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Research Paper

Improvement of seed shattering and dormancy in *Oryza sativa* L. 'Hokuriku 193' based on genetic information

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In this study, we investigated the genetic basis of seed shattering and dormancy in Hokuriku 193 and bred an NIL improved these traits. Analysis of an F_3 population from Hokuriku 193 × Koshihikari revealed a general correspondence between seed shattering and genotypes at the *qSH1* locus, suggesting a strong influence of this locus on the seed shattering in Hokuriku 193. An F_2 population from [ms-bo] Nekken 2 × Hokuriku 193 was also analyzed to identify quantitative trait loci (QTLs) for seed dormancy as measured by germination rate in the first December and March after seed harvest. The results revealed a concurrence QTLs of on chromosomes 1, 3, and 6 (*qSDo1*, *qSDo3*, *qSDo6*). In particular, *qSDo1* and *qSDo6* were considered regions worthy of active modification because they were QTL regions that promoted seed dormancy when carrying Hokuriku 193 genome regions around. SSDo_NIL, a near isogenic line (NIL) derived from Hokuriku 193 by introgressing Nekken 2 alleles only at the *qSH1* locus and *qSDo1*, did not shatter, and its germination rate was significantly higher than that of Hokuriku 193. Yield performance was similar between SSDo_NIL and Hokuriku 193, suggesting that improvement of seed shattering and dormancy does not affect yield.

Key Words: Hokuriku 193, near isogenic line (NIL), *Oryza sativa* L., quantitative trait loci (QTLs), seed dormancy, seed shattering.

Introduction

In recent years, the use of rice has expanded from a household staple to include uses in food service, food processing, and cattle feeding. Given the price gap with imported foodstuffs such as wheat and maize, non-staple uses of rice critically depend on reducing production costs. One effective measure is to increase yields, which encourages the use of high-yielding varieties. Meanwhile, an upward trend in summer temperatures in Japan is significantly affecting the growth of rice plants. Exposure of rice plant to high temperatures during the grain filling stage reduces starch accumulation and increases white immature grains, impairing appearance and quality (Nagato and Ebata 1960, Tashiro and Wardlaw 1991) in association with occasional yield losses (Morita 2008, Peng *et al.* 2004). High temperatures during the grain filling stage thus lead to a decline in the yield even of high-yielding varieties, thereby causing concerns about adverse effects on farmers' income. Therefore, the utmost attention should be given to breeding varieties that guarantee stable and high yields that are less sensitive to high temperatures during the grain filling stage.

'Hokuriku 193' is an *indica* rice cultivar bred in the National Agricultural Research Center (NARC) in Japan (Goto *et al.* 2009). Its high-yielding potential, with a record yield of more than 1000 g/m^2 (Kobayashi *et al.* 2014), is expected to contribute to the reduction of production costs. *Indica* cultivars such as Hokuriku 193 are also believed to be less susceptible to yield losses due to high temperatures than *japonica* cultivars (Nagata *et al.* 2012, 2015). These characteristics promise the extensive use of Hokuriku 193 as a stable, high-yielding, multipurpose rice cultivar resistant to yield losses due to high temperatures during the grain filling stage.

However, Hokuriku 193 has some disadvantages in comparison with common *japonica* cultivars, such as easy seed

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shattering during the harvest stage and deep seed dormancy. Seed shattering during the harvest stage reduces yields and allows volunteer rice to emerge from dropped seeds. Deep seed dormancy prevents adequate stand establishment after sowing, with potential adverse effects on transplantation and subsequent growth. One way to break seed dormancy is dry heating (Jennings and Jesus 1964), but this approach requires investment in equipment and is therefore unsuitable for general farm households. Accordingly, the seed shattering and dormancy in Hokuriku 193 are key traits to be improved by breeding.

Information on the quantitative trait loci (QTLs) for the seed shattering and dormancy in Hokuriku 193 is indispensable for efficient cross breeding to improve these traits, but clear information is as yet unavailable.

This study aimed to clarify the genetic basis of the seed shattering and dormancy in Hokuriku 193 and breed a near isogenic line (NIL) improved these traits according to the obtained information.

Materials and Methods

Evaluation of seed shattering and investigation of genetic factors

The *qSH1* locus on chromosome 1 is a major factor known to control seed shattering in *indica* cultivars (Konishi *et al.* 2006). We investigated the relationship between the genotype at the *qSH1* locus and the seed shattering in Hokuriku 193. An F_3 population derived from a cross between Hokuriku 193 and 'Koshihikari' was sown in a paddy field of the National Agricultural Research Center (NARC; Joetsu City, Niigata Prefecture: 37°6′59″N, 138°16′14″E) on April 23, 2008, and seedlings were transplanted on May 21. The basal fertilizer consisted of 5 g/m^2 each of N, P_2O_5, and K_2O with no additional fertilizer. Around the maturation stage, leaves were collected from 54 individual plants with few sterile spikelets and subjected to DNA extraction according to the method of Tabuchi *et al.* (2016). The collected DNA samples were genotyped according to the method of Hayashi *et al.* (2004): genotyping with single nucleotide polymorphism (SNP) markers at the *qSH1* locus (Forward: AGCAGCGGTAGCACACTAGC; Reverse: GCATTTGG TGGTACGTGTATCAGGC, which specifically amplifies the Hokuriku 193 allele, or GCATTTGGTGGTACGTGT ATCAGGA, which specifically amplifies the *japonica* allele). Forty-five individual plants with either the Hokuriku 193 or *japonica* allele fixed at the *qSH1* locus were tested for seed shattering according to Fukuta and Fukui (1995): a plant was considered to shatter if it dropped at least one grain when the panicle was grasped firmly.

Plant materials for QTL analysis for seed dormancy and heading trait

In 2009, an F_2 population derived from a cross between '[ms-bo] Nekken 2' and Hokuriku 193 was cultivated in the paddy field of NARC. [ms-bo] Nekken 2 is a *japonica* rice cultivar bred by introducing cytoplasmic male sterility into 'Nekken 2', which was derived from 'Akihikari' by incorporating a wide compatibility gene *S-5^n* to overcome sterility in *indica-japonica* hybrids (Ikehashi 2009). Hybrid sterility has been reported to be common in *indica-japonica* crosses (Kato 1930), which raised concerns that hybrid sterility would reduce uniformity within populations and interfere with the evaluation of crosses between Hokuriku 193 and *japonica* rice cultivars. For this reason, Nekken 2, which carries the wide compatibility gene *S-5^n*, was selected as the other parent for cross breeding. The F_2 seeds were sown on April 22, and seedlings were transplanted on May 20. The basal fertilizer consisted of 5 g/m^2 each of N, P_2O_5, and K_2O with no additional fertilizer. After maturation, 248 individual plants were selected randomly.

In 2010, F_3 lines derived from each F_2 individual were cultivated in order to investigate heading trait, because seed dormancy is influenced by temperatures during the ripening period (Ikehashi 1973). Seeds were sown on April 26, and seedlings were transplanted on May 26. The fertilizer was treated similarly according to the previously described method. The day on which panicles had emerged on about 50% of the plants belonging to a line was considered to be the heading date of the line. The number of days from the day of transplantation to the heading date was considered the days to heading, and used as indexes of heading trait.

Germination tests

Because most problems of seed dormancy are expected to manifest as poor stand establishment, the extent of release from dormancy at the sowing period in the next spring is crucial. Therefore, seeds were tested for dormancy in December, a time soon after harvest, and March, the time immediately before the sowing period. Harvested seeds were stored at room temperatures (10 ± 5°C) in a sealed desiccator with silica gel, and seeds from 221 individual plants that had produced enough seeds were tested for germination in December 2009 (the December test) and March 2010 (the March test). Fifty seeds each were imbibed with distilled water on a filter paper in a petri dish, and placed in a dark incubator at 25 ± 0.5°C. As an indicator of dormancy, germination rate was calculated from the number of seeds that germinated 4 and 7 days after sowing to evaluate the ability, speed, and uniformity of germination.

QTL analysis

DNA was extracted from each individual F_2 plant according to the method of Monna *et al.* (2002) and genotyped with 115 simple sequence repeat (SSR) markers with a genome-wide distribution (IRGSP 2005, McCouch *et al.* 2002). Linkage maps were constructed using MAPMAKER/EXP 3.0 (Lander *et al.* 1987). Intermarker distances were estimated using the Kosambi mapping function (Kosambi 1944). QTL analysis by composite interval mapping (CIM) was performed with Windows QTL Cartographer 2.5 (Wang *et al.* 2006). The logarithm of odds (LOD) threshold for

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QTL detection was determined by 1000 permutations at a significance level of 5%. Before analysis, germination rates were transformed to arcsine square root values to normalize the variance.

Breeding and evaluation of an NIL

Hokuriku 193 was backcrossed 4 times to [ms-bo] Nekken 2. Mother plants were selected according to the genotypes of SSR markers used in the QTL analysis, SNP markers at the *qSH1* locus, and sequence-tagged site (STS) markers at the semi-dwarf (*sd1*) locus (Forward: AGGATG GAGCCCAAGATCC, which specifically amplifies the Hokuriku 193 allele, or AAGCTCCCATGGAAGGAGAC, which specifically amplifies the Nekken 2 allele; Reverse: GGAGTTCCATGATCGTCAGC).

Individuals were selected from the BC_4F_2 generation in 2012, and fixation for the aforementioned markers was started in 2013. In 2015, all genomic regions in the BC_4F_5 generation were genotyped with 325 SNP markers (Ebana *et al.* 2010, Nagasaki *et al.* 2010). SSDo (Seed Shattering and Dormancy)_NIL, an NIL in which Nekken 2 alleles were introgressed only in genomic regions associated with seed shattering and dormancy, was selected and evaluated in terms of its agronomic characteristics by comparing them with those of Hokuriku 193. Seeds were sown on April 21, and seedlings were transplanted in the paddy field of NARC on May 20. The basal fertilizer consisted of 6 g/m^2 each of N, P_2O_5, and K_2O, and as the additional fertilizer, 3 g/m^2 of N, 0.8 g/m^2 of P_2O_5, and 3 g/m^2 of K_2O were applied about 25 days before heading. After maturation, stem length, panicle length, panicle number, brown rice yield, and 1000-brown grain weight were measured according to Sunohara and Horisue (1995) and Horisue (1995). Seed shattering was evaluated in the field in October 2015, by using the previously described method. Seed dormancy was assessed in seeds stored at room temperatures (10 ± 5°C) in a desiccator with silica gel, by the previously described 7-days germination test at 25 ± 0.5°C in March 2016.

Results

Relationship between seed shattering traits and genotypes at the qSH1 locus

QTL analysis for seed shattering is challenging because it requires quantitation of the extent of seed shattering, which is time-consuming and largely changes by environmental factors (Fukuta and Yagi 1998). Therefore, we considered it advisable to start by studying the effects of genetic loci known to be associated with seed shattering.

Table 1 shows seed shattering traits assessed for each genotype at the *qSH1* locus. Fisher's exact test showed no significant difference between seed shattering traits and genotypes at the *qSH1* locus.

QTL analysis for seed dormancy and heading trait

In contrast with seed shattering, numerous QTLs for seed dormancy have been reported from genetic analyses thus far conducted by making full use of diverse materials. Screening these QTLs for candidates that govern seed dormancy in Hokuriku 193 is difficult. Hence, we considered QTL analysis of Hokuriku 193 itself to be a better approach to identify chromosomal regions for modification. QTL analyses on seed dormancy reported thus far have often been carried out under conditions in which the difference was greatest between parents, and chronological analysis of the same samples has rarely been performed. Given that seed dormancy has its greatest practical impact during the sowing period in spring, the best genes to target are those at QTLs for dormancy that lasts into the next spring; as such, we placed importance on identifying chromosomal regions for modification through analysis of time-dependent QTL effects. In other words, we expected that by identifying QTL regions for seed dormancy that lasts into the next spring and by introgressing *japonica* alleles in these regions, the seed dormancy in Hokuriku 193 would be improved to a practically acceptable level.

Table 1. Relationship between seed shattering trait and genotype at the *qSH1* locus in the F_3 population from Hokuriku 193 × Koshihikari

Genotype	Seed shattering		Total	Significance	
	Shattering	Non-shattering		Between 50%:50%	Between 100%:0% or 0%:100%
Hokuriku 193	19	1	20	**	ns
Koshihikari	2	23	25	**	ns
Total	21	24	45		

** and ns: Significant at a level of 1% and not significant at a level of 5%, respectively, according to Fisher's exact test.

Fig. 1 shows the frequency distribution of germination rates and days to heading obtained by the December and March tests. All traits showed continuous distributions. However, germination rates showed continuous transgressive segregation towards a rate lower than that of Hokuriku 193. Statistically significant positive correlations were shown among germination rates and days to heading obtained under different test conditions (**Table 2**).

In the March test, the 7-days germination rate was 90% or higher in most samples and showed no clear differences (**Fig. 1**). Therefore, QTL analysis was based on December 4- and 7-days germination rates and March 4-days germination rates. The LOD threshold for CIM analysis determined by 1000 permutations at a significance level of 5% was 3.51 for December 4-days germination rates, 3.71 for December 7-days germination rates, 3.63 for March 4-days germination rates, and 3.51 for days to heading, and the number of QTLs identified was 6, 6, 5, and 6, respectively (**Fig. 2**, **Table 3**). QTLs on chromosomes 1, 3, and 6 were detected by all three germination tests and designated as *qSDo1* (*Seed Dormancy 1*), *qSDo3*, and *qSDo6*, respectively (**Fig. 2**).

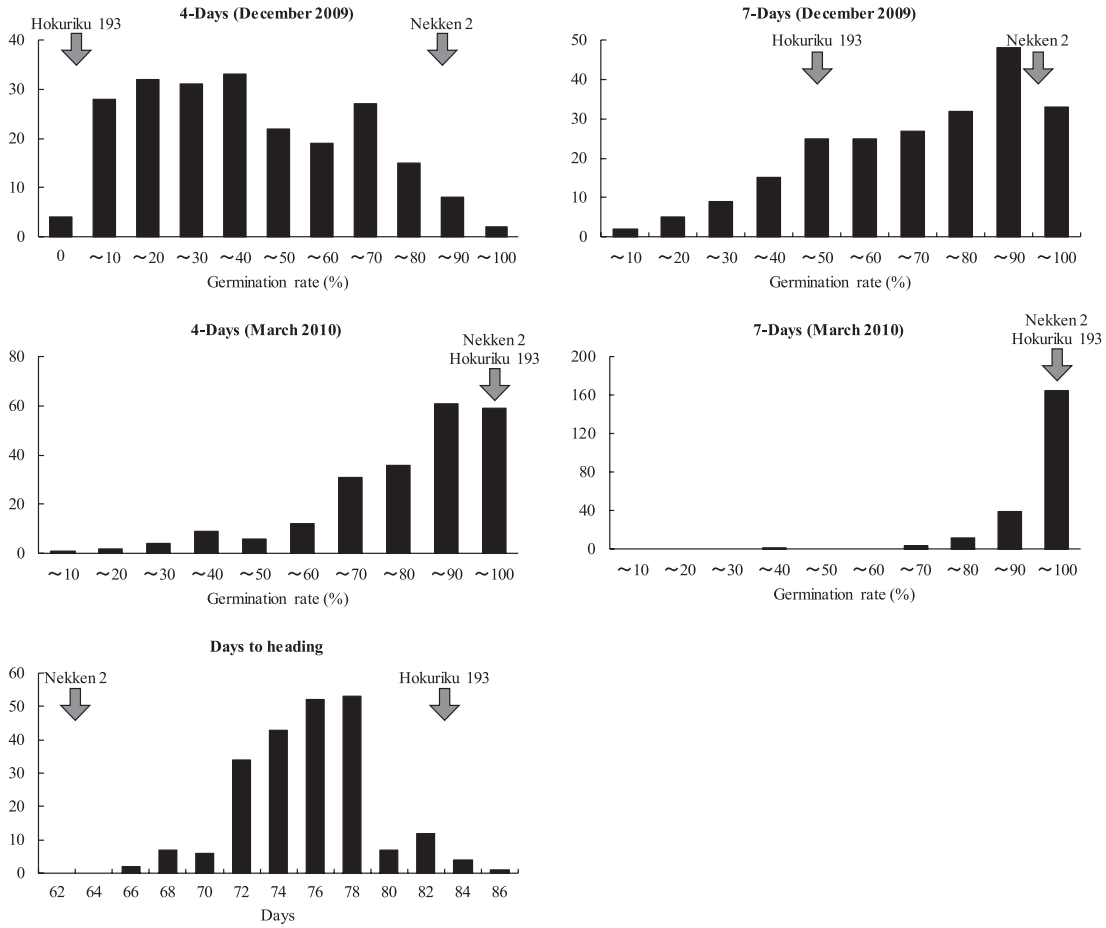

Fig. 1. Frequency distribution of germination rates in the F_2 population and days to heading in the F_3 lines from [ms-bo] Nekken 2 × Hokuriku 193. Y axes denote number of F_2 individuals or F_3 lines. Arrows indicate the values of parents.

Table 2. Correlation coefficients among germination rates and days to heading

	4-Days test, December 2009	7-Days test, December 2009	4-Days test, March 2010	7-Days test, March 2010
7-Days test, December 2009	0.85***			
4-Days test, March 2010	0.70***	0.70***		
7-Days test, March 2010	0.48***	0.61***	0.76***	
Days to heading	0.27***	0.20***	0.40***	0.30***

*** and **: Significant at a level of 0.1% and 1%, respectively.

Evaluation of an NIL improved with respect to seed shattering and dormancy

Fig. 3 shows the graphical genotype of SSDo_NIL. Nekken 2 alleles were introgressed at the *qSH1* locus and in the *qSDo1* region, whereas Hokuriku 193 alleles were found in other regions including *qSDo3*, *qSDo6*, and the *sd1* locus.

The agronomic characteristics, seed dormancy, and seed shattering trait of SSDo_NIL are shown in **Table 4**. SSDo_NIL did not shatter, and its germination rate was significantly higher than that of Hokuriku 193. Stem length, panicle length, panicle number, brown rice yield, and 1000-brown grain weight did not differ significantly between SSDo_NIL and Hokuriku 193.

Discussion

In this study, we aimed to clarify the genetic basis of the seed shattering and dormancy in Hokuriku 193—the disadvantageous traits of this cultivar—by using genetic information and breed an NIL by introgression in chromosomal regions encoding these traits.

Here we investigated whether the seed shattering in Hokuriku 193 was affected by the *qSH1* locus, a major factor known to regulate seed shattering in *indica* cultivars. Analysis of the F_3 population derived from Hokuriku 193 × Koshihikari revealed a general correspondence between seed shattering trait and genotype at the *qSH1* locus (**Table 1**). This suggested that the seed shattering in Hokuriku 193 is largely controlled by the *qSH1* locus. Accordingly, we surmised that the seed shattering could be improved by introgressing alleles from non-shattering *japonica* cultivars in this region. However, there were a few individuals which

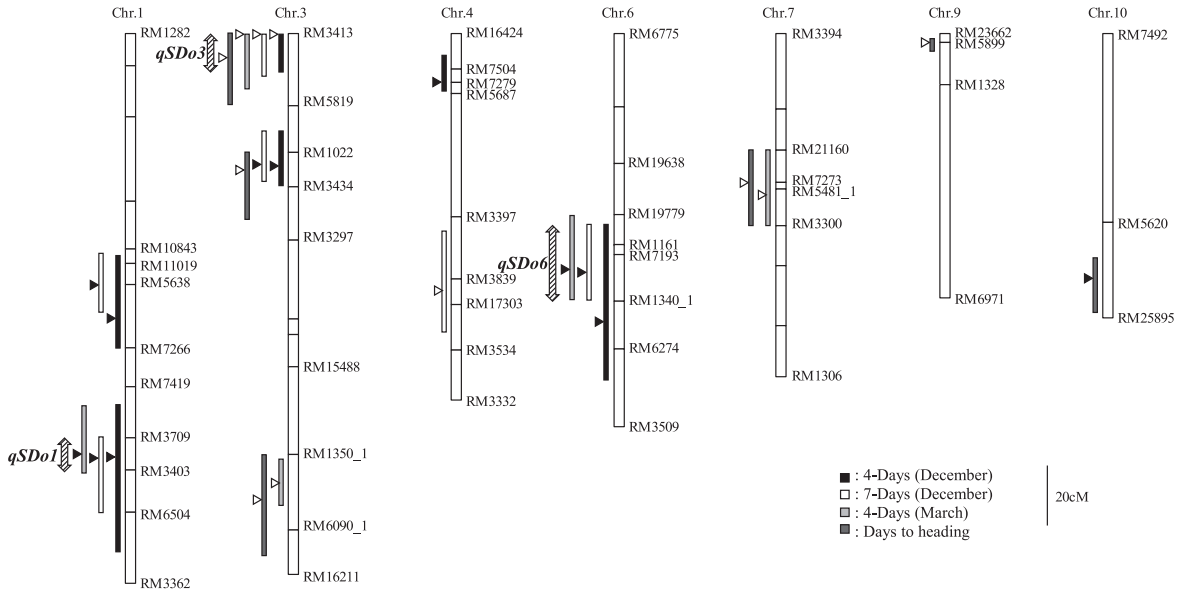

Fig. 2. Regions containing QTLs for seed dormancy and days to heading in Hokuriku 193. Important SSR markers are shown on the right side of each chromosome. Bars and triangles on the left side indicate intervals where LOD scores exceeded the threshold and corresponding LOD peaks, respectively. Closed and open triangles represent peaks of QTLs that additive effect was positive for Nekken 2 and Hokuriku 193 alleles, respectively. Hatched up/down arrows indicate regions thought to contain important QTLs for seed dormancy. Chromosomes with no QTLs detected are omitted.

Table 3. QTLs for the seed dormancy and days to heading in Hokuriku 193 detected by CIM analysis

	Chr.	Nearest marker	Peak position (cM)	LOD	Additive effect[a]	PVE[b] (%)	QTL renamed in this study	LOD threshold
4-Days test, December 2009	1	RM7266	95.0	5.60	7.76	12.0		3.51
	1	RM3403	142.2	9.37	7.80	15.9	*qSDo1*	
	3	RM3413	0.0	8.56	–9.44	15.3	*qSDo3*	
	3	RM1022	43.8	4.18	6.67	7.7		
	4	RM7279	16.5	4.22	5.33	6.8		
	6	RM1340_1	96.5	5.56	8.38	11.0	*qSDo6*	
7-Days test, December 2009	1	RM5638	84.0	4.41	4.78	6.0		3.71
	1	RM3403	143.2	10.69	7.65	17.6	*qSDo1*	
	3	RM3413	0.0	10.81	–8.91	16.1	*qSDo3*	
	3	RM1022	42.8	4.60	5.99	7.2		
	4	RM3839	86.3	7.42	–5.51	10.3		
	6	RM7193	79.9	6.56	7.42	11.4	*qSDo6*	
4-Days test, March 2010	1	RM3709	140.2	5.57	5.01	8.4	*qSDo1*	3.63
	3	RM3413	0.0	9.16	–7.04	12.5	*qSDo3*	
	3	RM1350_1	148.9	4.03	–4.59	7.2		
	6	RM7193	78.9	9.52	7.95	15.6	*qSDo6*	
	7	RM5481_1	54.0	7.53	–7.01	11.0		
Days to heading	3	RM3413	8.0	9.16	–2.11	13.9		3.51
	3	RM3434	46.8	5.37	–1.37	6.9		
	3	RM6090_1	156.9	5.55	–1.59	8.9		
	7	RM7273	49.7	23.52	–3.14	29.5		
	9	RM5899	2.7	3.88	–1.11	4.5		
	10	RM25895	82.2	4.12	2.04	10.0		

[a] Additive effect (%) was positive for Nekken 2 alleles.
[b] Percentage of phenotypic variation explained by each QTL.

seed shattering traits did not correspond with genotype at the *qSH1* locus (**Table 1**). We speculated that this resulted from errors or the effects of other genetic factors, but we are not able to discuss details in this study. It may be necessary to investigate the internal structure of rachis-branches.

In this study, QTL analysis for seed dormancy was based on germination tests in December 2009, the time soon after harvest, and March 2010, the time immediately before the sowing period. In both the December and March tests, germination rates of the F_2 population from [ms-bo] Nekken

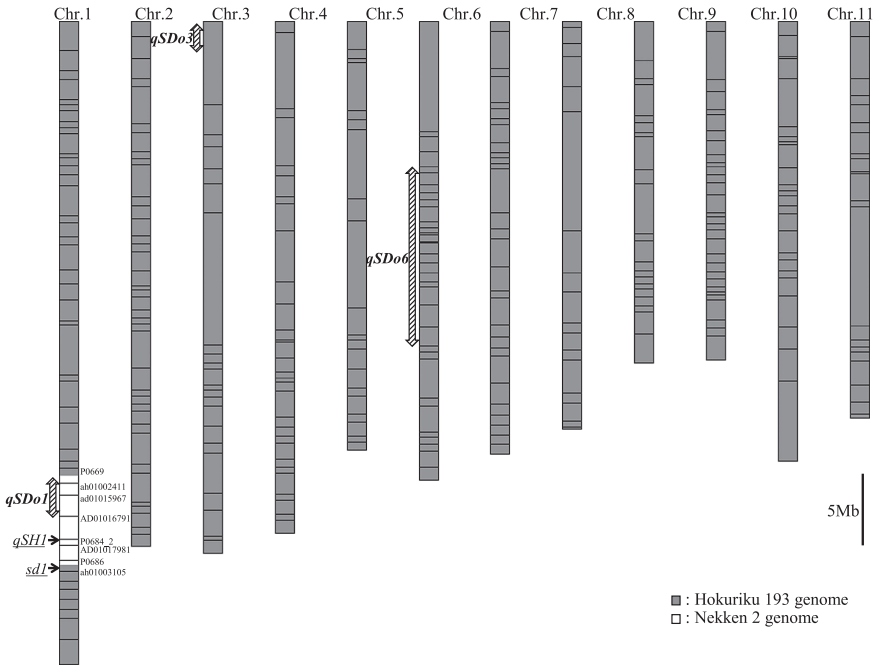

Fig. 3. Graphical genotype of SSDo_NIL. Important SNP markers are shown on the right side of each chromosome. The positions of *qSDo1*, *qSDo3*, *qSDo6*, and the *qSH1* and *sd1* loci are shown on the left side. Hatched up/down arrows indicate regions thought to contain QTLs. The position of each marker is according to the International Rice Genome Sequencing Project (IRGSP) Ver1.0.

Table 4. Agronomic characteristics, seed dormancy, and shattering habit of SSDo_NIL

	Heading date (M/D)	Culm length (cm)	Panicle length (cm)	Panicle number (/m^2)	Brown rice yield (g/m^2)	1000-brown grain weight (g)	Germination rate (%)	Seed shattering
SSDo_NIL	8/16	86.0 ns	29.4 ns	290 ns	827 ns	23.1 ns	81.3 *	Non-shattering
Hokuriku 193	8/17	85.9	28.8	282	794	23.4	46.7	Shattering

* and ns: Significant at a level of 5% and not significant at a level of 5%, respectively, according to t-test.

2 × Hokuriku 193 showed continuous segregation, and December 7-days and March 4-days germination rates, in particular, showed transgressive segregation towards rates lower than that of Hokuriku 193 (**Fig. 1**). These results suggested the involvement of multiple genes in seed dormancy in Hokuriku 193 and the possibility that Nekken 2 might carry genes that promote seed dormancy. The statistically significant positive correlation between December and March germination rates (**Table 2**) suggested the existence of common factors that strongly control seed dormancy in December and March. Indeed, germination rates significantly correlated with days to heading (**Table 2**), suggesting that heading trait affects seed dormancy.

QTL analysis based on December 4-days, December 7-days, and March 4-days germination rates identified 6, 6, and 5 QTLs for seed dormancy, respectively (**Fig. 2**, **Table 3**). QTLs on chromosomes 1, 3, and 6 (*qSDo1*, *qSDo3*, *qSDo6*) were detected by all three germination tests and were therefore thought to have effects lasting into the sowing period. Among these, *qSDo1* and *qSDo6* were QTLs at which Hokuriku 193 alleles reduced germination rates, and therefore considered to be key regions that needed modification to improve the seed dormancy in Hokuriku 193. Thus, these QTLs should be unrelated to the condition during the ripening period, because no QTL associated with days to heading was detected in these regions (**Fig. 2**, **Table 3**).

QTLs associated with seed dormancy have been reported, such as *qSdn-1* (Wan *et al.* 2006), in a region containing *qSDo1*, and *Sdr10* (Marzougui *et al.* 2012), in a region containing *qSDo6*, but differences in the materials and markers used preclude direct comparison with our results. Meanwhile, *qLTG3-1*, a gene for low-temperature germinability and viviparity identified in *japonica* cultivars (Fujino *et al.* 2008, Hori *et al.* 2010), has been reported in a region corresponding to *qSDo3*. The *qLTG3-1* gene may have variations because its effect has also been detected in non-*japonica* cultivars (Fukuda *et al.* 2014, Satoh *et al.* 2016). On the other hand, the detection of *qSDo3* may be the secondary effect of conditions during the ripening period, because a QTL associated with days to heading was detected in the same region (**Fig. 2**, **Table 3**). Anyhow, *qSDo3*, at which the Hokuriku 193 allele reduces seed dormancy, was considered to be less important than *qSDo1* and *qSDo6* in improvement of the seed dormancy in Hokuriku 193.

QTLs detected only by the December test and not by the

March test included one locus each on chromosomes 1 and 3 and two loci on chromosome 4. These QTLs, whose effect thus disappears by the next spring, were therefore excluded from factors to consider in improvement of seed dormancy. QTLs detected by the December 4- and 7-days germination tests shared generally the same position, suggesting that the ability, speed, and uniformity of germination are controlled by generally the same genetic factors.

QTLs detected only by the March test on chromosomes 3 and 7 may be responsible for the time-dependent decrease in germination rate, but this decrease occurred when they carried Nekken 2 alleles, and these QTLs were therefore dismissed as posing no obstacle to improvement of seed dormancy in Hokuriku 193. The detection of these QTLs may be the secondary effect of conditions during the ripening period, because QTLs associated with days to heading were detected in these regions (**Fig. 2**, **Table 3**). Moreover, in the regions corresponding to these two QTLs, the presence of QTLs for seed storability has also been reported (Hang *et al.* 2015, Li *et al.* 2012), but whether they have any effect on seed storability of Hokuriku 193 cannot be assessed from the results of this study.

Because [ms-bo] Nekken 2 does not possess *Rf-1*, a fertility restorer gene that is located on chromosome 10 (Komori *et al.* 2004), almost all genotypes around this gene may be Hokuriku 193 type or hetero in F_2 individuals. Therefore, the accuracy of detection of QTLs around this region should be decreased. However, that not a problem in improvement of seed dormancy in Hokuriku 193, because the additive effects and PVEs of *qSDo1* or *qSDo6* should be sufficient to improve seed dormancy in Hokuriku 193, and no QTL associated with seed dormancy have been reported around this region.

The above findings led to the idea that in Hokuriku 193, introgression of *japonica* alleles at the *qSH1* locus and into a region containing *qSDo1* and/or *qSDo6* would improve seed shattering and dormancy, respectively, in the Hokuriku 193 genetic background. The chromosomal positions of markers and genes revealed a very close genetic linkage between *qSDo1* and the *qSH1* locus. Therefore, an introgression across an entire region containing *qSDo1* and the *qSH1* locus would improve both seed shattering and dormancy simultaneously. On the other hand, *qSDo6* had a peak of LOD based on December 4-days germination rates separated from other peaks, and had a large interval where LOD scores exceeded the threshold, indicating that the results of this study were insufficient to narrow down the exact region to be introgressed. In light of these issues, this study aimed to derive an NIL from Hokuriku 193 by introgressing Nekken 2 alleles only at *qSDo1* and the *qSH1* locus.

However, the *qSH1* locus is closely linked to the *sd1* locus in *indica* cultivars (Oba and Kikuchi 1993). The allele at the *sd1* locus in Nekken 2 has its origin in Akihikari, i.e., the 'Reimei' (Sato 1982) allele. Consequently, in backcrossing, an attempt to introgress Nekken 2 alleles at *qSDo1* and the *qSH1* locus would also introgress the Reimei allele at the *sd1* locus in many individuals. Given that the Reimei *sd1* allele is less dwarfing than the *indica sd1* allele (Irie *et al.* 2009), the concern arose that the introgression of the Reimei allele at the *sd1* locus would increase stem length with resulting lodging. For this reason, in developing an NIL, an active effort was made to conserve the Hokuriku 193 allele at the *sd1* locus with the aid of DNA markers.

In SSDo_NIL, an NIL derived from Hokuriku 193, Nekken 2 alleles were introgressed only in the region containing *qSDo1* and the *qSH1* locus, and Hokuriku 193 alleles were conserved in other regions (**Fig. 3**). The absence of seed shattering in SSDo_NIL (**Table 4**) demonstrated that the seed shattering in Hokuriku 193 could be improved by introgression at the *qSH1* locus. Moreover, the germination rate measured in March 2016 was significantly higher in SSDo_NIL than in Hokuriku 193, showing that introgression at *qSDo1* alone could substantially improve the seed dormancy in Hokuriku 193. However, because the germination rate of SSDo_NIL was approximately 80%, it was considered desirable to further reduce seed dormancy to stabilize a high germination rate, benefiting from the use of a narrowed down region in *qSDo6*. Nevertheless, there are concerns that insufficient seed dormancy would cause pre-harvest sprouting, thereby reducing seed quality instead. Future studies are needed to investigate not only the individual effect of *qSDo6* but also the necessity of combining *qSDo1* and *qSDo6*.

Agronomic characteristics were similar between SSDo_NIL and Hokuriku 193 (**Table 4**) and indicated that introgression at *qSDo1* and the *qSH1* locus would not affect yield and other agronomic traits.

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