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Increased grain dormancy in white-grained wheat by introgression of preharvest sprouting tolerance QTLs

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Summary

White-grained wheat cultivars have long been recognized to be less resistant to preharvest sprouting (PHS) than the red-grained ones. Previously we identified two QTLs for grain dormancy, *QPhs.ocs-3A.1* (*QPhs-3AS*) and *QPhs.ocs-4A.1* (*QPhs-4AL*) in highly dormant Japanese red wheat, Zenkoujikomugi (Zen). Aiming at improvement of PHS tolerance in white-grained wheat, we examined the introgression effect of these two QTLs in a white-grained population consisting of forty recombinant inbred lines (RILs) developed from a cross between Zen and white-grained Spica. Random 20 RILs with red grains were also developed from the same cross and used as a control population. The RILs were grown in the field and in the glasshouse to evaluate the grain dormancy by germination test. Several SSR markers closely linked to the *QPhs-3AS* and *QPhs-4AL* were used to estimate the alleles at the QTLs. Dormancy variation in the RILs was significantly associated with the differences for grain colour and the alleles at *QPhs-3AS* over several years. Although allelic variation was detected in a SSR marker closely linked to *QPhs-4AL* there was no difference in germination data between Zen-allele and Spica-allele groups. As expected, the red-grained RILs with the Zen allele at *QPhs-3AS* were the most dormant. Some white-grained RILs with the Zen allele at *QPhs-3AS* showed higher dormancy compared to the red-grained RILs with the alternative allele. These results demonstrated that introgression of the *QPhs-3AS* gene could contribute to the increased grain dormancy in white-grained wheat.

Introduction

Millions of people in the world depend on wheat as a major food source. Out of many factors that limit the production of wheat, pre harvest sprouting (PHS) of grains has been a severe problem in many parts of the world. When the grains are exposed to prolonged wet or foggy conditions at ripening, they trigger the initial steps of germination along with the starch break-down. The flour of such grains is down graded and becomes unsuitable for baked foods, noodles or intended purpose due to high alpha amylase activity, which leads to the reduction of flour thickening power. In extreme cases the grain germinates in the head while attach to the plant and therefore growers and food processors get financial losses.

It is believed that white-grained wheat is generally more susceptible to PHS damage than red-grained wheat. The relationship between PHS tolerance and red grain color could be either due to pleiotropy or close linkage between the genes responsible for grain color (*R*) and genes affecting PHS (DePauw & Mcaig, 1983; Gale, 1990). Hence, red pigmentation of the seed coat has long been recognized as a genetic marker for tolerance to PHS. However, white-grained wheat has economic benefits to millers because it can be milled to a higher extraction rates (McCaig & Depauw, 1992). Flour from white wheat contains fewer visible bran specks, which is a critical factor to the appearance and acceptability of bread and noodles. Therefore having remarkable demand over red wheat by international customers it has been a dream for wheat breeders to develop PHS tolerant white-grained genotypes.

Recent mapping studies have indicated that the genetic control of grain dormancy in wheat is complex, and many chromosomes harbor factors affect it (Anderson et al., 1993; Zenetti et al., 2000; Kato et al., 2001; Mares & Mrva, 2001; Mares et al., 2002; Groos et al., 2002; Miura et al., 2002; Noda et al., 2002; Kulwal et al., 2004; Mori et al., 2005; Mares et al., 2005; Kulwa et al., 2005). Using near isogenic white and red-grained lines, Himi et al. (2002)

indicated that the dormancy effect conferred by the *R* gene was not large, suggesting that it plays a minor role in the development of grain dormancy. Furthermore, we identified a major dormancy QTL, *QPhs.ocs-3A.1* (*QPhs-3AS*), on the proximal end of the short arm of chromosome 3A (Osa et al., 2003; Mori et al., 2005). This QTL could explain a large portion of the phenotypic variation under diverse environmental conditions. The superior allele at *QPhs-3AS* that confers strong dormancy was found to inherit from a Japanese red cultivar, Zenkoujikomugi (Zen). Another promising QTL associated with grain dormancy, *QPhs.ocs-4A.1* (*QPhs-4AL*) has been found on the long arm of chromosome 4A (Kato et al., 2001; Torada et al., 2005; Mares et al., 2005). The effect of this QTL has also been monitored in Zen relative to the cultivar, Chinese Spring (CS) by Mori et al. (2005).

Looking into the commercial importance of the white-grained wheat, there is a need to provide white grained wheat with increased tolerance to PHS. One possible approach should be to target the dormancy QTLs which are not associated with the *R* genes. Aiming at improvement of PHS tolerance in white-grained wheat, we examined the introgression effect of *QPhs-3AS* and *QPhs-4AL* in Zen x white grain wheat cross. In the present paper, we described whether the superior alleles could be transferred into white-grained wheat and contributed to increase in grain dormancy.

Materials and methods

Planting materials

Zen was derived from the cultivar Igachikugo-Oregon by exposure of grains to γ radiation (Toda et al., 1972). It is a red-grained with an extreme level of dormancy (Osanai & Amano, 1993; Miura et al., 1997). Spica is Australian white-grained spring wheat, containing good milling qualities with a low level of grain dormancy. F_2 plants from the Spica x Zen cross segregated as 15:1 red-grained to white-grained since Zen carries the dominant red alleles at the

R-B1 and *R-D1* loci on chromosomes 3B and 3D respectively, and the white *R-A1a* allele on chromosome 3A (Miura et al., 2002). White-grained plants were segregated at F_3 grains derived from F_2 red plants because seed coat tissues of F_3 derived from genotype of F_2 seed. To produce a population of recombinant inbred lines (RILs), selected plants from F_2 and F_3 were self-pollinated up to F_8 generation. In total, 40 white-grained lines and 20 randomly selected red-grained lines were used for this study.

Evaluation of grain dormancy

The level of grain dormancy in the plant materials was evaluated for 2 years in glasshouse and 3 years in field, from 2002 to 2004. All trials were conducted at the research site of Obihiro University of Agriculture and Veterinary Medicine. The parents and the individuals of the each RIL were grown in plastic pots at glasshouse and in plots consisting of single 1 m rows at field with two replications. After anthesis, the experimental plots were covered with a transparent plastic roof to prevent rain damage. Spikes of each line in glasshouse and field were harvested at 45 days and 48 days post anthesis respectively. Spikes were dried under room condition for five or seven days until the moisture content of single grain was less than 15% and then gently hand threshed. Immediately the grains were utilized for germination test.

Germination test

Germination tests were performed at 20^{o}C and 15^{o}C in 90x15 mm disposable petri dishes. Fifty grains per line were tested with two replicates. The grains were sown on a single layer of filter paper wetted with distilled water. The dishes were incubated in the dark for 7 days. Germinated grains were counted every day and removed from dishes. Results were expressed as a weighted germination index (Walker-Simmons, 1988). This index provides maximum weight to the grains that germinate first and is calculated from the formula as follows. Germination index [GI]=(7x n1 + 6 x n2 +…..1 x n7)/ total days of the test x total grains, where n1, n2, n3

…….n7 are the number of grains that had germinated on day 1, day 2, …..day7. The maximum index is 1.0 if all grains germinate by the first day while lower indices show the increasing level of grain dormancy or reduced germinability.

DNA analysis

Genomic DNA was extracted from the 2-weeks-old leaves of Zen, Spica and each RIL. The PCR condition was same as used by Osa et al. (2003). Three SSR markers specific for *QPhs-3AS* on chromosome 3A, BARC321, BARC310 and BARC57 (Mori et al., 2005) were used to complete genotyping. Six SSR markers, BARC170, BARC269, gwm397, hbe3, hbe9, and hbe11, specific for the *QPhs-4AL* (Torada et al., 2005; Mares et al., 2005) were used to screen genotype and detect the effect of QTL on chromosome 4A. A 10 μl aliquot of the PCR mixture was analyzed by electrophoresis using 3% agarose or 10% polyacrilamide gel followed by CYBR Gold staining.

Statistical analysis

Germination index data were analyzed by ANOVA using SAS version 6 software (SAS Institute, 1991). The effects of *QPhs-3AS* allele, grain color and incubation temperature on germination and their interactions was determined for each year. Means were compared by the least significant difference ($LSD_{0.05}$).

Results

Variation of flowering date

In the glasshouse experiment, the days from planting to flowering of all RILs showed widely dispersed distribution ranging from 50 to 80 days. It was 57 and 63 days in Zen and Spica, respectively. In the field experiment, the days from planting to flowering date of all RILs were narrowly distributed from 67 to 78 days. It was 70 and 71 days in Zen and Spica, respectively.

No significant correlation between the germination rate and the flowering date was revealed in any trail.

RIL composition

Polymorphism between parental Zen and Spica was detected in all the three markers for *QPhs-3AS* (Figure 1). In the white population of 40 RILs, based on the results of BARC310 marker, 22 were classified into Zen type while 18 were Spica type. Markers, BARC57 and BARC321 co-segregated and generated 19 Spica types, showing one recombinant. In the red population, according to the BARC310 marker, 12 RILs carried the Zen allele and the remaining 8 RILs had the Spica allele, while other two markers again produced one recombinant, showing 9 Spica types. The markers close to the *QPhs-4AL*, hbe3, hbe9, hbe11, gwm397, BARC269 did not show polymorphism between Zen and Spica except BARC 170 (Figure 1). The genotype profile produced by BARC170 showed 18 RILs carrying the Zen allele and 22 RILs carrying the Spica allele in the white population where as each type was 10 in the red population. In every case segregation ratio did not deviate from the expected 1:1 ratio.

Source of variation

The results of analysis of variance shown in Table 1 revealed significant differences among the germinability of individual RILs in all years of field trials and, the effect of the temperature was highly significant in all trials. Variation in RILs was somewhat vague in 2004, where most RILs rapidly germinated at 15^{o}C. It may probably be due the higher temperature existed during the ripening period which gave rise higher germination in all RILs. In all trials of both glasshouse and field, the difference between Zen-allele group and Spica-allele groups based on the nearest marker, BARC310 of *QPhs-3AS* QTL, was highly significant. The mean germinability of Zen-allele group was significantly higher than that of the Spica-allelle group at both temperatures tested. Grain color was the most contributed variable for the dormancy variation in the field

trials. As expected, the mean germinability of red-grained RILs has shown lower values than that of white-grained. Grain color x allele interaction was detected in the 2004 trials only. In the glasshouse experiment, the germination temperature was the most influenced variable followed by the *QPhs-3AS* allele type.

Analysis of the effect ascribable to *QPhs-4AL* based on BARC170 marker did not reveal significant difference between the germinability of Zen-allele group and Spica-allele group statistically in any trial. In all three years of field trials and two years of glasshouse trials, the mean germinability of Spica-allele group was slightly lower than that of Zen-allele group though not significant.

Effect of allele type at *QPhs-3AS* on dormancy variation

In order to detect how strong that the allele at the *QPhs.ocs-3A.1* locus has affected in our interested white RIL population and the red population as a control, the 60 RILs were classified into four types, white-zen, white-spica, red-zen and red-spica based on the grain color and the genotype at *QPhs-3AS*. As shown in Figure 2, the most dormant type has been the red-zen while the least dormant type has been the white-spica. The anticipated white-zen type also has achieved considerable amount of grain dormancy. At 15 ^{o}C, the average GI of white-zen was around 0.6 while at 20^{o}C, it was around 0.5. In the glasshouse experiment, the mean GI of white-zen was almost equal to that of red-zen population at both temperatures (Figure 2).

In order to identify the ability and the stability of individual RILs, especially within the white-zen type, we examined the distribution pattern of the GI (Figure 3). The correlation coefficient between 15^{o}C and 20^{o}C was highly significant at or greater than $p<0.01$ level. Equally dispersed pattern between 15^{o}C and 20^{o}C was examined in 2002 and 2003, where as rather concentrated distribution at 15^{o}C was observed in the field trial of 2004. Decreasing trend of GI was clearly shown in white-zen type in all trials. Based on the results, several white-zen individuals, those which showed least germinability over all three years in the field were

identified and selected for further improvement. In the glasshouse condition, GI of those selected RILs had shown no difference comparative to the parental Zen.

Discussion

Grain dormancy is a complex trait and can be expressed as a result of several factors contributed by the seed coat, embryo and endosperm under the influence of surrounding environment (Gale, 1990). Generally it is thought that red wheat germplasm is more dormant than white because of the dormancy genes being tightly linked or pleiotropic with the seed coat color genes. In this study, although the difference between the GI of field grown red and white grained RILs was highly significant, such a big difference was not observed in the glasshouse experiment. This was mainly due to the drastic reduction in germination of white wheat grown in glasshouse as experienced by Amano & Torada (2002), whose findings confirmed that white wheat could not maintain sufficient dormancy in wet field environment.

The seed coat color is constituted with pigment of phlobaphens that is synthesized in the flavonoids synthetic pathway (Himi et al., 2002). Compared to white wheat grain, red wheat grain contains more than twice catkin and catkin-tannin that are precursors of phlobaphens. Those precursors have an ability to inhibit germination. Using harvest-ripe grain of white-grained mutants of cv. Chinese Spring (CS), Warner et al. (2000) has also showed that the *R* gene enhanced grain dormancy. One possible role of the *R* gene on grain dormancy has been suggested to be to accumulate germination inhibitors that are water-soluble precursors of the red pigment, catechin (Miyamoto & Everson, 1958). Therefore, once those inhibitors of the red wheat contacted with water got solubilized and might have triggered the inhibition activity which might persisted at germination test of our field trials. The mean GI of white wheat grown in glasshouse and red wheat grown in both glass house and field showed lower GI compared to the white wheat grown in the field. Hence the humid condition of the air can be a major factor that determines the susceptibility to PHS in white wheat.

Groos et al. (2002) analyzed the genetic basis of the relationship between grain color and PHS and found several QTLs co-localized with grain color leading to a large effect on dormancy variation. As an evidence to this, we also noticed that a large portion of dormancy variation was contributed by the grain color in the field trials and it may be due to the red color genes in Zen. However, it seems that accumulation of dominant red alleles is not the only way to improve the tolerance to PHS (Flintham et al., 2002; Groos et al., 2002; Mares et al., 2002). Accordingly, there are some QTLs associated with moderate grain dormancy that are governed by the alleles in white wheat also. Therefore, targeting QTLs that are independent of red color genes and QTLs associated with moderate dormancy are possible ways to improve white wheat for PHS tolerance.

QTL for grain dormancy, designated as *QPhs.ocs-3A.1*, was identified on the short arm of chromosome 3A. This QTL had explained the 23-38% phenotypic variation for grain dormancy under controlled environment in the RILs derived from Zen x CS cross (Osa et al., 2003). We have proved that the allele contributed for this particular dormancy in the CS x Zen RILs is the Zen allele. Furthermore this QTL was confirmed to be the predominant dormancy gene, since it strongly displayed under dormancy-breaking conditions in field experiments and at low germination temperatures (Mori et al., 2005). In the present study, this QTL could explain a large portion of phenotypic variation in the RILs derived from Zen x Spica also. Interestingly, the difference observed between the Zen-allele and Spica-allele groups was highly significant in all the trials. This particular effect was observed even in 2004, suggesting that the Zen allele has a striking effect in the white-grained genetic background on controlling grain dormancy though the temperature above 30^{o}C existed during ripening stage.

Another promising QTL associated with grain dormancy, *QPhs.ocs-4A.1*, found on the long arm of chromosome 4A, has been highlighted as an *R* independent QTL (Kato et al., 2001; Torada et al., 2005; Mares et al., 2005). The QTL is located between the SSR markers, gwm397 and hbe09 (Torada et al., 2005). Although the marker BARC170 is located between them we

could not find out any effect of either Zen or Spica allele. This can probably be due to the fact that Spica and Zen carry the identical *QPhs-4AL* allele so that no differential effect on germination appeared. According to Mares et al. (2005) and Torada et al. (2005), the BARC170 marker is located not on the peak of LOD score curves, but on a bit deviated lower position. However, whether this marker locus is located close to the QTL in our population is needed to be determined.

From the scatter diagram shown in Figure 3, it can be clearly noted that the white-zen type, selected based on the *QPhs-3AS* markers, has significantly improved dormancy regardless the other QTLs that are independent of color genes. Few white-zen RILs have maintained GI at around 0.3 throughout the tested 3 years in the field certifying their promising dormant nature. It can be clearly seen when the mean GI of red-spica type was compared with that of red-zen, red color alone does not have much strength to be resist against PHS unless the Zen allele at *QPhs-3AS* has been incorporated. Therefore further introgression of other QTLs by just simple crossing strategies may be given tremendous addition for the grain dormancy in white grained wheat.

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Figure Legends

Figure 1: PCR profile showing the polymorphism between the parental Zen (Z) and Spica (S) generated by SSR markers closer to the QTLs.

I: BARC310 for *QPhs-3AS* , II: BARC57 for *QPhs-3AS* , III: BARC321 for *QPhs-3AS* ,

IV: BARC170 for *QPhs-4AL*

Figure 2: Mean GI of RILs under 15^{o}C and 20^{o}C temperature treatments over different years. Different letters within each temperature show significant difference at $p< 0.05$.

* Four types of RILs classified based on the grain color, white and red, and the Zen and Spica allele types at *QPhs-3AS*.

Figure 3: Distribution pattern of GI in white and red grained RILs based on genotypes generated by BARC310 marker, showing contribution of the Zen allele at the *QPhs-3AS* locus, towards reduced germinability.

r = correlation coefficient, ** $p<0.01$, *** $p<0.001$

Table 1: Mean squires for the germination index of RILs in each trial.

	Field experiment						Glasshouse experiment			
	2002		2003		2004		2002		2003	
RILs	0.071	***	0.063	***	0.022	*	0.043	***	0.042	ns
Grain color (C)	2.283	***	1.958	***	0.486	***	0.143	**	0.010	ns
Allele at *QPhs-3AS* (A)[a]	0.436	***	0.546	***	0.237	***	0.645	***	0.885	***
C x A	0.034	**	0.007	ns	0.067	**	0.031	ns	0.019	ns
Temperature (T)	0.579	***	0.717	***	0.502	***	2.742	***	3.976	***
RIL x T	0.004		0.006		0.014		0.016		0.033	

[a] Based on BARC310 marker

[ns,] *, **,***; non significant, significant at 0.05, 0.01 and 0.001 levels respectively

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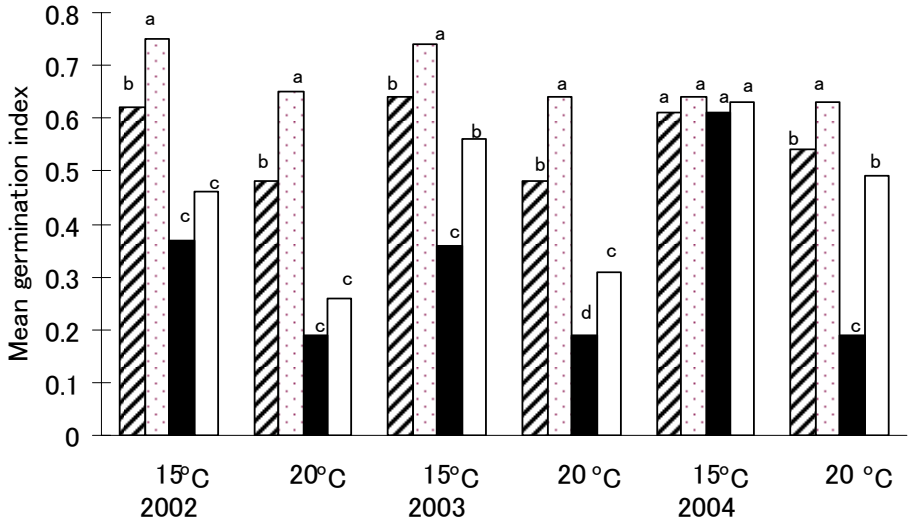
II: BARC57 for *QPhs-3AS*

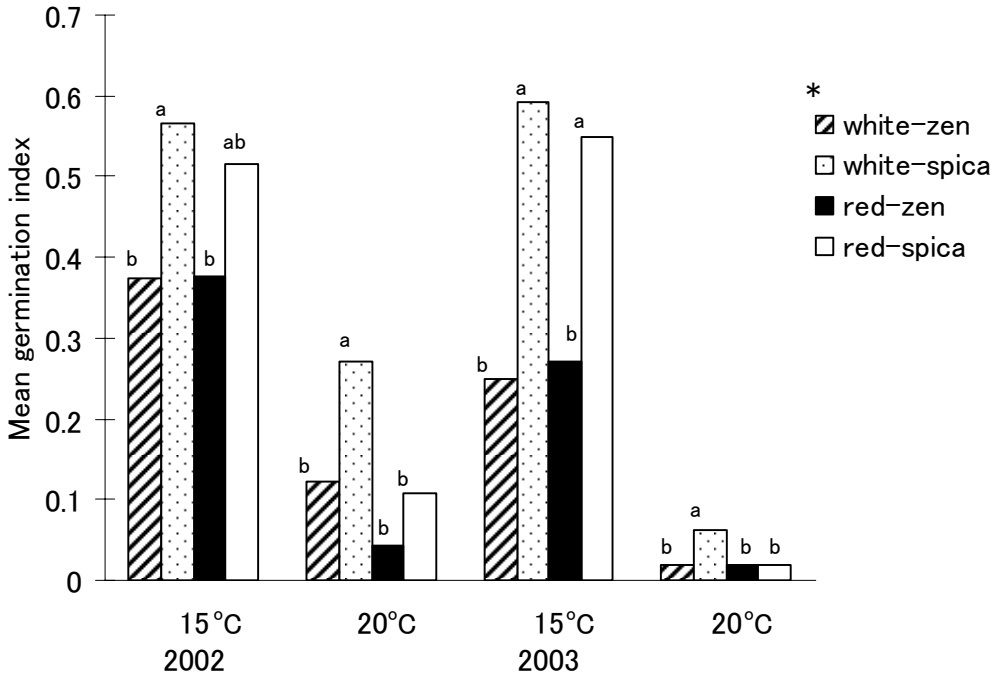
III: BARC321 for *QPhs-3AS*

IV: BARC170 for *QPhs-4AL*

A: Field experiment



B: Glasshouse experiment



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