Genetic Mapping of Pre-harvest Sprouting Resistance Loci in Bread Wheat (*Triticum aestivum* L.)

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Abstract

Pre-harvest sprouting (PHS) in bread wheat (*Triticum aestivum* L.) is the germination of mature grain while still in spike. PHS causes downgrading of grain quality which severely limits its end-use. In western Canada, cool and wet weather during harvest makes the crops susceptible to PHS. Breeding for PHS tolerance in wheat is challenging on phenotypic basis because PHS is inherited quantitatively and strongly affected by environmental conditions. A mapping population of one hundred and fifty one doubled haploid (DH) lines from a cross between two spring wheat cultivars ND690 (non-dormant) and W98616 (dormant) was developed for genetic mapping of PHS resistance loci. Initially, 20 dormant and 20 non dormant lines were used for genetic mapping with SSR (Simple sequence repeat) and AFLP (Amplified Fragment Length Polymorphism) markers. A total of 550 markers (300 SSR markers and 250 AFLP) markers have been mapped on different chromosomes. Five chromosomal regions on the chromosomes 1A, 3B, 4A, 5B and 6B associated with pre-harvest sprouting were identified in this study.

Introduction

Bread wheat (*Trticum aestivum* L.), is an allohexaploid (2n = 6x = 42, AABBDD), with a genome size of 16,700 Mb/1C (Bennet et al., 2000) of which about 90% repetitive DNA (Li et al., 2004). It originated from the hybridization of three different diploid progenitors from the *Triticum* and *Aegilops* genera. The first step involved the hybridization between *Triticum urartu* Thum. Ex Gandil. (source of A genome) and *Aegilops speltoides* (Tausch) Gren. or a closely related species (source of B genome). The resulting tetraploid wheat, *Triticum turgidum* (AABB), then hybridized with *Aegilops tauschii* Cross. (source of D genome) to produce the hexaploid bread wheat (Feldman, 2001).

Wheat forms a major part of the human diet and the factors that affect nutrition and quality of products derived from wheat are important from both economic and social perspectives. Wheat is utilized mainly as flour (whole grain or refined) for the production of a large number of leavened and flat breads, and for a wide variety of other baking products. In Canada, wheat is grouped into eight market classes that are based on kernel characteristics and end-uses. Hard white wheat is a new market class of spring wheat being developed for production in western

Canada. There is a world wide demand for white wheat because consumers prefer the taste and appearance of food prepared from white wheat. With fewer phenolic compounds and tannins in bran, white wheat also imparts a less bitter taste and a more favorable appearance to the final product. When milling wheat to flour color standard, hard white wheat has flour yield advantage over hard red wheat. Introduction of hard white wheat cultivars would allow Canada to compete more directly with Australia which grows white wheat and is the world's leading hard white wheat exporter.

For all its advantages, white wheat does have one drawback, pre-harvest sprouting (PHS). PHS is the germination of mature grain while still in spike. PHS in rain affected wheat crops causes downgrading of quality, severely limit end-use application, and results in substantial losses in price and crop yield. In western Canada, cool and wet weather during harvest makes the crops susceptible to PHS. Breeding for PHS tolerance in wheat is challenging on phenotypic basis because PHS is inherited quantitatively and strongly affected by environmental conditions. Moreover screening of PHS tolerance is hampered by existence of genotype x environment interactions. DNA based molecular markers have already proven to be extremely useful for a number of traits in wheat (Koebner & Summers, 2003). Thus, the objective of this study is to develop a genetic linkage map to identify the chromosomal regions conferring PHS resistance in bread wheat.

Materials and Methods

Plant Material:

A mapping population of one hundred and fifty one doubled haploid (DH) lines from a cross between two spring wheat (*Triticum aestivum* L.) cultivars ND690 (non-dormant) and W98616 (dormant) developed at the Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada was used for molecular mapping. The parent W98616 is a white kernel, dormant line selected from cross AUS1408/RL4137. AUS 1408, a white kernel wheat and RL4137, a red kernel wheat, are considered good sources of PHS resistance. The parent ND690, an American cultivar, commonly known as Argent, is a white seeded, non-dormant wheat having dark grain color.

Simple Sequence Repeat (SSR) & Amplified Fragment Length Polymorphism (AFLP) analysis:

For SSR analysis, a polymerase chain reaction (PCR) was performed in a 25 μl volume containing 100 ng of genomic DNA, 2.5 μl of 10x PCR buffer, 200 μM of each dNTP, 0.2 μM of each primer, 1.0 unit of *Taq* DNA polymerase (Sigma) in MyCycler^{TM} thermal cycler (Bio-Rad). The thermocycling program consisted of an initial denaturation at 95°C for 4.15 min, followed by 30 cycles of 45 sec at 95°C, 20 sec at 50-65°C, 1.30 min at 72°C and a final step of 10 min at 72°C.

AFLP analysis using *EcoR1*/*Mse1* primer/adaptor combination was performed as described by Vos et al. (1995). The PCR products were analyzed on 6% denaturing polyacrylamide gel electrophoresis to detect the polymorphism.

Genetic map construction & QTL analysis:

JoinMap® 3.0 (Van Ooijen and Voorrips, 2001) was used for linkage analysis of mapping data, with an LOD threshold of 3.0. Recombination fractions will be converted into genetic map distance (cM) using the Kosambi mapping function (Kosambi et al. 1944). Linkage groups were assigned to chromosomes via comparisons to reference maps of known SSR loci. MapQTL® 5 Van Ooijen JW (2004) software was use for QTL analysis. Kruskal-Wallis rank sum test was performed to find the association between individual marker and germination results.

Results and Discussions

Germination test:

The germination percentage of 151 DH lines from the W98616 x ND690 cross showed a normal distribution. The percentage germination varied from 8 to 97% for DH lines. The dormant genotype W98616 and non-dormant genotype ND690 showed a germination level of 4% and 97%, respectively. The genotypes RL4137 and AUS 1408, the parents of W98616, showed a germination level of 2% and 13%, respectively. The most dormant (20) and non-dormant (20) DH lines were selected for genetic mapping (Fig 1).

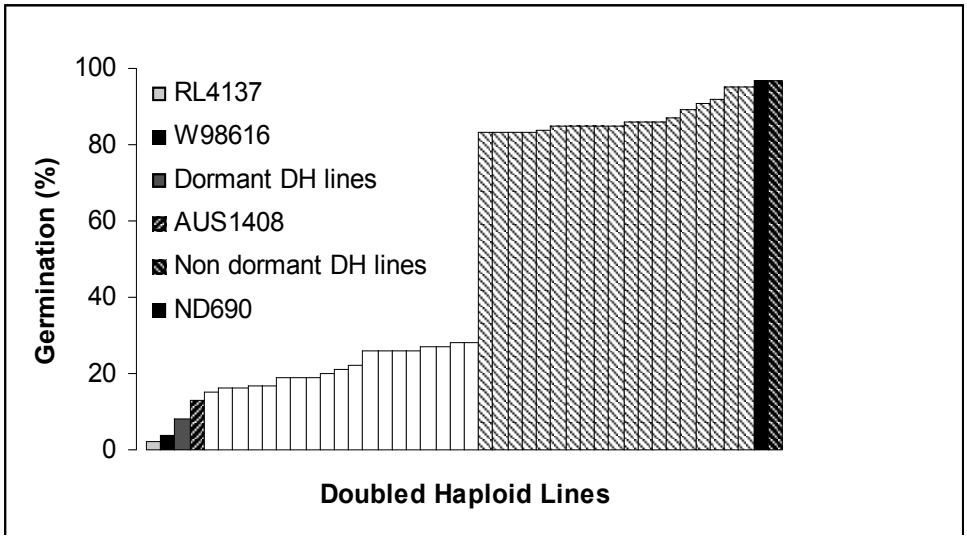

Figure 1. Germination percentage of Doubled Haploid (DH) lines

Marker analysis:

Molecular marker analysis with SSR markers were performed on 20 resistant and 20 susceptible lines. The resistant and susceptible lines were selected on the basis of mean germination percentage of two years, 2002 & 2003 (Fig 1). DH lines less than 30% germination were considered as dormant, whereas, DH lines more than 80% germination were considered as non-dormant in this study. The genotypes AUS 1408 and RL 4137, sources of dormancy were also

included in the screening. The polymorphic microsatellite markers were used to screen the mapping population. A total of 550 markers (300 SSR markers and 250 AFLP) markers were scored with the mapping population. A linkage map of 1610 cM was obtained in this mapping population. No linkage group was assigned on homoeologous chromosome group 2 and chromosome 4D. One linkage group was assigned to chromosome 1A, 1B, 1D, 3B, 3D, 4B, 6A and 6B, respectively, whereas, two linkage groups were assigned to other chromosomes.

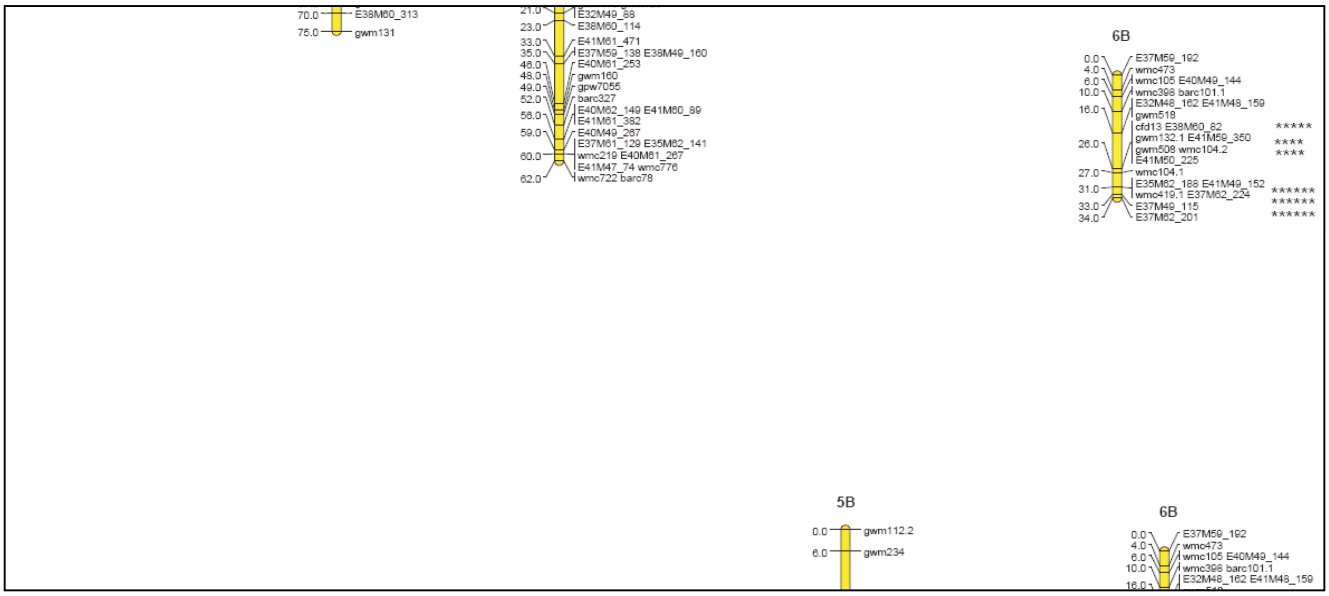

Figure 3. Genetic map of chromosomes involved in PHS resistance. Chromosomal regions implicated in PHS resistance from our study have been indicated by asterisk (*).

Five chromosomal regions on the chromosomes 1A, 3B, 4A, 5B and 6B were found to be associated with PHS resistance. Kruskal-Wallis rank sum test was performed to find the association between individual marker and germination data. Marker analysis identified a QTL located on chromosome 4A flanked by SSR markers DuPw004, barc170 and gwm565. A highly significant QTL associated with grain dormancy on 4A has been identified in two white grained wheat genotypes; AUS1408 & SW95-50213 and a Japanese red-grained wheat (Mares et al., 2005). Noda et al., (2002) have suggested that chromosome 4A has the major gene(s) for embryo sensitivity to abscisic acid and dormancy. The QTL on chromosome 6B was flanked by wmc104, gwm508 and cfd13 in this study. Roy et al. (1999) also reported the association of microsatellite wmc104 with PHS explaining 10.7% phenotypic variance. Flintham et al., (1999) reported a *Phs* locus on chromosome 7D based on bulk segregant analysis of DH lines derived from Boxer x Soleil cross. Subsequent analysis of additional RILs (Recombinant Inbred Lines) revealed that the initial QTL location to be a false QTL, and subsequently the *Phs* locus was relocated to the long arm of chromosome 4A. The *Phs* locus cosegregated with marker *Xpsr* 1327 in both DH and RIL populations, placing it in the region of an ancestral translocation/inversion point between chromosomes 4AS and 5AL (Flintham et al., 2002). The

three chromosomal regions on 1A, 3B and 6B also showed a strong association with PHS resistance. RFLP analyses revealed eight regions of the wheat genome were associated with PHS-resistance (Anderson et al., 1993) including 1A, 3B and 6B. In a different study, four QTL were mapped for resistance to PHS, all of them being co-localized with QTL for grain color (Groos et al. 2002). Three of these QTL's were located on the long arm of group 3 chromosomes, close to the position of the *R* loci. But in the present study the QTL on 3B was positioned on the proximal end of long arm of 3B. Work is in progress to find more markers in the regions associated with PHS resistance.

Acknowledgements

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