Theor Appl Genet (2007) 115:217–224
DOI 10.1007/s00122-007-0557-6

ORIGINAL PAPER

A novel *wx* mutation caused by insertion of a retrotransposon-like sequence in a glutinous cultivar of rice (*Oryza sativa*)

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Received: 11 August 2006 / Accepted: 14 April 2007 / Published online: 11 May 2007

Abstract DNA polymorphism of the *Wx* gene in glutinous rice cultivars was investigated by PCR-RF-SSCP and heteroduplex cleavage analysis using *Brassica* petiole extract, and the nucleotide sequence variations were identified. Most *japonica*-type glutinous rice was found to have a 23-bp duplication in the second exon, which causes loss of the function of granule-bound starch synthase (GBSS) encoded by the *Wx* gene. Without the 23-bp duplication, there was an insertion of 7,764 bp in the ninth exon of the *wx* allele of 'Oragamochi'. Expression analysis of the *wx* allele using RT-PCR and Northern blot analysis revealed that transcripts of the 'Oragamochi' *wx* allele are about 1-kb shorter and that the deduced amino acid sequence of the transcript lacks a motif important for GBSS. Therefore, this insertion was considered to be the cause of the glutinous trait of 'Oragamochi'. This 7,764-bp insertion had long terminal repeats, a primer binding site, and a polypurine tract, but no sequence homologous with *gag* and *pol*, suggesting that it is a non-autonomous element. Furthermore, it had a structure similar to *Dasheng* and may be a member of *Dasheng*.

Communicated by J. W. Snape.

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Introduction

Starch, which constitutes about three-fourths (w/w) of rice grains, is the most important component determining the taste of rice. Starch is composed of amylose and amylopectin, and the amylose content is controlled by the *Wx* gene encoding granule-bound starch synthase (GBSS). Mutation of the *Wx* gene causing the loss of GBSS results in rice grains of 0% amylose and 100% amylopectin, i.e., glutinous rice.

Many *wx* mutant lines have been obtained by induced mutation with gamma-ray irradiation or chemical mutagen treatment, and the point mutations in the *wx* gene have been identified (Inukai et al. 2000; Isshiki et al. 2001; Sato and Nishio 2003). However, there have been few reports on variation in the *wx* gene of glutinous cultivars obtained by spontaneous mutations. In waxy lines of maize (Wessler and Varagona 1985), barley (Domon et al. 2002), and wheat (Saito et al. 2004) obtained by spontaneous mutations, insertions of transposons or retrotransposons have been identified in their *Wx* genes, while only one spontaneous mutation caused by 23-bp duplication has been reported in rice (Inukai et al. 2000; Wanchana et al. 2003).

PCR-RF-SSCP, i.e., SSCP analysis of PCR products cleaved by restriction endonucleases, has been successfully used for the identification of point mutations in the rice *wx* gene (Sato and Nishio 2003). Detection of SNPs (single nucleotide polymorphisms) in bulked DNA samples using heteroduplex cleavage enzymes prepared from celery and *Brassica* have also been successfully applied to the selection of mutants in various plants (Till et al. 2004; Sato et al.

2006). These techniques are powerful tools to study nucleotide sequence variations among cultivars in crop species. Although these techniques cannot detect SNPs with 100% efficiency, almost all SNPs can be detected by their combined use.

In the present study, DNA polymorphism of the *Wx* gene in glutinous rice cultivars was investigated using PCR-RF-SSCP and the heteroduplex cleavage enzyme from *Brassica*, and the nucleotide sequence variations were identified. The causes of the loss of the gene function in most cultivars were revealed to be the same, but a *wx* allele having insertion of a retrotransposon-like sequence was found in two cultivars. The relationship between the newly identified retrotransposon-like sequence and a previously reported non-autonomous retrotransposon-like sequence is herein discussed.

Materials and methods

Materials

Sixteen glutinous paddy-rice cultivars and six glutinous upland cultivars listed in Table 1 were used as plant materials. 'Nipponbare,' 'Akihikari,' and 'Sasanishiki' were used as non-glutinous cultivars for comparison.

DNA polymorphism analysis

Genomic DNA was isolated from rice leaves by DNeasy Kit (Qiagen, Valencia, CA, USA). The *wx* gene of *ca.* 6 kb was amplified as six DNA fragments (Waxy-1, -2, -3, -4, -5a, and -5b) (Fig. 1) by PCR using the six pairs of primers shown in Table 2. PCR-RF-SSCP analysis was performed according to Sato and Nishio (2003) using *Mbo*I, *Alu*I, and *Hae*III as shown in Table 2. Heteroduplex cleavage analysis using *Brassica* petiole extract (Sato et al. 2006) was carried out as follows. Genomic DNAs of five cultivars were mixed and used as a template for PCR using the six primer pairs shown in Table 2. A heteroduplex was formed and cleaved with a *Brassica* petiole extract prepared from *Brassica rapa* according to Sato et al. (2006). The cleaved fragments were detected by agarose gel electrophoresis and stained with SYBR Green I Nucleic Acid Gel Stain (Cambrex Bio Science Rockland Inc., Rutherford, NJ, USA). As a positive control, a mutant line, R65, having a G to A transition mutation in the 11th exon, was used.

Sequencing

PCR products were directly sequenced using a nucleotide sequencer CEQ2000 (Beckman Coulter, Fullerton, CA,

Table 1 Polymorphism of the *wx* alleles of glutinous cultivars detected by PCR-RF-SSCP analysis

Cultivar name	Field (upland or paddy)	Waxy region						23 bp±*
		1	2	3	4	5a	5b	
Nipponbare	Paddy	A	A	A	A	A	A	–
Koganemochi	Paddy	B	A	B	A	A	A	+
Oragamochi	Paddy	B	A	A	–	–	A	–
Mochimusume	Paddy	B	A	B	A	A	A	+
Mangetsumochi	Paddy	B	B	B	A	A	A	+
Sakakimochi	Paddy	B	A	B	A	A	A	+
Yukimimochi	Paddy	B	A	B	A	A	A	+
Himenomochi	Paddy	B	A	B	A	A	A	+
Hidekomochi	Paddy	B	A	A	–	–	A	–
Kokonoemochi	Paddy	B	A	B	A	A	A	+
Kinunohada	Paddy	B	A	B	A	A	A	+
Calmochi 101	Paddy	B	A	B	A	A	A	+
Tohokumochi 161	Paddy	B	A	B	A	A	A	+
Hujikuramochi	Upland	B	A	B	A	A	A	+
Minomochi	Upland	B	A	B	A	A	A	+
Minamihatamochi	Upland	B	A	B	A	A	A	+
Rikutounourinmochi 1	Upland	B	A	B	A	A	A	+
Rikutounourinmochi 4	Upland	B	A	B	A	A	A	+
Toyohatamochi	Upland	B	A	B	A	A	A	+
Akamochi	Paddy	B	A	B	A	A	A	+
Kazenokomochi	Paddy	A	A	B	A	A	A	+
Hakuchoumochi	Paddy	A	A	B	A	A	A	+

A the same band pattern between a glutinous cultivar and 'Nipponbare', *B* different band pattern between a glutinous cultivar and 'Nipponbare', –: No amplification by PCR

*Presence (+) or absence (–) of 23-bp duplication in the second exon

Table 2 Primers and restriction endonucleases used for PCR-RF-SSCP analysis of the *Wx* gene

Waxy regions	Primer sequences				Restriction endonucleases	
	Name	Forward primers (5′-3′)	Name	Reverse primers (5′-3′)	1	2
Waxy-1	1-F	CTCTCTAGCTTATTACAGCC	1-R	GTATGAGACTACTTGTAAGG	*Alu*I	*Mbo*I
Waxy-2	2-F	TCATCAGGAAGAACATCTGC	2-R	TGAATTGTTTAAGGTTTGGTGAGCC	*Alu*I	*Mbo*I
Waxy-3	3-F	ATCTGATCTGCTCAAAGCTCTGTGCATCTC	3-R	TCCACGCTTGTAGCAATGGAAAAACCTCAC	*Hae*III	*Mbo*I
Waxy-4	4-F	GTTCTTGATCATCGCATTGG	4-R	ACTTGTCCTTGCTAGGATCC	*Alu*I	*Mbo*I
Waxy-5a	5-F	GAAGATCAACTGGATGAAGG	5-2-R	CATGTGGAAACCAGTCTTGC	*Alu*I	*Mbo*I
Waxy-5b	5-2-F	ATCTTTGTGCAATGCAATGC	5-R	GGCATGGTATAATATGGAAC	*Hae*III	*Mbo*I

USA) or ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). When the efficiency of DNA amplification was low, the PCR products were cloned using a TA cloning kit (Promega, Madison, WI, USA), and the sequences of more than two clones were determined.

Southern blot analysis

Genomic DNA, 2 μg, prepared by the CTAB method (Murray and Thompson 1980) was digested with *Pst*I, *Eco*RI, or *Dra*I (Takara Biochemicals, Shiga, Japan), electrophoresed on 1% agarose gel, and transferred to a nylon membrane (Nytran N, Whatman, Middlesex, UK). A region from the ninth exon to the tenth exon in the *Wx* gene of 'Nipponbare' was labeled with digoxigenin (Roche, Basel, Switzerland) by PCR and used as a probe. Hybridization and detection of signals were performed with a DIG nucleic acid detection kit (Roche).

Isolation of a genomic DNA clone

Genomic DNA of 'Oragamochi', 10 μg, was partially digested with *Sau*3AI (Takara Biochemicals) and ligated to the arms of lambda FIXII (Stratagene, La Jolla, CA, USA). Clones harboring the *wx* gene were isolated by plaque hybridization with the probe used for Southern blot analysis. *Sac*I fragments of the isolated clone were subcloned into pBluescript II SK(−) (Stratagene).

Detection of transcripts by RT-PCR

Total RNA was extracted from developing grains at 10 days after anthesis according to Shirzadegan et al. (1991). First strand cDNA was synthesized using 1 μg total RNA by a First-Strand Synthesis Kit (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) and used as a template of PCR.

Northern blot analysis

The total RNA, 10 μg, treated with glyoxal was electrophoresed on 1.2% agarose gel and transferred to a nylon membrane (Nytran N). A region from the second exon to the fourth exon and a region from the 10th exon to the 12th exon in the *Wx* gene were labeled with digoxigenin by PCR using primer pairs of w3F(5′-TCGCTCAGCGTGACGACCAG-3′)/3-R(5′-TCCACGCTTGTAGCAATGGAAAAACCTCAC-3′) and 5-1-F(5′-AAATCCCACTGATCGCGTTC-3′)/5-2-R(5′-CATGTGGAAACCAGTCTTGC-3′), respectively, and used as probes. Hybridization and detection of signals were performed with a DIG nucleic acid detection kit (Roche).

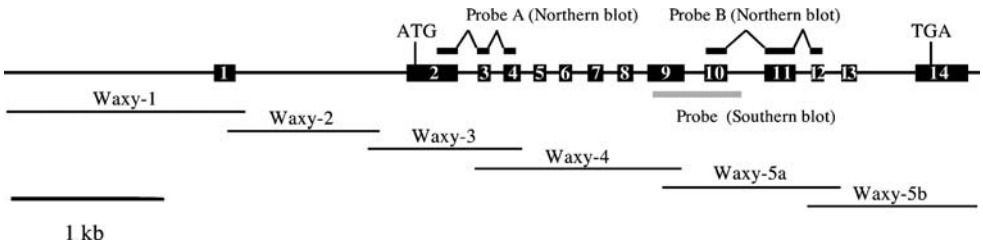

Fig. 1 A map of the rice *Wx* gene. *Black boxes* indicate exons. *Bars* under the map represent the DNA fragments amplified by PCR. Positions of the sequences used as probes for Southern blot and Northern blot analyses are also shown

Results

Detection of DNA polymorphism in the *wx* gene

In the region of Waxy-1, as shown by PCR-RF-SSCP analysis, all the glutinous paddy-rice cultivars except for 'Kazenokomochi' and 'Hakuchoumochi' showed a band pattern different from that of the non-glutinous cultivar 'Nipponbare'. The Waxy-2 region showed polymorphism only in 'Mangetsumochi'. The Waxy-3 region of all the glutinous cultivars except for 'Oragamochi' and 'Hidekomochi' were different from that of 'Nipponbare' (Fig. 2). No DNA polymorphism with 'Nipponbare' was detected in the Waxy-4, Waxy-5a, and Waxy-5b regions, but the Waxy-4 and Waxy-5a regions in 'Oragamochi' and 'Hidekomochi' were not amplified by PCR. Screening of DNA polymorphism using the heteroduplex cleavage enzyme prepared from *Brassica rapa* detected no polymorphism in the DNA fragments from Waxy-1 to Waxy-5b, although a mutation of R65 (Sato and Nishio 2003) was detected.

Nucleotide sequences of the regions having polymorphism with 'Nipponbare' were determined. The Waxy-1 and Waxy-3 regions, in which band patterns different from those of 'Nipponbare' were detected in many glutinous cultivars, were sequenced in several cultivars selected as representatives.

Nucleotide sequencing revealed the DNA polymorphism in the Waxy-1 region to be the numbers of CT repeats at a CT microsatellite: 18 in 'Nipponbare,' 'Kazenokomochi,' and 'Hakuchoumochi', and 17 in the other glutinous cultivars. Since the number of repeats is often misread in nucleotide sequencing, this difference was confirmed using polyacrylamide gel electrophoresis. The DNA polymorphism of the Waxy-2 region in 'Mangetsumochi' was a T-to-C substitution in the first intron. The DNA polymorphism in the Waxy-3 region was found to be a 23-bp duplication in the second exon as reported by Inukai et al. (2000). This 23-bp duplication was detected in all the glutinous paddy-rice cultivars except for 'Oragamochi' and 'Hidekomochi', as well as in all six glutinous upland cultivars. We also analyzed 13 other glutinous upland cultivars and detected the 23-bp duplication in all of them (data not shown). The *wx* allele of 'Oragamochi' and 'Hidekomochi' was considered to have a mutation different from the 23-bp duplication.

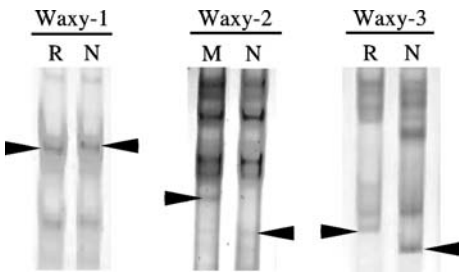

Fig. 2 PCR-RF-SSCP analysis of DNA fragments of the *Wx* gene. *R* Rikutou-Nourinmochi 4, *M* Mangetsumochi, *N* Nipponbare. *Arrow heads* indicate bands showing different patterns

Detection of a retrotransposon-like sequence in the *wx* allele of 'Oragamochi'

In Southern blot analysis of the genomic DNA of 'Oragamochi' after digestion with *Pst*I and *Eco*RI using the probe of the region covering the ninth exon to tenth exon, which includes an overlapping region of Waxy-4 and Waxy-5a, a detected DNA fragment was *ca.* 8 kb longer than those detected in 'Nipponbare' and 'Yukimimochi'. Southern blot analysis after digestion with *Dra*I showed two bands, 4 and 3.2 kb, in 'Oragamochi' and a single band of 3.2 kb in 'Nipponbare' and 'Yukimimochi'. The band pattern of 'Hidekomochi' was the same as that of 'Oragamochi' (data not shown). These results suggest that the *wx* allele of 'Oragamochi' and 'Hidekomochi' has an insertion of *ca.* 8 kb. Southern blot analysis after digestion with *Pst*I, *Eco*RI, and *Dra*I using a probe of the ninth exon showed the same band pattern as that shown in Fig. 3 (data not shown), indicating an insertion in the ninth exon.

Five clones of the *wx* gene were isolated from the genomic DNA library of 'Oragamochi'. Nucleotide sequencing of 7,799 bp (Acc. No. AB268120) revealed a 7,764-bp insertion in the ninth exon and 5-bp target site duplication (TSD) (Fig. 4). The 7,764-bp sequence contained long terminal repeats (LTRs) of 439 bp having 100% sequence identity and 32 direct repeats of 89–90 bp. The presence of a TSD and the LTRs suggests that the 7,764-bp sequence is a retrotransposon, but *gag* and *pol* sequences were not found in this sequence.

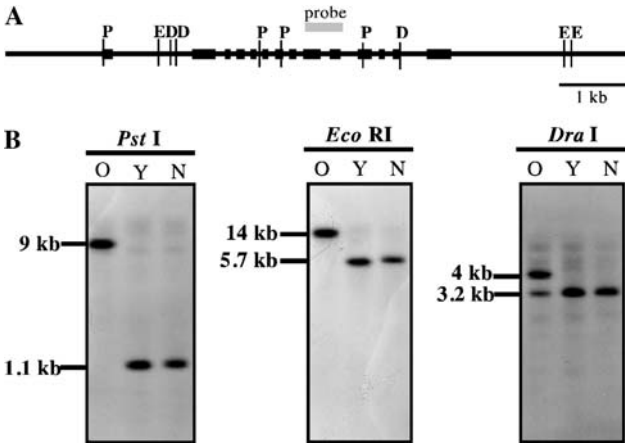

Fig. 3 Southern blot analysis of the *Wx* gene in three cultivars. **A** A restriction map of the *Wx* gene of 'Nipponbare'. P, *Pst*I; E, *Eco*RI; D, *Dra*I. *Gray box* indicates the position of a probe. **B** Band patterns of Southern blot analysis. *O* Oragamochi, *Y* Yukimimochi, *N* Nipponbare

Expression of the *wx* allele of 'Oragamochi' in developing seeds

Expression of the *wx* allele of 'Oragamochi' was analyzed by Northern blot analysis using probe A having the sequence of the second, third, and fourth exons, i.e., the region upstream of the insertion site, and probe B having the sequence of the 10th, 11th, and 12th exons, i.e., the region downstream of the insertion site. Probe A detected two major transcripts of 2.6 and 1.5 kb and one minor transcript of 3.4 kb in 'Oragamochi', and two major transcripts of 3.4 and 2.3 kb in the non-glutinous cultivar 'Akihikari' (Fig. 5a), while probe B detected very thin signals of 3.4 and 2.4 kb in 'Oragamochi'. Long exposure to X-ray film enabled detection of these minor transcripts in 'Oragamochi' (data not shown). Two transcripts of the *Wx-b* allele detected in 'Akihikari' are considered to be a normally spliced transcript and one without normal splicing of the first intron (Hirano et al. 1998). The two major transcripts detected in 'Oragamochi' were *ca.* 1 kb shorter than those in 'Akihikari', suggesting that mRNAs of the *wx* allele of 'Oragamochi' are truncated.

Nucleotide sequences of RT-PCR products were determined to identify the site of poly-A addition. In most of the transcripts, the ninth exon was followed by the terminal sequence of the 7,764-bp insertion at the insertion site, and a poly A tail was added at 277–347 bp from the end of the insertion sequence. A stop codon was generated at 157 bp from the insertion site (Fig. 5b). These transcripts are considered to correspond to the 2.6-kb band and the 1.5-kb band in Fig. 5a.

To determine the sequences of minor transcripts detected by Northern blot analysis, RT-PCR was performed using primers having the sequences of the ninth exon and the 12th exon. Two fragments of 768 and 467 bp were amplified (Fig. 6a). The 768-bp fragment contained a 109-nt terminal sequence of the 7,764-bp insertion, a downstream region of the 7,764-bp insertion in the ninth exon, and the 10th, 11th, and 12th exons. The 467-bp fragment had a 109-nt terminal sequence, and the 11th and 12th exons. The 109-nt insertion causes a frame shift, and a stop codon is generated in the tenth exon in the transcript detected as the 768-bp fragment. Although the transcript detected as the 467-bp fragment does not contain a stop codon, the deduced amino acid sequence of this transcript lacks the sequence encoded by the ninth and tenth exons and has a sequence different from that of the normal GBSS protein in the C-terminal region.

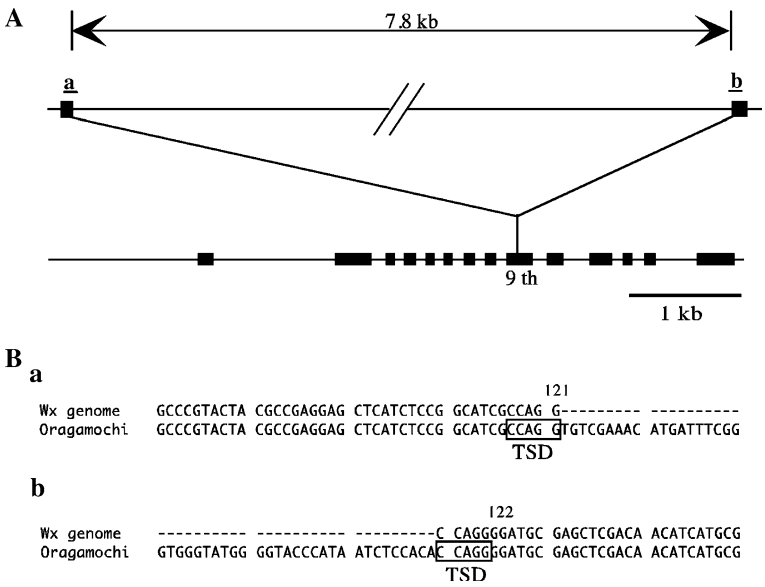

Fig. 4 Position of the inserted DNA fragment. **A** Schematic representation of the DNA insertion in the *wx* allele of 'Oragamochi'. **B** Nucleotide sequences of the upstream region (a) and the downstream region (b) of the insertion site. *Numbers* show nucleotide numbers in the ninth exon in the 'Nipponbare' *Wx* gene. Five bp duplication indicated by *squares* is inferred to be a target site duplication (*TSD*)

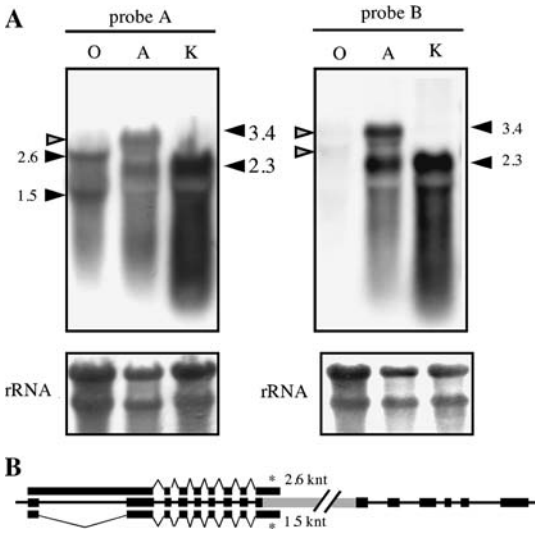

Fig. 5 Northern blot analysis of the 'Oragamochi' *wx* allele. Positions of the sequences of probe A and probe B are shown in Fig. 1. *Numbers on the left and right* indicate sizes of transcripts of 'Oragamochi' and 'Akihikari', respectively, in kilobases. *O* Oragamochi, *A* Akihikari (a *japonica*-type non-glutinous rice cultivar having *Wx-b*), *K* Kasalath (an *indica*-type non-glutinous rice cultivar having *Wx-a*)

Discussion

In the present study, variation of the number of CT repeats in the first exon, T to C transition in the first intron, 23-bp duplication in the second exon, and a 7,764-bp insertion in the ninth exon were identified as DNA polymorphism among the *japonica* glutinous cultivars and between the *japonica* glutinous cultivars and 'Nipponbare'. Since CT repeats are present in the 5′-untranslated region and variations of the number of the CT repeats from 8 to 20 have been reported in non-glutinous cultivars (Ayres et al. 1997), the variation of the CT repeats is not considered to be the cause of the glutinous trait. The T to C transition detected in 'Mangetsumochi' is not inferred to influence the function of the *Wx* gene either. The 23-bp duplication in the second exon resulting in a frame shift has been identified as the cause of the loss of the function of *Wx* in 'Kinoshitamochi' (Inukai et al. 2000). The presence of a premature stop codon in the second exon due to the frame shift has been reported to bring about degradation of mRNA by nonsense-mediated decay (Isshiki et al. 2001). The 23-bp duplication was detected in the *wx* allele of most of the glutinous cultivars used in the present study, including upland glutinous cultivars.

Without the 23-bp duplication, the *wx* allele of 'Oragamochi' had the insertion of 7,764 bp in the ninth exon. Expression analysis of the *wx* allele using RT-PCR and Northern blot analysis revealed shorter transcripts in 'Oragamochi' than those in a non-glutinous cultivar. The deduced amino-acid sequence of 335 amino-acid residues encoded by the shorter transcripts lacks a motif important for glycosyl transferase in the 166 aa of normal glycosyl transferase identified by UniProtKB/Swiss-prot (http://www.br.expasy.org./sprot/). The premature stop codon due to the 7,764-bp insertion is considered to be the cause of the loss of the function of the *Wx* gene. 'Hidekomochi' exhibited the same band pattern as that shown by PCR-RF-SSCP and Southern blot analyses in 'Oragamochi', suggesting that 'Hidekomochi' also has the same 7,764-bp insertion.

A 5-bp TSD, a 439-bp LTR starting and terminating with TG and CA, respectively, and the presence of a putative primer binding site (PBS) and a polypurine tract (PPT) in the 7,764-bp sequence suggest that this insertion is a retrotransposon. However, BlastX search revealed that the 7,764-bp sequence had no sequence homologous with *gag* and *pol* and contained repeats of 89–90 bp. Prediction of genes by RiceGAAS (http://www.ricegaas.dna.affrc.go.jp/) indicated no sequence for transposing activity in the 7,764-bp sequence, suggesting that the 7,764-bp sequence is a non-autonomous element. Furthermore, analysis using Repbase (GIRI, http://www.girinst.org/repbase/index.html) revealed the 7,764-bp sequence to be RIRE_X, which is considered to be a non-autonomous retrotransposon. Two ORFs having HGWP repeats, which are repeats of 30 amino acids containing HGWP (His-Gly-Trp-Pro) motif, were predicted. These repeat sequences are found in a number of plant proteins, the functions of which are unknown. *Dasheng* has been reported to be a non-autonomous element of a retrotransposon in rice (Jiang et al. 2002a).

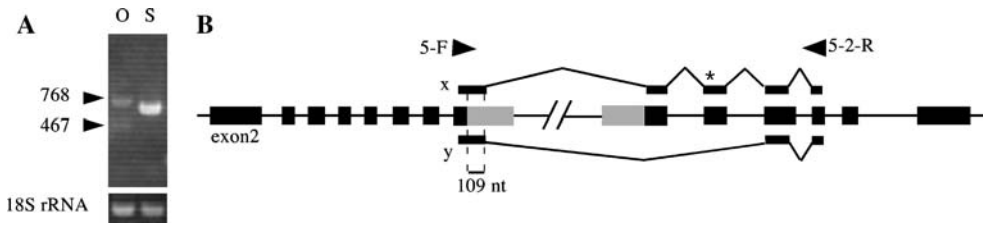

Fig. 6 Detection of the small amounts of transcripts by RT-PCR. **A** Two transcripts amplified from 'Oragamochi' RNA by RT-PCR using primers having the sequences of the ninth exon and the 12th exon. **B** Splicing patterns of the transcripts. *Black boxes* and *gray boxes* indicate exons of the *Wx* gene and LTR of the 7,764-bp insertion. *O* Oragamochi; *S* Sasanishiki. *Asterisks* represents a stop codon

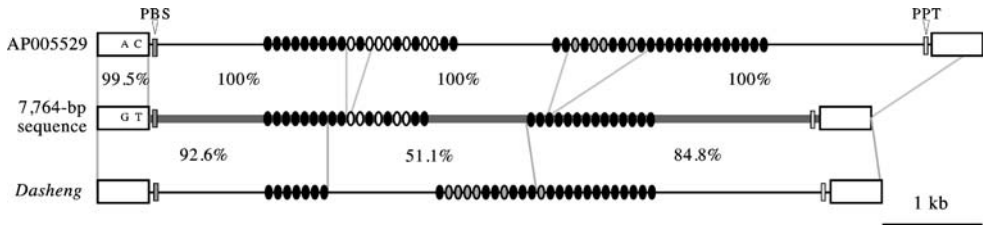

Fig. 7 Comparison of the 7,764-bp sequence with a putative original sequence (AP005529) and *Dasheng*. *Ovals* represent units of the repetitive sequence, and the *same color ovals* indicate similar repeat sequences. The nucleotide sequence of AP005529 was identical to that of the 7,764-bp sequence except for two SNPs in LTR and a difference of repeat number. The *number* indicates similarity of each region. *White boxes* indicate LTRs. The 7,764-bp sequence is reversed to align the sequences

Dasheng has 441-bp-LTRs, PBS, PPT, and a 5-bp TSD flanking the LTR. The region between LTRs contains 89–90-bp repeats, but no sequence of *gag* or *pol*. There are many copies of *Dasheng* in the rice genome. The characteristics of the 7,764-bp sequence correspond to those of *Dasheng*. Sequence comparison between the 7,764-bp sequence and *Dasheng* (http://www.retroryza.org/) revealed high similarities of both side regions of 1.5 and 4.5 kb, 92.6 and 84.8% identities, respectively, besides low similarity, 51.1%, in the central region (Fig. 7). This high similarity suggests that the 7,764-bp sequence is a member of *Dasheng*. *RIRE2* (*Rice Retroelement 2*, Ohtsubo et al. 1999), which is a *Ty3/Gypsy*-type LTR element, has been reported to be an autonomous element and may act to transpose *Dasheng* (Jiang et al. 2002b). The LTRs of *RIRE2* have 65–70% identity with the *Dasheng* LTRs and 57.2% identity with the LTRs of the 7,764-bp sequence. The 7,764-bp sequence may also be transposed by *RIRE2*.

The 100% sequence identity between the LTR sequences in the 7,764-bp sequence suggests a recent event of reinsertion (SanMiguel et al. 1998). The finding of the 7,764-bp sequence in the *wx* allele only of 'Oragamochi' and 'Hidekomochi' also suggests that the transposition of this sequence has occurred very recently. 'Oragamochi' was obtained by crossing between 'Anekomochi' having the 23-bp duplication and 'Hidekomochi' (Fig. 8). 'Hidekomochi' was obtained by crossing between a glutinous line derived from 'Koganemochi' having the 23-bp duplication (Fig. 8) and a non-glutinous cultivar. Insertion of the 7,764-bp sequence is inferred to have occurred in the wild-type *Wx* allele during or just before breeding of 'Hidekomochi'.

Although reinsertion of *Dasheng* has not been reported, the 7,764-bp sequence is likely to have transposing activity. A sequence highly similar to the 7,764-bp sequence was found at 109 cM (AP005529) from the end of the short arm of chromosome 8 by homology search using the published Nipponbare genome sequence (NCBI, http://www.ncbi.nlm.nih.gov/). Although there were differences in the number of the 89–90-bp repeats and two nucleotide differences in a LTR, the other regions were 100% identical between

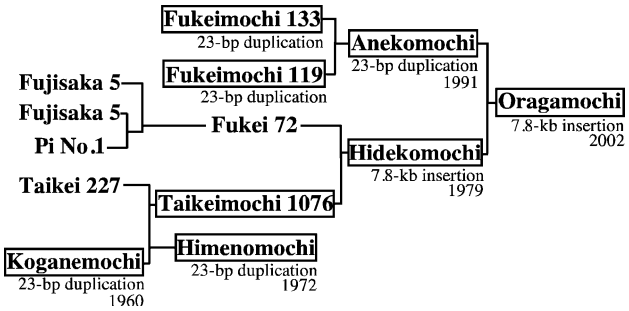

Fig. 8 Genealogy of 'Oragamochi' and 'Hidekomochi'. Glutinous cultivars are *boxed*, and registered years are shown

these sequences (Fig. 7), suggesting that the original sequence of the 7,764-bp insertion is located at the 109 cM site in chromosome 8. Since the sequence of 'Oragamochi' at the 109-cM site in chromosome 8 has not been obtained, whether the sequence alteration in the LTR and the 89–90-bp repeats occurred before, during, or after transposition cannot be ascertained.

Only *Tos17* has been identified as an LTR-type retrotransposon having transposing activity in rice (Hirochika et al. 1996). The finding of the transposing activity of the *Dasheng*-like sequence may contribute to the understanding of diversification of the rice genome. These retrotransposons may have caused mutations of the genes controlling the important traits of cultivars, as found in 'Oragamochi'. Transposon display analysis using the LTR sequence of the 7,764-bp insertion may lead to the finding of interesting genes in rice.

Acknowledgments We wish to express our thanks to Kuniaki Nagano at Furukawa Agriculture Research Station and Masayuki Yamaguchi at the National Agricultural Research Center for the Tohoku Region for providing seeds of the glutinous cultivars. This work was supported in part by funds from the Research Project for Utilizing Advanced Technologies in Agriculture, Forestry, and Fisheries.

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