

# Validation of QTL for resistance to pre harvest sprouting

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## ABSTRACT

Pre harvest sprouting (PHS) is a serious problem in many wheat growing regions of the world, particularly in north eastern Australia. The condition results from grain germinating before harvest, and occurs when humid conditions or rain events occur after grain maturity. PHS results in downgrading of grain quality and can cause total crop loss in extreme years. Resistance to PHS has proved to be difficult to breed for in white wheats as part of a non-dedicated selection program, and there are no current Australian varieties with an acceptable level of resistance. Screening for resistance to PHS is labour intensive and is strongly influenced by environmental conditions. It would be useful to be able to use molecular markers to enrich early generations for resistance to pre-harvest sprouting, to significantly increase the percentage of lines showing good resistance in phenotypic tests undertaken in later generations. One major source of resistance to PHS has been identified in the line AUS1408. Several studies have mapped quantitative trait loci (QTL) for PHS from this source. In this study, to identify user-friendly markers and to determine the best strategy for application of these markers fine mapping has been undertaken in the region identified on 4A to be associated with a QTL for resistance to PHS, in four populations derived from AUS1408. The best strategy for selection at this locus was found to be the application of two flanking markers, gpw2279 and barc170. Diversity Array Technology (DArT) has been applied to two of these populations to develop frame-work maps, to be used for QTL analysis for resistance to PHS. In each case two years of phenotypic data was used. This was provided by Dr Daryl Mares. In addition to the QTL on 4AL a major QTL was identified in one of these populations, close to the centromere, on chromosome 3B. This region had previously been identified by Mares et al. (2005). A less significant QTL was found on chromosome 4B. Only the major QTL on 4AL was evident in the second population. The expression of the QTL on 3B may be influenced by genetic background. The major QTL reported on chromosome 5BL in a study by Tan et al. (2006) was not detected in this study. The affect on population enrichment using markers for the QTL on 4A and 3B was assessed.

## INTRODUCTION

The Northern Australian wheat growing region often experiences high humidity during the grain maturation

period. Climate change is predicted to bring more extreme weather conditions to this region. This will increase the intensity of storm activity in wet years, and will encourage wheat growing in more northerly climates, where rainfall is more predictable. This means that resistance to pre harvest sprouting (PHS) will be an even more important trait for new wheat varieties in the future.

PHS is a complex trait, which is labour intensive to phenotype, subject to environmental variation and until recently (Lee Hickey, pers. comm.) has been difficult to screen under controlled conditions. One genetic source of dormancy is AUS1408, white-grained, hard wheat. Though being used in a number of breeding programs the PHS resistance has been difficult to maintain when screening only in later generations. A good strategy for developing PHS resistant breeding material would be to apply markers during segregating generations to enrich populations for resistance. That way, with limited phenotypic screening available, for a given number of phenotypic tests, a much higher proportion of resistant lines would be recovered.

Several studies have reported the mapping of quantitative trait loci (QTL) for PHS resistance from AUS1408. Mares et al (2005) identified a QTL for PHS resistance on 4AL from both AUS1408 and the Chinese line SW95-50213, which explained between 17% and 21% of the trait variation in crosses involving these parents. Transgressive segregation was observed for the trait in progeny from a cross between these two PHS resistant parents, which suggests that though these two lines both carry a QTL for PHS resistance on 4AL, they each carry alleles contributing to PHS resistance at other loci not in common. Their results suggested that on its own, the 4A locus confers intermediate dormancy, and that the other loci, which appear to have little effect in isolation, interact with the 4A locus to produce a dormant phenotype. Tan et al. (2006) reported two significant QTL from a study using AUS1408. These were on 4AL and on 5BL. The 4AL QTL was expressed in all three years of testing, with phenotypic variance ranging from 5 to 15%, indicating a strong genotype x environment interaction.

The dormancy QTL identified on 4AL has been identified in a number of other studies. Mares and Mrva (2001) identified a QTL for resistance to PHS from the unrelated wheat variety Halberd on the same region of chromosome 4AL. Mori et al. (2005) found QTL for dormancy on chromosomes 3AS and 4AL from the Japanese line Zenkoujikomugi. Kato et al (2001)

reported a major QTL for dormancy on 4AL and two minor QTL on 4B and 4D, from the wheat variety AC Domain.

This study identifies optimal selection methodologies for the 4AL locus, through closely linked flanking markers. We identified a second major QTL for dormancy on chromosome 3B in one of two populations, suggesting a significant epistatic effect on this locus of genetic background. We assessed the usefulness of including selection for alleles at the 3B locus from a breeding point of view. Surprisingly we found that inclusion of this locus as a selection tool may not have a major positive effect on breeding outcomes.

## MATERIALS AND METHODS

**Plant material:** Four F1 derived doubled haploid wheat populations were used in this study. They were SUN325A/QT8349 (n=151); SUN325A/QT9685 (n=54); SUN325C/QT8349 (n=148) and SUN325C/QT9685 (n=93). The QT lines are breeding material from the QDPI&F wheat breeding program. SUN325A and SUN325C are sister lines (AUS1408/4\*Vasco) produced by Frank Ellison, Sydney University.

**Phenotyping:** Screening of these populations for dormancy was conducted by Daryl Mares in 2002 and 2004 at the Waite Campus of the University of Adelaide using methods described elsewhere (Mares et al 2005). The maximum index is 1.0 whilst lower indices are indicative of increasing levels of grain dormancy.

**DNA extraction:** For DArT analysis the DNA was extracted from leaves of 2-week-old wheat seedlings using their recommended method (<http://www.triticarte.com.au/content/DNA-preparation.html>). For simple sequence repeat (SSR) analyses a protocol supplied by Simon Berry was used for DNA extraction. This used a Qiagen grinder to grind freeze dried leaf samples and extraction using Tris-HCl, EDTA, SDS and ammonium acetate. DNA was precipitated in iso-propanol.

**SSR markers:** SSR markers from the gwm (Röder et al., 1998), wmc (Agrogene), barc (USDA-ARS Beltsville Agriculture Research Station) and gpw (Sourdille, INRA) sets were used for genotyping the 4A region and in some other regions identified in the literature to carry QTL related to dormancy. PCR conditions for the SSRs were based on those described by Röder et al. (1998), using 10µl reactions, including fluorescent labels for capillary electrophoresis. Fragments were run on a Beckman CEQ8800 capillary electrophoresis machine.

**Enrichment:** Enrichment values were calculated as (no. of resistant selected lines/no. of lines selected using marker(s))/(total no of resistant lines/total no. of lines).

**DArT:** DNA samples were sent to Triticarte Pty Ltd, Australia ([www.triticarte.com.au](http://www.triticarte.com.au)), for the wheat DArT service.

**QTL analysis:** Maps were constructed using MultiPoint software (MultiQTL Ltd. <http://www.multiqtl.com>; Mester et al. 2003; Mester et al. 2004) in combination with RECORD (Van Os et al., 2005). QTL were located using Map Manager QTX (Manly et al., 2001).

## RESULTS AND DISCUSSION

**Fine mapping:** For SUN325C/QT8349 in the region of chromosome 4A associated with the QTL for resistance to PHS the marker order and map distances were:

gwm397–10.1cM–gpw2279–5.8cM–gwm269–5.6cM–  
32% 39% 45%

barc170–30.6cM–wmc468

35% 4%

Percentage variation explained is indicated under the marker name. Marker order was the same in all four populations tested and map distances and percentage variation explained similar.

In each population for both 2002 and 2004 the peak of the QTL trace fell between gwm397 and barc170 and between gpw2279 and barc170 when gpw2279 was included in the marker set using MapManager to locate the QTL.

**Enrichment:** From a breeding point of view, it is important to know the level of enrichment of a segregating population for the desirable trait that would be achieved with marker assisted selection, and how many lines or individuals with the desirable trait would be discarded. Scenarios were assessed for level of enrichment and percentage of phenotypically resistant lines discarded for selection based on gwm269 only, and on combinations of pairs of markers, where lines were selected if either or both markers expressed the SUN325 allele. The aim was to maximise enrichment while minimising discarded resistant lines.

Based on available information, and the fact that the gwm269 marker is not very reliable in our hands, the optimum flanking marker combinations were gwm397 and barc170 and gpw2279 and barc170, where lines were selected with either markers expressing the SUN325 allele. For example for SUN325C/QT8349 in 2004 a 1.54 fold enrichment with 2.0% of resistant lines discarded was achieved, based on a cut off PHS score of 7, by selecting lines with the SUN325 allele at either gwm397 or barc170. A 1.40 fold enrichment with 4.4% of resistant lines discarded was achieved by selecting lines with the SUN325 allele at either gpw2279 or barc170. For the breeder this means that they would only need to phenotype 91 instead of 133 to recover 44 of total 46 resistants, while discarding 2 resistant lines.

Gwm269 and barc170, and gwm269 alone also gave a good result, as did combinations of three markers such as gwm269, barc170 and gpw2279. In general enrichments were higher and percentage of discarded resistant lines lower in 2004 than 2002.

*DArT map:* Two framework maps were constructed based on the SUN325C/QT8349 and the SUN325C/QT9685 populations. These maps included DArT markers and SSR markers in regions reported to contain QTLs for PHS.

For the SUN325C/QT9685 population, only one highly significant QTL (LOD 10 and 15) was detected. It was flanked by gwm397 and gwm269 on chromosome 4A in both 2002 and 2004. QTL were not detected on chromosome 3B, 4B or 5A despite a reasonable marker density on 3B and 5A.

For the SUN325C/QT8349 population the highly significant QTL (LOD 9 and 23) was detected on chromosome 4A, being flanked by gwm269 and wPt-4290 in 2002 and by barc170 and gwm397 in 2004. In addition, a significant to highly significant QTL (LOD 4 and LOD 5) was detected on chromosome 3B flanked by wPt-0086 and gwm285, with a peak near gwm077 in each year. A significant QTL (LOD 3 and LOD 2) was detected on chromosome 4B distal to wPt-0391, with a peak near gwm107b each year. A suggestive QTL (LOD2) was detected on chromosome 5A between wPt-0605 and wPt-4131 in 2002 but not 2004. A suggestive QTL (LOD2) was detected on chromosome 7A between wPt-1928 and wPt-0205 in 2002 but not 2004. Again in this population no QTL was detected on chromosome 5B.

*Enrichment for second locus:* A chi-squared test for independence from PHS score is not significant for gwm077 alone for the 2004 results from SUN325C/QT8349, despite the Multipoint analysis identifying a highly significant QTL. If marker assisted selection was based on selecting the SUN325 allele at either barc170 or gpw2279 on chromosome 4A and the SUN325 allele at gwm077 on chromosome 3B, an enrichment of 1.86 fold would be achieved, but 17 of the resistant lines would be discarded. This level of error is probably unacceptably high. Thus, despite a major QTL being located in this region of chromosome 3B, from a breeding point of view applying selection based on this locus may not lead to a desirable result, and should be applied in the knowledge that a high number of resistant lines will be lost.

*Conclusion:* It is important to consider the outcomes from application of marker assisted selection in a breeding context. Selection based on markers associated with even highly significant QTL may not always result in the optimum outcome. Which markers to apply and in which combination should be decided in light of the

breeding generation, the desired selection pressure, the size of the population and the difficulty and cost involved in phenotypic screening.

## ACKNOWLEDGMENTS

Thanks to QDPI&F, Enterprise Grains Australia and GRDC for funding this work through the Australian Winter Cereals Molecular Marker Program.

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