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Identification and Characterization of New *Copia*-like Retrotransposon *Osr1* in Rice

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An insertion sequence identified as a solo long terminal repeat (LTR) of a new rice *copia*-like retrotransposon was detected in the ORF of the *Pi-b* gene from the rice cv. Nipponbare, and was designated as *Osr1*. *Osr1* consists of a 6386 bp nucleotide sequence including 965 bp LTRs on both ends with an 82% nucleotide sequence identity to the wheat *Tar1* retrotransposon on reverse transcriptase. Nucleotide divergence was noted among the individual LTRs, as well as the coding region of *Osr1*. Various restriction fragment length polymorphism (RFLP) of LTR were detected in *indica* cultivars, whereas, only a few could be detected in the *japonica* cultivars. The population of *Osr1* is lower in the wild-type rice compared with that in the domesticated cultivars. The insertion of LTR sequence in the *Pi-b* gene in the susceptible cultivar suggested that retro-transposon-mediated insertional mutation might play an important role in the resistance breakdown, as well as in the evolution of resistance genes in rice.

Keywords : LTR, *Osr1*, *Pi-b*, retrotransposon, RFLP, *Tar*.

Higher plant genomes undergo transposon-mediated structural changes during plant development, as well as over the evolutionary time period. It has been suggested that some of the ancient transposable elements may have played important role(s) in the evolution of plant genomes by altering the structures of coding regions, pattern of splicing, and regulation of gene expression (Finnegan, 1989; Weil and Wessler, 1990; Wessler et al., 1995). Since there is a strong hypothesis that the 12 rice chromosomes may have originated from one or more of the ancient chromosomes (Wang et al., 1999b), retrotransposon-induced polymorphism during the evolution may be used as a marker for genotyping and linkage analysis (Grandbastien et al., 1989; Wang et al., 1997).

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There are many transposon families which have common sequences and are ubiquitous in plant genomes (Voytas et al., 1992). Activation or movement of the transposon was investigated under various conditions. It has been reported that transposition and amplification of the retrotransposons could be induced through cell culture, wounding and infection by pathogen, and stress conditions (Grandbastien et al., 1989; Hirochika, 1993; 1997; Hirochika, et al., 1996; Takeda et al., 1998; Vernhettes, et al.). Microbial elicitors could also activate *Tn1* expression in tomato (Pouteau et al., 1994).

The genus *Oryza* has more than 20 species including 6 genomes, AA, BB, CC, BBCC, CCDD, EE, and FF, with 2n=24 or 48 (Vaughan, 1994). Various retrotransposons have been identified from *O. sativa* and wild rice species. *TrsB*, *TrsC*, and *RIRE1* have been previously identified in wild rice species using FF, CC, and EE genome, respectively (Nakajima et al., 1996). Also retrotransposon family such as *Tos1*, *Tos2*, *Tos3*, *Tos4*, and *Tos5* were isolated from *O. sativa*, and the induction and insertion of retrotransposon *Tos17* were activated through tissue culture (Hirochika, 1997). Wang et al. (1997) also reported the presence of *copia*-like retrotransposons using degenerated oligonucleotide primers.

This study reports a new retrotransposon *Osr1*, which was detected in the ORF of *Pi-b* gene from the rice cv. Nipponbare. This retrotransposon may have a potential role in the breakdown of resistance gene and evolution of *Oryza* species. The distribution of *Osr1* in rice chromosome was examined. A survey of a diverse rice cultivars suggested that *Osr1* may have been active in rice genome differently in *japonica* and *indica* cultivars.

Materials and Methods

Plant materials. Plant materials used in the experiments are listed in Table 1.

Cloning of *Osr1* and sequence analysis. A pair of primers was designed based on the genomic sequence data of 5th and 6th

Table 1. Plant materilas used in this study

Species	Cultivar	Origin	Varietal group	Genome
O. sativa cv.	Dongjinbyeo	Korea	*Japonica*	AA
	Chucheongbyeo	Korea	*japonica*	AA
	Ilpumbyeo	Korea	*japonica*	AA
	Hwaseongbyeo	Korea	*japonica*	AA
	Hwacheongbyeo	Korea	*japonica*	AA
	Ilmibyeo	Korea	*japonica*	AA
	Nakdongbyeo	China	*japonica*	AA
O. sativa cv.	Tong88-7	China	*japonica*	AA
	Caoshan	China	*japonica*	AA
	Gilokgaeng	China	*japonica*	AA
	China1039	China	*indica*	AA
	China20108	Japan	*japonica*	AA
O. sativa cv.	Homarenishiki	Japan	*japonica*	AA
	Kanto51	Japan	*japonica*	AA
	Akenohoshi	Japan	*japonica*	AA
	Nipponbare	Japan	*japonica*	AA
	Kinmaze	Vietnam	*japonica*	AA
O. sativa cv.	Tetep	United States	*indica*	AA
	Zenith	Philippine	*japonica*	AA
	Cemposelak	Philippine	*indica*	AA
O. sativa cv.	IR8	Philippine	*indica*	AA
	IR20	Philippine	*indica*	AA
	IR24	Philippine	*indica*	AA
	IR36	Philippine	*indica*	AA
	IR72	Philippine	*indica*	AA
	Tadukan	Cambodia	*indica*	AA
O. rufifogen		Africa	Wild type	AA
O. barthii		Philippine	Wild type	AgAg
O. minuta		Sri Lanka	Wild type	BBCC
O. eichingeri		Austrailia	Wild type	CC
O. officinalis		Africa	Wild type	CC
O. brachyantha			Wild type	FF

exons of *Pi-b* which is one of the identified rice disease resistance gene against fungal pathogen *Magnaporthe grisea* (Wang et al., 1999a). The LTR region of *Osr1* was amplified using genomic library clone 99-2-7 which contains susceptible *Pi-b* region with sense 5'GGTAGAAAATAGAGTCAGCA GTAAG-3' (LTR-F), and antisense 5'-TATGTACCT CTGATGTCTAGCA-3' (LTR-R) primers. The genomic DNA library of the rice cultivar Nipponbare was constructed through ligation of digested genomic DNA above 6 kb with *Hin*dIII into pBluescript vector (Stratagene, Lajolla, CA). The ligation mixture was transformed into *E. coli* DH10B, and screened by colony hybridization using PCR probe amplified with LTR-F and LTR-R primer pairs, and four clones were selected as described by Jwa and Lee (2000a). Each clone was characterized by sequencing analysis as new *copia*-like retrotransposon in rice and designated as *Osr1*. Open reading frame region of *Osr1* was amplified with the primer pairs [sense, 5'AGGCCAAGTTCGGAGTTTCTGACGC CGG-3' (OSR-5); antisense, 5'-CGCAGTTCATTAAGGATAGCTGGCACTGGTT-3' (OSR-3)] designed based on the nucleotide sequence of the *Osr1*, using genomic DNA of rice cv. Nipponbare. Thirty cycles of amplification were performed under the following conditions: Template rice cv. Nipponbare DNA was denatured for 1 minute at 94°C, annealed at 55°C for 1 minute, and DNA was synthesized at 72°C for 3 minutes. PCR products were sub-cloned using a TOPO TA cloning kit according to the protocol of the manufacturer (Invitrogen, Carlsbad, CA). Thirty clones were selected to see the variation among individual clones by the PE ABI prism 377 DNA sequencer (Perkin Elmer, CA) according to the protocol of the manufacturer. All sequencing data were analyzed using Genetyx software (Software development, Tokyo). Searches for information and homology of nucleotide and amino acid sequences were performed using a homology search with BLAST against the sequences in the GenBank and EMBL DNA database.

Phenogram was constructed based on the amino acid sequence similarity on reverse transcriptase domains from *copia*- and *gypsy*-like retrotransposons through the unweighted pair group method using the arithmetic mean (UPGMA) (Sneath and Sokal, 1973). The confidence limits of the branching arrangements of phenogram were determined through a bootstrap procedure (Efron and Gong, 1983) using 1000 re-sampling replicates of the reverse transcriptase homology data.

Restriction fragment length polymorphism and phylogenetic analysis. Rice genomic DNA was extracted according to the method of Rogers and Bendich (1985). One microgram each of genomic DNA from *japonica* and *indica* cultivars, and from wild rice species was digested with *Eco*RV and fractionated on a 0.7% agarose gel at 20 volts for 36 hours. DNA was denatured, neutralized, and blotted onto Hybond-N+ membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). Probe DNA was amplified with PCR reactions using two primers, LTR-F and LTR-R. Southern hybridization was carried out according to the protocol of Jwa and Lee (2000a).

Osr1-RFLP profile was generated from each rice cultivar and wild species based on the genomic Southern blot data using an *Osr1*-LTR probe. The data were subjected to the SIMQUAL program, and phenogram was constructed by the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) with the SAHN-clustering program of the NTSYS-pc package (Rohlf, 1992).

Northern hybridization analysis. Total RNA was extracted from rice cv. Hwacheong after inoculation with compatible and incompatible rice blast pathogen, *M. grisea* strains KJ201 and KJ401, respectively, according to the protocol of Jwa et al. (2000b). Rice cv. Nakdong was inoculated with *Xanthomonas oryza* pv. *oryza*, a pathogen for the rice bacterial leaf blight, and leaves were harvested every 24 hours for three days after inoculation and used for the RNA extraction. Northern hybridization was carried out according to the method of Pautot et al. (1993). Fifteen microgram of total RNA were fractionated through electrophoresis under a denaturing condition on 1.5% formaldehyde-agarose gel and transferred onto a Hybond N+ membrane (Sambrook et al., 1989). Activation of the *Osr1* was detected by PCR fragment amplified with LTR-F and LTR-R as a DNA probe in hybridization solution (50% Formamide, 5X SSPE, 0.5X Denhardt solution, 0.2% SDS with 0.1mg/ml salmon sperm DNA) at 42°C. After hybridization, the blots were washed twice with 2X SSC and 0.1% SDS for 10 minutes each at room

temperature, and washed twice with 0.2X SSC and 0.1% SDS at 45°C for 10 minutes each. Then, the membranes were exposed to X-ray film (Agfa-Gevaert, Germany) with intensifying screens at -70°C.

Results

Sequence analysis of *Osr1*. Complete unit of *Osr1* was cloned from the rice genomic library, and the nucleotide sequence was characterized (GenBank/DDBJ/EMBL accession number AB046118). The *Osr1* consists of two long terminal repeats (LTRs) with a large ORF in the middle, which made 6386 bp of a new retrotransposon. The *Osr1* ORF contains the genes of *gag*, *protease*, *integrase*, *reverse transcriptase*, and *RNAse H*. The order of genes contained in the coding region and amino acid conservation suggested that *Osr1* was categorized in a group of *copia*-like retrotransposon. Amino acid alignment of each putative protein sequence in *Osr1* was conducted with other retrotransposons, and the data indicated that all protein domains were conserved within them (Fig. 1). This new rice retrotransposon *Osr1* is homologous to *Tar1* which is a wheat retrotransposon in family 1 homologue, compared with the other homologous *Ty2-copia* group retrotransposons found so far from rice and other species.

Phylogenetic analyses were conducted to assess the relationship among the reverse transcriptases from *copia*- and *gypsy*-like retrotransposons (Fig. 2). Two large groups were distinguished based on *gypsy*- and *copia*-like retrotransposon families. Fungal retrotransposons including *Grasshopper, MAGGY, Cft-1, and Tf-1* were separately grouped among the *gypsy*-like retrotransposons. All the *copia*-like retrotransposons showed a higher sequence homology in amino acid sequences of the reverse transcriptase compared with those of the *gypsy*-like retrotransposons. Based on the characteristics of retrotransposons, *Osr1* belongs to copia-like retrotransposon.

Nucleotide divergence among individual *Osr1* was

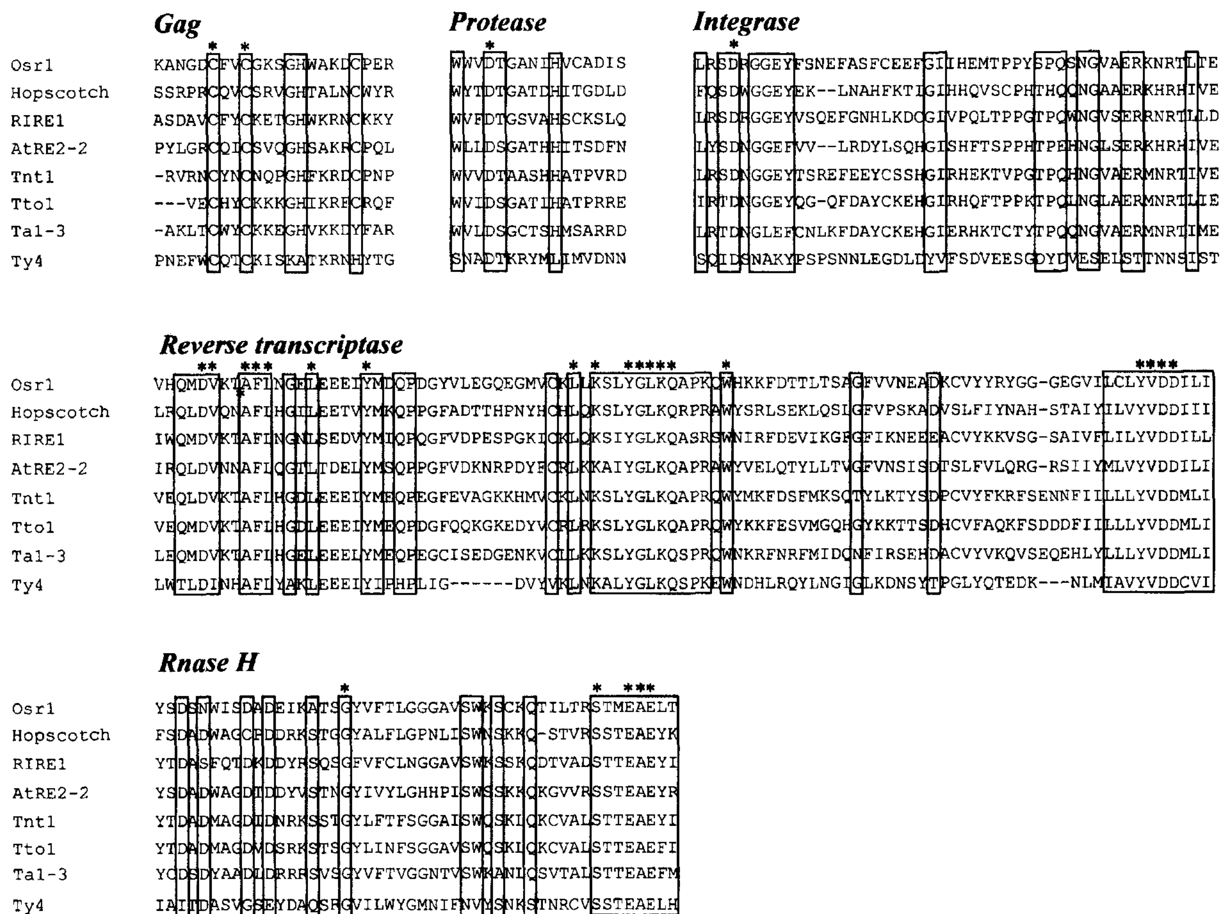

Fig. 1. Comparison of conserved domains in *copia*-like retrotransposons through an amino acid alignment of *Osr1* (AB046118), Hopscotch (AW154801), RIRE1 (D85597), AtRE2-2 (AB021267), Tnt1 (X13777), Tto1 (D83003), Tto1 (D83003), Ta1-3 (X13291), and *gypsy*-like retrotransposon Ty4 (M94164). Asterisk indicates the fully conserved residue and hyphen represents the alignment gap. Highly conserved motifs among many active sites are boxed.

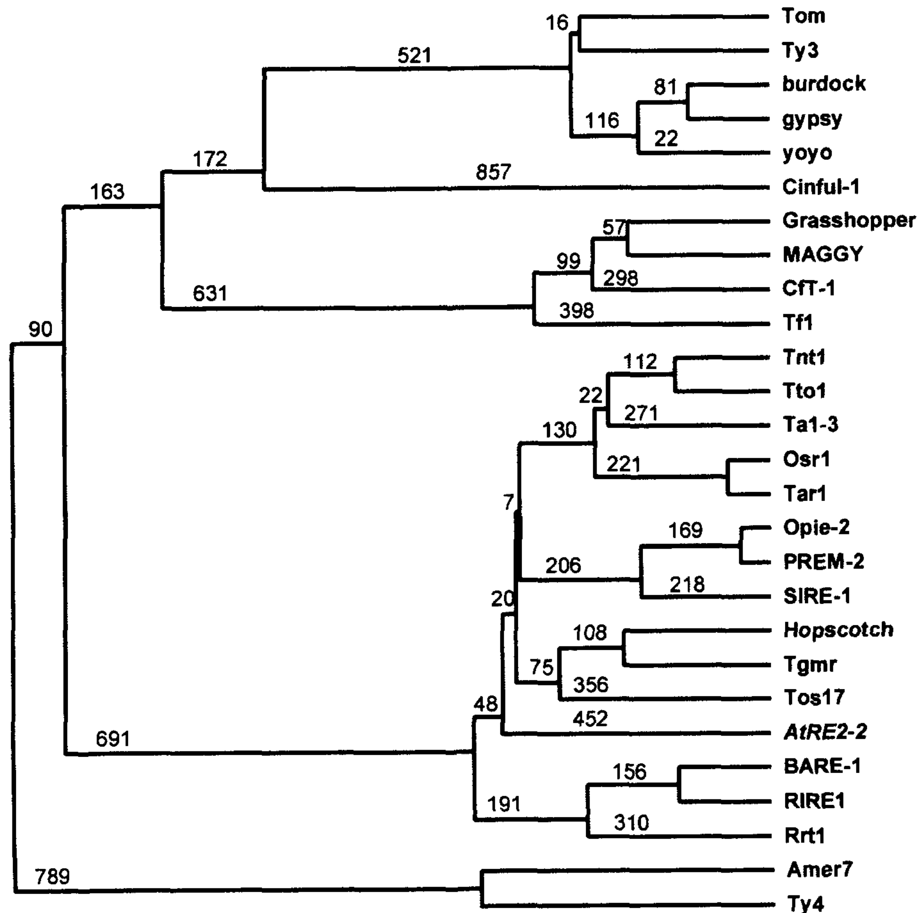

Fig. 2. Phylogenetic tree of reverse transcriptase among *copia-* and *gypsy-*like retrotransposons. Two groups belonging to *copia-* and *gypsy-*like retrotransposons were used. Tom (Z24451), Ty3 (M23367), burdock (U89994), *gypsy* (M12927), yoyo (U60529), Cinful-1 (AF049110), Grasshopper (M77661), MAGGY (L35053), CfT-1 (Z11866), Tf1 (M38526), Tnt1 (X13777), Tto1 (D83003), Ta1-3 (X13291), Osr1 (AB046118), Tar (AB008772), Opie-2 (U68408), PREM-2 (U41000), SIRE-1 (AF053008), Hopscotch (AW154801), Tgmr (U96748), Tos17 (D85876), AtRE2-2 (AB021267), BARE-1 (Z17327), RIRE1 (D85597), Rrt1 (Z75496), Amer7 (AJ006562), Ty4 (M94164). A tree was constructed through the UPGMA (unweighted pair group method, arithmetic means) using Genetyx program (Software Development, Tokyo, Japan). The number above the horizontal line is the frequency with which a given branch appeared in 1000 bootstrap replications.

detected from the sub-clones which were amplified from genomic DNA of rice cv. Nipponbare (data not shown).

Genomic Southern blot analysis. To understand genome-wide population of *Osr1*, genomic DNA was digested with *Eco*RV, ran on a 0.7% agarose gel, and transferred onto a Nylon membrane. The membrane was hybridized with LTR fragment amplified with PCR. The RFLP patterns of the *Osr1* among *japonica* cultivars were almost identical (Fig. 3A). Rice cvs. China20108, Dongjin, Homarenishiki, Tong88-7, and Nipponbare showed absence and/or addition of bands compared to with the other *japonica* cultivars (Fig. 3A). On the other hand, IR and typical *indica* cultivars exhibited significantly diverse RFLP patterns (Fig. 3B). In IR cultivars, IR24, 36, and 72 were quite similar to each other, but differed from IR8 and 20 cultivars. An interesting feature in the genomic Southern hybridization of wild *Oryza* species was that the population of retrotransposon in the wild-type rice species was much lower than that of the cultivars compared with the number of hybridizing signals. Only the distribution pattern of *Osr1* in the wild-type *O. barthii* was similar to the cultivated rice based on the major RFLP pattern (Fig. 3B), but the signal intensity of each band was significantly low.

These results imply that there is a possibility of genetic relationship between wild rice *Oryza barthii,* and *japonica* and *indica* cultivars which are widely cultivated. All *japonica* cultivars were separately grouped from the *indica* cultivars which approximately showed 75% *Osr1*-RFLP homology (Fig. 4). The *indica* cv. China1039 and Cemposelak were grouped with the *japonica* cultivars because each showed 75 and 88% homology, respectively. As a *japonica* cultivar, Dongjin was grouped at the 84% level of homology from

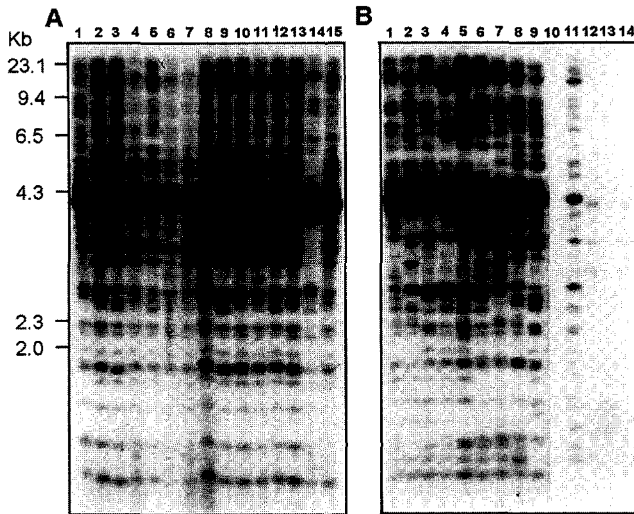

Fig. 3. Genomic Southern hybridization of *japonica* and *indica* cultivars and wild-type rice species with LTR probe of the *Osr1*. A: Lane 1, Homarenishiki; 2, Kanto51; 3, Akenohoshi; 4, Nipponbare; 5, Kinmaze; 6, Dongjinbyeo; 7, Chucheongbyeo; 8, Ilpumbyeo; 9, Hwaseongbyeo; 10, Ilmibyeo; 11, Tong88-7; 12, Caoshan; 13, Gilokgaeng; 15, China20108; and typical *indica* cv. China1039 was added in lane 14. B: Typical *indica* cultivars are lane 1, IR8; 2, IR20; 3, IR24; 4, IR36; 5, IR72; 6, Tadukan; 7, Tetep; 8, Zenith; 9, Cemposelak; 10, *Oryza rufipogen* (AA); 11, *O. barthii* (A^gA^g); 12, *O. minuta* (BBCC); 13, *O. officinalis* (CC); 14, *O. eichingeri* (CC); 15, *O. brachiantha* (FF).

other *japonica* cultivars. Wild-type rice species showed significant differences from both *japonica* and *indica* cultivars. Only *O. barthi* had a similar pattern to the major *Osr1*-RFLP, and the rest of the wild-type species of

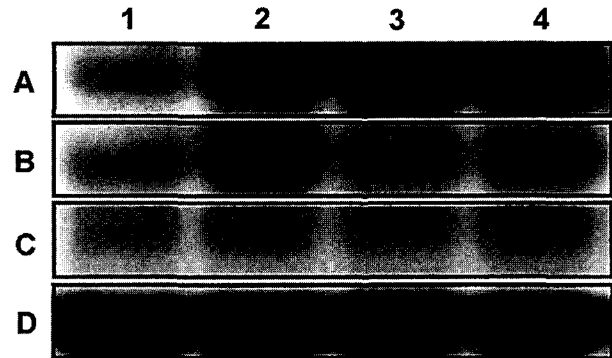

Fig. 5. Northern hybridization of *Osr1*. A; *Osr1* signal was detected using 100 ng of 1 Kb LTR probe labeled through ^{32}P-dCTP with Northern hybridization of 15 mg of total RNAs from the compatible interaction of rice cv. Hwacheong and *M. grisea* (KJ201). Size of the signal was longer than 6 Kb. B; Northern blot of the incompatible interaction of rice cv. Hwacheong and *M. grisea* (KJ401). C; Transcription level of *Osr1* was detected after the inoculation of *Xanthomonas oryzae* pv. *oryzae* on rice cv. Nakdong. D; signal with ribosomal DNA as a probe indicates equal loading of total RNAs. X-Ray film was exposed for 4 days in -80°C. (Lanes1, 2, 3, 4: 0, 1, 2, and 3 days after inoculation)

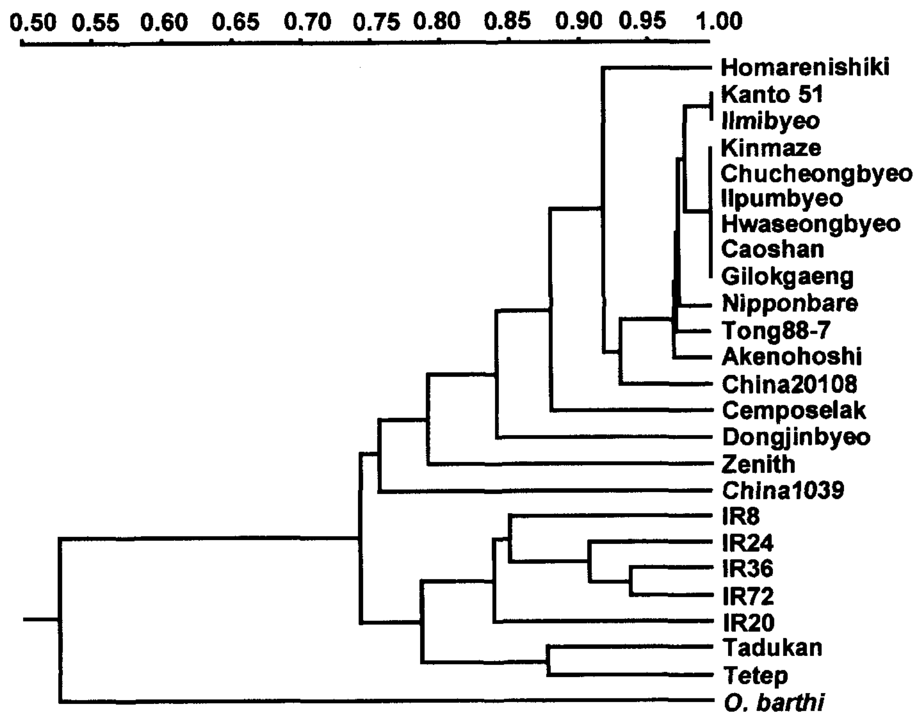

Fig. 4. Phenogram derived using UPGMA (unweighted pair group method, arithmetic mean) based on *Osr1*-restriction fragment length polymorphism (RFLP) data of each rice cultivar collected from Korea, China, Japan, India, Australia, and Philippines as well as from the wild-type rice. The RFLP data were obtained through the hybridization of *Eco*RV-digested genomic DNA with LTR of *Osr1* retrotransposon.

different genome types showed weak or no signals.

Regulation of *Osr1* through infection of *Magnaporthe grisea*. The mRNA transcripts of *Osr1* were detected from both compatible and incompatible interactions using combinations of *M. grisea* KJ201 and KJ401 with rice cv. Hwacheong, respectively (Fig. 5). The size of the *Osr1* transcript was similar to the full size of 6386 bp. The accumulation of *Osr1* mRNA implied RNA-to-DNA-mediated transposition. Transcription of *Osr1* was up regulated by the inoculation of 1×10^5 conidia of *M. grisea* KJ201 and KJ401 (Fig. 5A, B). However, no difference in the transcription level was found from inoculation with *X. oryzae* pv. *oryzae* which caused the bacterial leaf blight in rice (Fig. 5C). The transcription of *Osr1* reached the highest level of induction at 72 hours after inoculation in the compatible interaction, whereas, it was highest at 24 hours in the incompatible interaction.

Discussion

A new retrotransposon *Osr1* present as a solo LTR was characterized through a direct nucleotide sequence comparison of the ORF of the *Pi-b* region from both susceptible and resistant rice cultivars to the blast disease. The size of the *Osr1* was shorter than RIRE1, the first identified and fully characterized retrotransposon from the wild rice *O. australiensis* (Noma et al., 1997). Nucleotide divergence was also detected by a direct comparison with the LTRs of other clones selected from genomic library, as well as the internal region obtained by a random amplification with PCR primers and sub-cloning (data not shown). It is known that homologuous retrotransposons are present ubiquitously in many plant species (Flavell et al., 1992; Voytas et al., 1992). The *Osr1* was the most homologous to the wheat *Tar1* retrotransposon at the nucleotide sequence level. In wheat (*Triticum aestivum*), retrotransposons were classified into seven distinct families based on the nucleotide sequence similarities on the reverse transcriptase domain (Matsuoka and Tsunewaki, 1997). *Tar1* was classified into family 1 group on the basis of nucleotide sequence of its reverse transcriptase. Matsuoka and Tsunewaki (1997) also suggested the possibility of the presence of rice retrotransposons carrying a homologous reverse transcriptase gene to *Tar1*. In the evolutionary aspect of *Osr1*, the transfer of transposable element has been explained vertically from generation to generation and/or horizontally through the transmission of genetic material without sexual recombination (Xiong, and Eickbush, 1990). Both mechanisms can be applied to the data generated in this study by comparing *Osr1* and its homologous *copia*-like retrotransposons from monocots and dicots. For example, the reverse transcriptases of dicots (tobacco, Arabidopsis, soybean) show a high similarity among themselves, and those in monocots (rice, wheat, maize, barley) also have similar sequences, implying that they shared a common ancestor before speciation. Some retrotransposons from the same species (Opie-2 and PREM-2 of Maize) shared homologous retrotransposon with a distant taxa (SIRE-1 of soybean), suggesting horizontal transfer of retrotransposons between the genera (Fig. 2).

This also suggests that cultivation of rice might have activated the transposition and amplification of *Osr1*. There were almost no variations in copy numbers of the *Osr1* both in *japonica* and *indica* cultivars, but significant variation was detected in the wild-type rice species. Phenogram constructed using the *Osr1*-RFLP data indicated that *indica* and *japonica* rice cultivars were separately grouped at 75% similarity level (Fig. 2). However, the differences among individual cultivars were significantly higher in *indica* group than those detected in *japonica* group. Surprisingly, however, two *indica* cultivars (Cemposelak, China1039) were grouped as *japonica* type-RFLP, implying that *indica* cv. Cemposelak, China1039 shared a more recent common ancestor with the *japonica* cultivars.

Genetic differences in resistance to the same pathogen with different pathotype are very important in understanding the mechanism of pathogenesis and control of the disease (Flor, 1971). The insertional mutation was identified on the *Pi-b* genes in susceptible rice cultivars (Jwa and Lee, 2000a). Southern blot data with the LTR sequence demonstrated that the genetic resources of the *japonica* cultivar are almost uniform compared with the *indica* cultivar. Breeding brought in the *Pi-b* resistance gene into *japonica* cultivars from the *Pi-b* resistant *indica* cultivars such as Milek Kuning and Tjina of the tropical area where rice had originated (Yokoo et al., 1978). This signifies that the difference in the stresses against races of the pathogens could change the type of mutation caused by retrotransposons. As reported in tobacco by Vernhettes et al. (1997), transcriptional activation of the *Osr1* was also detected after the infection with the blast fungus *M. grisea*. McClintock (1984) also suggested that stresses activate transposable elements and have played important roles in generating new individual or species. In contrast to RIRE, copy number of the *Osr1* was significantly low in the wild-type species.

In conclusion, retrotransposon *Osr1* was identified as the major factor causing the insertional mutation on the rice blast resistant *Pi-b* gene, showing a RNA-to-DNA mediated transposition. Furthermore, mRNA transcripts were up regulated after the infection with compatible and incompatible races of *M. grisea*. Since, RFLP pattern of *Osr1* was polymorphic between *japonica* and *indica* cultivars, the transposition of *Osr1* might play a role in the generation of diverse varieties of rice. Thus, knowledge of the role of

transposition of retrotransposons including *Osr1* in plant genome will help to understand the evolution and/or generation of various plant species.

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References

Efron, G. and Gong, F. 1983. A leisurely look at the bootstrap, the jackknife and cross-validation. *Am. Stat.* 37:36-48.

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

Flavell, A. J., Dunbar, E., Anderson, R., Pearce, S. R., Hartley, R. and Kumar, A. 1992. Ty1-*copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. *Nucleic Acids Res.* 20:3639-3644.

Flor, H. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9:275-296.

Grandbastien, M. A., Spielmann, A. and Caboche, M. 1989. Tnt1, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. *Nature* 337:376-380.

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.

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.

Jwa, N. S. and Lee, Y. H. 2000a. Insertional mutation of the rice blast resistance gene, *Pi-b*, by long terminal repeat of a retrotransposon. *Plant Pathol. J.* 16:105-109.

Jwa, N. S., Park, S. G., Park, C. H., Kim, S. O., Ahn, I. P., Park, S. Y., Yoon, C. H. and Lee, Y. H. 2000b. Cloning and expression of a rice cDNA encoding a *Lls1* homologue of Maize. *Plant Pathol. J.* 16:151-155.

Matsuoka, Y. and Tsunewaki, K. 1997. Presence of wheat retrotransposons in Gramineae species and the origin of wheat retrotransposon families. *Genes Genet. Syst.* 72:335-343.

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

Nakajima, R., Noma, K., Ohtsubo, H. and Ohtsubo, E. 1996. Identification and characterization of two tandem repeat sequences (TrsB and TrsC) and a retrotransposon (RIRE1) as genome-general sequences in rice. *Genes Genet. Syst.* 71:373-382.

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

Pautot, V., Holzer, F. M., Reisch, B. and Walling, L. L. 1993. Leucine aminopeptidase: an inducible component of the defense response in *Lycopersicon esculentum* (tomato). *Proc. Natl. Acad. Sci. USA* 90:9906-9910.

Pouteau, S., Grandbastien, M. A. and Bpccara, M. 1994. Microbial elicitors of plant defense responses activate transcription of a retrotransposon. *Plant J.* 5:535-542.

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

Rohlf, F. 1992. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 1.7. Exter Software, New York.

Sambrook, J., Frisch, E. F. and Maniatis, T. 1989. Molecular cloning. A laboratory Mannual 3rd edn. Cold Spring Habor Laboratory Press, Cold Spring Harbor.

Sneath, P. H. A. and Sokal, R. R. 1973. Numerical Taxonomy: The Principle and Practice of Numerical Classification. W.H. Freeman and Co., San Francisco.

Takeda, S., Sugimoto, K., Otsuki, H. and Hirochika, H. 1998. Transcriptional activation of the tobacco retrotransposon *Tto1* by wounding and methyl jasmonate. *Plant Mol. Biol.* 36:365-376.

Vaughan, D. H. 1994. The relationship between the genus *Oryza* and other grasses. In *The Wild Relatives of Rice,* pp. 3-6. International Rice Research Institute. Philippines.

Vernhettes, S., Grandbastien, M. A. and Casacuberta, J. M. 1997. *In vivo* characterization of transcriptional regulatory sequences involved in the defence-associated expression of the tobacco retrotransposon Tnt1. *Plant Mol. Biol.* 35:673-679.

Voytas, D. F., Cummings, M. P., Koniczny, A., Ausubel, F. M. and Rodermel, S. R. 1992. *Copia*-like retrotransposons are ubiquitous among plants. *Proc. Natl. Acad. Sci. USA* 89:7124-7128.

Wang, S., Zhang, Q., Maughan, P. J. and Saghai, M. A. 1997. *Copia*-like retrotransposons in rice: sequence heterogeneity, species distribution and chromosomal locations. *Plant Mol. Biol.* 33:1051-1058.

Wang, Z. X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., Katayose, Y. and Sasaki, T. 1999a. The *Pi-b* 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.

Wang, S., Liu, N., Peng, K. and Zhang, Q. 1999b. 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.

Weil, C. F. and Wessler, S. R. 1990. The effects of plant transposable elements insertion on transcription initiation and RNA processing. *Ann 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. *Current Opinion of Genet. and Develop.* 5:814-821.

Xiong, Y. and Eickbush, T. H. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. *EMBO J.* 9:3353-3362.

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