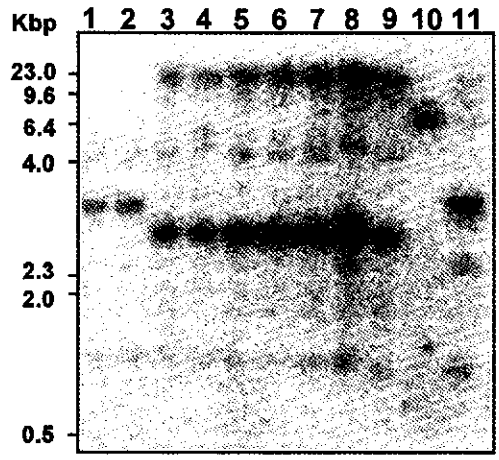

Fig. 1. Restriction fragment length polymorphism pattern of the near isogenic lines (NILs) and donor parents and susceptible cultivars. Genomic DNAs (1 μg) from susceptible *japonica, indica* cultivars and NILs were digested with *Eco* RI and fractionated, blotted and probed with 5' end of *Pi-b* cDNA probe (Fig. 2). Lane 1, Nipponbare; 2, Shimokita; 3, BL-1; 4, Tjina; 5, BL-5; 6, BL-3; 7, BL-7; 8, Milek Kuning; 9, Touhoku IL9; 10, Kasalath; 11, Tadukan

gene and resistant/susceptible reaction against *M. grisea* race *AVR-Pi-b*, genomic Southern blot analysis was conducted from *indica* and *japonica* cultivars with 5'-end of the *Pi-b* gene as a probe. As shown in Fig. 1, all resistant cultivars including *japonica* and *indica* rice exhibited two hybridization bands, suggesting two copies of the *Pi-b* gene exist. However, all susceptible cultivars exhibited a single hybridization band, although cv. Kasalath showed a different size of hybridization band (Fig. 1).

Characterization of insertion sequence in the *Pi-b* gene. Sequencing of *Pi-b* gene from susceptible cv. Nipponbare exhibited insertion sequence in the ORF of the *Pi-b* gene. Since the insertion sequence was located in exon 5, the insertion sequence was amplified by PCR with primers of Exn-4 and Exn-5 from cv. Nipponbare and resistant NIL, BL-1. As expected, the amplified band from cv. Nipponbare shifted about 1 Kbp compared to that from NIL, BL-1 (Fig. 2). The insertion sequence was further characterized by sequencing and homology search with a BLAST in the GenBank and EMBL databases. No specific ORF was found in the 965 bp inserted sequence.

To characterize the nature of this sequence, genomic library of cv. Nipponbare was constructed and screened by insertion sequence as a probe. Four positive clones were identified by colony hybridization. Sequencing of four clones revealed a *Ty1-copia*-like new retrotransposon (*Osr1*) in rice. This *Osr1* has two long terminal repeats (LTR, 965 bp) and a large ORF (3.8 Kb) in the middle. The insertion sequence in the *Pi-b* gene was identified as a solo LTR of the *Osr1*. Full sequence of this LTR is shown in Fig. 3. This sequence has two dinucleotides TG and CA at 5' and 3' ends as consensus sequences, respectively.

To examine the distribution of LTR sequence of the *Osr1* in the rice genome, genomic Southern blot analysis was carried out with LTR as a probe. LTR was widely distributed in the rice genome. Various types of restriction fragment length polymorphism (RFLP) of LTR were detected in *indica* cultivars, whereas a single type was detected from *japonica* cultivars (Fig. 4).

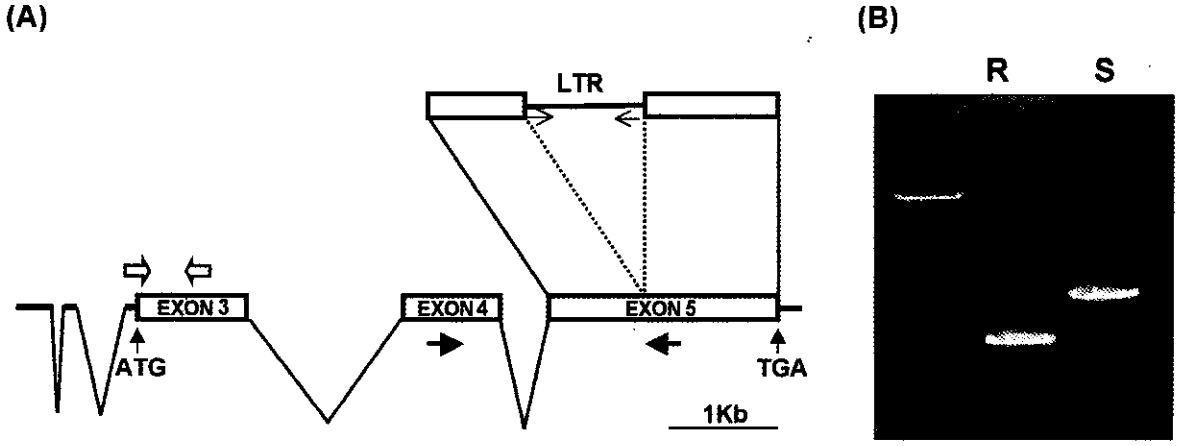

Fig. 2. PCR-amplification of 4th and 5th exon fragment with internal primer pairs. A. Genomic structure of the *Pi-b* gene. Exons are indicated as bald horizntal lines and open boxes, and lines angled downward indicate introns. Closed arrows represent pair of primers used for PCR-amplification ORF including insertion sequence (Exn-4 and Exn-5). Insertion sequence identified as LTR are indicated as bald line in the diagram. Open arrows are primer pairs used for amplification of 5' end of *Pi-b* cDNA clone (PIB5-F and PIB5-R). Two arrows located under the LTR are primers for amplification of LTR-specific probe(LTR-5 and LTR-3). B. PCR-product length polymorphism profile of the NIL and susceptable cv. Nipponbare. PCR-product from susceptible cv. Nipponbare shifted up 1Kb compared to that of resistant NIL (BL-1). PCR-products were separated by electrophoresis on the 0.8% agarose gel.

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1   TGTTAGATTT AATTGGGTAA TTAATCCATT TAAATCAATT AAATCAAATA AATTTCAAGG
61  CTCATTATGC TAGGAATTCA TGTGAATTCA TTCTTCTATG GGATATCAAT GGGATGAAGA
121 GTTTTGAGAA TTAATCCATT TGATTAAGGA ATTGGTAACT TATGTCAATT AGTCCTAATT
181 GATGGATGGT TGATGGTTGT GTAGTGGAGG ATGGTTCATG GCTAGTTGAT GACATTTAGT
241 TGCTCTATTC CTCTTCCTAT TCCATTGGTA ACTTACATCA ATTACTCTTA ATTGATTGTT
301 GGTTGATGGT TGTGTAGTGG AGGATGGTTC ATGGCTAGTT GATGACAATT AGTTGCTCCA
361 TTCCTCTTCC TATTCCATGA CTCTTACTCT TCATCTTCCA TTCCTCTTAT AAAATGGGAA
421 TGGATTTGAT CTCCCGCGAG AAAAAGAAGA CACACTTTCA TCCATTTTCC AAAGCTGTTG
481 TTGCTACGGT AATCCCATCC CGACGAGTGT GTGCACACGC GTTGGGAGAG TAGGCCTCCG
541 AAACCACGCG TTGCTGCGAC GTTTGCACAG ACGGGCGGGC GATCAGGTTT TTGGGGAGCG
601 CAAGGCGCGA CTACTCACTG TTCGTCAACA TCTACTTCAT CTTCACCAAC ATGTCGAACA
661 CTGGAGACAA GGAGAAGGAG ACTCCCGTCA ACACCAACGG AGGCAATACT GCCTCAAACT
721 CCAGCGGAGG ACCATTCTTG GGGTATAACC TTATTACATT ATTTCAATTA GAAGTTTTAC
781 TGTTAATGTT CATCGCAATG TCAACATTGT GTCATTATGT GATTGTTGAT GCTTATTCAA
841 CGTTAAGCAT GCTCATGTTG ATTACATTCA ACACTATCAC TGGATCAAAT CCTATTGTAA
901 ATATCATGTT TATTATCTTG TTATTTTGGA TTAAAATATG CCGAATTATG ACCAAATTTC
961 CAACA
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Fig. 3. The nucleotide sequence of LTR of the new rice retrotransposon. One unit of LTR is consist of 965 bp nucleotide and 5'-LTR had 99.8% identity with 3'-LTR. Two dinucleotide TG, CA were present at 5' and 3' ends as consensus sequences and indicated in the boxes.

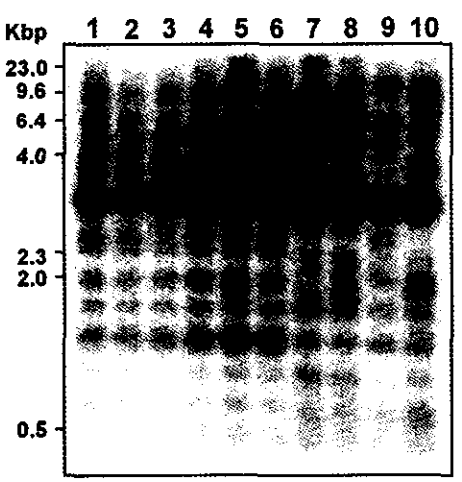

Fig. 4. Genomic Southern blot analysis of the LTR of the new rice retrotransposon. Genomic DNAs from susceptible cultivars and resistant NILs and donor cultivars were digested with *Eco* RV and fractionated, blotted and hybridized with an PCR-amplified LTR specific probe (Fig. 2). Lane 1, Nipponbare; 2, Shimokita; 3, BL-1; 4, BL-3; 5, BL-7; 6, Touhoku IL9; 7, Tjina; 8, Milek Kuning; 9, Tadukan; 10, Kasalath

Discussion

Resistant *indica* cultivars against *M. grisea AVR-Pi-b* had two copies of the *Pi-b*, but susceptible cultivars had a single copy of the *Pi-b* gene. The similar phenomenon was observed in *japonica* cultivars and NILs. Two copies of the *Pi-b* in NILs of *japonica* background could be explained by the fact that *Pi-b* was introgressed from *indica* into *japonica* cultivars.

We have identified LTR insertion in the ORF of the *Pi-b* gene in susceptible cv. Nipponbare, but not in resistant cultivars. This is the first report that retrotransposon-mediated insertional mutation may be involved in resistance-breakdown in rice against the rice blast fungus. Similar phenomena have also been reported in maize and soybean (Multani et al., 1998; Bhattacharyya et al., 1997). Two insertions, *Tourist* and *Drone*, were identified in intron and exon of *hm*1 locus in maize, respectively. These insertions caused the loss of resistance in maize against *Cochliobolous carbonum* infection. In soybean, a *copia-like* retrotransposon Tgm*r* was also closely linked to *Rps1-K* allele that confers race specific resistance against *Phytophthora sojae* infection.

Recently, the sequence of new rice retrotransposon *Osr1* was registered in the form of total sequence of PAC clone (6630680) as well as EST partial sequence (AP000969) on the GenBank database. This indicates that this element is active and may play a role on genome rearrangement in rice. This proposal is further supported by the fact that the *Osr1* is widely distributed in the rice genome, rendering genetic rearrangement by homologous recombination between closely or distantly located retrotransposons. A solo LTR of *Osr1* was remained on the *Pi-b* ORF in the susceptible cultivars. It suggests that the polymerase region (*pol*) of the *Osr1* was deleted by recombination, and LTR was left as foot print. It has also been reported that several LTR-retrotransposons in other plants are being defective or existing as solo LTR, resulted by internal deletions, rearrangements or replacements (Hu et al., 1995; Jin and Bennetzen, 1994).

The *Osr1*, a new rice *Ty1-copia*-like retrotransposon, consists of two 965 bp LTRs and 3.8 Kbp ORF including the reverse transcriptase region. The nucleotide sequence of *Osr1* was 81% homologous to wheat *Tar1* retrotransposon (Matsuoka and Tsunewaki, 1997). The size of *Osr1* is relatively long compared to other retrotransposons, but shorter than *RIRE1* retrotransposon of *Oryza australiensis* (Noma et al., 1997).

Although the *Osr1* TS widely distributed in rice genome, more polymorphisms of LTR-RFLP were found from *indica* compared to *japonica* cultivars. This is further supported by the fact that *japonica* cultuvars in Japan, China and Korea exhibited almost identical pattern of LTR-RFLP (unpublished data). Another explanation may be available on various types of LTR-RFLP in *indica* cultivars. *Japonica* cultivars were originated from one of LTR-RFLP types in *indica* culativars. It suggests that *Osr1* might have been

more active in *indica* than in *japonica* cultivars, but the mechanisms involved in activation of this retrotransposon are not known. The transcription of *Osr1* was activated when the suceptible cv. Nipponbare was infected by a virulent *M. grisea* race (unpublished data). McClintock (1984) also suggested that stress activated transposable elements in plants and played an important role in generation of new individual or species. It is tempting to speculate that activation of *Osr1* may be regulated by infection with different races of *M. grisea*. In conclusion, retrotransposon-mediated insertional mutation in resistance genes may be another factor for resistance breakdown in rice in addition to new race appearance.

References

Bhattacharyya, M. K., Gonzales, R. A., Kraft, M. and Buzzell, R. I. 1997. A *copia*-like retrotransposon Tgm*r* closely linked to the *Rps1*-k allele that confers race-specific resistance of soybean to *Phytophthora sojae*. *Plant Mol. Biol.* 34:255-264.

Bonman, J. M., Khush, G. S. and Neson, R. J. 1992. Breeding for rice resistance to pests. *Annu. Rev. Phytopathol.* 30:507-528.

Bureau, T. E. and Wessler, S. R. 1992. *Tourist*: a large family of small inverted repeat elements frequently associated with maize genes. *Plant Cell* 4:1283-1294.

Finnegan, D. J. 1989. Eukaryotic transposable elements and genome evolution. *Trends in Genet.* 5:103-107.

Genovesi, A. D. and Magill, C. W. 1976. Heterokaryosis and parasexuality in *Pyricularia oryzae* Cavara. *Can. J. Microbiol.* 22:531-536.

Gierl, A. and Saedler, H. 1992. Plant-transposable elements and gene tagging. *Plant Mol. Biol.* 19:39-49.

Hirochika, H., Fukuchi, A. and Kikuchi, F. 1992. Retrotransposon families in rice. *Mol. Gen. Genet.* 233:209-216.

Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H. and Kanda, M. 1996. Retrotransposons of rice involved in mutations induced by tissue culture. *Proc. Natl. Acad. Sci.* USA 93:7783-7788.

Hirochika, H. 1993. Activation of tobacco retrotransposons during tissue culture. *EMBO J.* 12:2521-2528.

Hirochika, H. 1997. Retrotransposons of rice: their regulation and use for genome analysis. *Plant Mol. Biol.* 35:231-240.

Hu, W., Das, O. P. and Messing, J. 1995. *Zeon-1*: a member of a new maize retrotransposon family. *Mol. Gen. Genet.* 248:471-480.

Jin, Y. K. and Bennetzen, J. L. 1994. Integration and nonrandom mutation of a plasma membrane proton ATPase gene fragment within the *Bs1* retroelement of maize. *Plant Cell* 6:1177-1186.

Kameswar Row, K. V. S. R., Aist, J. R. and Crill, J. P. 1985. Mitosis in the rice blast fungus and its possible implications for pathogenic variability. *Can. J. Bot.* 63:1129-1134.

Kiyosawa, S. 1982. Gene analysis for blast resistance. *Oryzae* 18:196-203.

Matsuoka, Y. and Tsunewaki, K. 1997. Presence of wheat retrotransposons in *Gramineae* species and the origin of wheat retrotransposon families. *Genes Genet. Syst.* 72:335-43.

McClintock, B. 1948. Mobile loci in Maize. *Carnegie Inst. Wash. Yearb.* 47:155-169.

McClintock, B. 1984. The significance of responses of the genome to challenge. *Science* 226: 792-801

Miyamoto, M., Ando, I., Rybka, K., Kodama, O. and Kawasaki, S. 1996. High resolution mapping of the *Indica*-derived rice blast reisistance genes. I. *Pi-b*. *Mol. Plant-Microbe. Interact.* 9:6-13.

Mochizuki, K., Umeda, M., Ohtsubo, H. and Ohtsubo, E. 1992. Characterization of a plant *SINE*, p-SINE1, in rice genomes. *Jpn. J. Genet.* 67:155-166.

Monna, L., Miyao, A., Zhong, H. S., Yano, M., Iwamoto, M., Umehara, Y., Kurata, N., Hayasaka, H. and Sasaki, T. 1997. Saturation mapping with subclones of YACs: DNA marker production targeting the rice blast disease resistance gene, *Pi-b*. *Theor. Appl. Genet.* 94:170-176.

Multani, D. S., Meeley, R. B., Paterson, A. H., Gray, J., Briggs, S. P. and Johal, G. S. 1998. Plant-pathogen microevolution: molecular basis for the origin of a fungal disease in maize. *Proc. Natl. Acad. Sci.* USA 95:1686-1691.

Noma, K., Nakajima, R., Ohtsubo, H. and Ohtsubo, E. 1997. *RIRE1*, a retrotransposon from wild rice *Oryza australiensis*. *Genes Genet. Sys.* 72:131-140.

Ohtsubo, H., Kumekawa, N. and Ohtsubo, E. 1999. *RIRE2*, a novel *gypsy*-type retrotransposon from rice. *Genes Genet. Syst.* 74:83-91.

Rogers, S. O. and Bendich, A. J. 1985. Extraction of DNA miligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.* 5:69-76.

Suzuki, H. 1965. *The Rice Blast Disease*. pp. 111-149, Johns Hopkins Press, Baltimore, USA.

Valent, B. and Chumley, F. G. 1994. Avirulence genes and mechanisms of genetic instability in the rice blast fungus. In: *Rice Blast Disease*, ed. by R. S. Zeigler, P. S. Teng, and S. A. Leong, pp. 111-134, CAB International, Wallingford, UK.

Yokoo, M., Kikuch, F., Fujimaki, H. and Nagai, K. 1978. Breeding of blast resistant lines (BL-1 to 7) from *indica-japonica* corsses of rice. *Jpn. J. Breed.* 28:359-385.

Wang, S., Liu, N., Peng, N. and Zhang, Q. 1999a. The distribution and copy number of *copia*-like retrotransposons in rice (*Oryza sativa* L.) and their implications in the organization and evolution of the rice genome. *Proc. Natl. Acad. Sci.* USA 96:6824-6828.

Wang, Z. X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna. L., Hayasaka, H., Katayose, Y. and Sasaki, T. 1999b. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J.* 19:55-64.

Weil, C. F. and Wessler, S. R. 1990. The effects of plant transposable elements insertion on transcription initiation and RNA processing. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:527-552.

Wessler, S. R., Bureau, T. E. and White, S. E. 1995. LTR-retrotransposons and MITEs: important players in the evolution of plant genomes. *Curr. Opin. Genet. Develop.* 5:814-821.