# Molecular diversity at QTLs for pre-harvest sprouting resistance in spring wheat using microsatellite markers

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## ABSTRACT

Preharvest sprouting (PHS) resistance is an important wheat breeding objective. We have characterized 32 wheat accessions using 33 microsatellite markers flanking PHS quantitative trait loci on chromosomes of group 3, 4, 5, and 6. A total of 229 alleles, with an average of 6.94 alleles per marker, were observed among the 32 wheat lines. The polymorphic information content ranged between 0.25 and 0.90, with an average of 0.67. A cluster analysis revealed 3 main clusters and three singlet wheat lines, which agreed closely with pedigree-based relationships, seed coat colour and accession origin. Canadian wheat accessions could be subdivided into four sub-clusters based on pedigree and end-use classification. Results of this research have been previously published (1).

## INTRODUCTION

In wheat, pre-harvest sprouting (PHS) can result in significant losses in grain yield, and decreased milling and baking quality (2). Thus, improved pre-harvest sprouting resistance is a highly desired characteristic. However, resistance to pre-harvest sprouting is derived from a combination of factors including water uptake, ear drying rate, grain dormancy, and storage reserve mobilization during germination. Thus, it is difficult to obtain resistance to PHS because the trait is quantitatively inherited (3, 4).

In wheat, several studies have reported quantitative trait loci (QTLs) associated with pre-harvest sprouting resistance, and these QTLs have been found throughout the genome. To date, at least 22 QTLs, derived from 9 mapping populations have been found to be associated with the expression of PHS in wheat (see 1). Recently, 21 QTLs including 9 QTL clusters for PHS resistance, defined using three PHS parameters: germination index (GI), sprouting index (SI) and Hagberg falling number (FN), have been identified in two Canadian doubled haploid (DH) mapping populations (5, 6).

Microsatellite (SSR) markers are often used in QTL mapping and genetic diversity studies because SSR markers are highly polymorphic, reliable and reproducible (7). SSR markers have been used to evaluate haplotype diversity (8) and genetic relationships (9). Through information obtained from genetic mapping studies and pedigree relationships of wheat germplasm, breeders can better understand their breeding material, and more efficiently exploit available genetic resources (10).

This study was undertaken to investigate relationships between Canadian white wheat germplasm and other PHS resistance sources using SSR markers flanking PHS resistance QTLs located on group 3, 4, 5, and 6 chromosomes, and to identify wheat lines that will be useful in breeding white wheat for PHS resistance.

## MATERIALS AND METHODS

Thirty-two hexaploid wheat accessions (23 white and 9 red) sourced from various countries were selected for evaluation based on their seed coat colour and reported PHS resistance status. Field trials were conducted in three environments [Glenlea (2003 & 2005) and Winnipeg (2005)]. For each site-year, 30 wheat accessions were grown using a 5x6 lattice design with three replicates. Spikes were collected at physiological maturity (PM), air dried for 5 days and stored at -5 to -20˚C until processing. PHS response for the 30 wheat lines was determined using germination index (GI) and sprouting index (SI); and for amylase activity, using the Hagberg falling number (FN) (see 1).

The 33 microsatellite markers used in this study were selected from group 3, 4, 5, and 6 chromosomes based on mapping data reported in two spring wheat DH populations (5, 6). The markers were those that mapped closest to PHS QTLs in these DH populations. Thirty-two wheat lines were genotyped including 30 wheat accessions characterized for PHS and two white DH lines with high breeding potential. DNA was extracted from individual plant using the Qiagen DNeasy 96 Plant Kit and PCR reactions were carried out using a method modified from Röder et al. (11) with PCR data collected using an ABI 3100 genetic analyzer and converted to a gel-like image using Genographer. Microsatellite locations were determined using the wheat microsatellite consensus map (12) and allelic sizes determined and scored manually in Genographer. Chromosome-specific marker data with uncertain of allele size were not considered.

Phenotypic data were analysed using SAS v8.2. For each PHS parameter, a one-way analysis of variance was performed using a PROC MIXED model. Wheat lines effects were fixed and replication, block, location and

their interactions effects were random. Genetic diversity parameters including gene diversity and polymorphic information content (PIC) were estimated using PowerMarker V3.25 (13). Gene diversity is defined as the probability that two randomly chosen alleles from the population are different; whereas; PIC is defined as the probability that the marker genotype of a given offspring will allow deduction, in the absence of crossing-over, of which of the two marker alleles of the affected parent it received (14). Genetic relationships among wheat accessions were estimated using pairwise genetic distances determined using Nei's method (15), and a Neighbour Joining tree was constructed (16).

## RESULTS

Pre-harvest sprouting was assessed on 32 wheat accessions using germination index (GI), sprouting index (SI), and Hagberg falling number (FN). Correlation analysis showed that GI and FN were the most consistent PHS indices across environments and significant differences ($P<0.0001$) were observed between wheat accessions for all three PHS indices. GI and SI were positively correlated ($r=0.83$) across all the three environments while FN was negatively correlated with GI ($r=-0.86$) and SI ($r=-0.91$). The germplasm was classified as susceptible, tolerant, or resistant to PHS principally based on overall mean FN. Each PHS resistance class was observed for both red and white seeded wheat germplasm. Generally, an increase in mean FN with increasing levels of PHS resistance was mirrored by a reduction in mean SI and GI value consistent with the correlation analysis.

In this study, 33 microsatellite markers were used to assess genetic diversity of the 32 wheat accessions. The microsatellite markers detected a total of 229 alleles, with an average of 6.94 alleles per marker. The observed PIC ranged from 0.25 for marker *wmc*232 on chromosome 4A to 0.90 for marker *gwm*497 on chromosome 5B, with an average PIC of 0.67. Based on the pairwise matrix of distance, the neighbour joining tree grouped the wheat germplasm into three main clusters with three singlets (Fig. 1).

In general, wheat lines tended to group by origin, pedigree-based relationships and PHS resistance status. Cluster 1 consisted of the New Zealand PHS susceptible line Kotuku and the two Canadian PHS susceptible lines RL4452 and ES34. Cluster 2 consisted solely of accessions from Australia and New Zealand. Cluster 3 was the largest cluster and contained mainly the Canadian germplasm, with the exception of Monad and Kenya321. All Cluster 3 accessions, except HY475 and Roblin, were found to have good PHS resistance. Cluster 3 could be divided in to four sub-clusters, which were designated: 3-1, 3-2, 3-3, and 3-4. Sub-cluster 3-1 consisted of PHS resistant germplasm derived from Red-RL4137 and White-RL4137. Sub-cluster 3-2 consisted of AC Karma and AC Karma-derived Canada Prairie Spring (CPS) wheat varieties. Sub-cluster 3-3 included the Canadian red seeded cultivars Roblin and AC Majestic, and the USA cultivar, Thatcher.

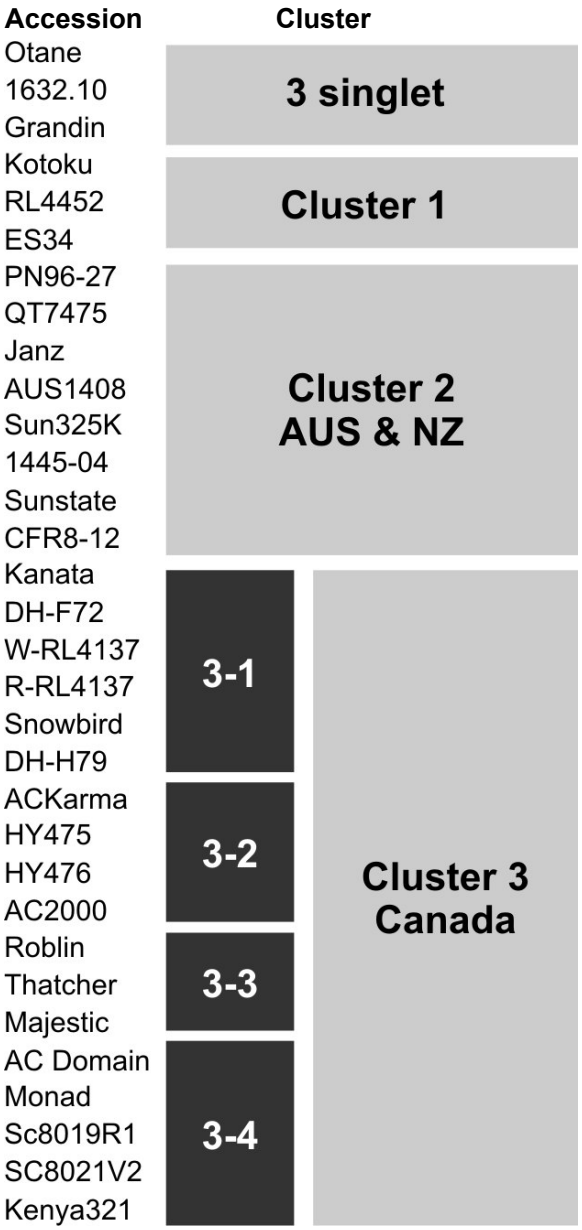


**Figure 1:** Clusters obtained using the neighbour joining method for 32 wheat accessions based on matrix of genetic distance (see 15) determined from microsatellite allele frequency.

Cluster 3-4 consisted of three Canadian PHS resistant lines (AC Domain, SC8019-R1 and SC8021-V2), the New Zealand cultivar Monad, and Kenya 321 of Kenyan origin. The latter two also had good PHS resistance. Wheat accessions in clusters 2, 3-1 and 3-4 had generally similar haplotype-based allele diversity as those observed for the PHS resistant accessions

AUS1408; Red-RL4137 and White-RL4137; and Kenya 321 respectively (data not shown, see 1).

## DISCUSSION

In this study, GI, SI, and FN were used to assess pre-harvest sprouting resistance in 32 accessions. These indices have been used previously (3, 4). While, high correlations were observed between the three methods, FN was generally the most consistent measure across environments indicating that FN determination alone could be sufficient for an accurate and reliable assessment of PHS (4, 6).

Genetic diversity is the backbone of plant breeding programs. The gene diversity revealed by the 33 markers used in this study ranged between 0.27 and 0.90, with an average of 0.72. For polyploid organisms, gene diversity is an estimate of the probability that two randomly chosen genes from a population are different and is not related to the expected proportion of heterozygotes, as for diploid organisms in Hardy-Weinberg equilibrium (17). The gene diversity was found to increase with the allele number, which is consistent with previous studies (8). PIC values, a measure closely related to gene diversity, varied from 0.25 to 0.90 were similar to those previously reported (18, 19) although higher allele numbers have been reported in studies where wheat genotypes with more divergent genetic backgrounds were used (8, 19).

The genetic relationships between germplasm are a useful means to identify structure within a set of genotypes (20). Our study identified three mains clusters and three singlet lines. The germplasm within the main clusters was generally related based on pedigree, origin, and PHS status. The power of the genetic structure in a cluster can be resolved based on the number of alleles subjected to clustering (21), bootstrap indices, but also on the genetic relatedness of the investigated taxonomic units. Our data were obtained from relatively closely related wheat germplasm and from 229 alleles. The New Zealand cultivars Monad, Kotuku and 1632.10 were found to be isolated from other New Zealand germplasm that have AUS1408 in their genetic background. This observation is not surprising because 1632.10, Monad, and Kotuku have unrelated pedigrees. The grouping of the New Zealand lines in the phylogram (Fig. 1) maybe related to ancestral relationships with North American wheat cultivars, which should provide guidance to wheat breeders as to choice of PHS resistant parents.

Three PHS resistance sub-clusters were identified within Canadian white wheat germplasm: the RL4137 lineage, the AC Karma lineage, and the Kenya321 lineage. Crosses between white wheat germplasm within and between lineages, as sources for PHS resistance, should be beneficial because pleiotropic effects between red seed coat colour and PHS resistance genes would be removed. Although narrow genetic variation may exist between lines of the same lineage, a wide range of variability for PHS resistance could be expected in germplasm derived from crosses between lineages. Finally, the AUS1408-derived germplasm in Cluster 2, offer the potential for broadening the genetic background of Canadian white wheat germplasm.

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