VALUE ADDED WHEAT CRC PROJECT REPORT

Molecular Marker Development for seed dormancy in white wheat

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1. Background

Pre-harvest sprouting causes an accumulation of α-amylase in wheat grains resulting in downgrading of wheat quality and loss of premiums to farmers, which can range from a few percent to the loss of the entire crop. A major factor contributing to pre-harvest sprouting is an inadequate level of seed dormancy. It has been established that there is considerable genetic variation in grain dormancy in both white- and red-grained wheat germplasm. The Australian wheat industry is based almost exclusively on white-grained cultivars and Aus1408 is currently being used as a major source of tolerance in Australian breeding programs.

Molecular marker development for seed dormancy in Aus1408 was first embarked on a F2 population derived from Janz X Aus1408. This population was found to possess an insufficient level of polymorphisms for the generation of molecular marker maps for correlation with the trait. A second attempt with a doubled haploid population derived from Janz X Aus1408 also failed due to 'contamination' (presence of progenies not derived from the parents) in the population. The mapping population in this report is a doubled haploid population derived from Cascade and Aus1408.

Previous work by Dr. Mares suggested the location of two dormancy genes on chromosome 3DL Research outcomes reported by various research groups (see manuscript attached) have also implicated the possible involvement of loci on group 3 chromosomes with seed dormancy.

However, intensive genotyping and mapping of group 3 chromosomal markers did not uncover any major QTLs on group 3 chromosomes with seed dormancy in Aus1408. The report of a grain dormancy locus on chromosome 3DL by Dr. D. Mares was based on the Oxley/Aus1408 substitution lines. Mapping of 3DL specific markers revealed the presence of recombination in these 3DL substituted lines, leaving the correct identification of these lines in doubt.

A skeletal mapping of molecular markers on the whole genome was thus conducted to search for linkages with seed dormancy in Aus1408. The attached manuscript reported on the significant QTLs linked to seed dormancy in Aus1408 and the

comparative mapping to putative orthologous seed dormancy QTLs in barley and rice. It also discussed the confounding factor of black point expression with sprouting tolerance. A validation report is also attached to show the results of the screening of these QTLs with some selected wheat lines with known dormancy phenotype.

2. Manuscript

Mapping quantitative trait loci associated with grain dormancy in white wheat

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ABSTRACT

A white-grained wheat genotype, Aus1408, is currently being used as a major source of pre-harvest sprouting tolerance in Australian breeding programs. A doubled haploid population of 83 lines, derived from the cross; Cascade X Aus1408, was tested for sprouting tolerance in field trials over 4 years, 1999-2002. Six QTLs derived from Aus1408, located on chromosome arms 1A, 2AL, 3AL, 4AL, 5BL and one from Cascade (on chromosome 7BS) contributed to sprouting tolerance, and collectively accounted for 40-63% of the phenotype. A contribution to pre-harvest sprouting tolerance by a QTL on 2DS has been observed in environments where the black point phenotype has been expressed. This QTL on 2DS is primarily linked to its genetic susceptibility to black point, and has no role in the seed dormancy mechanism. Most QTLs accounted for <10% of the phenotype and were not expressed in all years of testing, indicating a genotype X environmental interaction. There was possible conditional epistasis in the expression of a major QTL on chromosome 4AL, which was reduced by the presence of black point affected grain. Comparative mapping has enabled the detection of orthologous QTLs in other closely related grass species (rice and barley)

and provided added evidence to the possible genetic effects of these QTLs to the trait. Identification of these 5 QTLs from Aus1408 should enable sprouting tolerance derived from this source to be incorporated into advanced breeding lines of white wheats, with the use of molecular markers, reducing the requirement of multi-year field testing for sprouting tolerance.

INTRODUCTION

Pre-harvest sprouting (PHS) in bread wheat in response to rain prior to harvest leads to a reduction in grain quality (Buchanan & Nicholas 1980) and significant grain yield and economic losses for growers. PHS is common in parts of Australia and many other wheat growing areas of the world, and losses can range from a few percent to the loss of the entire crop. A major component of PHS in wheat, and other cereals, is grain dormancy and it has been established that there is considerable genetic variation for this trait in both white- (Mares 1987, Jiang et al. 1995) and red-grained wheat germplasm (Derera 1989). The Australian wheat industry is based almost exclusively on white-grained cultivars and since PHS represents a limitation to the reliable production of high quality grain for export and domestic use, there is considerable incentive to improve tolerance to PHS through incorporation of grain dormancy and/or other PHS tolerance mechanisms. Breeding for wheats with an adequate level of dormancy has been hampered by the lack of knowledge on the number of putative genetic factors involved in its expression and their interaction. This complex quantitative trait is also confounded by environmental factors.

Screening and selection on the basis of phenotype is thus difficult and DNA markers linked to genes involved in PHS represent a more reliable, environment-insensitive tool for selecting genotypes more resistant to PHS.

PHS has been shown to be a complex quantitative trait affected by several genetic factors on different chromosomal regions in barley (Oberthur et al. 1995, Feng et al. 1999), wheat (Anderson et al. 1993, Roy et al. 1999, Mares & Mrva 2001, Groos et al. 2002; Zanetti et al. 2000), rice (Lin et al. 1998) and sorghum (Lijavetzky et al. 2000).

Red grain colour has long been associated with seed dormancy, although it is now clear that red colour *per se* is not sufficient to guarantee dormancy (Mares 1999, Flintham et al. 1999). Combining dominant *R* alleles (*R-A1, R-B1 and R-D1;* McIntosh et al. 1999) at two or three loci has been reported to confer an additive effect on dormancy (Flintham 1993). The *R* genes have been mapped some 60cM from the centromere on the long arms of the group 3 chromosomes in wheat (Flintham & Gale 1996). These genes would be expressed through a coat-imposed dormancy mechanism (Gale, 1989; Flintham et al, 1999) in keeping with the conclusion from inheritance studies that dormancy in wheat involves at least one factor from the maternal seed coat (Mares 1993).

Mares (1996) reported grain dormancy loci on chromosome 3DL of a white-grained wheat genotype, Aus1408, that is currently being used as a major source of tolerance in Australian and overseas breeding programs. Genetic studies have suggested that a gene controlling sensitivity to germination inhibition by ABA (ABA-sensitivity) and the seed coat factor are both recessive and largely independent, but may be on the same

chromosome. The presence of orthologous embryo dormancy genes independent of the grain colour on homoeologous group 3 chromosomes had been suggested in work of Miura et al. (1996).

Flintham et al. (1999) suggested a putative relationship between the locus reported by Mares (1996) affecting embryo dormancy on chromosome 3D and the maize seed dormancy related gene, *Vp1* (McCarthy et al. 1989). The wheat orthologous gene, *taVp1*, has been mapped on the long arms of wheat chromosomes 3A, 3B and 3D (Bailey et al. 1999), some 30cM proximal to the red grain (R) loci that control seed colour.

The *Vp1* gene plays a critical role in the induction and maintenance of dormancy in maize (McCarty et al. 1991). An association between PHS and *Abi3*, a gene with a high level of homology with *Vp1* has been observed in *Arabidopsis* (Giraudat et al. 1992, Van der Schaar et al. 1997). The sequence information of the 3 homoeologous *taVP1* genes (Bailey et al 1999) were utilised to analyse for a correlation, if any with PHS in the Cascade X Aus1408 DH population.

Anderson et al. (1993) and Sorrells and Anderson (1996) reported several RFLP markers for pre-harvest sprouting were located on chromosomes of groups 1, 2, 3, 4, 5 and 6 using QTL analysis of wheat. More recently, a huge QTL associated with differences in grain dormancy or PHS have been identified on the proximal portion of chromosome 4AL (Kato et al. 2001, Flintham et al. 2002, Mares and Mrva 2001), which is homeologous to one of the 4 QTLs controlling seed dormancy in barley (Oberthur et al. 1995).

Wheat is allohexaploid with 42 chromosomes and having been subjected to domestication and intensive selection during breeding, presents great challenges to the search for genetic polymorphisms that could be used for marker-assisted selection of agronomically important genes or QTLs in wheat breeding. Comprehensive RFLP (restriction fragment length polymorphism) linkage maps (McGuire and Qualset 1997) have been developed for all seven homeologous groups in allohexaploid wheat. Use of these RFLP markers for mapping important agronomic traits is severely limited, however, by the low level of polymorphism in wheat.

Microsatellites, or simple sequence repeats (Tautz, Trick and Dover 1986) with a basic motif of <6 bp, have served as an important source of ubiquitous genetic markers for many eukaryotic genomes (eg Wang et al. 1994). Microsatellites show a much higher level of polymorphism in hexaploid wheat than RFLPs (Röder et al. 1995). Microsatellite markers [gwm, (Roder et al. 1998); wmc (Agrogene)] have been utilised in this study to search for polymorphic genetic markers associated with resistance to PHS in white-grained wheat. These markers must be easy to implement in a high throughput system for aided selection in wheat breeding.

MATERIALS AND METHODS

Genetic Stocks:

A doubled haploid (DH) population (83 lines) derived from Cascade and Aus 1408 (obtained from P Williamson, Leslie Research Centre, Toowoomba, Qld, Australia) Australia, was used for the mapping of the linkage groups. AUS1408, a dormant white-grained wheat from the Transvaal region of South Africa was obtained from the Australian

Winter Cereals Collection, Tamworth, NSW, Australia (Mares 1987). Cascade is a white-grained, non-dormant cultivar grown commercially in Australia.

Characterisation of grain dormancy phenotype

Genotypes and breeding of doubled haploid lines were sown as small plots (single rows by 0.5m) in the field at Narrabri in 1999, 2000 and at the Waite campus in 2001. During the later stages of ripening, from just prior to physiological maturity until harvest was completed, the replicated experiments consisting of small plots of DH lines and parents were covered with translucent, white plastic to avoid confounding effects of rain (Mares 1989; Trethowan 1995). The progress of ripening in all trials was monitored visually at 2-day intervals taking note of the loss of chlorophyll from the leaves, stems, and spikes. Experience with wheat ripening in this location indicated that the complete loss of green colour from vegetative structures corresponded to the cessation of transpiration and grain moisture content of 16 – 18%. (Mares 1989). Spikes were harvested from each line and parent cultivar when all green colour had just disappeared from the leaves and stems, stored under cover at ambient temperature for 5 days, and then grain recovered by gentle hand-threshing (Mares 1989). During the 5-day storage, moisture content declined to below 12% (harvest-ripeness). After 5 days the grain was transferred to – 20^0C to preserve dormancy (Mares 1983) until all plots had been harvested and all the seed required for germination testing was available.

Germination test

A germination test to determine the Germination Index (GI) for each line in the population in the various field trials was as published in Mares and Mrva (2001). This index gives maximum weight to grains that germinate

rapidly. The maximum index is 1.0 if all grains germinate by day 1 whilst lower indices are indicative of increasing levels of grain dormancy or reduced germinability.

Germination Index, GI1 was performed on grains from field trials in 1999 at Narrabri, GI2 and GI3 on grains from 2 replicate trials in 2000 at Narrabri and GI4 on grains from trial in 2001 at Waite Campus, University of Adelaide, South Australia.

DNA Extraction

Wheat leaf fragments (5 x 1cm lengths) were placed in a small microfuge tube and ground to a fine powder in liquid nitrogen using a pellet pestle (Sigma Z35,994-7). DNA was extracted from the fine leaf powder using the DNeasy Plant Mini Kit (Qiagen).

Typing with microsatellite markers

Microsatellite markers from Röder *et al.* (1998) and the Wheat Microsatellite Consortium (Agrogene) were used as described. Fragments were run on either 10-12% polyacrylamide gels (20 X 20 cm) or 6% denaturing polyacrylamide gels and visualized using silver staining.

Map construction

Linkage analyses were performed using MapManager QTXb15 (Manly and Olson 1999) based on the likelihood ratio statistic (LRS). Permutation tests were carried out on associations suggestive of QTLs, with Map Manager set for 1 cM intervals and 1000 iterations. Linkage groups developed from microsatellite markers (*gwm* and *wmc*) were

assigned to chromosomes via comparisons to reference maps using microsatellite loci (Röder et al. 1998, Chalmers et al. 2001).

Map manager files were also exported to the Cartographer file format for analysis with the QTL Cartographer v1.16 (Basten et al. 1994, 2002). Programs used to dissect the various quantitative traits included stepwise regression, interval mapping, composite interval mapping and multiple interval mapping. Analysis was undertaken using program Zmapqtl which implemented interval and composite interval mapping (CIM) of a prior output file from program SRmapqtl (FB option) which searched QTLs using stepwise regression. The default values of 5 for n_p (number of markers to control for the genetic background) and 10 cM for w_s (window size for analysis) were used in the Zmapqtl program. The program MImapqtl which implements QTL mapping analysis for multiple QTL in multiple intervals for a single trait in a single environment, was ran using the option smprtSeC which searched for QTLs starting with an initial model containing no QTL (MIM). The output model was then refined and tested for significance using the option sMPRTseC. Results for each environment were presented in Table 2. Dormancy data obtained under different environments were separately analysed.

RESULTS

Variation of Phenotype

The germination indices (see 'Methods') of the Cascade X Aus1408 DH population ranged from <0.1 to >0.7 (Fig.1). Frequency distributions of the indices for GI1 and GI2 appeared to be near normal but not that of GI3 and GI4 (Fig. 1).

Mapping and analysis of correlation of taVp1 gene

Two pairs of primers, *Vp1f1/Vp1r1* and *Vp1f3/Vp1r3*, were designed from *T. aestivum* Vp1 gene (*taVp1*) to achieve amplification of 3 fragments corresponding to the 3 genomes. The primer pair, *Vp1f1/Vp1r1* amplified 3 fragments of 480bp, 519bp and 571bp and the *Vp1f3/Vp1r3* pair amplified 3 fragments of 275bp, 314bp and 386 bp corresponding to the 'A', 'B' and 'D' genome respectively.

Typing of the primer pairs on the Cascade/Aus1408 population revealed that the polymorphic allele that segregates using both of the primer pairs were from the 3A genome. The alleles from the 3D and 3B genome were found to be non-polymorphic.

The *taVp1* gene was found to have a correlation with PHS in three out of the four trials (Table 1). The LRS values ranged from 6.4 to 7.2 ($p<0.01$) (only suggestive with permutation tests of 1000 reiterations), with an almost constant phenotype contribution of 8% in all three environments. QTL Cartographer analysis suggested a significant association of *taVp1* with seed dormancy only for GI3 and a weak association for environment GI4.

Typing of microsatellite markers

A total of 115 polymorphic microsatellite markers was typed in the Cascade X Aus1408 DH population. Analysis using the additive regression model in Map Manager QTXb15 with chi square statistics of at least $p=0.05$ (Table 1) identified QTLs on chromosome arms 1A, 2AL,

2DS, 3AL, 3BL, 4AL, 4BL, 5BL and 7BS. The left flanking markers of the respective QTLs and their corresponding statistics from the analysis were presented in Table 1. Permutations with 1000 reiterations at $p<0.01$ indicate suggestive, significant and highly significant LRS values respectively for each of the environments as GI1(7.1, 13.3, 25.1); GI2(7.2, 13.2, 21.7); GI3(7.2, 13.9, 21.2) and GI4(7.2, 14.0, 22.8).

The program QTL Cartographer v1.16 identified similar QTLs on the same chromosomes except 3BL and 4BL (Table 2). The significance of the same QTLs differed in the two analyses.

The most significant QTL is the marker gwm397, which is about 10-15 cM from the centromere on 4AL. This QTL was expressed very strongly (LRS: 23.5, 29% phenotype contribution, $p = 0$) only in one trial (GI4) out of the 4 trials measured in 3 years. It was expressed weakly (LRS=5-6% phenotype contribution, $p=0.025$) in 1999 (GI3). This QTL was not detected in the other two trials. QTL Cartographer gave similar results with higher LRS scores and greater phenotypic variance contribution for the same 2 trials (Table 2).

The next most significant QTL is the marker gwm513 on 4BL. It was expressed (14% phenotypic contribution) in only one (GI1) out of the four environments, with a LRS of 12.2 and a $p=0.001$. QTL Cartographer using programs as listed under 'Materials and Methods' did not detect any QTL on 4BL.

The other QTLs, *Xgwm 164* on 1A, *Xgwm 356-Xgwm294* on 2AL, *Xgwm108* on 3BL, *Xgwm 604-Xgwm639a* on 5BL were all suggestive with low LRS values (Table 1) using Mapmanager QTXb15. However,

their contributions of between 5% - 9% were observed in three out of four trials observed except for *Xgwm108* on 3BL which was observed only in two.

A weak QTL (LOD 2.03) on 1AS, *Xgwm*33 (Table 2) was detected for the trial GI2. QTL Cartographer analysis suggested that the QTL on 2AL (*Xgwm294*) was significant (LRS 12.0) in the trial GI3. Similarly, the QTL on 5BL(*Xgwm604-Xgwm639a*) was significant (LRS=12) in the trial GI1.

QTLs with contribution of PHS from Cascade were identified with *gwm484*, which is about 45cM from the centromere on 2DS. This was observed in three trials, GI1, GI2, GI3, with LRS values of 4.3 (p=0.04), 12.9 (p=0.017), and 12.6 (0.00038) respectively. Another QTL for PHS from Cascade was identified in the region *Xgwm297-Xgwm333* on 7B (Table 1 and 2).

DISCUSSION

Group 3 and taVP1 loci

The *taVp1* locus was mapped to approximately 30- 40cM from the centromere on 3AL (Fig. 2). Groos et al. (2002) located significant QTLs for pre-harvest sprouting tolerance on 3AL, 3BL and 3DL of red kernel, Renan to co-localize with the *R* genes for red grain colour. The QTL for the *R-A1* gene on 3AL (Fig. 4 in Groos et al. 2002) is distal to the marker Xgwm 155 (Fig. 2). Thus the *taVp1* locus in Aus1408 is proximal to the

QTL for *R-A1* in Renan. The relative position of the *taVp1* locus and the *R-A1* gene thus agrees with the result of Bailey et al (1999).

The *taVp1* gene was found to have a linkage ($p<0.05$) with seed dormancy in AUS1408 but contributed to only about 8% of the phenotypic variance (Table 1) in three of the four trials. Differential expression of the *taVp1* gene has been reported between dormant and non-dormant wheat genotypes (Nakamura & Toyama 2001). The degree of differences was however not mentioned. In contrast, Osa et al. 2003 reported that the *taVp1* gene did not play a role in seed dormancy in the red wheat Zen.

McKibbin et al. (1999) has shown that only a slight loss of embryo dormancy was associated with a decrease in *taVp1* gene expression. Expression analyses of the *taVp1* gene in wheat embryos have shown that the majority of transcripts are incorrectly spliced, a genetic defect that has been inherited from ancestral species (McKibbin et al. 2002). This compromised regulation of the *taVp1* expression may explain for the low contributions of this gene to PHS as is also evidenced from analysis of the Cascade x Aus1408 data which suggested low LOD scores and low phenotypic variance (Table 1 and 2).

On the contrary, a significant role in the control of dormancy has been reported for the *afVp1* gene from *Avena fatua* (Jones et al. 1997), *Vp1* from maize (McCarty et al. 1991), *Abi3* from *Arabidopsis thaliana* (Giraudat et al. 1992) and the *vp1* gene of sorghum (Lijavetzky et al. 2000, Carrari et al. 2001).

The *taVp1* gene is orthologous to the maize *VP1* gene (McCarty et al. 1991) on chromosome 3 and the rice *osVp1* on rice chromosome 1 (Bailey et al. 1999). The locations of QTLs for PHS on rice chromosome 1 reported by Cai and Morishima (2000) mapped to positions near the centromere and are proximal to the *osVp1* gene determined by Bailey et al. 1999. Hence it appeared that the *osVp1* gene is not linked to seed dormancy in the populations examined so far.

The chromosomal region, *wmc264-wmc428* near the centromere on 3AL and proximal to the *taVp1* locus was found to have some linkage ($p<0.05$) to PHS with a phenotypic contribution between 2.5% to 9% in three of the four environments tested (Table 1). This genetic region apparently maps to the same vicinity as the QTL on 3AL identified in the red grain wheat Zen (Osa et al. 2003). Comparative maps showed that this region is also possibly linked to the rice seed dormancy QTL on chromosome 1 proximal to *osVp1*(Cai and Morishima 2000, Fig. 2).

Another more significant QTL associated with seed dormancy of the red grain wheat, Zen was identified on the terminal region of 3AS (Osa et al. 2003). No markers on 3AS have been mapped in Cascade X Aus1408 and thus further mapping is required to determine the presence of any QTL for seed dormancy on 3AS of Aus1408.

Due to the non-polymorphism of fragments derived from the 3B and 3D genome between the cultivars, Aus1408 and Cascade, it was not possible to infer whether homeologous *taVp1* alleles on 3B and 3D also correlate with dormancy. Further work involving other regions of the *Vp1* gene are being explored for the possible mapping of these alleles.

A minor QTL has been suggested for marker *Xgwm108* on chromosome 3BL (Table 1) of Aus1408. A QTL for PHS proximal to the R-B1 gene has been suggested (Fig. 4,Groos et al. 2002), which appears to be in the same vicinity as the locus, *Xgwm108*.

Fifteen polymorphic 3DL-specific microsatellite markers were typed on the Cascade x AUS1408 DH population. Seven markers (*Xgwm)* were linked into one linkage group (Fig. 4) by Map Manager Mqtxb15. The *R-D1* gene on 3DL in 'Renan' was mapped to the region *Xgwm314-Xgwm3-Xcfd9* (Fig. 4, Groos et al. 2002).

There are seven *Xgwm* loci including *Xgwm314,* which are proximal to the *R-D1* gene (Fig. 4). The *taVp1* allele on 3D would presumably be on this chromosomal region. However, no QTL linked to PHS has been suggested in this region. There thus appears to be no contribution by the 3D allele of the *taVp1* gene.

Highly significant/Significant QTls on Group 4

A major QTL for PHS was detected for Aus1408 in the region *Xgwm397-Xwmc161* on chromosome 4AL. This QTL is proximal to the marker *Xwmc161* (Fig.2) as well as proximal to the marker *Xpsr115* and is likely to be in the same chromosomal position as the major QTL on chromosome 4AL identified for the red wheat, AC Domain (Kato et al. 2001). A QTL on 4AL was also identified in the Cranbrook X Halberd DH population but this was contributed from the very susceptible parent, Cranbrook (Mares and Mrva 2001).

This QTL was expressed in only two of the four environments examined. It has a highly significant LRS value of 23.5 with a phenotypic contribution of 29% in GI4 (Table 1 and 2, Fig.3) but only suggestive (LRS =6.5) with a greatly reduced contribution of 7% in GI1 and not detected in the other 2 trials. QTL Cartographer reported significance of the QTL on 4AL in the same two trials (Table 2). The 4AL QTL in the red wheat, AC Domain (Kato et al. 2001) was similarly reported to be highly significant accounting for phenotypic variance from 33% to 86%. This QTL is homeologous with one of the barley QTLs for seed dormancy on chromosome 4H (Kato et al. 2001) and possible a seed dormancy QTL on chromosome 6 of rice (Fig. 2).

Marker gwm 513 on 4BL was found to have a significant association (LRS=12.2) with PHS only in one environment, GI1, with a phenotypic contribution of about 14%. This QTL appears to be located on the same terminal region of 4BL where a minor QTL has been reported for AC Domain (Kato et al. 2001). However, no QTL on 4B associated with seed dormancy was suggested by analysis using the program QTL Cartographer. A minor QTL on 4DL (Table 1) was observed in Aus1408 and further mapping is required to confirm its correlation with a QTL reported on 4DL on AC Domain (Kato et al. 2001).

Embryo dormancy is related to sensitivity to ABA and a gene on the middle region of 4AL was reported to have the highest sensitivity (Noda et al. 2002). Further work is thus required to determine the association if any of the seed dormancy QTL on 4AL with this ABA-sensitive gene on 4AL.

Suggestive QTLs on 1A, 2AL and 5BL

A QTL around the centromere of 1A around the locus, *Xgwm164* was observed in 3 environments with a small phenotypic contribution of around 7-9% (Table 1). This QTL apparently maps to the same genetic region of one of the QTL linked to the CDO431 locus for a white-grained wheat (Anderson et al. 1993; Sorrells & Anderson 1993).

A QTL linked to marker, *gwm33* on chromosome 1AS was suggested in only one of the four trials, GI2 (Table 2) with a phenotypic contribution of about 8.5%. This region has been comparatively mapped to a rice QTL for seed dormancy on chromosome 5, whose phenotypic contribution (about 7%) was in the same range (Lin et al. 1998). A QTL on 1AS (*Xbcd1434*) had also been reported in a white kernel wheat with a similar range of phenotypic variance of about 10% (Anderson et al. 1993).

A QTL linked to a region between the markers *gwm294* and *gwm356*, about 40-65 cM from the centromere on chromosome 2AL was observed in Aus1408. Map Manager QTXb15 gave a suggestive LRS value ranging from 3.8 to 8 in three of the four trials. QTL Cartographer suggested a significant association (LRS 12) in environment GI3. A significant QTL was also observed on chromosome 2AL in the Cranbrook X Halberd DH population with the allele contribution from the non-dormant parent, Cranbrook (Mares & Mrva 2001). Further mapping is required to determine if these 2 positions were in the same chromosomal region. A region near the centromere on 2AL is syntenous to regions on rice chromosome 7 (Wheat Cornell Synthetic X Opata 1995 and Rice Cornell RFLP 2001 in http://www.gramene.org). Two QTLs on rice chromosome 7 (Lin et al. 1998) have been reported but more

mapping is required to determine if the QTLs are linked to the wheat QTL on 2AL.

A QTL on 5BL is located between *gwm 639a-gwm604*, about 25 cM from the centromere. Map Manager and QTL Cartographer attributed different significance to the QTL (Table 1 and 2). Map Manager analysis gave low LOD scores with phenotypic variance from 5-7% (Table 1). The QTL Cartographer suggested that the QTL on 5BL is significant (LRS=12) with phenotypic contribution of 10-20% (Table 2). A QTL mapped to chromosome 5L (using *BCD1874* and *BCD450*) in white wheat has also been reported (Sorrells and Anderson 1995) but further mapping is required to determine if the QTL on 5BL is homeologous to *Xbcd1874-Xbcd450*, which is on 5DL. This QTL has been cross-mapped to major seed dormancy QTLs in barley on chromosome 5 (Ullrich et al. 1993, Han et al. 1999) and in rice on chromosome 3 (Lin et al. 1998, Cai and Morishima 2000, Fig. 2).

Contributions to PHS from Cascade

A very significant QTL on 2DS with marker *gwm484* for PHS was observed with contribution from Cascade. This QTL was significant in two trials, GI1 and GI3 (Table 1) with contributions estimated to be around 15%. In the environment GI2, it is insignificant and not detected in GI4.

Analysis of the genotype data with the black point data (Mares D. personal communication) uncovered the same genetic region, *Xgwm112b-Xgwm 484* on 2DS to be significantly linked to the expression of black point by Aus1408. Correlation of the 2DS genetic region to black point

phenotype data is highly significant (LRS=18 for black point data at Narrabri; LRS=31 for that at Toowoomba). It has been reported that black point has been associated with a reduction in the rate of germination and seedling emergence (Zhang et al. 1990). Thus the QTL on 2DS is primarily linked to its genetic susceptibility to black point, and has no role in the seed dormancy mechanism. Hence, a contribution to pre-harvest tolerance by the 2DS QTL is only observed in the environments where the black point phenotype has been expressed.

A significant QTL for PHS from Cascade was identified with the genetic region *Xgwm333-Xgwm297* on 7B. This may thus represent a valuable source of PHS in addition to the primary source from Aus1408 in wheat breeding. This contribution could possibly explain for the presence of transgressives with higher PHS tolerance than Aus1408 in the DH population.

CONCLUSION

Reports on major QTLs for seed dormancy were disproportionately from red grain wheat (Kato et al. 2001, Groos et al. 2002, Osa et al 2003, Miura et al. 2002). This work reported on major QTLs from white wheat cultivars, Aus1408 and Cascade. Variation in grain dormancy in the Cascade X Aus1408 DH population is caused by segregation at multiple QTLs. This has similarly been reported for white kernel wheat (Anderson et al. 1993, Sorrells and Anderson 1996) and other species like sorghum, rice, maize and barley. A small number of genetic loci had been found to contribute significantly to grain dormancy in Aus1408. These include the QTLs associated with marker *Xgwm 397* on 4AL, with markers *Xgwm294-Xgwm356* on 2AL, and markers *Xgwm604-Xgwm639a* on

5BL. Analysis of genotype and phenotype data in the four trials suggested very strong genotype-environment interactions and other environmental variance on expression of these QTLs. Minor QTLs were located on chromosome 1A, 3A and 4B. The total phenotypic variance is thus attributed to components that are additive, dominant, epistatic, genotype-environment interactions and other environmental variance. This work is confined to only estimates of the heritability with no control of environmental interactions.

Comparative mapping has enabled the detection of orthologous QTL in other closely related grass species (e.g. rice and barley), provided added evidence to the possible genetic effects of these QTLs to the trait. However the factors governing their expression and regulation of seed dormancy are not understood. A small number of different genetic loci contributed to a significant proportion of the variance of the trait providing a feasible target for further molecular investigation. Matching the QTL to a genetic locus requires high resolution recombination or linkage disequilibrium mapping to nominate putative candidate gene followed by genetic and/or functional complementation and gene expression analyses.

These QTLs have been validated with some selected wheat lines with Aus1408 as the dormancy source. Validation has confirmed the preferential selection of the Aus1408 alleles on 4AL and 5BL in the sprout tolerant lines and their absence in the non-tolerant lines (see Validation report pg. 39). Thus the QTLs on 4AL and 5BL have been validated to be useful for implementation in marker-assisted selection for the breeding of Australian wheat germplasm with the appropriate level of seed dormancy at harvest ripeness.

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Table 1: Putative QTLs of seed dormancy in Aus1408 and Cascade from regression analysis using Map Manager QTXb15 as explained under 'Methods'.

Chrom	NML[a]	LRS	LOD	Effect %[b]	Probability	GI1	GI2	GI3	GI4
Aus1408									
1A	Xgwm164	5.6	1.2	7	0.018		♦		
1A	Xgwm164	7.7		9	0.006			♦	
1A	Xgwm164	6.3	1.4	9	0.012				♦
2AL	Xgwm294-Xgwm356	3.8	0.8	5	0.050		♦		
2AL	Xgwm294-Xgwm356	7.1		8	0.008			♦	
2AL	Xgwm294-Xgwm356	4.6	1	5	0.032	♦			
3AL	Xwmc264	6.8	1.5	9	0.009		♦		
3AL	Xwmc428	5.4	1.2	6	0.019	♦			
3AL	*Xwmc428*	5.1		6	0.02				
3AL	*taVp1*	7.2	1.6	8	0.007	♦			
3AL	*taVP1*	6.4	1.4	8	0.012		♦		
3AL	*taVP1*	5.5		7	0.019			♦	
3BL	Xgwm108	5.4	1.2	6	0.020	♦			
4AL	Xgwm397-Xwmc161	6.5	1.4	7	0.011	♦			
4AL	Xgwm397	23.5	5.1	29	0.000				♦
4BL	Xgwm165a	10.4	2.3	12	0.001	♦			
4BL	Xgwm513	12.2	2.6	14	0.001	♦			
4BL	Xgwm368	10.5	2.3	12	0.001	♦			
4DL	Xwmc48	9.2		13	0.002				♦
5BL	Xgwm604-Xgwm639a	4.2	0.9	5	0.041	♦			
5BL	Xgwm604	3.9		5	0.05			♦	
5BL	Xgwm604-Xgwm639a	5.4	1.2	7	0.020				♦
Cascade									
2DS	Xwmc112b-Xgwm484	12.6	2.7	14	0.0004	♦			
2DS	Xwmc112b-Xgwm484	4.3	0.9	6	0.038		♦		
2DS	Xwmc112b-Xgwm484	11.4		13	0.0007			♦	
7BS	Xgwm297-Xgwm333	4.9	1.1	6	0.03	♦			
7BS	Cfa2174b-Xgwm297	13.5	2.9	16	0.0002		♦		
7BS	Xgwm297	6.8		8	0.009			♦	

[a] Nearest marker locus of putative QTLs or flanking markers

[b] Phenotypic variation explained by each QTL

Table 2: Putative QTLs of seed dormancy in Aus1408 and Cascade from analysis using QTL Cartographer programs as explained under 'Methods'.

Chrom	NML[a]	GI1			GI2			GI3			GI4		
Cascade		CIM	MIM		CIM	MIM		CIM	MIM		CIM	MIM	
		LRS	LRS	%[b]	LRS	LRS	%[b]	LRS	LRS	%[b]	LRS	LRS	%[b]
1AL	Xgwm33				12	7	9						
1AL	Xgwm164												
2AL	Xgwm294							12	12	15			
3AL	Xwmc050-Xwmc428				7	3	6						
3AL	Xwmc264-*taVp1*	16	10	9									
4AL	Xgwm397-Xwmc161	14	10	15							36	45	63
4BL	Xgwm513-Xgwm368												
5BL	Xgwm639a	12	11	18				5	4	10			
2DS	Xwmc112b-Xgwm484	28	20	21				14	25	30			
7BS	Xgwm333-Xgwm297				27	23	21						

[a] Nearest marker locus of putative QTLs

[b] Phenotypic variation explained by each QTL as determined by composite interval mapping (CIM) and multiple interval mapping (MIM) with their corresponding likelihood ratio statistic (LRS)

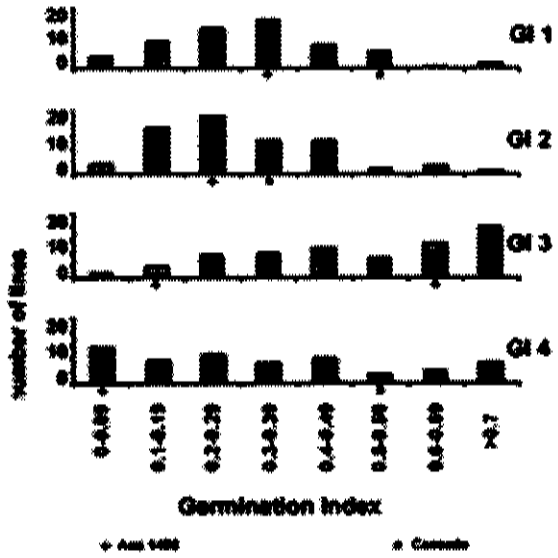
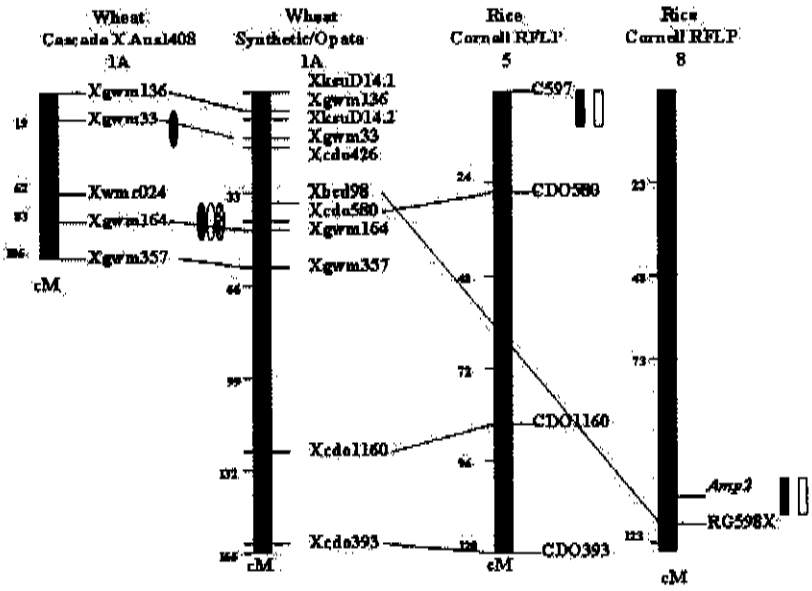


Fig. 1: Frequency distributions of germination indices, GI1 to GI4, of Cascade x Aus1408 measured between 1999 and 2001. The germination indices of Cascade and Aus1408 are marked as indicated in the appropriate intervals for each of the field trials.

Fig. 2. Wheat linkage groups 1A, 2A, 3A, 4A and 5B (constructed from Cascade X Aus1408 DH cross) bearing putative QTLs for PHS tolerance from Aus1408 were compared with homeologous and orthologous loci on the wheat map of Cornell Synthetic/Opata 1995 (Nelson et al. 1995a,b; Marino et al. 1996; Van Deynze et al. 1995), the rice map of Cornell RFLP 2001 (Causse et al. 1994, Wilson et al. 1999) and the barley map of NABGMP Steptoe x Morex 1993 (Kleinhofs et al. 1993).

The QTLs for PHS in barley () were based on the work of Oberthur et al. 1995 and Han et al. 1999 while that of the rice QTLs were from Lin et al. 1998 () and Cai & Morishima 2000 (). The QTLs for the Cascade X Au1408 population have been derived from four environments, GI1:[RS5 (2000) ]; GI2: [RS6 (2000) ]; GI3:[Rep1 (1999) ] and GI4: [GI(waite) ] .


Wheat
Cascade X Ausl408
1A
Wheat
Synthetic/Opata
1A
Rice
Cornell RFLP
5
Rice
Cornell RFLP
8
Xgwm136
Xgwm33
Xwmc024
Xgwm164
Xgwm357
XksuD14.1
Xgwm136
XksuD14.2
Xgwm33
Xcdo426
Xbcd98
Xcdo580
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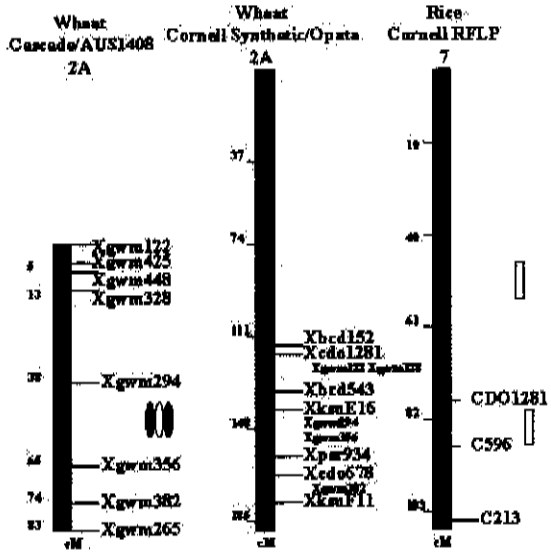
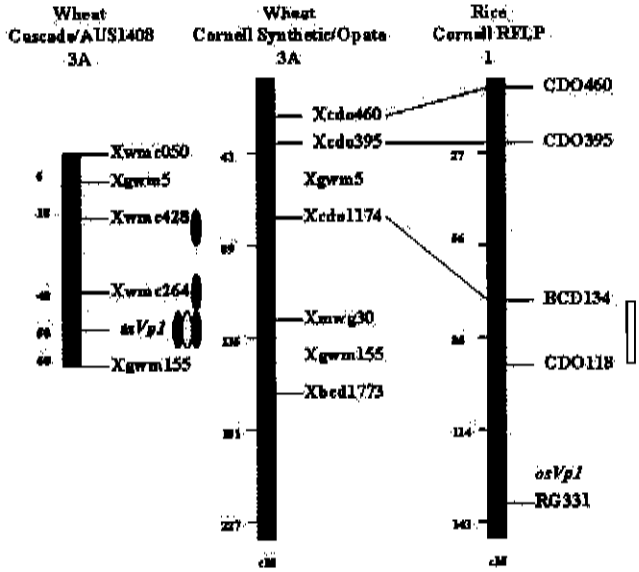
CDO580
CDO1160
CDO393
Amp2
RG598X
cM


Wheat
Cascade/AUS1408
2A
Wheat
Cornell Synthetic/Opata
2A
Rice
Cornell RFLP
7
Xgwm122
Xgwm425
Xgwm448
Xgwm328
Xgwm294
Xgwm356
Xgwm382
Xgwm265
Xbcd152
Xcdo1281
Xbcd543
XksmE16
Xpsr934
Xcdo678
XksmF11
CDO1281
C596
C213
cM


Wheat
Cascade/AUS1408
3A
Wheat
Cornell Synthetic/Opata
3A
Rice
Cornell RELP
1
Xwmc050
Xgwm5
Xwmc428
Xwmc264
osVp1
Xgwm155
Xcdo460
Xcdo395
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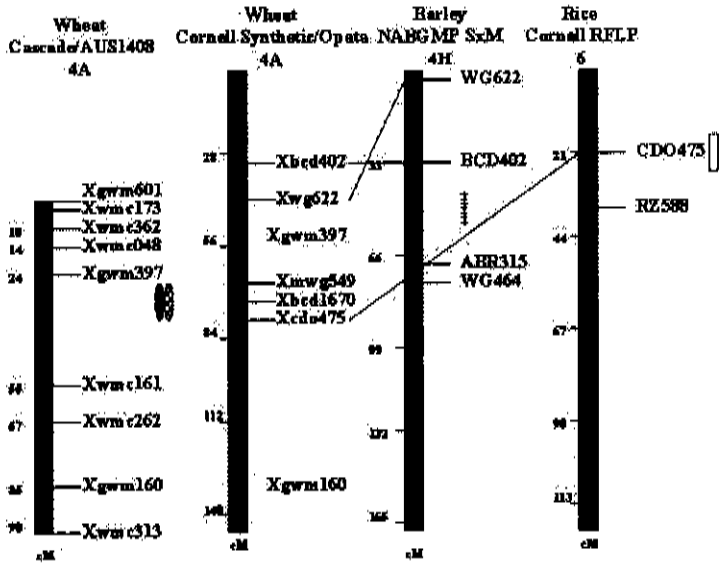
CDO460
CDO395
BCD134
CDO118
osVp1
RG331


Wheat
Cascade/AUS1408
4A
Wheat
Cornell Synthetic/Opata
4A
Barley
NABGMP SxM
4H
Rice
Cornell RELP
6
Xgwm601
Xwmc173
Xwmc362
Xwmc048
Xgwm397
Xwmc161
Xwmc262
Xgwm160
Xwmc313
Xbcd402
Xwg622
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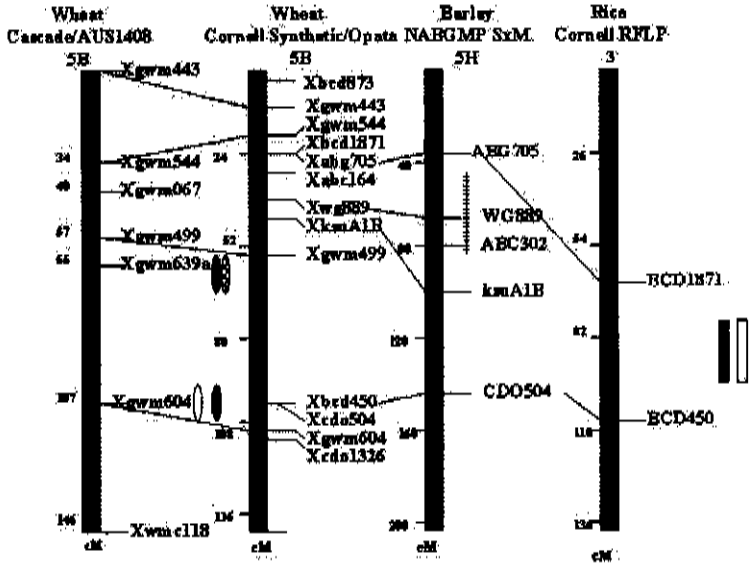
WG622
BCD402
AHR315
WG464
CDO475
RZ588


Wheat
Cascade/AUS1408
Wheat
Cornell Synthetic/Opata
Barley
NABGMP StrM.
Rice
Cornell RFLP
5B
5B
5H
3
Xgwm443
Xgwm544
Xgwm067
Xgwm499
Xgwm639a
Xgwm604
Xwmc118
Xbcd873
Xgwm443
Xgwm544
Xbcd1871
Xabg705
Xabc164
Xwg889
XksmA1B
Xgwm499
Xbcd450
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ksmA1B
CDO504
BCD1871
BCD450
cM

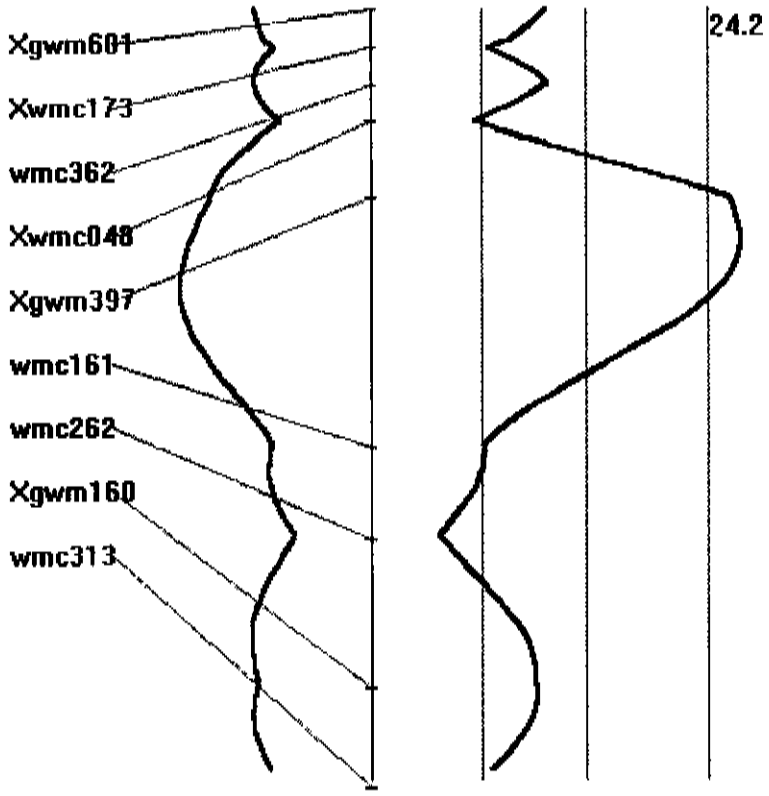

Fig. 3. Location of a major QTL for PHS on 4AL linkage group ($p<0.001$) for the Cascade X Aus1408 DH cross. Interval mapping scan (Mapmanager QTXb15) and the thresholds for significant ($P<0.05$) and highly significant ($p<0.001$) association as determined using permutation tests for 1000 iterations and 1 cM intervals.

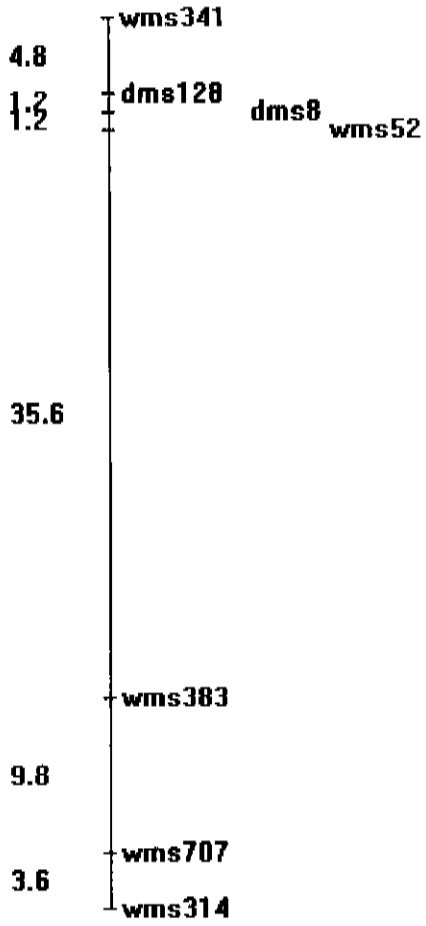

Fig 4: Linkage group of chromosome 3DL constructed from the Cascade X Aus1408 DH cross.

3. Validation of putative QTLs for seed dormancy

Putative seed dormancy QTLs of Aus1408 have been located on 1A, 2AL, 3AL, 4AL and 5BL(see manuscript above). The molecular markers (Table 1) corresponding to the QTLs were screened on selected wheat lines from the following crosses:-

- Janz3*/Aus1408
- Rosella2*/Aus1408
- Seri82/Aus1408
- 2*HTG/Vasco///Aus1408
- 2*URES/Jun/KAUZ//Aus1408
- Sunco/2*QT7475

Five of the six crosses have Aus1408 as the dormancy source. The wheat line, QT7475 was derived from Aus1408 and was postulated to have lost one of the dormancy genes. Hence it has intermediate dormancy compared to Aus1408.

The results (Table 1) showed that the wheat viviparous gene (*Vp1*) was not important for seed dormancy in the selected lines. The ratios of Aus1408 allele : non-Aus1408 alleles on 4AL and 5BL were both 49:1 for the tolerant lines (Table 1) and 0:14 for the non-tolerant lines. This suggested that the Aus1408 alleles on 4AL and 5BL were preferentially selected in the dormant lines, compared to their absence in the non-tolerant lines.

Only one of the 2 flanking markers for the 4AL QTL was polymorphic in the Sunco X QT7475 lines. Fisher's exact test of the data also suggested that the 4AL QTL is significant in the selection for dormancy in these lines. The 5BL QTL is entirely absent in the Sunco x QT7475 lines and this may explain for the intermediate dormancy of QT7475.

Table 1: Validation of putative QTLs for seed dormancy on wheat lines

		Aus1408 as dormancy source		QT7575 as dormancy source	
		Ratio: Aus1408:non-Aus1408 alleles		Ratio: QT7475: non-AT7475 alleles	
chromosome	locus	Tolerant (50 lines)	Non-tolerant (14 lines)	Tolerant (20 lines)	Non-tolerant (20 lines)
1A	*Xgwm164*	0:50	1:11	No polymorphism	
2AL	*Xgwm312*	31:16	2:12	14:5	12:7
2AL	*Xgwm356*	No polymorphism		15:5	13:7
3AL	*Vp1*	26:24	2:12	5:15	7:13
4AL	*Xgwm397*	48:2	0:14	10:10	3:17
4AL	*Xwmc161*	49:1	0:14	No polymorphism	
5BL	*Xgwm604*	49:1	0:14	0:20	0:20
3BL	*Xgwm108*	21:29	0:14	No polymorphism	

Major seed dormancy QTLs of Aus1408 have been located and validated on chromosome 4AL and 5BL.