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Mori M, Uchino N, Chono M, Kato K, Miura H

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

Mori M, Uchino N, Kato K, Miura H

Department of Crop Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, 080-8555 Hokkaido, Japan,

Chono M

Department of Wheat and Barley Research, National Institute of Crop Science, National Agriculture and Bio-oriented Research Organization, 2-1-18 Kannondai, Tsukuba, 305-8505, Ibaraki, Japan

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Corresponding author;

Dr. Hideho Miura

Department of Crop Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, 080-8555,

Tel 81 155 49 5476, Fax 81 155 49 5479

E-mail address; miurahm@obihiro.ac.jp

Abstract A major QTL for grain dormancy, *QPhs.ocs-3A.1*, derived from the highly dormant wheat Zenkoujikomugi (Zen), has been identified under a controlled environment. Further investigations were needed to dissect the precise position and expression of *QPhs.ocs-3A.1* in different field conditions because the ability to detect genetic loci for grain dormancy trait is compromised by the effects of environment and genotype × environment interaction. Group 4 chromosomes are also verified to be possible sites of QTLs for grain dormancy. The objectives of this study were to (i) locate additional molecular markers in the *QPhs.ocs-3A.1* region, (ii) identify QTLs on group 4 chromosomes and (iii) elucidate their combined effect. We examined the recombinant inbred lines (RILs) from a cross between Chinese Spring (CS) and Zen over a three-year period in one location and one-year in a different location. By an interval mapping study *QPhs.ocs-3A.1* was mapped within the 4.6-cM region flanked by *Xbarc310* and *Xbcd907* at the proximal end of the short arm of 3A. *QPhs.ocs-3A.1* was confirmed to be most predominant and important since this QTL explained a large portion (11.6-44.8%) of the phenotypic variation, and displayed greatly under a dormancy-breaking condition or at a low germination temperature. For *QPhs.ocs-4A.1* identified on the long arm of 4A and *QPhs.ocs-4B.1* on the centromeric region of the long arm of 4B, the LOD peak positions and the desirable allele were almost consistent among the trials while the LOD scores and contribution to the phenotypic variation were 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 being superior to Zen.

Introduction

Pre-harvest sprouting (PHS) in bread wheat, *Triticum aestivum* L., severely limits end-use application of flour and results in substantial losses in crop yield and price. It is thus a major

problem during wheat harvesting in different parts of the world and 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 interaction (Hagemann and Ciha 1987; Anderson et al. 1993). Therefore, screening for tolerance to PHS on phenotypic basis in segregating populations has been difficult during breeding exercises.

Recently, however, much progress has been made in the development of molecular genetic maps, and thus 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 in these maps, there is little information available for improving tolerance to PHS. One reason is that mapping populations used in the construction of those genetic maps may not be profitable because parental materials of the mapping populations were chosen for different aims but not for analyses of tolerance to PHS or grain dormancy. In quantitative trait loci (QTL) analysis for PHS tolerance and grain dormancy, a mapping population is so prepared or chosen 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 to be profitable 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 potent tolerance to PHS of Zen is an object of attention in Japanese breeding programs, because PHS tolerant lines have been derived from the progeny of Zen by cross breeding. Those lines received attention as breeding materials to develop new cultivars that are more tolerant to PHS than Zen. Thus QTLs associated with grain dormancy specific to the Zen genome is attractive not only for practical breeding but also for analyzing biological mechanisms of grain dormancy. Using a backcross

reciprocal monosomic method, we previously found that for a high level of grain dormancy in Zen, chromosome 3A and homoeologous group 4 chromosomes were possible sites of QTLs (Miura et al. 2002). Furthermore a molecular-marker linkage mapping of chromosome 3A was conducted using recombinant inbred lines derived from a cross between Zen and Chinese Spring, 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 phenotypic variation for grain dormancy under a controlled environment and the Zen allele had a striking effect on maintaining dormancy. While chromosome 3A carries the wheat orthologue (*TaVp1*) of maize viviparous gene *Vp1* and the seed-color *R-A1* gene, it was concluded that the high dormancy associated with chromosome 3A of Zen is ascribable to *QPhs.ocs-3A.1* but is not due to the direct contribution of either the *TaVp1* or *R-A1* locus (Osa et al. 2003). However, 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 trait is compromised by the effects of environment and genotype×environment interaction.

There are several reports that homoeologous 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 doubled haploid population derived from AC Domain with a high level of dormancy x Haruyutaka with a low level of dormancy, and the AC Domain alleles at all detected QTLs increased grain dormancy. As mentioned previously, homoeologous group 4 chromosomes of Zen are possible sites of genes controlling strong dormancy. Therefore, these chromosomes would be important targets of candidate QTLs for MAS in 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 plagued by genotype×genotype and genotype×environment interactions that make it difficult to predict which putative QTLs or which allelic combination 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 to (1) 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 recombinant inbred lines derived from a cross between Zen and Chinese Spring.

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 recombinant inbred lines (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 self-pollination during eight successive generations (F_8). To determine the chromosome or chromosome arm location of RFLP fragments and SSR markers, CS and its aneuploid stocks including CS ditelosomics 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 three-year field trials in Obihiro and one-year trial in Tsukuba. The spring-sown trials were carried out under natural field conditions at the research field, 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 National Institute of Crop Science in 2003 (TK'03). In the experiments in Obihiro the parents and 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 managements. 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, 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 one 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℃ in 90 x 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℃ 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 two-week-old leaves in each of the parents, the 125 RILs and 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 in 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. PCR conditions were the same as those used by Osa et al. (2003).

After screening of the parents for marker locus polymorphisms, the RFLP and SSR markers screened with the aneuploid lines were assigned chromosomes. By using the markers that were proved 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 were 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

Germinability at 20 and 15℃

There were clear differences in germination rate at 20℃ between CS and Zen over 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 CS grains in OB'01 and OB'03 germinated indicating a slight level of dormancy. Consequently, these results confirmed a distinctive difference in the level of grain dormancy between CS and Zen.

Across the four trials, the germination rates of 125 RILs were ranged from 0% to 100% and the distribution was continuous with a skew toward lower germination rate in Obihiro but with a skew toward higher germination in Tsukuba. Transgressive RILs with lower dormancy than CS appeared in OB'01 and OB'03

Reduced grain dormancy in the parents and the RILs was detected in the results of a germination test at 15℃ (Fig. 1B), but again we found a distinctive difference in the level of grain dormancy between the parents. In both 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 OF'02 and from 3.0% to 97.0% in OF'03, and followed a nearly normal distribution. In TK'03 more than half of RILs and CS lost dormancy. Inversely in the germination tests at 20℃, many transgressive RILs that were more dormant than Zen were detected. The transgressive variation suggested that not only the Zen alleles at several loci but the CS alleles at different loci augmented the dormancy in the RIL population, when germinated at 15℃.

Linkage map of *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 3A (Fig. 2). *Xbarc310* was mapped in the 10-cM region flanked by two RFLP markers, *Xbcd907* and *Xfbb370*, while *Xbarc321* was closely linked to *Xfbb370* by 1.7 cM.

Linkage maps of group 4 chromosomes

Of the 43 RFLP clones screened, 25 clones were polymorphic in the parents. An aneuploid test using the CS ditelosomic lines revealed that eleven of the 25 polymorphic RFLP clones were associated with homoeologous 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 chromosomes 4A and 4B, illustrated in Figs. 2 and 3, were constructed on the basis of genotypic classifications for the RIL population. As a result, six marker loci covered approximately 50 cM in chromosome 4A. RFLP analyses of ditelosomic stocks available in CS demonstrated that the centromere was assigned within the marker interval between *Xcdo189* on the short arm and *Xcdo795* on the long arm of chromosome 4A. Thus two loci of RFLP markers were mapped on the short arm and three were on the long arm. For chromosome 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. On the other hand, the linkage map of chromosome 4D was not completed because polymorphic markers were not linked with each other.

Detection of grain dormancy QTLs

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

In the germination test at 20℃, a QTL with LOD scores ranging from 3.39 (OB'01) to 16.11 (TK'03) was detected and mapped in the terminal region of the chromosome 3A short arm (Fig. 2). This QTL, designated as *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 those carrying the CS allele. Thus the Zen allele was responsible for increasing grain dormancy. The *Xcdo795 / Xbcd808* region on the long arm of chromosome 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 experiments, the peak of QTL-likelihood curve was found in the *Xcdo795* region but that did not exceed the LOD score 2. For chromosome 4B, one QTL was

identified within the marker interval between *Xgwm495* and *Xgwm375*, proximal to *Xgwm495* in OB'01 and OB'02. This putative QTL, designated as *QPhs.ocs-4B.1* was also detected in OB'03 as a suggestive QTL with a LOD score of 2.13. In the 0B'01 experiment, *QPhs.ocs-4B.1* was the most predominant with a LOD score of 6.18 which explained 19.8% of the phenotypic variation. In the three-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, inversely to the QTLs on chromosomes 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℃ when the RILs were grown in Obihiro. This QTL had a LOD score of 6.63 in OB'02 and 13.91 in OB'03 which explained 21.7 % and 40.1% of the phenotypic variation, respectively. In Tsukuba this QTL was also predominant having 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 as differences for 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 significantly detected in OF'02 and was suggested to be a QTL (LOD=2.25) in OB'03. Similar to the germination test at 20℃, the desirable allele at this QTL to increase dormancy was from Zen. On the other hand, LOD scores of *QPhs.ocs-4B.1* were less than 2 in both OB'02 and OB'03 when grains were germinated at 15℃. Again no QTL effect associated with chromosomes 4A and 4B was detected in the TK'03 experiment.

QTL×environment interactions

Using three seasons data under different germination temperatures, the presence of QTL× environment was deduced. A significant environmental interaction was revealed for *QPht.ocs-3A.1* by QGENE analysis (LOD=13.18), caused by changes in magnitude wherein the

allelic differentiation at this QTL was greatest in the germination test at 15℃ such as OB'03. But 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 environments were negligible (LOD scores < 1.0).

Combined effects of the three QTLs

As transgressive variation in the mapping population were observed in Fig. 1, we tentatively classified the 125 RILs into eight groups that were constructed by the different alleles for individual markers linked to the QTLs on chromosome 3A, 4A and 4B. The results of mean germination rates in the eight groups are presented in Table 2. The allelic combination of the Zen alleles at *Xbcd907* and *Xcdo795*, and the CS alleles at *Xgwm495*, tentatively called Zen-Zen-CS group, was responsible for the highest dormancy in Obihiro since the mean germination rates of this group were low and consistent over the three years, being around 12% and 25% in the 20℃ and 15℃ 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℃. This was also a 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 caused by the combining effects of desirable alleles not only from Zen but also from CS. The Zen-Zen-Zen group showed inferior dormancy than Zen itself, suggesting that the Zen alleles at these three loci are not perfect determinants of the high dormancy of Zen and is in part responsible for other undetectable QTLs.

Discussion

The grain dormancy in wheat is generally known to be a complex trait controlled by a number of QTLs, and in a few cases these genes have been mapped in specific chromosome regions. In

the present study, we examined the RIL population from the cross of Zen×CS in the field conditions over three years and identified three putative QTLs associated with grain dormancy on chromosomes 3A, 4A and 4B. While the LOD scores and contribution to the phenotypic variation varied with the trial, 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* seems to be the most predominant and important in different locations and different years since this 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 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 at this chromosome region have been found in other molecular-marker studies on PHS or grain dormancy (Anderson et al. 1993; Roy et al. 1999; Zanetii et al. 2000; Mares and Mrva 2001; Groos et al. 2002; Kulwal et al. 2004). Furthermore, the homoeologous regions in barley 3H (Oberthur et al. 1995) and rice chromosome 1 (Lin et al. 1998; Cai and Morishima 2000; Takeuchi et al. 2003) do not carry genes associated with grain dormancy. *QPhs.ocs-3A.1* may 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 embryo to ABA (Miura unpublished data). The desirable dormancy effect of the Zen allele was displayed greatly at 15℃ in Obihiro and at 20℃ in Tsukuba , resulting in a significant QTL×environment interaction. In general QTLs that are most 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 desired (Osanai and Amano 1993). Hence the Zen allele at *QPhs.ocs-3A.1* could have a potential for PHS tolerance breeding. Therefore, to elucidate the biological function and potential as a target gene in breeding programs for improving PHS tolerance, fine mapping and development of nearly isogenic lines for *QPhs.ocs-3A.1*, using

molecular markers, are now in progress in our laboratory.

QPhs.ocs-4A.1 identified on the long arm of chromosome 4A was supposed to be identical to the QTL mapped by Kato et al. (2001) in a doubled 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 only 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 homoeologous 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 a 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. Noda et al. (2002) similarly suggested two separate dormancy genes on 4AL using chromosome deletion stocks available in 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 detected in the centromere region of the long arm of chromosome 4B and the CS allele at this QTL contributed to a high dormancy. This QTL is not synteny with *QPhs.ocs-4A.1*, since the *QPhs.ocs-4A.1* region including *Xcdo795* has homology with 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 LOD scores were less than 2 at the germination temperature of 15℃. There was no QTL at this region of chromosome 4B in other studies. Only we had identified a QTL (*QPhs.ocs-4B.2*) with a minor effect in the telomere region of the long arm of chromosome 4B (Kato et al. 2001).

Before this study, we were interested in the dormancy-associated effects of *TaVp1* on 3AL, since this gene was 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* transcripts 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 and 3) and thus a 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, thereby being far 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 detection of different QTLs. However, when a desirable allele at *QPhs.ocs-3A.1* would be transferred to a new genetic background, we should keep in mind the possibility of linkage between this QTL and yield trait QTLs.

Information on the position of QTLs relative to marker loci provides a basis for MAS for 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 of 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*. Nineteen RILs possessed this type of allelic combination, and most of them exhibited dormancy levels equivalent or superior to Zen. Usually, in breeding programs for PHS tolerance, a large-scale phenotyping often varies depending on the environmental conditions, thereby preventing effective selection. However, the present results demonstrated that it was possible to identify RILs with three desirable alleles using molecular markers closely linked with each of the dormancy QTLs. Consequently MAS for multiple loci can 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 in MAS of PHS tolerance

Osanai and Amano (1993) 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 were more tolerant to PHS than Zen, and have received much attention as breeding materials to develop cultivars. In fact, the Osanai lines have been 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 Zen can be expected when the desirable QTL alleles are combined into new genetic backgrounds. However, it remains impossible to predict confidently whether 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 proved, MAS will be more useful in improving the quantitative traits and promoting practical breeding. Breeders will be able to apply it 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 use of radiochemicals in Southern analysis, practical application of MAS requires molecular markers with a high level of accuracy and efficiency, which would 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.

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Legends of figures

Fig. 1. Frequency distribution of cumulative percentage germination for 10 days in 125 RILs incubated at 20℃ (A) and 15℃ (B) in three-year trials in Obihiro, 2001 (OB'01), 2002 (OB'02) and 2003 (OB'03), and in Tsukuba (TK'03).

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

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

Table 1. Putative QTLs for grain dormancy in five trials detected by interval mapping of the QGENE application, and their LOD scores, phenotypic variation explained (R^2) and mean germination rates in the allele class

Trials	QTLs	Marker interval	LOD score	R^2	Mean germination rate (%)		
					CS[a]	Zen[a]	Diff.
Germinability at 20℃							
OB'01	*QPhs.ocs-3A.1*	*Xbarc310/Xbcd907*	3.39	0.116	28.8	17.6	11.1
	QPhs.ocs-4B.1	*Xwms495/Xwms375*	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	*Xwms495/Xwms375*	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℃							
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

[a] CS and Zen represent the means of RILs carrying the Chinese Spring alleles and Zenkoujikomugi alleles, respectively.

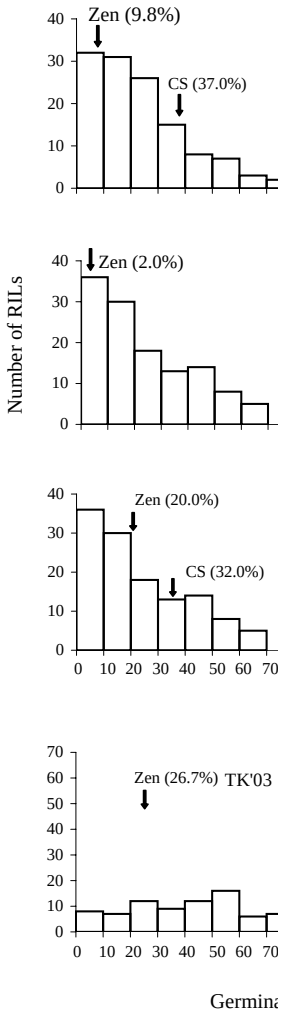
Table 2. The number of RILs and their mean germination rates in the eight marker-genotype groups

Markers			No. of RILs	Mean germination rate (%)						
				at 20℃				at 15℃		
Xbcd907	*Xcdo795*	*Xwms495*								
3AS	4AL	4BL		OB'01	OB'02	OB'03	TK'03	OB'02	OB'03	TK'03
CS[a]	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

[a] CS and Zen represent the Chinese Spring alleles and Zenkoujikomugi alleles at the marker loci, respectively.

%	OF'01	OF'02	OF'03	OF'02-15	OF'03-15	TK'03	TK'03-15
0∽	32	20	36	2	3	8	0
10∽	31	28	30	14	16	7	0
20∽	26	24	18	14	23	12	3
30∽	15	17	13	12	15	9	2
40∽	8	23	14	22	19	12	7
50∽	7	8	8	16	12	16	6
60∽	3	3	5	17	13	6	8
70∽	2	0	0	20	12	7	11
80∽	1	1	1	6	10	16	21
90∽	0	1	0	2	2	32	68

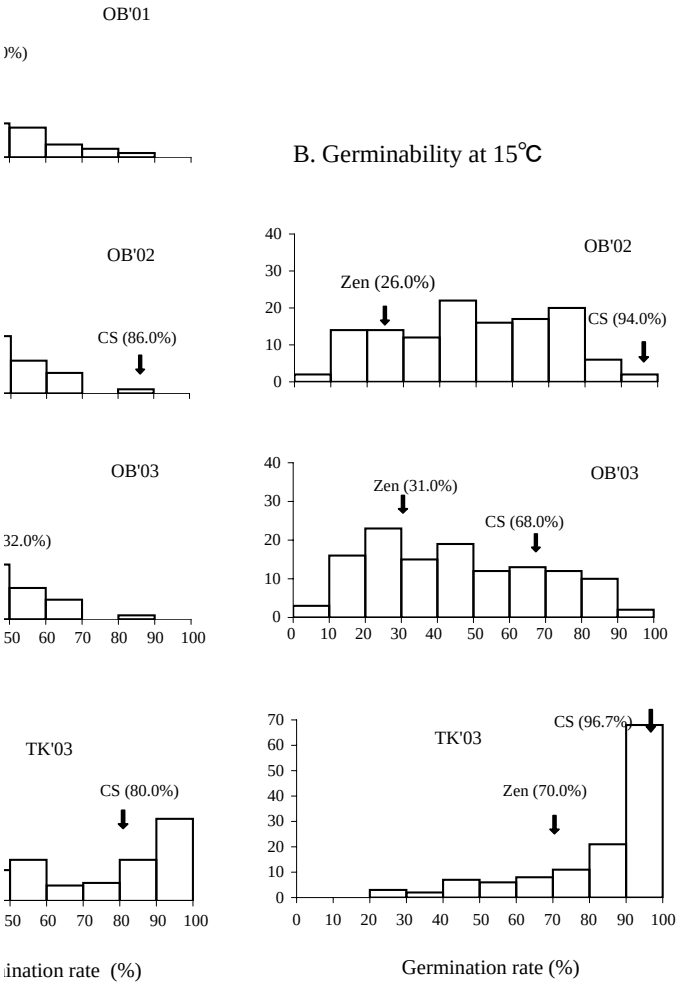
A. Germinability at 20°



Germina

Fig. 1. Frequency distr
of cumulative percenta

C



stribution of cumulative percentage germination for 10 days in 125
)℃(A) and 15℃ (B) in three-year trials obihiro, 2001 (OB'01), 2002

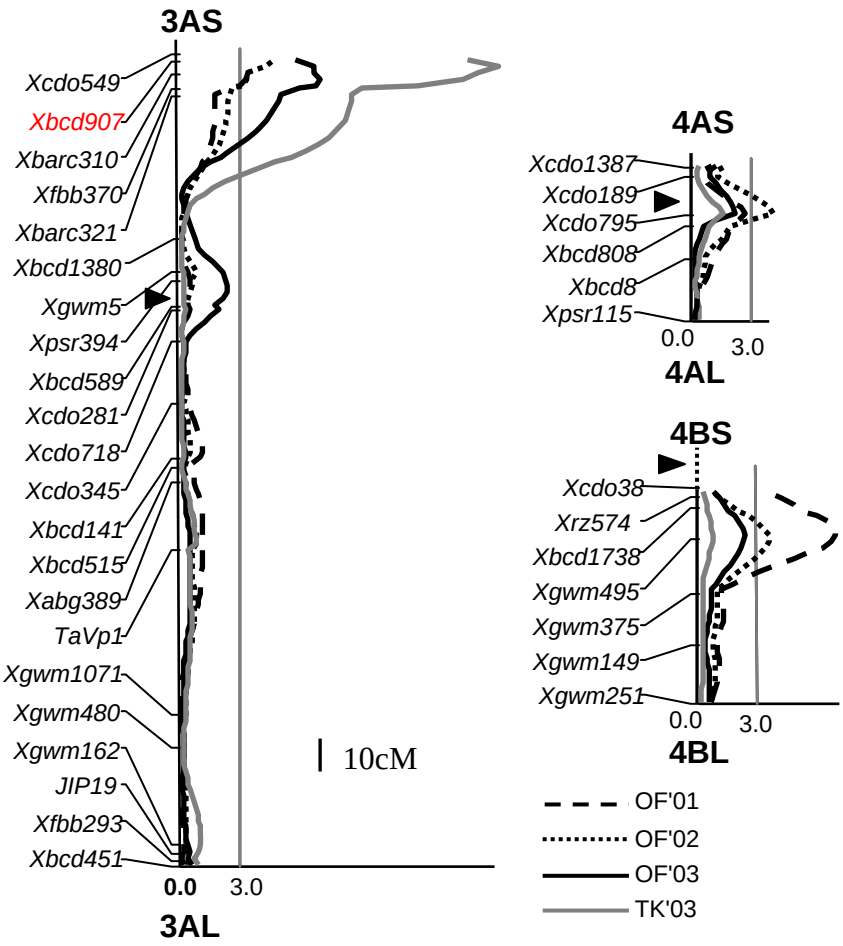


Fig. 2. QTL-likelihood curves of LOD scores showing the locations of QTLs on chromosomes 3A, 4A and 4B for grain dormancy in the germination tests at 20℃ in the three-year trials in Obihiro, 2001(OB'01), 2002 (OB'02) and 2003 (OB'03), and in Tsukuba (TK'03). *Arrow* Centromere

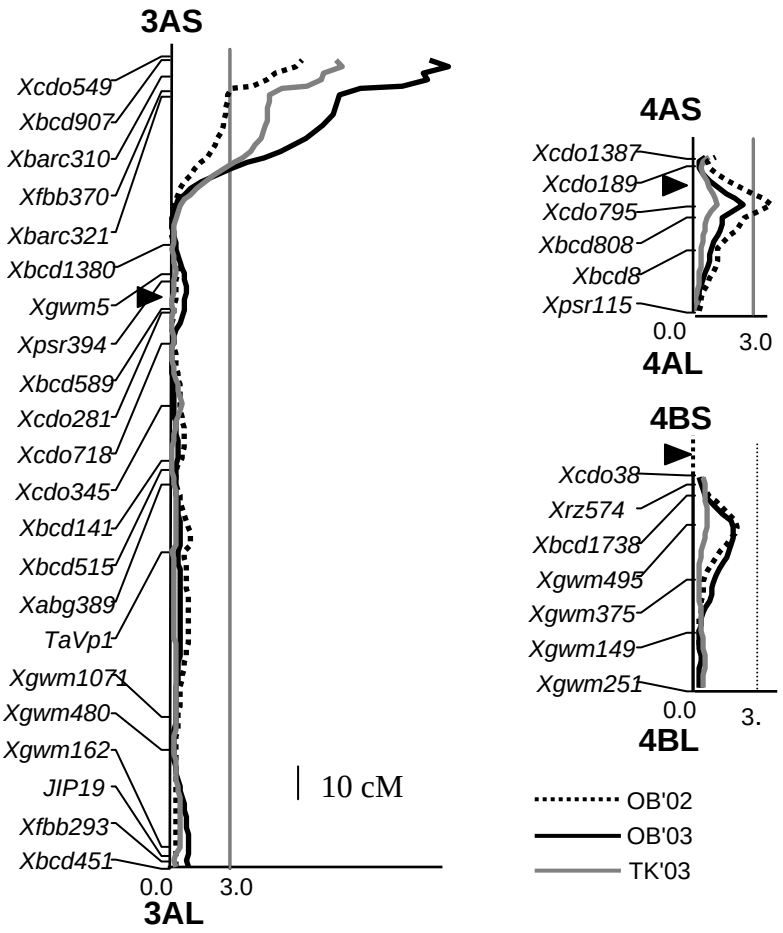


Fig. 3. QTL-likelihood curves of LOD scores showing the locations of QTLs on chromosomes 3A, 4A and 4B for grain dormancy in the germination tests at 15℃ in the two-year trials in Obihiro, 2002 (OB'02) and 2003 (OB'03), and in Tsukuba (TK'03). Arrow Centromere

Table 2. The number of RILs and their mean germination rates in the eight marker-genotype groups

Markers			No. of RILs	Mean germination rate (%)						
				at 20℃				at 15℃		
Xbcd907 3AS	*Xcdo795* 4AL	*Xwms495* 4BL		OB'01	OB'02	OB'03	TK'03	OB'02	OB'03	TK'03
CS[a]	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
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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

a CS and Zen represent the Chinese Spring alleles and Zenkoujikomugi alleles at the marker loci, respectively.