Theor Appl Genet (2005) 110: 1315–1323
DOI 10.1007/s00122-005-1972-1

ORIGINAL PAPER

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Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect

Received: 2 August 2004 / Accepted: 15 November 2004 / Published online: 1 April 2005

Abstract A major QTL for grain dormancy, *QPhs.ocs-3A.1*, derived from the highly dormant wheat Zenkoujikomugi (Zen), has been identified in a study made under a controlled environment. Further investigations were needed to dissect the precise position and expression of *QPhs.ocs-3A.1* under different field conditions because the ability to detect genetic loci for grain dormancy traits is compromised by environmental effects and genotype/environment interactions. Group 4 chromosomes have also been shown to be possible sites of QTLs for grain dormancy. The objectives of this study were (1) to locate additional molecular markers in the *QPhs.ocs-3A.1* region, (2) to identify QTLs on the group 4 chromosomes and (3) to elucidate their combined effects. We examined the recombinant inbred lines (RILs) from a cross between Chinese Spring (CS) and Zen over a 3-year period in one location and 1 year in a different location. In an interval mapping study *QPhs.ocs-3A.1* was mapped to within the 4.6 cM region flanked by *Xbarc310* and *Xbcd907* at the proximal end of the short arm of chromosome 3A. *QPhs.ocs-3A.1* was confirmed to be the predominant dormancy QTL since it explained a large portion (11.6–44.8%) of the phenotypic variation, and was strongly displayed under dormancy-breaking conditions or at low germination temperatures. For *QPhs.ocs-4A.1*, identified on the long arm of chromosome 4A, and *QPhs.ocs-4B.1*, on the centromeric region of the long arm of Chr 4B, the LOD peak positions and the desirable allele were consistent between the trials, while the LOD scores and contribution to the phenotypic variation varied. Transgressive segregants were observed among the 125 RILs and most of them had a combination of the three alleles conferring a higher dormancy: the Zen alleles at *QPhs.ocs-3A.1* and *QPhs.ocs-4A.1* and the CS allele at *QPhs.ocs-4B1*. This demonstrated a combined effect of the desirable alleles on accelerating grain dormancy, with their total effect being superior to that of Zen.

Communicated by R. Hagemann

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Introduction

Pre-harvest sprouting (PHS) in bread wheat, *Triticum aestivum* L., severely limits its end-use application for flour and results in substantial losses in crop yield and price. It is thus a major problem during wheat harvesting worldwide and is often the target for improvement of grain quality. The main component of the observed genetic variation for PHS appears to be the level of grain dormancy present at the time of tolerance assessment (Mares 1987). PHS and grain dormancy in wheat are expressed as a quantitatively inherited trait that is strongly influenced by the environment as well as by genotype × environment interactions (Hagemann and Ciha 1987; Anderson et al. 1993). Therefore, screening for tolerance to PHS on a phenotypic basis in segregating populations has been difficult during breeding exercises.

Recently, much progress has been made in the development of molecular genetic maps of wheat, and the identification of DNA markers linked to PHS tolerance genes should facilitate indirect marker-assisted selection (MAS) of genotypes that have certain levels of PHS tolerance. However, within these maps there is little information available for improving tolerance to PHS. One reason for this is that the mapping populations used for the construction of the available maps may not be appropriate for the analysis of tolerance to PHS or

dormancy, because the parental materials of the mapping populations were chosen for other aims. In quantitative trait loci (QTL) analysis for PHS tolerance and grain dormancy, a mapping population must be chosen so that the parents of the population exhibit two extremes of the phenotype. At least four different mapping populations (Anderson et al. 1993; Kato et al. 2001; Groos et al. 2002; Osa et al. 2003) have been found to satisfy the above criteria and thus to be useful for detecting QTL effects on tolerance to PHS or grain dormancy.

Japanese spring wheat, Zenkoujikomugi (Zen) is known to be highly tolerant to PHS (Osanai and Amano 1993; Miura et al. 1997). Recently, the high PHS tolerance of Zen has become the object of attention in Japanese breeding programs, because PHS-tolerant lines have been derived from the progeny of Zen by cross-breeding. These lines have received attention as breeding materials for the development of new cultivars that are more tolerant to PHS than Zen. Thus QTLs associated with grain dormancy specific to the Zen genome are attractive not only for practical breeding but also for analyzing the biological mechanisms of grain dormancy. Using a backcross reciprocal monosomic method, we previously found that chromosome 3A and homologous group 4 chromosomes were possible sites of QTLs for the high level of grain dormancy in Zen (Miura et al. 2002). Furthermore, a molecular-marker linkage mapping of Chr 3A was conducted using recombinant inbred lines (RILs) derived from a cross between Zen and Chinese Spring (CS), and a QTL for grain dormancy, designated as *QPhs.ocs-3A.1*, was identified on the short arm. *QPhs.ocs-3A.1* explained 23–38% of the phenotypic variation for grain dormancy under a controlled environment and the Zen allele had a striking effect on maintaining dormancy. While Chr 3A carries the wheat orthologue (*TaVp1*) of the maize viviparous gene *Vp1* and the seed color *R-A1* gene, it was concluded that the high dormancy associated with Chr 3A of Zen is ascribable to *QPhs.ocs-3A.1* but is not due to a direct contribution of either the *TaVp1* or the *R-A1* loci (Osa et al. 2003). However, further investigations are needed to dissect the precise position and expression of *QPhs.ocs-3A.1* under different field conditions, since the ability to detect genetic loci for grain dormancy is greatly affected by environmental effects and genotype/environment interactions.

There are several reports that the homologous group 4 chromosomes are sites of QTLs associated with PHS tolerance (Anderson et al. 1993) or grain dormancy (Mares and Mrva 2001; Kato et al. 2001; Miura et al. 2002; Noda et al. 2002). Kato et al. (2001) detected three QTLs for grain dormancy on Chromosomes 4A, 4B and 4D in a double haploid population derived from AC Domain (high level of dormancy) × Haruyutaka (low level of dormancy), and the AC Domain alleles at all detected QTLs increased grain dormancy. As mentioned previously, the homologous group 4 chromosomes of Zen are possible sites of genes controlling strong dormancy. Therefore, these chromosomes may be important targets in the search for candidate QTLs for MAS breeding programs for PHS tolerance. However, the identity of the QTLs on group 4 chromosomes in Zen and AC Domain was unclear. Moreover QTL studies are prone to genotype × genotype and genotype × environment interactions that make it difficult to predict which putative QTLs or which allelic combinations at different loci are the most stable when transferred to a new genetic background and/or evaluated in different environments. The objectives of this study were (1) to locate additional molecular markers in the *QPhs.ocs-3A.1* region, (2) identify QTLs on group 4 chromosomes and (3) elucidate their combined effect, using RILs derived from a cross between Zen and CS.

Materials and methods

Plant materials

Zen is a Japanese red spring wheat showing an extremely high level of grain dormancy (Osanai and Amano 1993; Miura et al. 1997). Zen was derived from the cultivar Igachikugo-Oregon by exposure of seeds to γ rays (Toda et al. 1972). CS is a Chinese soft red spring wheat with some dormancy (Warner et al. 2000).

A mapping population in the form of RILs, developed from the cross between Zen and CS by the single-seed decent method, was utilized. One hundred and twenty-five RILs were allowed to self-pollinate during eight successive generations (F_8). To determine the chromosome or chromosome arm location of the RFLP fragments and SSR markers, CS and its aneuploid stocks including the CS ditelosomic 4AS, 4AL, 4BS, 4BL, 4DS and 4DL lines (Sears 1954) were used.

Evaluation of grain dormancy

The level of grain dormancy in the RIL population was evaluated in 3-year field trials in Obihiro and a 1-year trial in Tsukuba. The spring-sown trials were carried out under natural field conditions at the research field of Obihiro University of Agriculture and Veterinary Medicine in 2001 (hereafter OB'01), 2002 (OB'02) and 2003 (OB'03). The autumn-sown trial in Tsukuba was conducted at the research field of the National Institute of Crop Science in 2003 (TK'03). In the experiments in Obihiro the parents and the RILs were grown in plots consisting of single 1-m rows with two replications. The plots were sown in late April, and were grown under standard field management. After anthesis, the experimental plots were covered with a transparent plastic roof to prevent rain damage. In the TK'03 experiment in Tsukuba, the genotypes were grown in single 1-m rows without replication. The plots were sown in late October.

In all trials, the flowering date was recorded on a genotype basis to examine the degree of grain dormancy at a given number of days post anthesis (DPA). To minimize the variation in physiological maturity across the trials, all plant materials were harvested at 45 DPA. Harvested spikes were allowed to air-dry for about 1 week until the moisture content of grain was approximately 14%, and dried spikes were gently hand-threshed.

Germination tests were performed at 15 and 20°C in 90×15 mm disposable Petri dishes containing 50 grains per line in Obihiro and 30 grains in Tsukuba. The grains were sown on a single layer of filter paper wetted with distilled water. The dishes were incubated in the darkness for 10 days. Germinated grains were counted every day and removed from the dishes. Results were presented as the mean germination rate of two replicates. Germinability at 15°C was not recorded in OB'01.

RFLP and SSR analyses of group 4 chromosomes

For DNA extraction, the plant materials were grown in a growth chamber. Genomic DNA was extracted from 2-week-old leaves from each of the parents, the 125 RILs and the aneuploid lines using a modified CTAB method (Murray and Thompson 1980). Southern blotting was performed according to the method described by Kato et al. (1998). Forty-three RFLP clones already known to hybridize with DNA fragments located on wheat group 4 chromosomes were used as probes. Thirty-two SSR primer sets, specific for group 4 chromosomes (Röder et al. 1998) were screened for this analysis. The PCR conditions were the same as those used by Osa et al. (2003).

After screening the parents for marker locus polymorphisms, the RFLP and SSR markers screened in the aneuploid lines were assigned chromosomes. By using the markers that were proven to be polymorphic between CS and Zen, 125 RILs were genotyped and the marker location on the group 4 chromosomes was analyzed.

For locating additional molecular markers in the *QPhs.ocs-3A.1* region, we used the BARC markers, which were developed for the US Wheat and Barley Scab Initiative, to map and characterize genes for *Fusarium* resistance (Song et al. 2002). The primer sequences are available in the public domain (http://www.scabusa.org).

Linkage and QTL analysis

Linkage analyses were performed with the program Mapmaker/EXP 3.0 (Lander et al. 1987), and the recombination frequencies between two markers were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944).

For QTL analyses, the mean germination rate was transformed to arcsine. The chromosomal location of QTLs for grain dormancy was determined by the simple interval mapping method using the QGENE program (Nelson 1997). A log likelihood (LOD) score threshold of 3.0 was used to identify regions containing a putative QTL associated with grain dormancy.

Results

Germination rates at 20°C and 15°C

There were clear differences in the germination rate at 20°C between CS and Zen over the four trials (Fig. 1a), especially in OB'02 and TK'03. CS showed a very high germination rate of 80% or more and Zen showed almost perfect dormancy (2%) in OB'02. Around 30–40% of the CS grains germinated in trials OB'01 and OB'03, indicating a slight level of dormancy. Consequently, these results confirmed a distinct difference in the level of grain dormancy between CS and Zen. Across the four trials, the germination rates of 125 RILs ranged from 0 to 100% and the distribution was continuous with a skew towards a lower germination rate in Obihiro but with a higher germination rate in Tsukuba. Transgressive RILs with lower dormancy than CS appeared in trials OB'01 and OB'03.

Reduced grain dormancy in the parents and the RILs was detected in the germination test at 15°C (Fig. 1b), but again we found a distinctive difference in the level of grain dormancy between the parents. In trials OB'02 and OB'03, Zen displayed a germination rate of around 30%, while CS had a germination rate of 70% or more. The germination rate of the 125 RILs ranged from 8.0 to 99.0% in trial OF'02 and from 3.0 to 97.0% in trial OF'03, and followed a nearly normal distribution. In trial TK'03 more than half of the RILs as well as CS lost dormancy. Conversely, in the germination tests at 20°C, many transgressive RILs that were more dormant than Zen were detected. This degree of transgressive variation suggested that not only the Zen alleles at several loci, but also the CS alleles at different loci augmented the dormancy in the RIL population when germinated at 15°C.

Linkage map of the *QPhs.ocs-3A.1* region

Two new BARC microsatellite markers (*Xbarc 310* and *Xbarc321*) were located at the proximal end of the short arm of Chr 3A (Fig. 2). *Xbarc310* was mapped to within the 10 cM region flanked by two RFLP markers, *Xbcd907* and *Xfbb370*, while *Xbarc321* was linked to *Xfbb370* by 1.7 cM.

Linkage maps of the group 4 chromosomes

Of the 43 RFLP clones screened, 25 were polymorphic in the parents. An aneuploid test using the CS ditelosomic lines revealed that 11 of the 25 polymorphic RFLP

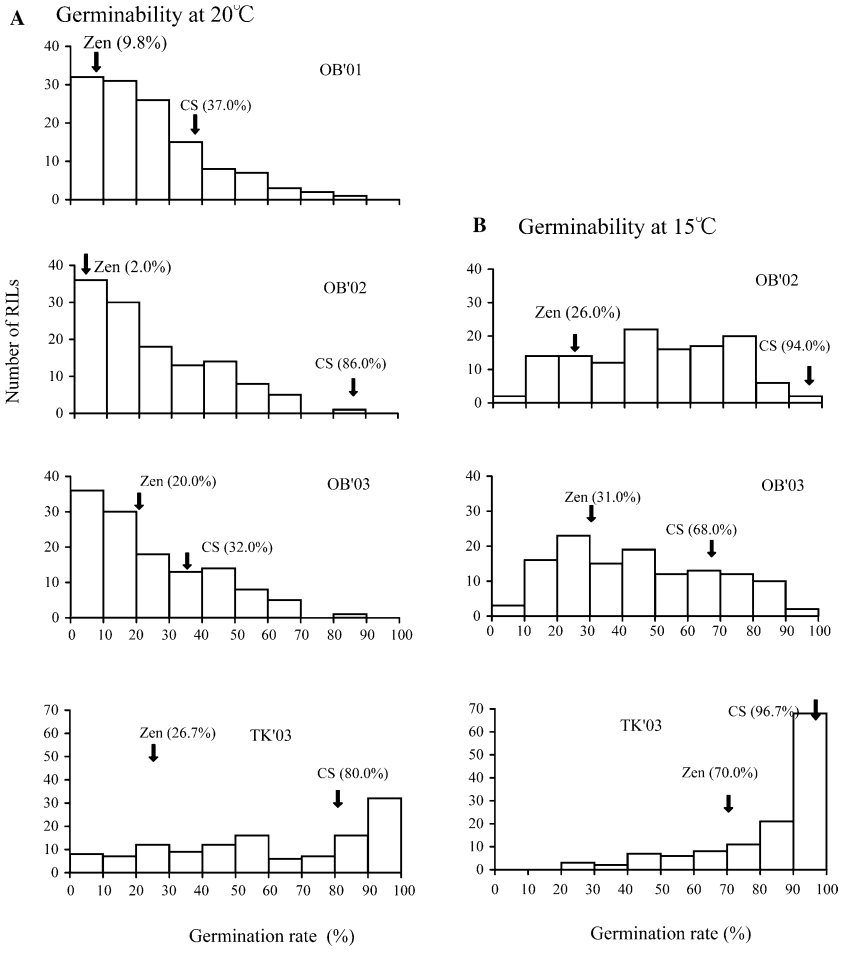

Fig. 1 Frequency distribution of the cumulative percentage germination for 10 days in 125 RILs incubated at 20°C (**a**) and 15°C (**b**) in the 3-year trials in Obihiro 2001 (*OB'01*), 2002 (*OB'02*) and 2003 (*OB'03*), and in Tsukuba (*TK'03*)

clones were associated with homologous group 4 chromosomes. On the other hand, CS and Zen were polymorphic in seven SSRs and all of those were assigned to group 4 chromosomes. The genetic linkage maps of Chr 4A and 4B, shown in Figs. 2 and 3, were constructed on the basis of the genotypic classifications of the RIL population. As a result, six marker loci covered approximately 50 cM of Chr 4A. RFLP analyses of the ditelosomic stocks available for CS demonstrated that the centromere was assigned to the marker interval between *Xcdo189* on the short arm and *Xcdo795* on the long arm of Chr 4A. Thus, two loci of RFLP markers were mapped to the short arm of Chr4A and three on the long arm. For Chr 4B, seven marker loci consisting of three RFLP markers and four SSR markers were mapped. Ditelosomic analysis indicated that all seven markers resided on the long arm and covered about 70 cM in length. The linkage map of Chr 4D was not completed because the polymorphic markers were not linked with each other.

Detection of grain dormancy QTLs

The results of the putative QTLs detected in the RIL population from the CS × Zen cross are summarized in Table 1. In total, three genomic regions in the three chromosomes examined were significantly associated with grain dormancy.

A QTL with LOD scores ranging from 3.39 (OB'01) to 16.11 (TK'03) was detected in the germination test at 20°C and mapped within the terminal region of the short arm of Chr 3A (Fig. 2). This QTL, designated *QPhs.ocs-3A.1*, was closely linked to the RFLP marker *Xbcd907* and accounted for 11.6–44.8% of the phenotypic variation (Table 1). The mean germination rate of the RILs carrying the Zen allele at *QPhs.ocs-3A.1* was 11.1–17.1% lower in Obihiro and 38.0% lower in Tsukuba than in those carrying the CS allele. Thus the Zen allele was responsible for increasing grain dormancy. The *Xcdo795*/*Xbcd808* region on the long arm of Chr 4A had a significant effect in the OB'02 experiment, with a LOD score of 3.98 explaining 13.6% of the variation. Again, the Zen allele increased grain dormancy. In the OB'01, OB'03 and TK'03 trials, the peak of QTL-likelihood curve was found in the *Xcdo795* region, but did not exceed a LOD score of 2.0. For Chr 4B, one QTL was identified within the marker interval between *Xgwm495* and *Xgwm375*, proximal to *Xgwm495* in the OB'01 and OB'02 trials. This putative QTL, designated *QPhs.ocs-4B.1,* was also detected in the OB'03 exeperiment as a possible QTL with a LOD score of 2.13. In the 0B'01

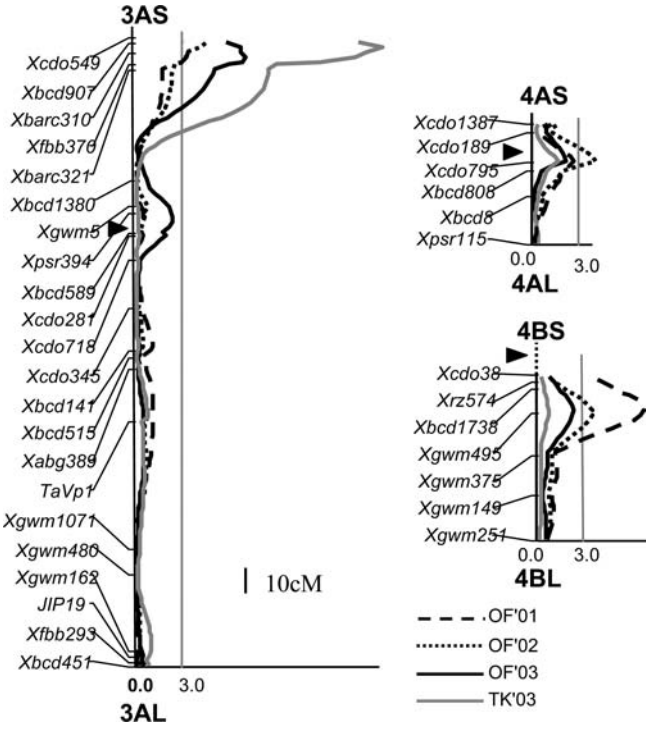

Fig. 2 QTL-likelihood curves of LOD scores showing the location of QTLs for grain dormancy on Chr 3A, 4A and 4B in the germination test at 20°C in the 3-year trials in Obihiro 2001 (*OB'01*), 2002 (*OB'02*) and 2003 (*OB'03*), and in Tsukuba (*TK'03*)

experiment, *QPhs.ocs-4B.1* was the most predominant QTL with a LOD score of 6.18, which explained 19.8% of the phenotypic variation. In the 3-year trials in Obihiro, the mean germination rate of the RILs carrying the CS allele was about 5% less than that of the RILs with the Zen allele. Therefore, conversely to the QTLs on Chr 3A and 4A, it was the CS allele at the *QPhs.ocs-4B.1* locus that was responsible for increasing grain dormancy.

The expression of *QPht.ocs-3A.1* was greatly accelerated in the germination test at 15°C when the RILs were grown in Obihiro. This QTL had a LOD score of 6.63 in the OB'02 trial and 13.91 in trial OB'03, which explained 21.7 and 40.1% of the phenotypic variation, respectively. In Tsukuba this QTL was also predominant, with a LOD score of 8.61, which explained 27.2% of the phenotypic variation. Compared to the RILs carrying the CS allele, the RILs carrying the Zen allele were more dormant since the differences for the mean germination rates between the Zen allele group and the CS allele group were 21.2% in OF'02, 28.5% in OB'03 and 18.1% in TK'03. The effect of *QPhs.ocs-4A.1* was detected significantly in trial OF'02 and was suggested to be a QTL (LOD = 2.25) in trial OB'03. Similar to the germination test at 20°C, the desirable allele for increasing dormancy was that from Zen. On the other hand, LOD scores of *QPhs.ocs-4B.1* were less than 2.0 in both trials OB'02 and OB'03 when the grains were germinated at 15°C. Again, no QTL effect associated with Chr 4A and 4B was detected in the TK'03 experiment.

QTL × environment interactions

The presence of QTL × environment effects was deduced using data from three seasons under different germination temperatures. A significant environmental interaction was revealed for *QPht.ocs-3A.1* by QGENE analysis (LOD = 13.18), caused by changes in magnitude where the allelic differentiation at this QTL was greatest in the germination test at 15°C, such as in OB'03. However, this interaction was much smaller than the corresponding main effect (LOD = 55.54). *QPhs.ocs-4A.1* and *QPhs.ocs-4B.1* were not sensitive to different environments, since their interactions with the environments were negligible (LOD scores < 1.0).

Combined effects of the three QTLs

As transgressive variations were observed in the mapping population (Fig. 1), we tentatively classified the 125

Table 1 Putative QTLs for grain dormancy in five trials detected by interval mapping using QGENE, their LOD scores, the phenotypic variation explained (R^2), and the mean germination rates in each allele class. *CS* and *Zen* represent the means of the RILs carrying the Chinese Spring and Zen alleles, respectively

Trials	QTLs	Marker interval	LOD score	R^2	Mean germination rate (%)		
					CS	Zen	Difference
Germinability at 20°C							
OB'01	*QPhs.ocs-3A.1*	*Xbarc310/Xbcd907*	3.39	0.116	28.8	17.6	11.1
	QPhs.ocs-4B.1	*Xgwm495/Xgwm375*	6.18	0.198	16.6	23.1	−6.5
OB'02	*QPhs.ocs-3A.1*	*Xbcd907/Xcdo549*	4.58	0.155	35.4	21.2	14.2
	QPhs.ocs-4A.1	*Xcdo795/Xbcd808*	3.98	0.136	33.3	28.2	5.1
	QPhs.ocs-4B.1	*Xgwm495/Xgwm375*	3.39	0.117	22.9	28.2	−5.3
OB'03	*QPhs.ocs-3A.1*	*Xbarc310/Xbcd907*	7.09	0.230	32.6	15.5	17.1
TK'03	*QPhs.ocs-3A.1*	*Xbarc310/Xbcd907*	16.11	0.448	78.0	40.0	38.0
Germinability at 15°C							
OB'02	*QPhs.ocs-3A.1*	*Xbcd907/Xcdo549*	6.63	0.217	60.1	38.9	21.2
	QPhs.ocs-4A.1	*Xcdo795/Xbcd808*	3.74	0.129	56.0	49.5	6.5
OB'03	*QPhs.ocs-3A.1*	*Xbarc310/Xbcd907*	13.91	0.401	59.2	30.7	28.5
TK'03	*QPhs.ocs-3A.1*	*Xbarc310/Xbcd907*	8.61	0.272	91.7	73.6	18.1

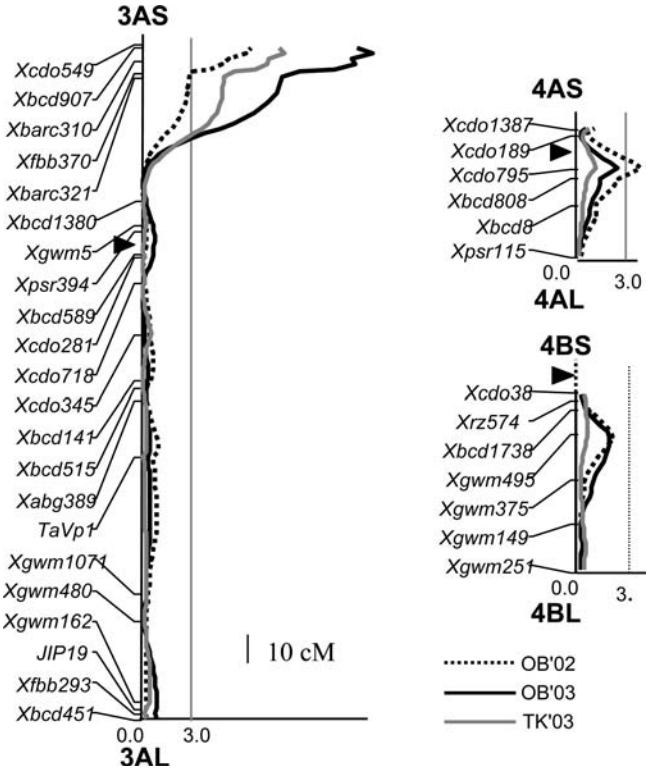

Fig. 3 QTL-likelihood curves of LOD scores showing the location of QTLs for grain dormancy on Chrs 3A, 4A and 4B in the germination test at 15°C in the 3-year trials in Obihiro 2001 (*OB'01*), 2002 (*OB'02*) and 2003 (*OB'03*), and in Tsukuba (*TK'03*)

RILs into eight groups that were constructed by the different alleles for individual markers linked to the QTLs on Chrs 3A, 4A and 4B. The results of the mean germination rates in the eight groups are presented in Table 2. The allelic combinations of the Zen alleles at *Xbcd907* and *Xcdo795* and the CS alleles at *Xgwm495*, tentatively called Zen-Zen-CS group, were responsible for the highest dormancy in Obihiro since the mean germination rates of this group were low and consistent over all 3 years, being around 12% and 25% in the 20°C and 15°C germination tests, respectively. Inversely, the alternative group of the allelic combination, CS-CS-Zen, showed the lowest dormancy, especially in the germination test at 15°C. This was also the case in Tsukuba as the mean germination rate of Zen-Zen-CS was the lowest among the eight groups at both germination temperatures. Hence it was found that one of the reasons for the observed transgressive variation was the combined effect of the desirable alleles not only from Zen but also from CS. The Zen-Zen-Zen group showed inferior dormancy than that of Zen itself, suggesting that the Zen alleles at these three loci are not perfect determinants of the high dormancy of Zen, and are in part responsible for other undetectable QTLs.

Discussion

Grain dormancy in wheat is generally considered to be a complex trait controlled by a number of QTLs, and in a few cases these have been mapped to specific chromosome regions. In the present study, we examined the RIL population from a cross between Zen and CS under field conditions over 3 years and identified three putative QTLs associated with grain dormancy on Chrs 3A, 4A and 4B. While the LOD scores and contributions to the phenotypic variation varied between the trials, the LOD peak positions and the desirable allele at each of the three QTLs were almost consistent.

Of the three QTLs, *QPhs.ocs-3A.1* seemed to be the most important in the different locations and over the different years, since it was detected above the threshold LOD score in all of the trials. This QTL was located within the 4.6 cM region flanked by *Xbarc310* and *Xbcd907* at the proximal end of the short arm of Chr 3, and the Zen allele contributed to the high dormancy, confirming the results we obtained previously in a controlled environment (Osa et al. 2003). No QTLs have been found within this chromosomal region in other molecular marker studies of PHS or grain dormancy (Anderson et al. 1993; Roy et al. 1999; Zenetti et al. 2000; Mares and Mrva 2001; Groos et al. 2002; Kulwal et al. 2004). Furthermore, the homologous regions on barley Chr 3H (Oberthur et al. 1995) and rice Chromosome 1 (Lin et al. 1998; Cai and Morishima 2000;

Table 2 The number of RILs and their mean germination rates in the eight marker-genotype groups. *CS* and *Zen* represent the Chinese Spring alleles and Zen alleles at the marker loci, respectively

Markers			No. of RILs	Mean germination rate (%)						
Xbcd907 3AS	*Xcdo795* 4AL	*Xgwm495* 4BL		At 20°C				At 15°C		
				OB'01	OB'02	OB'03	TK'03	OB'02	OB'03	TK'03
CS	CS	CS	24	25.0	35.6	31.8	80.7	58.7	57.9	93.5
CS	CS	Zen	18	41.6	45.5	42.1	87.2	74.1	71.2	93.9
CS	Zen	CS	11	9.4	15.5	15.2	57.3	40.9	42.8	83.3
CS	Zen	Zen	9	36.9	38.8	37.1	77.8	59.5	58.8	92.6
Zen	CS	CS	19	15.0	21.6	14.3	46.7	43.1	30.5	72.6
Zen	CS	Zen	17	26.9	30.3	11.0	44.1	47.3	38.0	80.6
Zen	Zen	CS	19	11.9	12.5	11.9	28.6	27.2	23.0	67.5
Zen	Zen	Zen	8	17.8	21.1	19.5	42.9	39.2	33.8	75.8

Takeuchi et al. 2003) do not carry genes associated with grain dormancy. *QPhs.ocs-3A.1* may thus be a new dormancy QTL specific to wheat (Osa et al. 2003), and our preliminary study further suggested that this QTL would be associated with the sensitivity of embryos to ABA (Miura, unpublished data). The desirable dormancy effect of the Zen allele was strongly displayed at 15°C in Obihiro and at 20°C in Tsukuba, resulting in a significant QTL × environment interaction. In general, QTLs that are more consistent in various environments are probably more useful. For improving PHS tolerance, however, new cultivars that still have an appropriate level of dormancy when the temperature during the harvesting stage is low are highly desirable (Osanai and Amano 1993). Hence the Zen allele at *QPhs.ocs-3A.1* has potential for PHS tolerance breeding. Therefore, to elucidate its biological function and potential as a target gene in breeding programs for improving PHS tolerance, fine mapping using molecular markers and development of nearly isogenic lines for *QPhs.ocs-3A.1* are now in progress in our laboratory.

QPhs.ocs-4A.1, located on the long arm of Chr 4A, was thought to be identical to the QTL mapped by Kato et al. (2001) in a double haploid population derived from a cross between AC Domain (Canadian red-grained wheat with a high level of dormancy) and Haruyutaka (Japanese red-grained wheat with a low level of dormancy), because the same molecular marker, *Xcdo795*, was tightly linked to this QTL. By comparative mapping across wheat, barley and rice, we have suggested that the wheat *QPhs.ocs-4A.1* was homologous to the barley gene *SD4* because these QTLs were linked to *Xcdo795* (Kato et al. 2001). Anderson et al. (1993) identified one QTL for PHS that was linked to the RFLP marker *Xcdo545* on the long arm of 4A. *Xcdo545* is located on the translocation segment from 7BS in the terminal region of 4AL (Nelson et al. 1995), indicating that 4AL carried at least two QTLs for grain dormancy. Similarly, Noda et al. (2002) have identified two separate dormancy genes on 4AL using chromosome deletion stocks available for CS. In our genetic linkage map, unfortunately, there were some regions on 4AL we could not cover. Thus more saturated maps covering those regions should be constructed by further mapping studies. *QPhs.ocs-4B.1* was located to within the centromeric region of the long arm of Chr 4B and the CS allele at this QTL contributed to a high dormancy. This QTL is not syntenic with *QPhs.ocs-4A.1*, since the *QPhs.ocs-4A.1* region, including *Xcdo795*, shows homology to parts of the short arms of 4B and 4D due to inversion (Nelson et al. 1995). Compared to the other two QTLs detected in this study, expression of *QPhs.ocs-4B.1* was somewhat temperature-dependent, as the LOD scores were less than 2.0 at the germination temperature of 15°C. No QTLs have previously been detected at this region of Chr 4B in other studies. However, we have identified a QTL (*QPhs.ocs-4B.2*) with a minor effect located in the telomere region of the long arm of Chr 4B (Kato et al. 2001).

Prior to this study, we were interested in the dormancy-associated effects of *TaVp1* on 3AL, since this gene has been suggested to be responsible for the high level of PHS or grain dormancy (Nakamura and Toyama 2001; Groos et al. 2002; Wilkinson et al. 2002). McKibbin et al. (2002) demonstrated that missplicing of the *TaVp1* transcript results in a high level of PHS and that transgenic wheat carrying *Avena fatua Vp1* were less susceptible to PHS. However, our results indicated that there were no LOD peaks in the *TaVp1* region (Figs. 2, 3) and thus the high level of grain dormancy in Zen was not due to a direct effect of *TaVp1*. Linkage of grain dormancy QTLs and QTLs for traits of primary importance such as yield and grain protein content is also interesting. Groos et al. (2003) detected a QTL for grain protein content on 3AS, but this QTL seems to reside on the middle of the short arm, and thus is distant from *QPhs.ocs-3A.1*. On the other hand, the 10 cM *Xbcd907* region is the site of QTLs for grain yield and its components (Campbell et al. 2003). In our mapping population, however, the *Xbcd907*-linked QTLs for yield components escaped detection, perhaps because they were the same QTL alleles in Zen and CS. It is logical to assume that different parental materials would allow the detection of different QTLs. However, when a desirable allele at *QPhs.ocs-3A.1* is to be transferred to a new genetic background, the possibility of linkage between this QTL and yield trait QTLs should be kept in mind.

Information on the position of QTLs relative to marker loci provides the basis for MAS of quantitative traits. MAS allows selection of genotypes with a desirable trait in a segregating population at any plant growth stage based on linked DNA markers. In this study, we found that combining the three desirable alleles provided a high level of dormancy. This allelic combination had the Zen alleles at *Xbcd907* and *Xcdo795* and the CS allele at *Xgwm495*. Twenty RILs possessed this type of allelic combination, and most of them exhibited dormancy levels equivalent to or superior to that of Zen. In breeding programs for PHS tolerance, large-scale phenotyping often varies depending on the environmental conditions, thereby preventing effective selection. However, the present results demonstrate that it is possible to identify RILs with three desirable alleles using molecular markers closely linked with each of the dormancy QTLs. Consequently, MAS for multiple loci may provide a more efficient and reliable selection system for developing new cultivars with tolerance to PHS, and these DNA markers will be very effective tools for the MAS of PHS tolerance.

Osanai and Amano (1993) have demonstrated the effectiveness of recurrent selection for low temperature germinability to improve PHS tolerance. Their breeder's lines, designated as OS (Osanai's spring wheats) and OW (Osanai's winter wheats), were derived from the progeny of Zen by cross-breeding. These lines are more tolerant to PHS than Zen, and have received much attention as breeding materials to develop cultivars. In fact, the Osanai lines are used in current breeding programs in

Japan, especially in Hokkaido. As deduced in the present study, it is possible that the Osanai lines carry accumulated desirable QTL alleles for deeper dormancy, and some of them are from the inferior parent. A higher level of PHS tolerance than that shown by Zen can be expected when the desirable QTL alleles are combined into new genetic backgrounds. However, it remains impossible to predict confidently whether the QTLs detected in one mapping population can effectively be manipulated by selecting for specific marker genotypes in other breeding populations. Therefore, we need to clarify if MAS for the combining of the three QTL alleles has made it possible to identify progeny with strong grain dormancy from different cross combinations.

Once this issue is resolved, MAS will be more useful in improving the quantitative traits and promoting practical breeding. Breeders will be able to apply this technique to a wider range of materials. However, the candidate markers for selecting the grain dormancy QTLs on 3AS and 4AL are RFLP markers, and thus they are somewhat difficult to use in MAS. Because MAS based on RFLP has some disadvantages, such as being laborious, time-consuming and cost ineffective, as well as the necessity for the use of radiochemicals in Southern analysis, the practical application of MAS requires molecular markers with a high level of accuracy and efficiency, which must be cost effective and easy to use. PCR-based markers can offer these advantages. Thus we need to convert the RFLP markers to PCR-based markers.

Acknowledgements RFLP clones were kindly provided by the USDR-ARS Central Probe Repository, Albany, Calif., USA; Dr. M.E. Sorrells, Cornell University, N.Y., USA,and Dr. M.D. Gale, John Innes Centre, UK. The aneuploid stocks of the CS ditelosomic lines for group 4 chromosomes were a kind gift from Dr. K. Noda, Research Institute of Bioresources, Okayama University, Japan. This work was supported by funds from the Ministry of Agriculture, Forestry and Fisheries of Japan for the project 'New cultivar breeding for high quality and early maturation, and development of techniques controlling high quality in wheat and barley'.

References

Anderson JA, Sorrells ME, Tanksley SD (1993) RFLP analysis of genomic regions associated with resistance to pre-harvest sprouting in wheat. Crop Sci 33:453–459

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

Campbell BT, Baenziger PS, Gill KS, Eskridge KM, Budak H, Erayman M, Dweikat I, Yen Y (2003) Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci 43:1493–1505

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet G (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white × red grain bread wheat cross. Theor Appl Genet 104:39–47

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032–1040

Hagemann MG, Ciha AJ (1987) Environment × genotype effects on seed dormancy and after-ripening in wheat. Agron J 79:192–196

Kato K, Miura H, Akiyama M, Kuroshima M, Sawada S (1998) RFLP mapping of three major genes, *Vrn1*, *Q* and *B1* on the long arm of chromosome 5A of wheat. Euphytica 101:91–95

Kato K, Nakamura W, Tabiki T, Miura H, Sawada S (2001) Detection of loci controlling seed dormancy on group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. Theor Appl Genet 102:980–985

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kulwal PL, Singh R, Balyan HS, Gupta PK (2004) Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. Func Integr Genomics 4:94–101

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lin SY, Sasaki T, Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L, using backcross inbred lines. Theor Appl Genet 96:997–1003

Mares DJ (1987) Pre-harvest sprouting tolerance in white grained wheat. In: Mares DJ (ed) Proceedings of the 4th international symposium on pre-harvest sprouting in cereals. Westview, Boulder, pp75–84

Mares DJ, Mrva K (2001) Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. Aust J Agric Res 52:1257–1265

McKibbin RS, Wilkinson MD, Bailey PC, Flintham JE, Andrew LM, Lazzeri PA, Gale MD, Lenton JR, Holdsworth MJ (2002) Transcripts of *Vp-1* homologues are misspliced in modern wheat and ancestral species. Proc Natl Acad Sci USA 99:10203–10208

Miura H, Fukuda Y, Sawada S (1997) Expression of seed dormancy in diallel F_1 and F_2 seed of wheat ripened under a controlled environment. J Genet Breed 51:195–200

Miura H, Sato N, Kato K, Amano Y (2002) Detection of chromosomes carrying genes for seed dormancy of wheat using the backcross reciprocal monosomic method. Plant Breed 121:394–399

Murray MG, Thompson WF (1980) The isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nakamura S, Toyama T (2001) Isolation of a *VP1* homologue from wheat and analysis of its expression in embryos of dormant and non-dormant cultivars. J Exp Bot 52:875–876

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson MD, Bernard M, Leroy P, Faris JD, Anderson JA (1995) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5 and 7. Genetics 141:721–731

Noda K, Matsuura T, Maekawa M, Taketa S (2002) Chromosome responsible for sensitivity of embryo to abscisic acid and dormancy in wheat. Euphytica 123:203–209

Oberthur L, Drey W, Ullrich SE, Blake TK (1995) Genetic analysis of seed dormancy in barley (*Hordeum vulgare* L.). J Quant Trait Loci http://probe.nalusda.gov:8000/otherdocs/jptl1995-05/dormancy.html.1995

Osa M, Kato K, Mori M, Shindo C, Torada A, Miura H (2003) Mapping QTLs for seed dormancy and the *Vp1* homologue on chromosome 3A in wheat. Theor Appl Genet 106:1491–1496

Osanai S, Amano Y (1993) Selection of tolerant lines to low temperature germinability in wheat. In: Walker-Simmons MK, Ried JL (eds) Pre-harvest sprouting in cereals 1992. American Association of Cereal Chemists, St. Paul, Minn., pp 76–82

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of microsatellite on chromosomes 6B and STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. Theor Appl Genet 99:336–340

Sears ER (1954) The aneuploids of common wheat. Mo Agric Exp Sta Res Bull 572:1–59

Song QJ, Fickus EW, Cregan PB (2002) Characterization of trinucleotide SSR motifs in wheat. Theor Appl Genet 104:286–293

Takeuchi Y, Lin SY, Yano M (2003) Fine linkage mapping enables dissection of quantitative trait loci for seed dormancy and heading date in rice. Theor Appl Genet 107:1174–1180

Toda M, Nakata T, Miki S, Tsukada A (1972) Studies on mutation breeding in barley and wheat plants. I. Breeding for new variety and desirable short-culm strains in wheat by gamma-ray irradiation. Jpn J Breed 22:43–49

Warner RL, Kudrna DA, Spaeth SC, Jones SS (2000) Dormancy in white-grain mutants of Chinese Spring wheat (*Triticum aestivum* L). Seed Sci Res 10:51–60

Wilkinson MD, McKibbin RS, Bailey PC, Flintham JE, Gale MD, Lenton JR, Holdworth MJ (2002) Use of comparative molecular genetics to study pre-harvest sprouting in wheat. Euphytica 126:27–33

Zenetti S, Winzeler M, Keller M, Keller B, Messmer M (2000) Genetic analysis of pre-harvest sprouting resistance in a wheat × spelt cross. Crop Sci 40:1406–1417