Breeding Science 64: 199–205 (2014)
doi:10.1270/jsbbs.64.199

Effect of quantitative trait loci for seed shattering on abscission layer formation in Asian wild rice *Oryza rufipogon*

Than Myint Htun, Chizuru Inoue, Orn Chhourn, Takashige Ishii and Ryo Ishikawa*

Laboratory of Plant Breeding, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe, Hyogo 657-8501, Japan

Asian cultivated rice *Oryza sativa* L. was domesticated from its wild ancestor, *O. rufipogon*. During domestication, the cultivated rice lost its seed-shattering behaviour. Previous studies have shown that two major quantitative trait loci (QTLs; *qSH1* and *sh4*) are responsible for the seed-shattering degree. Here, we produced introgression lines carrying non-functional alleles from *O. sativa* 'Nipponbare' at the two major QTLs in the genetic background of wild rice *O. rufipogon* W630, and examined the effects of the two QTLs on seed shattering and abscission layer formation. The introgression lines, with Nipponbare alleles at either or both loci, showed complete or partial abscission layer formation, respectively, indicating that other unknown loci might be involved in enhancing seed shattering in wild rice. We detected a single QTL named *qSH3* regulating seed-shattering degree using an F_2 population between Nipponbare and the introgression line carrying Nipponbare alleles at the two QTLs. Although we generated an introgression line for *qSH3* alone, no effects on seed shattering were observed. However, a significant effect on seed-shattering degree was observed for the introgression line carrying Nipponbare alleles at *qSH3* and the two QTLs, suggesting an important role of *qSH3* on seed shattering in coordination with the two QTLs.

Key Words: wild rice, *Oryza rufipogon*, seed shattering, abscission layer, QTL.

Introduction

Seed shattering is one of the most important characteristics enabling the efficient propagation in wild plants. In many monocot plants, seed shattering is achieved by degradation of the abscission layer formed at the basal part of grains. After fertilisation, plants transfer nutrients to seeds, but as soon as the seed matures, the abscission layer begins to degrade. Since seed shattering causes reduction in the yield, hunter-gatherers and early agriculturists may have selected non-shattering plants to harvest the seeds more efficiently. Therefore, loss of seed shattering is proposed to be one of the most important traits selected for in early crop domestication (Fuller 2007, Harlan 1975).

Asian cultivated rice *Oryza sativa* L. is known to have been domesticated from its wild ancestor *O. rufipogon* about 10,000 years ago (Fuller 2007, Oka 1988). During domestication, rice plants with weak or no seed shattering were preferably selected and an increase in yield was proposed to have contributed human civilisation in Asia. Among rice cultivars, a wide variation in seed-shattering degree has been observed (Konishi *et al.* 2006), suggesting that seed shattering is a quantitatively regulated trait. Genomic loci involved in seed shattering have been identified based on quantitative trait locus (QTL) analyses using segregating populations between natural variations (Cai and Morishima 2000, Ishikawa *et al.* 2010, Lee *et al.* 2005, Li *et al.* 2006a, Onishi *et al.* 2007a, Thomson *et al.* 2003, Xiong *et al.* 1999). A major QTL for seed shattering between *O. rufipogon* and *O. sativa* Indica was *sh4*, which explained 69% of the total phenotypic variance in their segregating populations. A gene responsible for *sh4* was identified and shown to encode a Myb-type transcription factor. A cultivated allele with a non-functional mutation was found to inhibit the normal development of the abscission layer (Li *et al.* 2006b). Another major QTL is *qSH1*, which was identified using a segregating population between *O. sativa* Indica cv. Kasalath and Japonica cv. Nipponbare explaining 68.6% of the phenotypic variance. A causative mutation at *qSH1* was shown to be an SNP located upstream of the rice homolog of the *Arabidopsis REPLUMLESS* (*RPL*) gene, that is involved in the formation of a dehiscence zone in the *Arabidopsis* silique. The Nipponbare allele causes the reduction of rice *RPL* gene expression, resulting in the complete absence of abscission layer formation (Konishi *et al.* 2006). Since the non-functional mutation at *sh4* is commonly observed in cultivated rice, it is widely accepted that the mutation played an important role in rice domestication (Lin *et al.* 2007, Onishi *et al.* 2007b, Zhang *et al.* 2009). A reverse genetic approach was also used to identify genes involved in seed shattering. *SHAT1* encoding an APETALA2 transcription factor was

Communicated by N. Mori
Received February 5, 2014. Accepted April 23, 2014.
*Corresponding author (e-mail: r-ishika@port.kobe-u.ac.jp)

identified by the suppressor mutagenesis of cultivated rice cv. Guangluai 4 with strong seed shattering due to a wild *sh4* allele (Zhou *et al.* 2012). Mutant analysis of seed-shattering cultivars identified *OsCPL1*, encoding a nuclear phosphatase, as a repressor of abscission layer formation (Ji *et al.* 2010).

We previously evaluated the two major seed-shattering loci, *qSH1* and *sh4*, using a backcross population between cultivated rice, *O. sativa* Japonica cv. 'Nipponbare', as a donor parent, and wild rice, *O. rufipogon* acc. W630, as a recurrent parent, aiming to elucidate the effects of the Nipponbare alleles with non-functional mutations (Htun *et al.* 2011, Ishikawa *et al.* 2010). Interestingly, non-shattering behaviour was not observed, even though the Nipponbare non-functional allele at either the *qSH1* or *sh4* locus was introgressed into the wild genetic background. Further, we found that the introgression line that had the Nipponbare non-functional alleles at both loci showed weak shattering. These results suggest that wild rice still had additional effective gene(s) controlling seed shattering. In this study, we carried out a histological analysis to understand the role of the non-functional alleles at seed-shattering loci on abscission layer formation using these introgression lines. Subsequently, QTL analysis was performed to explore novel loci involved in seed shattering in wild rice.

Materials and Methods

Plant materials

A Japonica rice cultivar, *O. sativa* cv. Nipponbare, and an annual wild accession, *O. rufipogon* acc. W630, originating from Myanmar, were used in this study. *O. sativa* cv. Nipponbare has non-shattering behaviour while *O. rufipogon* W630 has strong shattering behaviour. Introgression lines of wild rice carrying the Nipponbare alleles at either the *sh4* or *qSH1* locus have previously been produced (Ishikawa *et al.* 2010). We also produced the introgression line containing the Nipponbare alleles at both the *qSH1* and *sh4* loci in the genetic background of wild rice by backcrossing (Htun *et al.* 2011). This introgression line was further crossed with Nipponbare and their F_2 plants were grown in pots in the greenhouse at Kobe University. To minimise the variation in heading date, F_2 individuals were genotyped for *Hd5* (chr. 8) and *Hd6* (chr. 3) using dCAPS and CAPS markers, respectively (Table 1), and short day treatment was given to the plants with late-flowering genotypes. Further, introgression lines of the newly identified shattering locus were generated in the genetic background of wild rice by using two flanking simple sequence repeat (SSR) markers (Table 1). Chromosomal information of the Nipponbare segment covering *qSH1*, *sh4*, and *qSH3* loci in the five introgression lines was also analysed (Supplemental Table 1).

Histological analysis of abscission layer formation

Histological samples for abscission layer analysis were collected from pedicel tissue of rice grains just after flowering. Samples were fixed in an FAA solution (5% v/v formaldehyde, 5% v/v acetic acid and 63% ethanol) with vacuum infiltration. These samples were kept at 4°C and then were dehydrated through an ethanol series (50%, 70%, 80%, 90%, 95%, 99.5% and 100%) for 1 h each. Then, they were embedded in Technovit 7100 resin (Heraeus Kulzer, Germany) according to the manufacturer's instruction. Samples were cut into 3-μm-thick sections by using a rotary microtome, RM2125RT (Leica Biosystems) and stained with toluidine blue O solution. These sections were observed under a microscope.

Evaluation of seed shattering degree

To precisely evaluate the shattering degree in plants in an F_2 segregating population, a digital force gauge (FGP 0.5, Nidec-Shimpo Co., Japan) was employed. The seed-shattering degree was evaluated by measuring the breaking tensile strength (BTS) required to detach the seeds from the pedicels. At the maturation stage, approximately 35 days after flowering, shattering degree was measured using 125 spikelets (25 randomly chosen spikelets from 5 panicles of each plant), and their average BTS values were calculated.

SSR marker genotyping and QTL analysis

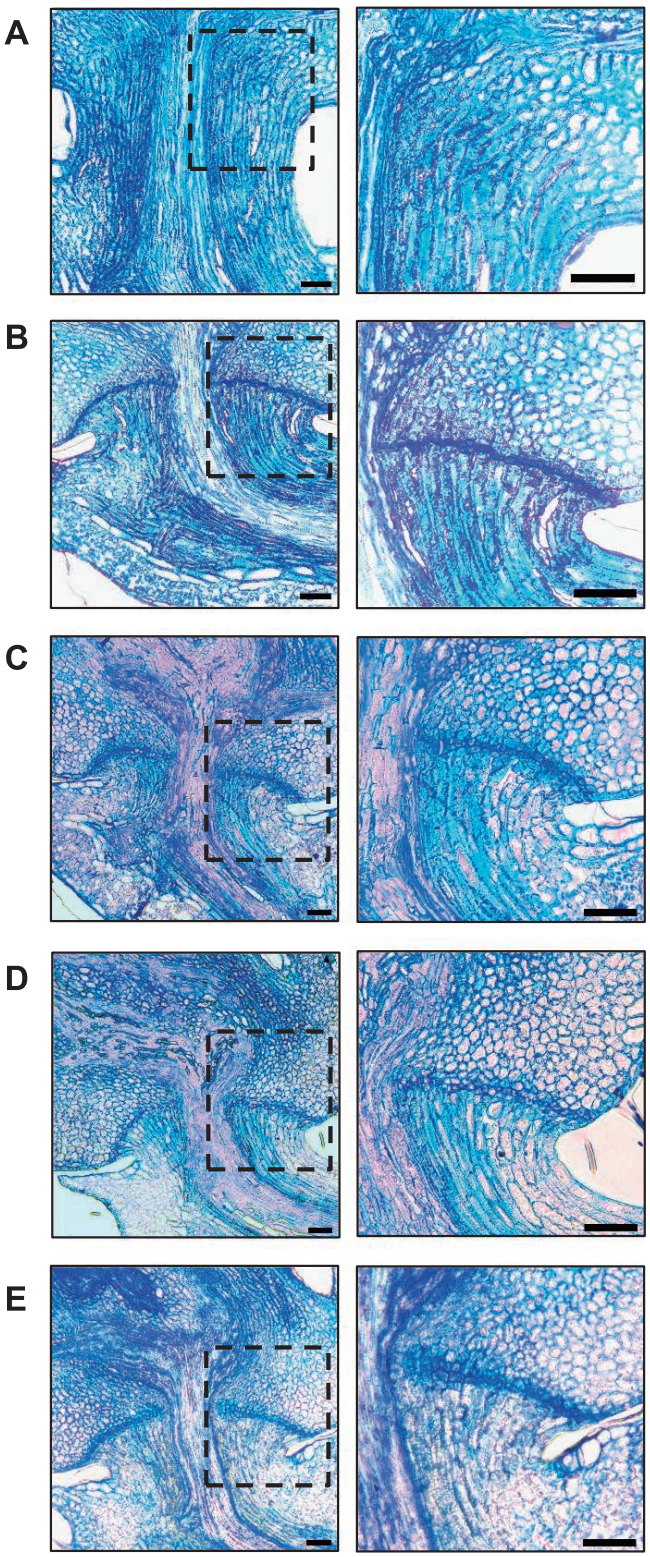
To determine the genotypes at 179 polymorphic marker loci covering the whole rice genome (Thanh *et al.* 2010), genomic DNA was extracted from 90 F_2 plants and PCR was conducted as previously described (Ishikawa *et al.* 2010). The amplified products were electrophoresed in 4.0% polyacrylamide gels and banding patterns were visualised by the silver staining method of Panaud *et al.* (1996). QTL analysis for seed shattering was carried out with

Table 1. List of dCAPS, CAPS, and flanking SSR markers used in this study

Chr.	Locus	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Restriction Enzyme	Reaction condition
1	*qSH1*	TGATGGTATTGATGTATACTGGACAaT[a]	TCGAAATGTGGAGACAGCTC	*Eco*T22I (*Ava*III)	37°C
4	*sh4*	AACGCCGGCGCGGCGGTCGTCGTCCAGtCG[a]	ACGGGCACCTGACTGCTAC	*Taq*I	60°C
3	*Hd6*	ACCTGGCAGCATGTTATGAC	CTACAGATCCACAGAACAGG	*Hin*d III	37°C
8	*Hd5*	GCGCCGATGGCGTCGTCATCGACGGGaATT[a]	ACGCCTTGTTCCCTGACG	*Eco*RI	37°C
3	*qSH3*				
	RM16	CGCTAGGGCAGCATCTAAA	AACACAGCAGGTACGCGC		
	RM3513	TACTCCTATCCTGCCATGGC	TGTAGTAGACGAGAGGCCGG		

[a] Nucleotide with a small letter indicates the primer mismatch creating restriction endonuclease-sensitive polymorphism.

phenotypic data (BTS values) by composite interval mapping (CIM) using WinQTL cartographer ver. 2.5 (Wang *et al.* 2007). The optimal log of odds (LOD) threshold values (with the permutation value set at 1000) were used to determine the presence of a putative QTL, and the percentage of variation explained by the QTL was estimated. Information on the candidate genes at *qSH3* was obtained from RAP-DB (IRGSP-1.0, Rice Annotation Project, http://rapdb.dna.affrc.go.jp/) (Sakai *et al.* 2013).



Results

Effects of two major quantitative trait loci (QTLs) for seed shattering on abscission layer formation in wild rice

In our previous study, we generated and characterised backcross plants individually having the Nipponbare non-functional alleles at *qSH1* or *sh4* in the genetic background of wild rice. We found that these two introgression lines showed strong seed-shattering habits similar to those of wild rice, indicating that a single mutation at either the *qSH1* or *sh4* locus is not sufficient to confer the non-shattering phenotype (Ishikawa *et al.* 2010). We also observed partial seed shattering in the introgression line having the Nipponbare alleles at both the *qSH1* and *sh4* loci in the genetic background of wild rice. This line could keep mature seeds on the panicles, giving small BTS values to detach the seeds (Htun *et al.* 2011, Ishikawa *et al.* 2010). To better characterise the shattering habits in these plants, we observed their abscission layer formation at the flowering stage (Fig. 1). In the parental lines, non-shattering cultivated rice (*O. sativa* cv. Nipponbare) formed no abscission layer, while wild rice (*O. rufipogon* acc. W630) formed a complete abscission layer across the pedicel region (Fig. 1A, 1B). In both introgression lines having the Nipponbare alleles at *qSH1* or *sh4*, complete abscission layers across the pedicel, as in wild rice, were observed (Fig. 1C, 1D). Partial inhibition of the abscission layer was observed for the introgression line carrying both the Nipponbare alleles at *qSH1* and *sh4* (Fig. 1E), although the complete loss of the abscission layer was observed for Nipponbare having non-shattering alleles at *qSH1* and *sh4*. Taken together, these results agree with previous observations of seed shattering and indicate that some other minor gene(s) are still involved in promoting abscission layer formation in wild rice.

Distribution of seed-shattering degree in an F_2 population produced between Nipponbare and the introgression line carrying both the Nipponbare alleles at qSH1 and sh4

To identify novel gene(s) controlling seed shattering in wild rice, we crossed Nipponbare and the introgression line carrying both the Nipponbare alleles at *qSH1* and *sh4*. Since these two loci are fixed with the Nipponbare alleles,

Fig. 1. Abscission layer formation of the introgression lines carrying non-functional alleles from *O. sativa* cv. Nipponbare at one or both major QTLs for seed shattering in the genetic background of wild rice *O. rufipogon* W630. (A) A Japonica rice cultivar *O. sativa* cv. Nipponbare. (B) Wild rice *O. rufipogon* W630. (C, D) Introgression lines carrying Nipponbare alleles at *qSH1* (C) or *sh4* (D). (E) The introgression line carrying Nipponbare alleles at both *qSH1* and *sh4* loci. Magnification of the boxed abscission layer on the left is shown on the right-hand side. Bar = 50 μm.

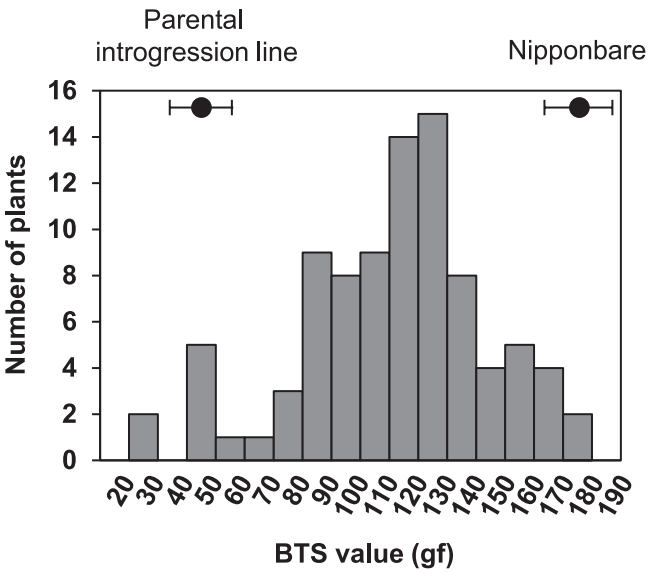


Fig. 2. Frequency distribution of breaking tensile strength (BTS) values for 90 F_2 individuals between cultivated rice *O. sativa* cv. Nipponbare, and wild introgression line carrying Nipponbare alleles at both *qSH1* and *sh4* loci. BTS values for parental lines are shown with black dots with s.d. (n = 125).

variation of seed shattering-degree in the F_2 population was not affected by them. QTL analysis of seed shattering was carried out to search for the additional loci. We used 90 F_2 individuals, however, the heading date was expected to segregate depending on the genotypes at *Hd5* and *Hd6*, as these two loci were previously shown to influence the flowering time of Nipponbare and *O. rufipogon* W630 (Thanh *et al.* 2010). To minimise the environmental effects on seed-shattering degree, these F_2 lines were required to flower in a similar period. Therefore, we screened the F_2 plants for late flowering by PCR genotyping at two loci, *Hd5* and *Hd6* (Table 1). We performed short-day treatment for plants with late-flowering genotypes and induced their flowering in the period from Aug. 9 to 16, 2011. We measured BTS values for all the plants at approximately 35 days after flowering. A continuous frequency distribution for BTS values was observed ranging from 28 to 180 gf, and a few plants showed transgressive segregation (Fig. 2). These observations indicate that seed shattering in wild rice is still regulated by QTLs apart from *qSH1* and *sh4*.

Identification of additional QTL involved in the regulation of seed shattering in wild rice

QTL analysis for shattering degree was carried out with 90 F_2 plants using 179 polymorphic markers in the linkage map previously constructed between *O. sativa* Nipponbare and *O. rufipogon* W630 (Thanh *et al.* 2010). A QTL was detected near RM16 on chr. 3 with an LOD score of 5.8. It explained 16.0% of the total phenotypic variance with the Nipponbare allele enhancing BTS value and the dominant effect was 4.9 (Table 2). We named this QTL, '*qSH3*', a name previously given for the QTL close to RM16 (Onishi *et al.* 2007b). No additional loci were detected over threshold score (LOD = 3.8) in this QTL analysis.

Evaluation of qSH3 in the genetic background of wild rice

On chr. 3, QTLs for seed shattering were reported in several studies (Cai and Morishima 2000, Onishi *et al.* 2007a, 2007b). However, as mentioned in Onishi *et al.* (2007b), the effect of the allele at *qSH3* from wild rice on seed shattering is small in the genetic background of cultivated rice. This could be due to the interactions among seed-shattering loci in rice. To evaluate the effect of the allele at *qSH3* on seed shattering, we generated an introgression line with the Nipponbare allele at *qSH3* in the genetic background of wild rice. This line showed strong seed-shattering behaviour with complete abscission layer formation, as observed in wild rice (Fig. 3A). This indicates that the Nipponbare allele at *qSH3* alone did not contribute at all to abscission layer formation in the genetic background of wild rice. We next evaluated shattering degree of introgression lines carrying the Nipponbare alleles at two (*qSH1* and *sh4*) and three loci (*qSH1*, *sh4*, and *qSH3*). A significant difference was observed for the BTS value between them, namely, the latter showed approximately 4 times higher value (116 ± 37 gf) than the former (29 ± 9 gf), although the latter is still lower than Nipponbare (180 ± 8 gf) (Fig. 3B). We further carried out a histological analysis with these introgression lines, and found that abscission layer formation is partially blocked outside the region of pedicel due to the additional Nipponbare allele at *qSH3* (Fig. 3C). Inhibition of abscission layer formation outside the region of pedicel might contribute to the significant increase in the BTS value (Fig. 3B), suggesting an important role of the Nipponbare allele at *qSH3*. Taken together, our results indicate that the Nipponbare allele at *qSH3* alone does not show significant effects on abscission layer formation in the genetic background of wild rice, however, its additive effect was observed in coordination with the Nipponbare non-functional alleles at *qSH1* and *sh4*.

Discussion

Loci contributed to loss of seed shattering in cultivated rice

Wild rice has strong seed-shattering habits while

Table 2. Characteristics of the QTL for seed-shattering degree detected in this study

Chr.	QTL region	Nearest marker	Source[a]	LOD	PV[b] (%)	Additive effect (gf)	Dominant effect (gf)
3	RM16-RM3513	RM16	Nipponbare	5.8	16.0	19.0	4.9

[a] Allele source increasing trait values.

[b] Percentage of total phenotypic variance explained by the QTL.

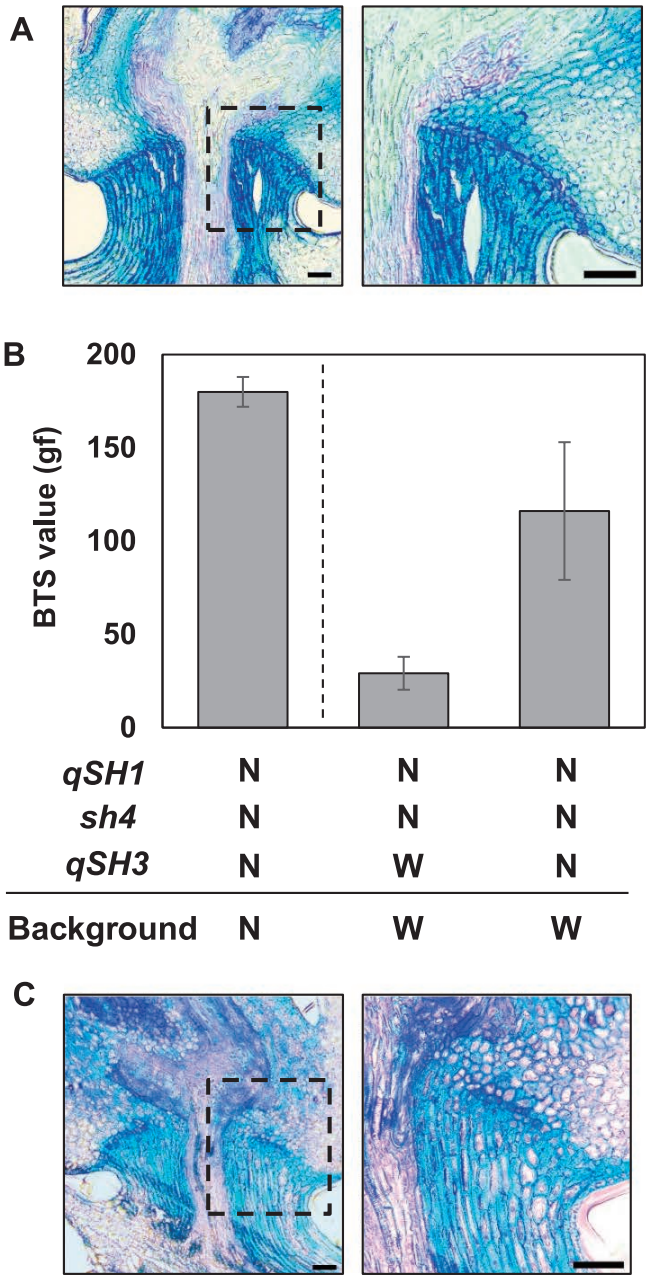


Fig. 3. Evaluation of *qSH3* locus in the genetic background of wild rice. (A) Abscission layer formation of introgression line carrying Nipponbare allele at *qSH3*. (B) Breaking tensile strength (BTS) values of introgression lines carrying Nipponbare alleles at two (*qSH1* and *sh4*) and three loci (*qSH1*, *sh4*, and *qSH3*). Genotypes at *qSH1*, *sh4*, and *qSH3* and background information are as follows: N, *O. sativa* cv. Nipponbare; W, *O. rufipogon* W630. BTS value for Nipponbare is shown as control on the left. Data are mean ± s.d. ($n = 75$). (C) Abscission layer formation for the introgression line carrying Nipponbare alleles at *qSH1*, *sh4*, and *qSH3*. Bar = 50 μm.

cultivated rice has non-shattering habits. A wide range of natural variation in seed-shattering degree is observed for cultivated rice, suggesting that several genes or alleles might be involved in their quantitative regulation. Using these variations, seed shattering has been extensively studied and two major QTLs have been identified as the *qSH1* and *sh4* loci in rice (Konishi *et al.* 2006, Li *et al.* 2006b). In most cases, these loci were evaluated in cultivated rice (Ishikawa *et al.* 2010, Konishi *et al.* 2006, Onishi *et al.* 2007b). A *qSH1* mutation was detected in the variation between *O. sativa* Indica and Japonica cultivars, and the causative mutation was proposed to be selected among rice cultivars. Since introgression or transformation of a functional allele at *sh4* into cultivated rice causes the shattering phenotype and a non-functional mutation at *sh4* is widely conserved in cultivated rice (Lin *et al.* 2007, Onishi *et al.* 2007b), it is widely accepted that the mutation at *sh4* plays an important role in changing seed-shattering degree and was selected in rice domestication. However, we do not know how many genes or mutations were involved in the complete loss of seed shattering as observed in Japonica cultivars. To precisely study seed shattering in rice, we evaluated the effects of the Nipponbare non-functional alleles in the genetic background of wild rice. We did not find either of the Nipponbare alleles at *qSH1* and *sh4* to be sufficient in conferring loss of the abscission layer (Fig. 1C, 1D). This finding confirms that other loci are involved in promoting complete seed shattering in wild rice. In this study, *qSH3* was found to be necessary for establishment of non-shattering in coordination with *qSH1* and *sh4*. However, the BTS value of the introgression line carrying the Nipponbare alleles at *qSH1*, *sh4*, and *qSH3* (116 ± 37 gf) is still lower than that of Nipponbare (180 ± 8 gf), implying these three loci are not enough to explain the complete non-shattering habit. It is likely that some additional minor loci might be involved in the complete loss of shattering as observed in Nipponbare. In our recent study, we found that the closed panicle trait regulated by *OsLG1* was selected for in the early phase of rice domestication to increase gathering efficiency (Ishii *et al.* 2013). Interestingly, a closed panicle trait was also shown to promote self-pollination, suggesting that recessive mutations may have easily been fixed. Therefore, several mutations at seed-shattering loci might have accumulated under artificial selection during rice domestication.

We have successfully identified a novel QTL for seed shattering by fixing two major loci previously detected in the segregating population. A similar approach was previously tested for identifying two new QTLs regulating internode elongation in deepwater rice (Nagai *et al.* 2012). Therefore, an approach that involves fixing the major QTL(s) in the segregating population is very effective to explore and uncover the additional QTL(s) involved.

Role of qSH3 on abscission layer formation

Previously, interaction at three seed-shattering loci from wild rice was studied in the genetic background of cultivated rice, *O. sativa* Japonica cv. A58 (Onishi *et al.* 2007b). The results showed that *qSH1* and *qSH4* (same as *sh4*) played significant roles in seed shattering. In contrast, the effect of *qSH3* on seed shattering was relatively small compared to those of *qSH1* and *qSH4*. In the present study, we

detected a strong effect of the Nipponbare allele at *qSH3* on abscission layer formation with the Nipponbare alleles at *qSH1* and *sh4* in the genetic background of wild rice. The difference in the effects of *qSH3* can be due to the unidentified mutations at seed-shattering loci potentially harboured in cultivated rice. Thus, evaluation of non-functional alleles for seed shattering in the genetic background of wild rice may reveal the role of *qSH3* more clearly. Complete abscission layer formation was observed for introgression lines carrying any one of the Nipponbare alleles at *qSH1*, *sh4*, or *qSH3* (Figs. 1C, 1D, 3A), suggesting that abscission layer formation in wild rice is redundantly regulated by these loci. We found that abscission layer formation was disturbed around vascular bundles in the introgression line carrying the Nipponbare alleles at *qSH1* and *sh4* (Fig. 1E). Moreover, the introgression line carrying the Nipponbare alleles at *qSH1*, *sh4*, and *qSH3* showed additional loss of abscission cells outside of the pedicel, resulting in a large increase in the BTS value (Fig. 3B, 3C). These observations suggest that redundancy and gradient regulation may exist in the regulation of abscission layer formation in rice. Therefore, tissue-specific analysis of the abscission cells and expression domain of the genes controlling seed shattering will be an important scope of future study. It would be of interest to analyse genetic interaction between *qSH3* and two major loci of *qSH1* and *sh4* in the genetic background of wild rice. These genetic studies will shed light on how non-shattering cultivated rice was established during rice domestication.

Candidate genes related to qSH3

In our QTL analysis, *qSH3* was identified on chr. 3 between two SSR markers, RM16 and RM3513 (Table 2). We surveyed candidate genes involved in seed shattering or abscission layer formation reported around this genomic region. One candidate gene is *OsSh1* (Os03g0650000), a rice homolog of the sorghum *Shattering 1* (*Sh1*) gene, encoding YABBY transcription factor and controlling cell numbers at the abscission layer (Lin *et al.* 2012). Interestingly, the genomic region harbouring *OsSh1* was shown to be under strong artificial selection (He *et al.* 2011, Xu *et al.* 2012), suggesting that *OsSh1* may have been involved in the loss of seed shattering during rice domestication (Lin *et al.* 2012). However, studies have not shown any of the selected causative mutations of *OsSh1*, because the phenotypic effect of *OsSh1* on seed shattering was examined only by using an artificially induced non-shattering mutant SR-5 (Lin *et al.* 2012). To explore the involvement of *OsSh1* in this study, we compared nucleotide sequences of the *OsSh1* gene between *O. sativa* Nipponbare and *O. rufipogon* W630. Among several mutations detected in the genic region, one SNP was found located on exon 1 of *OsSh1*. This SNP contributes to an amino acid change from leucine (L) in W630 to phenylalanine (F) in Nipponbare (Supplemental Fig. 1A). Recently, another gene, *SpWRKY*, located approximately 300 kb away from *Sh1* in sorghum, was also shown to be involved in seed shattering (Tang *et al.* 2013). We also found the homologue of the rice *WRKY* gene (Os03g0657400), that is located approximately 450 kb away from the *OsSh1* gene. Sequence analysis between *O. sativa* Nipponbare and *O. rufipogon* W630 identified two amino acid changes; one amino acid (glycine: G) deletion in Nipponbare and one amino acid change from alanine (A) in W630 to threonine (T) in Nipponbare (Supplemental Fig. 1B). We do not know whether these changes are associated with the seed-shattering degree between wild and cultivated rice. Further fine mapping analysis and transformation study are required to identify the causative mutation at *qSH3*. Moreover, the additional effect of *qSH3* identified in this study may be important in the breeding process, used to fine-tune the shattering degree for efficient harvesting of cultivated rice in coordination with the two major QTLs at *qSH1* and *sh4*.

Acknowledgements

The wild rice accession used in this study was kindly provided from the National Institute of Genetics (Japan) supported by the National BioResource Project (MEXT, Japan). This work was supported in part by a Grant-in-Aid (No. 23580006 to T.I. and No. 23580473 to R.I.) from Japanese Society for Promotion of Science.

Literature Cited

Cai, H.W and H. Morishima (2000) Genomic regions affecting seed shattering and seed dormancy in rice. Theor. Appl. Genet. 100: 840–846.

Fuller, D.Q. (2007) Contrasting patterns in crop domestication and domestication rates, recent archaeobotanical insights from the Old World. Ann. Bot. 100: 903–924.

Harlan, J.R. (1975) Crops and Man. American Society of Agronomy, Madison.

He, Z., W. Zhai, H. Wen, T. Tang, Y. Wang, X. Lu, A.J. Greenberg, R.R. Hudson, C.I. Wu and S. Shi (2011) Two evolutionary histories in the genome of rice: the roles of domestication genes. PLoS Genet. 7: e1002100.

Htun, T.M., T. Ishii and R. Ishikawa (2011) Temporal changes of seed shattering degree of substitution lines having non-shattering alleles from cultivated rice (*Oryza sativa*) in the genetic background of wild rice (*O. rufipogon*). J. Crop Res. 56: 39–44.

Ishii, T., K. Numaguchi, K. Miura, K. Yoshida, P.T. Thanh, T.M. Htun, M. Yamasaki, N. Komeda, T. Matsumoto, R. Terauchi *et al.* (2013) *OsLG1* regulates a closed panicle trait in domesticated rice. Nat. Genet. 45: 462–465.

Ishikawa, R., P.T. Thanh, N. Nimura, T.M. Htun, M. Yamasaki and T. Ishii (2010) Allelic interaction at seed-shattering loci in the genetic backgrounds of wild and cultivated rice species. Genes Genet. Syst. 85: 265–271.

Ji, H., S.R. Kim, Y.H. Kim, H. Kim, M.Y. Eun, I.D. Jin, Y.S. Cha, D.W. Yun, B.O. Ahn, M.C. Lee *et al.* (2010) Inactivation of the CTD phosphatase-like gene *OsCPL1* enhances the development of the abscission layer and seed shattering in rice. Plant J. 61: 96–106.

Konishi, S., T. Izawa, S.Y. Lin, K. Ebana, Y. Fukuta, T. Sasaki and M. Yano (2006) An SNP caused loss of seed shattering during rice domestication. Science 312: 1392–1396.

Lee, S.J., C.S. Oh, J.P. Suh, S.R. McCouch and S.N. Ahn (2005) Identification of QTLs for domestication-related and agronomic traits in an *Oryza sativa* × *O. rufipogon* BC_1F_7 population. Plant Breed. 124: 209–219.

Li, C., A. Zhou and T. Sang (2006a) Genetic analysis of rice domestication syndrome with the wild annual species, *Oryza nivara*. New Phytol. 170: 185–194.

Li, C., A. Zhou and T. Sang (2006b) Rice domestication by reducing shattering. Science 311: 1936–1939.

Lin, Z., M.E. Griffith, X. Li, Z. Zhu, L. Tan, Y. Fu, W. Zhang, X. Wang, D. Xie and C. Sun (2007) Origin of seed shattering in rice (*Oryza sativa* L.). Planta 226: 11–20.

Lin, Z., X. Li, L.M. Shannon, C.-T. Yeh, M.L. Wang, G. Bai, Z. Peng, J. Li, H.N. Trick, T.E. Clemente *et al.* (2012) Parallel domestication of the *Shattering1* genes in cereals. Nat. Genet. 44: 720–724.

Nagai, K., T. Kuroha, M. Ayano, Y. Kurokawa, R.B. Angeles-Shim, J.-H. Shim, H. Yasui, A. Yoshimura and M. Ashikari (2012) Two novel QTLs regulate internode elongation in deepwater rice during the early vegetative stage. Breed. Sci. 62: 178–185.

Oka, H.I. (1988) Origin of Cultivated Rice. Elsevier, Tokyo, p. 254.

Onishi, K., Y. Horiuchi, N. Ishigoh-Oka, K. Takagi, N. Ichikawa, M. Maruoka and Y. Sano (2007a) A QTL cluster for plant architecture and its ecological significance in Asian wild rice. Breed. Sci. 57: 7–16.

Onishi, K., K. Takagi, M. Kontani, T. Tanaka and Y. Sano (2007b) Different patterns of genealogical relationships found in the two major QTLs causing reduction of seed shattering during rice domestication. Genome 50: 757–766.

Panaud, O., X. Chen and S.R. McCouch (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet. 252: 597–607.

Sakai, H., S.S. Lee, T. Tanaka, H. Numa, J. Kim, Y. Kawahara, H. Wakimoto, C.C. Yang, M. Iwamoto, T. Abe *et al.* (2013) Rice Annotation Project Database (RAP-DB): an integrative and interactive database for rice genomics. Plant Cell Physiol. 54: e6 (1–11).

Tang, H., H.E. Cuevas, S. Das, U.U. Sezen, C. Zhou, H. Guo, V.H. Goff, Z. Ge, T.E. Clemente and A.H. Paterson (2013) Seed shattering in a wild sorghum is conferred by a locus unrelated to domestication. Proc. Natl. Acad. Sci. USA 110: 15824–15829.

Thanh, P.T., P.D.T. Phan, R. Ishikawa and T. Ishii (2010) QTL analysis for flowering time using backcross population between *Oryza sativa* Nipponbare and *O. rufipogon*. Genes Genet. Syst. 85: 273–279.

Thomson, M.J., T.H. Tai, A.M. McClung, X.H. Lai, M.E. Hinga, K.B. Lobos, Y. Xu, C.P. Martinez and S.R. McCouch (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor. Appl. Genet. 107: 479–493.

Wang, S., C.J. Basten and Z.B. Zeng (2007) Windows QTL cartographer 2.5. (http: //statgen.ncsu.edu/qtlcart/WQTLCart.htmZ). Department of Statistics, North Carolina State University, Raleigh, NC.

Xiong, L.Z., K.D. Liu, X.K. Dai, C.G. Xu and Q. Zhang (1999) Identification of genetic factors controlling domestication-related traits of rice using an F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor. Appl. Genet. 98: 243–251.

Xu, X., X. Liu, S. Ge, J.D. Jensen, F. Hu, X. Li, Y. Dong, R.N. Gutenkunst, L. Fang, L. Huang *et al.* (2012) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat. Biotechnol. 30: 105–111.

Zhang, L.B., Q. Zhu, Z.Q. Wu, J. Ross-Ibarra, B.S. Gaut, S. Ge and T. Sang (2009) Selection on grain shattering genes and rates of rice domestication. New Phytol. 184: 708–720.

Zhou, Y., D. Lu, C. Li, J. Luo, B.F. Zhu, J. Zhu, Y. Shangguan, Z. Wang, T. Sang and B. Zhou (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor *SHATTERING ABORTION1*. Plant Cell 24: 1034–1048.