Identification and validation of QTLs associated with pre-harvest sprouting tolerance in bread wheat.

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Abstract

Pre-harvest sprouting (PHS) is the in-spike germination of physiologically mature grain in response to relatively high humidity due to untimely rains prior to harvest. PHS in bread wheat (*Triticum aestivum* L.) results in substantial economic loss, as it decreases the functional quality of wheat grain. The Canadian Grain Commission sets the limit of percentage severely sprouted and total sprouted grain depending on the grade and wheat classes. Pre-harvest sprouted wheat is reduced in grade and value, depending on the quantity of sprouted kernels present in a sample. Breeding for PHS tolerance in wheat is challenging on phenotypic basis because PHS is inherited quantitatively and highly influenced by environmental conditions. Seed dormancy is the main factor responsible for conferring the PHS resistance to the grains of bread wheat. The objectives of this study were to identify and validate the major quantitative loci (QTL) for pre-harvest sprouting (PHS) resistance in bread wheat. A F_1-derived doubled haploid (DH) population of 151 lines from a cross between two spring wheat cultivars ND690 (non-dormant) and W98616 (dormant) was used to identify the genomic regions associated with PHS tolerance. A total of 950 polymorphic markers (369 SSR, 306 AFLP, 267 DArT and 8 EST) have been used to develop a genetic map and to identify QTLs for PHS tolerance. Interval mapping revealed a major QTL on chromosome 4A explaining 25% phenotypic variation in this mapping population. Forty two Canadian wheat cultivars and germplasm lines were screened with the DNA marker in the QTL region on chromosome 4A for validation. 113 BC_1F_1 plants from four different backcrosses were screened with the marker associated with PHS resistance. Marker assisted back crossing reduced the population size in BC_1F_1 generation by 40.7%. This information will help the plant breeders to pyramid this QTL with other QTLs from different PHS resistance sources.

Keywords: Wheat, Pre-harvest sprouting, molecular marker, QTL, Marker validation.

Introduction

Pre-harvest sprouting (PHS) is the in-spike germination of physiologically mature grain in response to relatively high humidity due to untimely rains prior to harvest. PHS in bread wheat (*Triticum aestivum* L.) results in substantial economic loss, as it decreases the functional quality of wheat grain. In western Canada, the estimated economic losses due to PHS were up to $400 million for the period 1978-1988 (Derera, 1990). PHS causes yield loss due to a diminution of grain weight but major affect is deterioration of the bread and noodle quality. The flour from sprouted wheat leads to a sticky dough, and breads baked from this have a sticky crumb, dark-coloured crust and loaves with large holes that are difficult to slice. Noodles made from sprouted wheat showed a five-fold greater number of spots as compared with sound flour alkaline noodles (Hatcher and Symons, 2002). Breeding for PHS tolerance in wheat is challenging on phenotypic basis because PHS is inherited quantitatively and highly influenced by environmental conditions. Seed dormancy is the main factor responsible for conferring the PHS resistance to the grains of bread wheat. Lack of knowledge of the genetic factors involved in seed dormancy expression and their interactions hampered the wheat.Red grain color is a traditional marker for resistance to sprouting in wheat improvement programs. White grained wheat has been reported to be more susceptible than red grained wheat, although both groups vary in PHS (Bassoi and Flintham, 2005). Molecular markers linked to PHS resistance trait represent a more reliable tool for selecting PHS resistant genotypes at early stages in wheat breeding programs.

Once a target trait has been identified and tagged, breeders can use the molecular markers to efficiently and effectively accelerate the crop improvement programs by tracing the favorable alleles in the genomic background of genotype to be improved and insuring the presence of elite alleles at the selected loci through repeated cycle of selection (Dreher et al, 2003). Effectiveness of molecular markers should be validated by determining the target phenotype in independent populations and different genetic backgrounds which is referred as marker validation (Collins et al., 2003; Sharp et al., 2001). There is no guarantee that molecular markers identified in one population will be useful in different populations, when the populations originate from distantly related germplasm (Yu et al. 2000). Marker-assisted selection (MAS) is the selection based on genetic information retrieved through the application of molecular markers. MAS involves using the presence/absence of a marker as a substitute for or to assist in phenotypic selection, in a way which may make it more efficient, effective, reliable and cost-effective compared to the conventional plant breeding methodology Marker-assisted selection has several advantages; selection of genotypes at early growth stage, elimination of environment effect, selection for traits with low heritability and gene pyramiding (Collard et al.2005). Backcross breeding is a well-known procedure for the introgression of a target gene from a donor line into the genomic background of an elite recipient line. The objective is to increase the recipient genome content of the progenies, by repeated backcrosses to the recipient line. In this context, markers can be used to control the target gene (foreground selection) and/or to hasten the return to the recipient genotype on chromosomal regions outside the target gene (background selection). The efficiency of such marker-assisted introgression programs has been analyzed in a series of theoretical works (Visscher et al.,

1996). The quantitative trait loci (QTLs) with larger effects are very useful for corresponding trait improvement through MAS. Enabling favourable allele frequency to be increased in early generation through molecular markers would deliver substantial efficiency gains in a crop improvement program (Koebner and Summers, 2003).

Materials and Methods

Plant Material

A mapping population of one hundred and fifty one doubled haploid (DH) lines from 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 and commonly known as Argent, is a white seeded, non-dormant wheat having dark grain color.

Genetic mapping and QTL analysis

SSR (Simple Sequence Repeats), AFLP (Amplified Fragment Length Polymorphism) DArT (Diversity Array Technology) markers were used for genetic mapping. JoinMap® 3.0 was used for linkage analysis of mapping data, with a LOD threshold of 3.0. Linkage groups were assigned to chromosomes via comparisons to reference maps of known SSR loci. MapQTL® 5 software was used for QTL analysis.

Marker validation and marker assisted backcrossing

42 different wheat genotypes were grown in four replicate trial in RCBD experimental design at Seed Farm, University of Saskatchewan, Saskatoon, Canada in 2006. At Zadoks' Growth Stage 92, 50 spikes per plot were harvested. One hundred seeds per genotype were assayed for germination percentage at 20°C. The genotypes were also screened for DNA marker on chromosome 4A associated with PHS resistance. A total of 113 BC_1F_1 plants from different backcrosses were screened with the marker associated with PHS resistance.

Results and discussion

Genetic mapping & QTL analysis

Molecular marker analysis with SSR and AFLP 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. 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. A total of 950 polymorphic markers (369 SSR, 306 AFLP, 267 DArT and 8 EST) were used to develop a genetic map and to identify QTLs for PHS tolerance. 39 linkage groups covering 2138 cM of genome were obtained in this mapping population. One linkage group was assigned to chromosome 1A, 1D, 2B, 3A, 3D, 4A, 5B, 6B, and 7B, respectively, whereas, two to three linkage groups were assigned other chromosomes.

Interval mapping revealed a major QTLs on chromosome 4A explaining 25% in this mapping population (Fig.1). 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. 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).

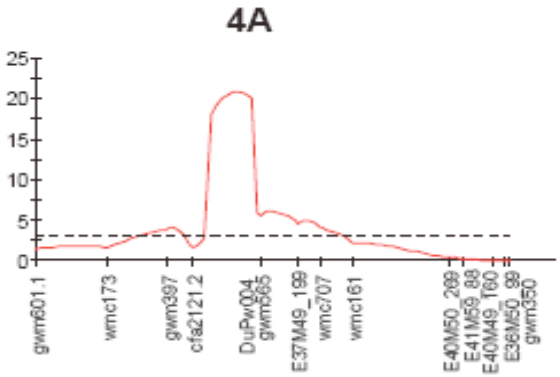

Fig.1 Quantitative trait locus 0n chromosome 4A identified by interval mapping

Marker validation:

The dormant parent ('W98616') and non-dormant parent ('ND690') amplified PCR products of 200 and 300 base pairs (bp) size, respectively (Fig 2). In order to validate this marker in different genetic backgrounds, initially we selected some genotypes for which information about seed dormancy was available in previous studies (Hucl and Matus-Cádiz, 2002; Osanai and Amano, 1993; Hucl, personal communication). All the genotypes amplified either PHS resistance (200 bp) or PHS susceptible allele. A PCR product of 200 bp was amplified from all the PHS resistant wheat lines. The same marker produced a 300 bp allele from PHS susceptible wheat lines. The cvs. 'Columbus' and 'AC Domain' had 'RL4137' in their parentage as the source of PHS resistance. The cv. 'Columbus' was in the pedigree of cv. 'AC Majestic', whereas, cv. 'AC Domain' was in the pedigree of cvs. 'PT434', 'PT435' and 'KANE'. Similarly the source of PHS resistance in line 'CDC EMRD9' was 'AUS1408' and in cv. 'BW384' was 'AC Superb' ('BW252'), which had 'AC Domain' in its pedigree. The cvs. '5500HR', '5600HR', '5601HR', 'AC Barrie', 'AC Superb', 'Harvest', 'McKenzie', 'PT559' and 'Snowbird' amplified 200 bp PCR product have 'RL4137' in their ancestry. 'Losprout' is the source of dormancy in 'AC Vista'. 'AC Eatonia' has two sister lines Lancer and Leader as

source of PHS resistance. Thus 'RL4137' is the major source of elevated PHS resistance in number of Western Canadian wheat cultivars. The ability of the marker to detect the appropriate allele in these different backgrounds supports its usefulness for selecting PHS resistant genotypes within breeding programs.

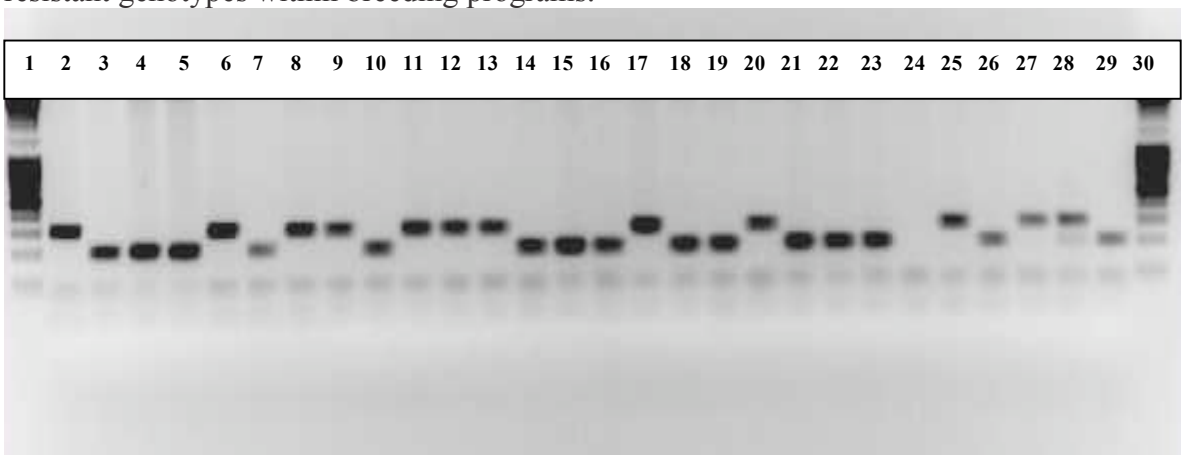

Fig 2. Screening of different cultivars and germplasm lines with PHS resistance marker. 1- MassRuler, 2- ND690, 3- W98616, 4- AUS1408, 5- RL4137, 6- AC Abbey, 7- Columbus, 8- AC Interpid, 9- CDC Imagine, 10- Harvest, 11- AC Elsa, 12- AC Cadillac, 13- CDC Bounty, 14- AC Barrie, 15- AC Domain, 16- PT435, 17- ES41, 18- AC Superb, 19- PT434, 20- AC Crystal, 21- 5000HR, 22- AC Vista, 23- McKenzie, 24- CDC Kendell, 25- 99SPELT, 26- Snowbird, 27- AC Infinity, 28- Lillian, 29- Eatonia, 30- MassRuler.

Screening of BC_1F_1 and BC_2F_1 with the PHS resistance marker:

As PHS is inherited quantitatively and highly influenced by environmental conditions, selecting PHS resistant genotypes on phenotypic basis at early stages is not possible. DNA-based markers have gained wider recognition as tools for increasing the selection efficiency for plants of desirable genotype. The presence of molecular marker linked to the economically important trait allows one to select plants with desirable allele and to discard those with undesirable allele in each generation. A total of 113 BC_1F_1 plants from different backcrosses were screened with the marker associated with PHS resistance. Marker assisted back crossing reduced the population size in BC_1F_1 generation by 40.7%. This information will help the plant breeders to pyramid this QTL with other QTLs from different PHS resistance sources.

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