Mapping of a QTL in Chromosome 3B for Grain Dormancy in White-Grained Wheat Population

Nisha Sulari Kottearachchi[1], Shinichi Takao[2], Kiyoaki Kato[2] and Hideho Miura[2]

ABSTRACT

White-grained wheat is usually more susceptible to preharvest sprouting than red-grained wheat. Identification of QTLs affecting grain dormancy is one of the promising ways of developing PHS tolerant white grained-wheat. A white-grained recombinant inbred line (RIL) population was developed from a cross between highly dormant Japanese red-grained wheat cultivar, 'Zenkoujikomugi' ('Zen'), and a low dormant white-grained wheat cultivar 'Spica'. The 3B chromosome was assessed with SSR and EST markers covering most of the chromosomal areas. A highly significant quantitative trait locus (QTL) associated with grain dormancy on chromosome 3B was identified in this cross and the allele contributed to higher dormancy was derived from 'Zen'. Linkage map of 3B short arm was developed showing the location of the QTL linked with *Xwocs207* marker. The results of the *t*-test revealed that the QTL was significant in 5 environments in the field and 3 environments in the glasshouse. This QTL was detected only in the white-grained RIL population and it was not detected if the phenotypic variation of the population was affected by major red color genes. Mapping of the QTL with QGENE also proved that this QTL, located close to the centromere region of 3B, can be a major influential factor for the total dormancy variation of this cross in the field.

Keywords: grain dormancy, pre-harvest sprouting, QTLs, SSR markers, white-grained wheat

[1]*Department of Biotechnology,*
Faculty of Agriculture & Plantation Management,
Wayamba University of Sri Lanka,
Makandura, Gonawila

[2] *Obihiro University of Agriculture and Veterinary Medicine,*
Obihiro,
Hokkaido,
Japan

INTRODUCTION

White-grained wheat is susceptible to preharvest sprouting (PHS) in rain affected areas of the world. It is the major constraint for the production of high quality white-grained flour and, therefore wheat breeders give much attention to implement strategies to overcome the problem. Red-grained wheat seems to be rather tolerant even they are grown in PHS prone areas where there are cold and moist environment during harvest ripening stage which is the ideal condition for PHS. Therefore red-grained wheat was the choice of many farmers in the past. Nowadays however white-grained wheat is becoming popular in all over the world due to many advantages. It has been proved that the red color testa or red polyphenolic pigments increase the dormancy though the actual reason has not been elucidated yet. The relationship between red color and PHS tolerance could be due either to pleiotropy or to close linkage between the red color genes and the genes affecting PHS (DePauw and McCaig, 1983). In the white-grained wheat population also, dormancy variation among germplasms seems much wider. This simply gives an idea that not only red pigment cause for the dormancy variation but other factors independent from red colour genes may also contribute to the dormancy variation. There are many reports proving that prevalence of color independent genes which can be manipulated to improve the tolerance to PHS in white-grained wheat (Anderson et al., 1993; Groos et al., 2002; Mori et al., 2005; Mares et al., 2005; Torada et al., 2005). Therefore, it is important to identify those color independent genes from red dormant germplasms and introduced into white-grained wheat in order to produce PHS tolerant white-grained cultivars.

Since long time, prevalence of many dormancy quantitative trait loci (QTL) in wheat has been reported from various germplasms although many of their functions have not been characterized so far. A major QTL has been mapped on the long arm of chromosome $5A^m$ using diploid population derived from a cross between *Triticum monococcum* (Tm) and *Triticum boeoticum* (Tb) (Nakamura et al. 2007). Another major QTL and two minor QTLs were identified on chromosomes 4A, 4B and 4D respectively using a population derived from 'AC-Domain' x 'Haruyutaka' cross (Kato et al. 2001). The QTL reported on 4A was detected also in other populations. 'Zenkoujikomugi' x 'Chinese Spring' (CS) by Mori et al. (2005), 'Janz' x 'AUS1408' by Mares et al. (2005), 'Kitamoe' x 'Munstertaler' by Torada et al. (2005) and 'Totomai A' x 'Siyang 936' by Chen et al. (2007). The white-grained wheat accession, 'AUS1408' is one of the major sources of PHS tolerance in Australian breeding programs and it is reported for being associated with several grain dormancy QTLs (Tan et al. 2006).

Presently molecular markers are being utilized for various applications in wheat breeding including mapping genes for marker assisted selection (MAS). Most of these markers have not been developed from the genes themselves because the cloning of genes in wheat has been complicated because of its allo-hexaploid nature and large genome size. However, their use in wheat breeding programs on PHS tolerance is highly effective because the selection based only on dormancy phenotype may change with time due to environmental factors. Hence, molecular markers linked with dormancy QTLs need to be investigated to improve the PHS tolerance. Present paper describes the investigation of useful SSR markers linked with dormancy QTLs and their potential to be used in the genetic background of white-grained wheat.

MATERIALS AND METHODS

Plant Materials

A white-grained recombinant inbred line (RIL) population was developed using red-grained 'Zenkojikomugi' (Zen) derived from the cultivar 'Igachikugo-Oregon' by exposure of grains to radiation (Toda et al. 1972) which has extreme tolerance to PHS. Using low dormant white-grained 'Spica', 41 white-grained RIL population was selected from 'Zen' x 'Spica' cross. 20 red RILs also were developed from the same cross to use as control. F_2 plants from the 'Zen' x 'Spica' 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). 'Spica' express white-grained color because all of the *R* genes on group 3 chromosomes are recessive with the genotype of *R-A1a* : *R-B1b* : *R-D1a*. To produce a population of RILs, forty one white-grained plants from F_2 and F_3, were self-pollinated and established up to F8 generation by single seed descent method.

Evaluation of Grain Dormancy

The level of grain dormancy in the 'Zen' x 'Spica' white RILs was evaluated for 3 years in the field and glasshouse of Obihiro University of Agriculture and Veterinary Medicine from 2002 to 2004. After anthesis, the experimental plots in the field were covered with a transparent plastic roof to prevent rain damage. Spikes of each line from the glasshouse and field were harvested at 45 days and 48 days post-anthesis. Germination tests were performed at 20°C and 15°C in each year, in 90x15 mm disposable petridishes. Fifty grains per line were tested with two replicates. Germination index (GI) was expressed as a weighted germination index (Walker-Simmons, 1988).

Analysis of DNA Markers

Micro-satellite markers were selected based on the previously published reports, Somers et al. (2004) and Song et al. (2005) specific for chromosome 3B. A total of 13 SSR markers, about 15cM apart from each other, were selected to screen the polymorphic markers between two parental genotypes. Four wheat EST markers developed based on the exon sequences of open reading frames on chromosome 1 of rice were also utilized for screening. Two extreme RIL groups, 8 RILs showing most dormant phenotype and another 8 RILs showing least dormant phenotype were selected from 41 RILs, to screen the makers that show apparent linkage with grain dormancy. Then using genotypic data, GI of 'Zen' and 'Spica' allele groups was compared by the student *t* test for field and glasshouse environments with 15^{o}C and 20^{o}C treatments.

DNA Isolation, Amplification and Visualization

Leaves of 2 week- old rice seedling were used for the extraction of genomic DNA. About 3-5 leaves were cut into small pieces and inserted into 1.5 ml ependorf tube. The leaf pieces were homogenized using a micro pipette tip containing 300 µl DNA extraction buffer (1M KCl, 1M Tris HCl pH8, 5M EDTA). Capped ependorf tube was incubated at 70^{o}C for 25-30 minutes. Extracts were centrifuged at 15000 rpm for 15 minutes under room temperature. The supernatant was transferred into new ependorf tube and 100 µl of ice cold iso-proponol was added to precipitate the DNA. After mixing gently tubes were kept at 4^{o}C for 15-30 minutes and centrifuged at 15000 rpm at 4^{o}C temperature for 15 minutes. After removing the supernatant, DNA pellets were washed with150 µl of 70% ice cold ethanol by centrifuging at 15000 rpm under 4 ^{o}C temperature for 10 minutes. Supernatants were removed and pellets were air dried in dark. Pellets were dissolve in 1/10 th TE buffer (10 mM Tris, 1 mM EDTA) and used for the experiments.

DNA amplification was carried out in a Perkin-elmer Thermal Cycler using 15µl reaction mixtures. Each mixture contained about 50ng of template DNA, 1X PCR buffer, and 25mM each dNTP, 10µM of each primer and 0.15µl of TakaraTaq polymerase (5units/µl) (Takara). The temperature profile consisted of an initial denaturing at 95^{o}C for 5 min, followed by 35 cycles of 0.30 min at 95^{o}C, 0.30 min at 54-64^{o}C (annealing temperature), 1 min at 72^{o}C and final cycle of 5 min at 72^{o}C. A 5 µl aliquot of the PCR mixture was separated by electrophoresis using either 2.5%-4% agarose or 10% poly acrylamide gel followed by ethidium bromide staining.

Statistical Analysis

At each marker locus GI of 'Zen' and 'Spica' allele groups were compared by the student *t* test to find the linked markers with grain dormancy. Linkage maps were constructed using MAPMAKER/EXP 3.0 (Lander et al. 1987) to locate the position of the significant markers in the genome. The recombination frequencies were converted to centiMorgans (cM) using Kosambi mapping function (Kosambi, 1944). The chromosomal location of the QTLs for seed dormancy was further confirmed by the simple interval mapping method using QGENE programe (Nelson, 1997). A log-likely LOD scores were developed for GI of each trial at 20^{o}C and 15^{o}C treatments.

RESULTS AND DISCUSION

Frequency Distributions

In this study frequency distributions were examined to detect the extent of phenotypic variation occurred by high dormant 'Zen' and low dormant 'Spica'. As expected all distributions were normally spread proving that dormancy is affected not by a single gene but by poly genes. Frequency distribution of GI in red-grained RILs and white-grained RILs at

15°C and 20°C was illustrated in Figure1. At both germination temperatures and in both years the grain dormancy in red-grained RILs was higher than that of white-grained population and this fact confirms that red pigment has contributed for higher dormancy as reported by Groos et al. (2002) and Kottearachchi et al. (2006). However some white-grained RILs showed moderate dormancy. In these distributions inferior genotypes than 'Spica' and superior genotypes than 'Zen' can be identified. Hence it can be speculated that some alleles inherited from 'Spica' also has contributed for those genotypes to be superior to 'Zen'. In white RIL distributions, few RILs being closer to the GI of 'Zen' proved that they contained color independent genes that have the ability to maintain grain dormancy at 15 °C and 20 °C in the field condition.

Comparison of GI with DNA Markers

Least dormant and most dormant RILs were selected from the 41 white grained population considering the GI for three years at two different temperature treatments. Of the used 17 primers, 9 SSR and all 4 EST primers were shown reproducible polymorphism between the two parental genotypes. Polymorphism % of chromosome 3B between 'Zen' and 'Spica' was fairly high. Of 9 SSR markers, marker *Xgwm566* and *Xgwm285* and, of four EST markers, marker *Xwocs207* showed apparent linkage with grain dormancy when the most dormant RILs and least dormant RILs were compared (Table 1). According to the genotype at marker locus of *Xwocs207* seven most dormant RILs exhibited 'Zen' allele while seven least dormant RILs exhibited 'Spica' allele. These data give evidences to prove the relationship between grain dormancy and the allele at *Xwocs207*. Hence, all 41 white RILs were genotyped and the effectiveness of the locus was analyzed by the student *t* test. The *t* test data revealed that the association between marker *Xwocs207* and GI was highly significant in five of all six environments in the field experiment (Table 2). The level of significance was greater than $P<0.001$ under 20°C in three years of field experiment. In the glasshouse, 3 of 4 environments were significant with greater than $P<0.001$. These data proved the possibility of locating grain dormancy QTL at *Xwocs207* locus.

Linkage Mapping

Linkage map was developed with the neighboring markers in order to investigate the location of EST marker in chromosome 3B. Wheat EST marker, *Xwocs207* was developed based on the exon sequences of the open reading frames of chromosome 1 in *Oryza sativa* and therefore marker locus in *Triticum aestivum* genome was not known. By linkage mapping, *Xwocs207* was positioned closer to the centromere region and it exhibited linkage with the markers, *Xgwm285* and *Xgwm566* (Figure 2). Linkage map also exhibited same marker orders and similar distance among SSR markers as those of the linkage maps previously developed by Somers et al. (2004) and Song et al. (2005).

The putative QTL linked with *Xwocs207* appeared to be an extremely effective and reliable as it was expressed in 5 environments out of 6 in the field (Tables 2). Previously, in 'Zen', chromosomes carrying genes for grain dormancy were detected in comparison with 'Chinese Spring' (CS) homologue by Miura et al. (2002). According to their results, chromosome 3B of 'Zen' has produced very low germination % although the difference between 'CS' and 'Zen' was not significant due to the factor that 'CS' homologue also produced low germinations. This indicated that some alleles that have affected on maintaining grain dormancy could be present in 3B of 'Zen'. In this study, as the comparison was made with respect to 'Spica', the difference was highly significant in many environments suggesting that 'Spica' may contain the allele that could not contribute to dormancy as that of 'Zen'. Mrva and Mares (2001) also have investigated a QTL close to the centromere

region of the 3B short arm associated with "high pI" (based on iso-electric point-amylase (malt, germination type) activity in wheat. Further experiments are necessary for fine mapping of this QTL in order to relate this QTL with those previously reported in above.

QTL Mappi*ng*

QGENE mapping data also prove that a dormancy QTL can be existed at the mapping position of *Xwocs207* locus. Interval analysis at each and every trial indicated that marker *Xwocs207* flanked by *Xgwm566* and *Xgwm285* is the peak position (Figure 2). In 2002 and 2004 at 20^{o}C treatment of field environment the QTL was significant with greater than LOD 3. This QTL can be identified in all the years at 15^{o}C and also in 2003 at 20^{o}C of field experiment with the same map position of *Xwocs207* although LOD score of the QTL peaks were smaller. The map position of *Xwocs207* explained up to 37%, 26% and 29% (R2=0.37, 0.26 and 0.29) phenotypic variation at 20^{o}C treatment of 2002, 2003 and 2004 respectively. In the glasshouse experiment, LOD score values of the QTL was significant in 2002 and 2003 at 15^{o}C treatment and explained up to 34% and 29% (R2=0.34 and 0.29) phenotypic variation respectively (Table 3).

General Remarks

Seed dormancy in wheat is generally governed by many genes and only a few of these genes have been mapped to specific chromosome regions. From this study a major QTL located in 3B short arm was investigated in a 'Zen' x 'Spica' white grained wheat RIL population. Usually grain dormancy is a polygenic character which is highly varied with the environment. In some environments, the dormancy phenotype was not significantly associated with marker genotype of this study and it may be due to the genotype-environment interaction. It is reported that low temperature (10^{o}C) during seed development induce deep and prolonged dormancy while high temperature during development stage tend to have shallow dormancy (Osanai et al., 2005). Usually 15^{o}C is ideal temperature for wheat germination and germination ability decrease or grains remain dormant upon increasing the temperature. In the field experiment 3B QTL has affected on maintaining dormancy when the temperature was increased up to 20^{o}C than it was exposed to 15^{o}C treatment suggesting the effect of the environment. Therefore, many genes or QTLs should be identified from different populations and accumulated into a one line in order to develop a cultivar having the desired trait with least variation for the environment. In the 'Zen' x 'Spica' cross both white and red grained RILs were obtained. However the putative QTL located at *Xwoc207* was visualized by QGENE mapping within only the white grained population and it was not visualized when the both red and white populations were combined. This may be due to the masking effect of red color genes which have highly contributed for maintenance of grain dormancy. However, this study has shown the prevalence of color independent grain dormancy QTLs and hence the QTL detected in 3B chromosome has led to the foundation for further breeding studies to practice effective MAS for PHS tolerance in white grained wheat.

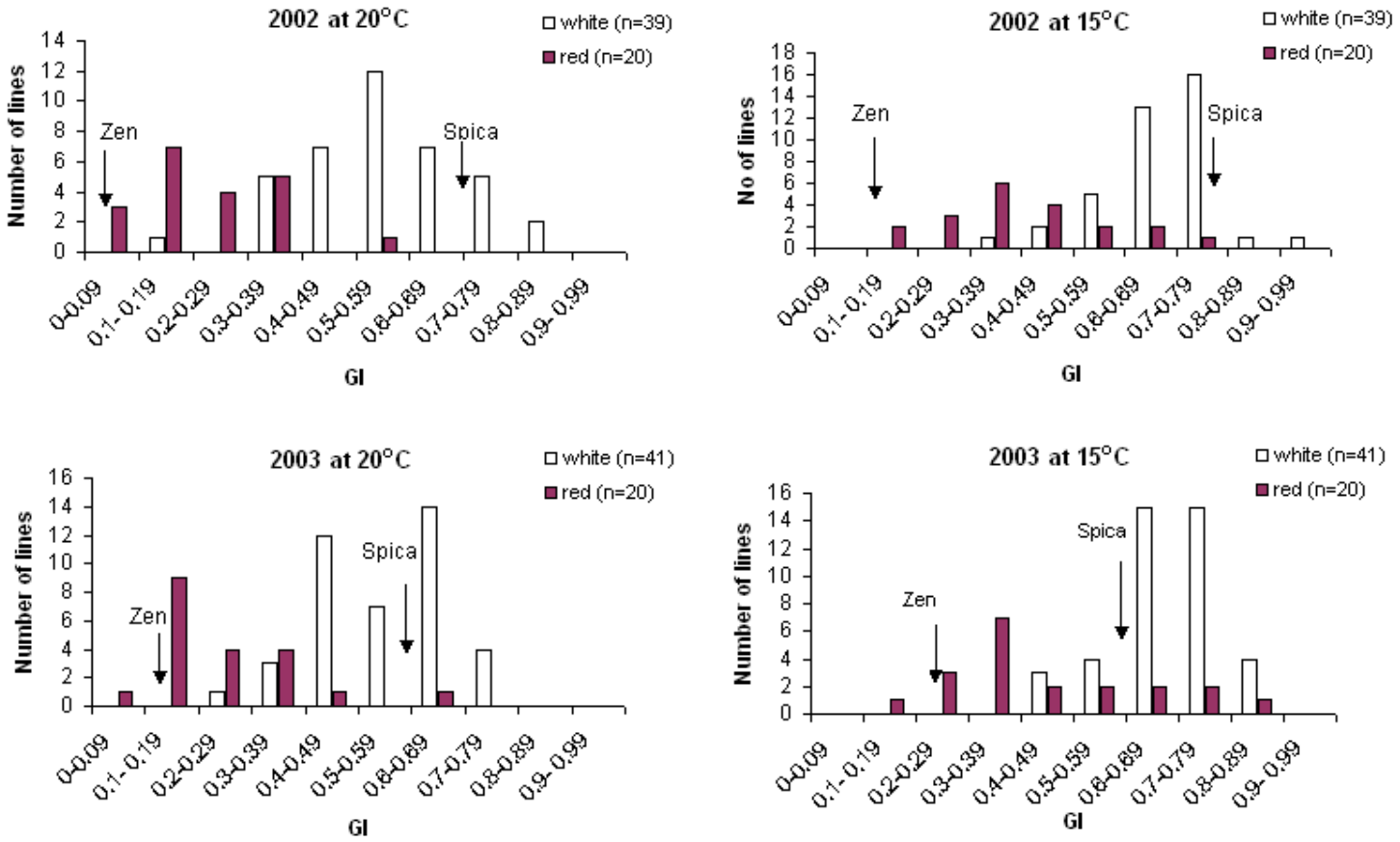

Figure 1: Frequency distribution of the GI of red and white-grained RILs in the field experiment

Table 1: Distribution of the 'Zen' and 'Spica' alleles at the loci of *Xgwm285*, *Xwocs207and Xgwm566* between most dormant and least dormant RIL groups with their GI at 20°C in the field experiment

	GI in 2002	GI in 2003	GI in 2004	Genotype at *Xgwm285*	Genotype at *Xwocs207*	Genotype at *Xgwm566*
Most dormant types						
wRIL5	0.46	0.36	0.54	Z	Z	S
wRIL8	0.37	0.41	0.46	Z	Z	Z
wRIL17	0.38	0.44	0.41	Z	Z	Z
wRIL19	0.37	0.29	0.57	Z	Z	S
wRIL29	0.46	0.45	0.37	Z	Z	S
wRIL30	0.41	0.54	0.53	S	Z	Z
wRIL37	0.58	0.46	0.57	S	S	S
wRIL42	0.15	0.36	0.41	S	Z	Z
Least dormant types						
wRIL18	0.62	0.78	0.73	S	S	S
wRIL28	0.74	0.66	0.62	S	S	S
wRIL31	0.75	0.69	0.68	S	S	S
wRIL36	0.63	0.69	0.79	S	S	S
wRIL39	0.75	0.68	0.61	S	S	S
wRIL47	0.82	0.62	0.48	S	S	S
wRIL49	0.72	0.60	0.70	S	S	S
wRIL15	0.61	0.78	0.66	Z	Z	Z

Table 2: Effect of 'Zen' and 'Spica' alleles at marker, *Xwocs207* on germination index at 15°C and 20°C temperatures in the field

Experimental location	Year	Temperature	t values
Field	2002	15	-2.08*
		20	-4.48***
	2003	15	-2.99**
		20	-3.74***
	2004	15	-1.37ns
		20	-4.23***
Glasshouse	2002	15	-3.41***
		20	-3.41***
	2003	15	-4.40***
		20	-0.50^{ns}

^{ns,} *, **,***; non significant, significant at 0.05, 0.01 and 0.001 levels, respectively.*

Table 3: LOD scores and r^2 values detected by QGENE in the field experiment and glasshouse, for the QTL close to the marker *Xwocs207,* located on chromosome 3BS

Experimental year	Experimental Temperature-^oC	LOD	r^2
Field			
2002	15	1.09	0.12
	20	3.69	0.37
2003	15	2.01	0.20
	20	2.71	0.26
2004	15	0.38	0.04
	20	3.01	0.29
Glasshouse			
2002	15	3.70	0.34
	20	0.76	0.08
2003	15	02.95	0.29
	20	0.18	0.02

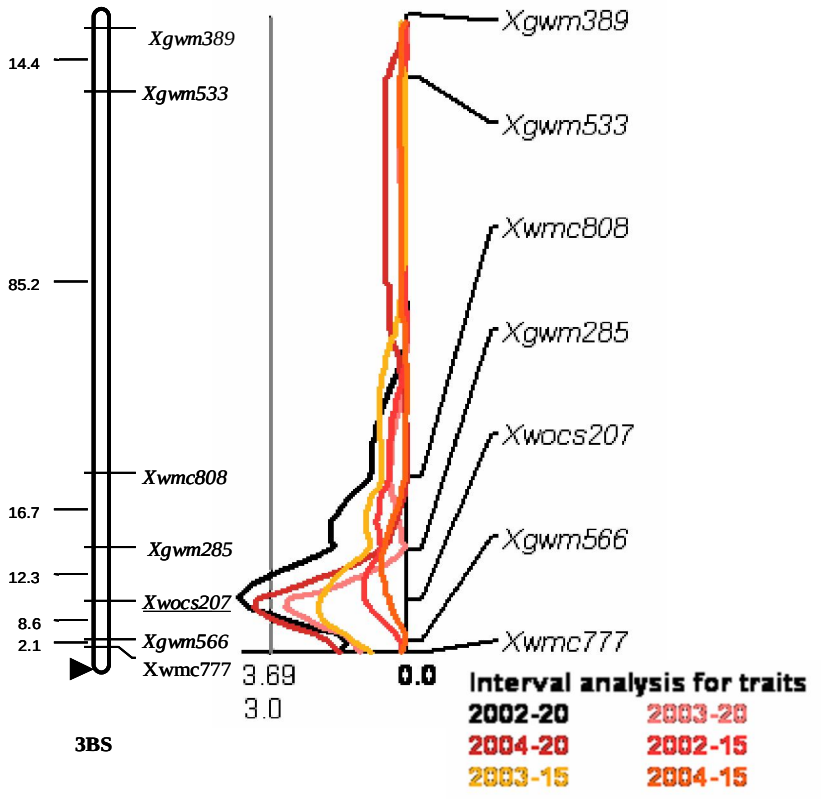

Figure 2: Genetic map of chromosomal arm 3BS and QTL likelihood curves of LOD scores for the GI at 20^{o}C and 15^{o}C treatments in three year trials of the field experiment. Location of QTL is indicated by LOD score peaks. Genetic distances (cM) are indicated on the left side of the 3BS and marker associated with PHS tolerance was underlined.

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