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IDENTIFICATION OF GRAIN DORMANCY QTLS IN A WHITE-GRAINED WHEAT POPULATION DERIVED FROM 'ZEN' X 'SPICA' CROSS

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ABSTRACT

White-grained wheat is usually more susceptible to preharvest sprouting (PHS). Accumulation of color independent grain dormancy QTLs is one of the promising ways of developing PHS tolerance in white-grained wheat. A white grained recombinant inbred line (RIL) population was developed from a cross between high dormant Japanese red grained wheat, 'Zenkoujikomugi' ('Zen'), and a white cultivar 'Spica'. White-grained RILs demonstrated a severe variation proving that many genes have involved in maintaining grain dormancy. Hence, to identify those dormancy QTLs of 'Zen' x 'Spica', whole genome was assessed with SSR and EST markers. Five novel grain dormancy QTLs, linked with *Xwocs207, Xcfa2163, Xbarc243, Xbarc44* and *Xgwm577* markers were detected located on chromosomal arms 3BS, 5AL, 5BL, 5DL and 7BL, respectively. In all instances, allele contributed to comparatively higher dormancy was derived from 'Zen'. The QTL linked with *Xwocs207* marker appeared to be extremely effective, stable and reliable. Usually QTLs rendering minor effects were difficult to be detected if the phenotypic variation of the population was affected by major red color genes. In the present study, since only the white-grained RILs were used, minor QTLs were also able to be investigated.

***Key words**:* Grain dormancy QTLs, EST markers, SSR markers, Preharvest sprouting, White-grained wheat

INTRODUCTION

Preharvest sprouting (PHS) of wheat (*Triticum aestivum*) is a world wide problem causing downgrading of grain quality that leads to a significant economic loss for farmers and food producers. White-grained wheat cultivars have long been recognized to be less resistant to PHS than red-grained ones. 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). However, white-grained wheat is comparatively more preferred nowadays as it produces lighter color in whole grain bread, better color stability in noodles and higher flour extraction rate. Hence, development of white-grained cultivars tolerant to PHS has been one of the main objectives in world breeding programmes.

PHS depends on the level of grain dormancy present at the time of ripening of each wheat genotype. Grain dormancy is regulated by complex interaction between environment and genetic factors. Over a long period, prevalence of many dormancy QTLs in wheat have 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 and 4B and 4D, respectively using a population derived from 'AC-Domain' x 'Haruyutaka' cross (Kato *et al*., 2001). The QTL reported on 4A was also detected 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 contained several grain dormancy QTLs (Tan *et al*., 2006).

In *Arabidopsis* many genes associated with seed dormancy have been identified and many of them belong to abscisic acid, gibberelic acid, ethylene or brassinosteroid related gene products. Kucera *et al*. (2005) have described about 32 genes/ loci related to above four hormone groups that involve in seed dormancy of many plants. All of them seem to be seed coat color independent genes. Therefore, it may be worthwhile to pyramidise all

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of these genes into a one genotype to produce a PHS tolerant white-grained line. In the present study a white-grained recombinant inbred line (RIL) population was genotyped with DNA makers for the whole genome and several useful markers linked to high dormancy phenotype were investigated. Their positions in the genome were confirmed by mapping with neighboring markers. The investigated useful SSR markers linked with putative dormancy QTLs and their potential to be used in the genetic background of white-grained wheat are described.

MATERIALS AND METHODS

Plant materials: A white-grained RIL population was developed using red-grained Zenkojikomugi (Zen) derived from the cultivar Igachikugo-Oregon by exposure of grains to g radiation (Toda *et al.*, 1972), which has extreme tolerance to PHS. Using low dormant white-grained Spica, 41 white-grained recombinant inbred line (RIL) population was selected from Zen x Spica cross. Twenty red RILs were also developed from the same cross to be used as the control. F_2 plants from the 'Zen' x 'Spica' cross segregated as 15 red-grained to 1 white-grained, as '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' expresses white-grained color because all of the *R* genes on group 3 chromosomes are recessive in this line with the genotype of *R-A1a* : *R-B1b* : *R-D1a*. To produce a population of recombinant inbred lines (RILs), fourty one white-grained plants from F_2 and F_3 populations, were self-pollinated and generations were advanced up to F8 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 trial in 90x15 mm disposable petri dishes. Fifty grains per line were tested in two replications. Germination index (GI) was expressed as a weighted germination index (Walker-Simmons, 1988).

Analysis of DNA markers: Micro-satellite markers specific for each of 21 wheat chromosomes were selected based on the previously published reports of Somers *et al.* (2004) and Song *et al.* (2005). Several "gpw" primers were also provided by Dr. Pierre Sourdille, Institute National de la Recherche Agronomique (INRA), France (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/). A total of 258 SSR markers, about 20cM apart from each other, were selected to screen the polymorphic markers between two parental genotypes. Four wheat Expression Sequence Tag (EST) markers developed based on the exon sequences of open reading frames on chromosome 1 of rice were also utilized for screening. Comparatively more markers were screened for the areas of the chromosomes where there was no polymorphism. Of the above 258 SSR primers, 131 and all 4 EST primers were showing reproducible polymorphism between the two parental genotypes. Many areas in chromosome 2D could not be covered when screening because low polymorphism was observed. In these assessment areas where the *QPhs-3AS* present in chromosome 3A, was not used as it was previously characterized (Kottearachchi, 2006).

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 131 polymorphic markers at the initial step. Only the markers that showed apparent linkage with the dormancy phenotype from these two RIL groups were continued to be genotyped with the rest of RILs. Markers that showed the Zen allele in minimum of 5 RILs in most dormant group and the Spica allele in minimum of 5 RILs in least dormant group were selected from first screening. Markers that represented alternative allele in extreme groups were also considered. Then using genotypic data, GI of Zen and Spica allele groups was compared by the student test for field and glasshouse environments under 15°C and 20°C.

DNA isolation, amplification and visualization: Genomic DNA of RILs, parents and selected lines was extracted from the 2-weeks-old leaves using the method described in http://rgp.dna.affrc.go.jp/rgp/protocols/QTL.pdf. DNA amplification was carried out in a Perkin-elmer Thermal Cycler using 15ml reaction mixtures. Each mixture contained about 50ng of template DNA, 1X PCR buffer, and 25mM each dNTP, 10mM of each primer and 0.15ml of TakaraTaq polymerase (5units/ml) (Takara). The temperature profile consisted of an initial denaturing at 95°C for 5 min, followed by 35 cycles of 0.30 min at 95°C, 0.30 min at 54-64°C (annealing temperature), 1 min at 72°C and final cycle of 5 min at 72°C. A 5 ml aliquot of the PCR

mixture was separated by electrophoresis using either by 2.5%-4% agarose or 10% polyacrilamide gel followed by ethidium bromide staining.

Statistical analysis: Genotype data recorded from amplification profile using each primer pair and germination index data obtained from 2002 to 2004 under 15°C and 20°C were analyzed using student *t* test available in Excel Program, to identify the primers showing association with PHS tolerance. Linkage maps were constructed using MAPMAKER/EXP 3.0 (Lander *et al*., 1987) to locate the position of the significant markers in the genome using both white and red RILs. The recombination frequencies were converted to centiMorgans (cM) using Kosambi mapping function (Kosambi, 1944).

RESULTS

In this study five novel dormancy QTLs were detected by markers, *Xwocs207*, *Xcfa2163*, *Xbarc243*, *Xbarc44* and *Xgwm577* located on chromosomes 3B, 5A, 5B, 5D and 7B, respectively. The association between those markers and germination index was significant in several environments in field (Table 1) as well as in glasshouse (Data not shown).

The major putative dormancy QTL or strongest association was observed in chromosome 3B by EST primer WOCS207. The association between this marker and grain dormancy data was significant in five out of six environments in the field experiment. The level of significance was greater than $P<0.001$ under 20°C in all three years of field experiment. EST marker *Xwocs207* was positioned closer to the centromere region and it exhibited linkage with the markers, *Xgwm285* and *Xgwm566* (Fig 1).

The association between *Xcfa2163* marker and grain dormancy was significant in 2002 and 2003 under 15°C. The location of the marker *Xcfa2163* in 'Zen' x 'Spica' population was similar to the map location reported in Somers *et al.* (2004) and it was positioned in the middle of the long arm. A significant association between *Xbarc243* and grain dormancy was found only under 20°C of 2003. However, the mean germination index of the 'Zen' allele group at *Xbarc243* locus was lower than that of the 'Spica' group in each and every environment in the field. The location of the marker, *Xbarc243* in 'Zen' x 'Spica' cross seems to be compatible with previously published reports (Song *et al.*, 2005) where it has been located close to the telomere region of 5BL. The locus seems to have some linkage with neighboring markers of *Xbarc59* and *Xgwm497* in 'Zen' x 'Spica' map, which showed homology with the same markers in the map reported by Tan *et al.* (2006). The QTL detected with *Xbarc44* was significant in two environments in the field. In 'Zen' x 'Spica' cross *Xbarc44* was located close to the centromere region of the chromosome 5D long arm. This data well matched with the map reported by Song *et al.* (2005) where the marker *Xbarc44* was positioned towards the centromere region of the 5D long arm. The QTL detected with *Xgwm577* was significant in one environment in the field. Marker, *Xgwm577* was positioned towards the 7B long arm in this population showing linkage with *Xbarc182* and *Xgwm611* (Fig 1). The location of *Xgwm577* in 'Zen' x 'Spica' RILs, seems to be compatible with maps reported by Somers *et al*. (2004) and Song *et al*. (2005).

Table 1: Effect of 'Zen' (Z) and 'Spica' (S) alleles at several DNA markers on germination index (GI) examined under 15°C and 20°C temperatures in the field experiment

Marker	Allele	Temperature											
		20 °C						15 °C					
		2002		2003		2004		2002		2003		2004	
		Mean	*t* value	Mean	*t* value	Mean	*t* value	Mean	*t* value	Mean	*t* value	Mean	*t* value
Xwocs207	Z	0.49		0.50		0.53		0.64		0.65		0.61	
(3B)	S	0.67	-4.48***	0.63	-3.74***	0.66	-4.23***	0.71	-2.08*	0.73	-2.99**	0.63	-1.37^{ns}
Xcfa2163	Z	0.53		0.52		0.57		0.63		0.64		0.62	
(5A)	S	0.56	-0.74^{ns}	0.57	-1.39 ^{ns}	0.58	-0.10 ^{ns}	0.69	-1.96*	0.70	-2.20*	0.62	-0.03^{ns}
Xbarc243	Z	0.52		0.49		0.55		0.64		0.65		0.61	
(5B)	S	0.57	-1.30 ^{ns}	0.59	-2.61**	0.60	-1.09 ^{ns}	0.69	-1.46 ^{ns}	0.69	-1.47^{ns}	0.63	-1.02 ^{ns}
Xbarc44	Z	0.51		0.52		0.59		0.63		0.64		0.61	
(5D)	S	0.58	-1.44 ^{ns}	0.57	-1.16 ^{ns}	0.57	0.57 ^{ns}	0.69	-1.79*	0.70	-1.99*	0.62	-0.42 ^{ns}
Xgwm577	Z	0.54		0.53		0.56		0.66		0.65		0.62	
(7B)	S	0.58	-0.94 ^{ns}	0.60	-1.87*	0.62	-1.57 ^{ns}	0.66	0.14 ^{ns}	0.71	-1.60^{ns}	0.61	0.51 ^{ns}

ns: Not significant at 5% probability level

*, **,***: Significant at 5%, 1% and 0.1% probability levels, respectively

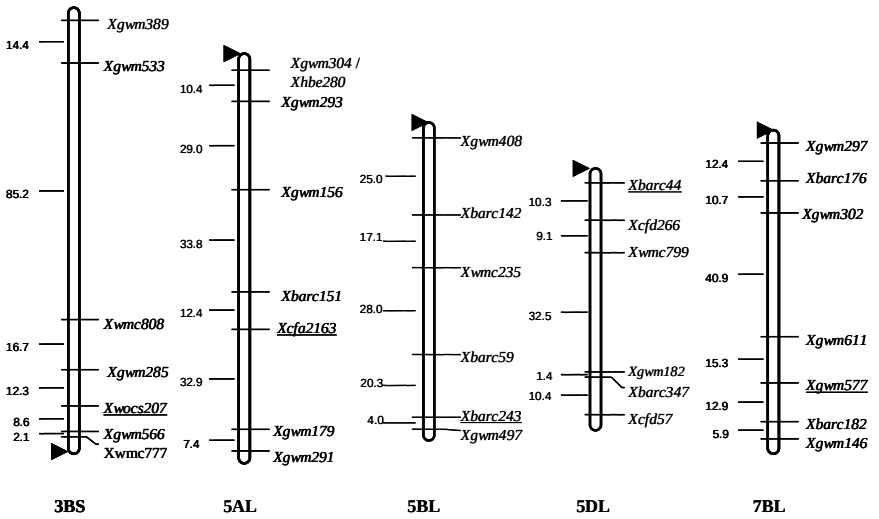

Figure 1: Genetic maps of chromosomal arms 3BS, 5AL, 5BL, 5DL and 7BL of Zen x Spica cross. Markers associated with grain dormancy have been underlined. Arrow head indicates centromere region. Genetic distances (cM) are indicated on the left side of each chromosome

DISCUSSION

The QTL linked with *Xwocs207* appeared to be an extremely effective and reliable as it was expressed in five environments out of six (Table 1). Previously, in 'Zen', chromosomes carrying genes for grain dormancy were detected in comparison with '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 fact 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 respective 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) have investigated a QTL close to the centromere region of the 3B short arm associated with high pI (malt, germination type) a-amylase activity in wheat. They have implied that although this locus is not related to the place where the structural genes of a-amylase are present, this QTL may be associated with the gene/s that modifies the expression of a-amylase under cool temperature. 'Spica' is reported to be a cultivar prone for the expression of a-amylase under many environments. Therefore, there is a possibility that the QTL detected on 3B chromosome of this study to be related to the expression of a-amylase as reported by Mrva and Mares (2001).

The QTL detected on chromosome 5A by linkage with marker *Xcfa2163* demonstrated a minor effect on the dormancy variation. Although many markers assigned to different locations on chromosome 5A were analyzed, no any major QTL was detected and it may due to the fact that 'Spica' and 'Zen' carry the identical allele in those locations so that no differential effect on germination appeared.

The QTL found on the chromosome 5B telomere region was significant only in one environment. The same locus was identified by Tan *et al.* (2006) as an effective QTL in PHS tolerance of white-grained lines, inherited from high dormant Australian white cultivar 'AUS1408'. It may be the genotype-environment interaction that causes the association to be non significant in many environments of this study. The QTL detected on chromosome 5D by the SSR marker *Xbarc44*, was located close to the centromere region. In barley, SD1 QTL detected near the centromere region had a large effect on grain dormancy in 'Steptoe' x 'Morex' cross (Han *et al*., 1996; Li *et al*., 2003, Prada, *et al*., 2004). Nakamura *et al*. (2007) have proved that common causal genes affect grain dormancy regardless of the origin of two genera, *Triticum* and *Hordeum.*

According to a previous study of Miura *et al*. (2002), 7B in 'Zen' was identified as a possible site for the availability of dormancy genes. Hence, it can be speculated that chromosome 7B might contain important genes affecting grain dormancy validating the QTL detected on 7B of the present study in 'Zen' x 'Spica' cross. Mrva and Mares (2001) have investigated a QTL for late maturity a-amylase (LMA) located on the long arm of 7B, distal to the *a-Amy2* gene. Carter (2006) has studied details about the 7B QTL reported on above mentioned LMA. According to his study, this QTL has

been associated with two peaks of which the short peak was laid around SSR marker, *Xgwm577* while the high peak was laid just above the short peak. As the QTL detected on chromosome 7B of the present study also was located near the marker *Xgwm577*, it may be worthwhile to carry out further experiments to examine whether those two QTLs represent the same.

Availabily of dormancy QTLs in chromosome 4A have been reported in many populations. Although polymorphic differences were observed covering most of the locations of chromosome 4A, allelic differences with respect to GI could not be detected, suggesting that 'Zen' and 'Spica' may contain functionally similar dormancy alleles in 4A. The QTLs linked to the markers, *Xcfa2163*, *Xbarc243*, *Xbarc44* and *Xgwm577* seemed to exhibit minor effects, in this population. These QTLs could be detected only under white-grained RIL population of 'Zen' x 'Spica' cross and they could not be detected when red-grained RILs of 'Zen' x 'Spica' were included. These observations prove that under the influence of *R* genes, which give a severe impact on grain dormancy, such minor QTLs could not be detected. Kucera *et al*. (2005) have explained the combined networks of regulating grain dormancy and germination functioned by the involvement of many genes in the interconnected signal transduction pathway. Hence it may be difficult to visualize the effect of each and every gene phenotipically within the present range of GI or germination % even there is allelic differences exist between two parents. Moreover, hexaploid nature of the wheat genome has made the identification of such genes difficult. On the other hand this trait is highly sensitive to environmental factors. Therefore, phenotypic expression of the grain dormancy depends on the interaction between the expressed gene and environmental factors which can exist from development stage to maturation stage of the grain. Under such interactions and under the narrow range of phenotypic evaluation methods those QTLs may not be significant in the same way in every trial. The present study has investigated five putative novel grain dormancy QTLs which lead to the foundation for further breeding studies to practice effective marker-assisted selection aiming at producing PHS tolerant white-grained lines.

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