ORIGINAL PAPER

D. Mares · K. Mrva · J. Cheong · K. Williams
B. Watson · E. Storlie · M. Sutherland · Y. Zou

A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin

Abstract Improved resistance to preharvest sprouting in modern bread wheat (*Triticum aestivum*. L.) can be achieved via the introgression of grain dormancy and would reduce both the incidence and severity of damage due to unfavourable weather at harvest. The dormancy phenotype is strongly influenced by environmental factors making selection difficult and time consuming and this trait an obvious candidate for marker assisted selection. A highly significant quantitative trait locus (QTL) associated with grain dormancy and located on chromosome 4A was identified in three bread wheat genotypes, two white- and one red-grained, of diverse origin. Flanking SSR markers on either side of the putative dormancy gene were identified and validated in an additional population involving one of the dormant genotypes. Genotypes containing the 4A QTL varied in dormancy phenotype from dormant to intermediate dormant. Based on a comparison between dormant red- and white-grained genotypes, together with a white-grained mutant derived from the red-grained genotype, it is concluded that the 4A QTL is a critical component of dormancy; associated with at least an intermediate dormancy on its own and a dormant phenotype when combined with the *R* gene in the red-grained genotype and as yet unidentified gene(s) in the white-grained genotypes. These additional genes appeared to be different in AUS1408 and SW95-50213.

D. Mares (✉) · K. Mrva
School of Agriculture and Wine, University of Adelaide,
Waite Campus, Glen Osmond, SA 5064, Australia
E-mail: daryl.mares@adelaide.edu.au
Tel.: +61-8-83037262
Fax: +61-8-83037109

J. Cheong · K. Williams
SARDI, GPO Box 397, Adelaide, SA 5001, Australia

B. Watson · E. Storlie · M. Sutherland
Faculty of Sciences, University of Southern Queensland,
Toowoomba, QLD 4350, Australia

Y. Zou
Sichuan Academy of Agricultural Science,
Chengdu City, Sichuan 610066, China

Introduction

Pre-harvest sprouting in rain-affected wheat (*Triticum aestivum* L. em Thell.) represents a major constraint to the consistent production of high quality wheat in many regions of the world. This is particularly important in Australia where wheat production is restricted to white-grained cultivars that are generally considered to be more susceptible to sprouting, where grain ripening is usually completed under warm, dry conditions that do not favour development of dormancy, and where up to 80% of the annual crop may be exported to quality conscious markets for use in a wide range of end-products. Resistance to pre-harvest sprouting is a complex trait that is determined by genotype together with a large number of other factors that include: stage of maturity; environmental conditions, particularly temperature and rainfall, during grain ripening; temperature and humidity that accompany rainfall events; spike, plant and crop morphology; and both biotic and abiotic stress. A major component of genetic variation in resistance is the level of grain dormancy (Mares 1987, Mares and Mrva 2001) and this has been the focus of recent work to improve sprouting resistance both in Australia and in other sprouting-prone wheat growing areas of the world. Most cultivars that are currently grown in Australia are non-dormant and at harvest-ripeness the grain is capable of rapid germination if placed in moist conditions at 20°C (Mares 1984). Dormant white-grained wheat genotypes are relatively rare, however for those that do have significant levels of grain dormancy, germination of harvest-ripe grain at 20°C is significantly slower or inhibited, and this confers resistance to preharvest

sprouting similar to, but probably not as good as, the best red-grained genotypes.

In red-grained wheats, dormancy is associated with the red colour (R) genes located on the homoeologous group 3 chromosomes (Flintham and Gale 1996), although it appears that the dormant phenotype requires the interaction between the R-gene(s) and at least one other gene (Mares 1993, 1999; Flintham et al. 1999). Mares (1999) isolated both dormant and non-dormant, red-grained progeny from dormant, red-grained×non-dormant, white-grained crosses. By contrast, the dormancy phenotype of white-grained progeny in the same population ranged from intermediate to non-dormant. Mutation of the R-gene to yield white-grained derivatives resulted in the loss of much of the dormancy (Mares 1999; Warner et al. 2000). Kato et al. (2001) and Flintham et al. (2002) examined dormancy and sprouting in spikes subjected to a standard wetting treatment respectively using populations derived from dormant red×non-dormant red wheat crosses. Both groups identified a significant Quantitative Trait Loci (QTL) located on chromosome 4A that was associated with dormancy in red-grained wheats. More recently, Mori et al. (2005) confirmed the association between the 4A QTL and dormancy in a Japanese red-grained wheat.

In white-grained wheats, such as 'AUS1408', dormancy has been attributed to two or more independent genes (Mares 1996). In other cultivars, a larger numbers of genes may be involved (Paterson et al. 1990; R. DePauw personal communication, 1999). QTL analysis of pre-harvest sprouting resistance in two North American white winter wheat crosses (moderately resistant×resistant, and moderately resistant×susceptible) identified loci on chromosome arm 1AS and one of the homoeologous group 2 chromosomes in the first population, and on chromosome arms 3BL, 4AL, 5DL, and 6BL in the second population, respectively (Anderson et al. 1993). Mares and Mrva (2001) identified a significant QTL on chromosome 4A that was associated with an intermediate level of dormancy in the white-grained cultivar Halberd.

The objectives of this study were to identify QTLs associated with higher levels of grain dormancy in two white-grained wheats and one red-grained wheat of diverse origin, to compare these with those previously reported QTL and to develop molecular markers suitable for marker assisted selection in wheat breeding programs.

Materials and Methods

Plant material

Doubled haploid (DH) populations, developed using the wheat×maize (*Zea mays*) system (Kammholtz et al. 2001), parental cultivars and inbred lines used in this study are described in Tables 1 and 2. Janz, Cunningham and Cascades are Australian bread wheat cultivars

developed in Queensland and Western Australia. AUS1408 was identified in a germplasm survey of white-grained *T. aestivum* accessions held in the Australian Winter Cereal Collection, Tamworth, NSW, Australia, and originates from the Transvaal region of South Africa (Mares 1987). SW95-50213 (AUS26906) is a landrace collected in China and supplied by Y. Zou. AUS1490 is a red-grained genotype, containing a single gene for seed coat colour on chromosome 3A (*R-A1b,R-B1a, R-D1a*, McIntosh et al. 1995), which was also identified during a germplasm survey of bread wheat accessions held at the Winter Cereals Collection. SUN325 sister lines (A, B, C), and QT7475 are AUS1408 derived breeding lines. AUS1490-EMS mutant was selected on the basis of grain colour from an M_2 population derived from EMS-treated grain of AUS1490 (Mares 1999).

Seed coat colour in AUS1490, AUS1490-EMS mutant and doubled haploid lines from Janz×AUS1490 was determined by soaking grains in 0.1 M NaOH containing Tween20 (two drops per 100 ml solution).

Populations, together with parental cultivars, were grown in the field at the Waite Campus, South Australia as 0.5 m single row unreplicated plots in 2001 and as 0.5 m single row plots, replicated in two separate blocks, in 2002. Standards, Janz (non-dormant), QT7475 (intermediate), AUS1408 and SW95-50213 (dormant) were included at 20 plot intervals. Parents, inbred lines and putative transgressive segregants from the AUS1408×SW95-50213 population were re-tested using the same replicated trial format in 2003 used in 2002. A number of precautions were taken during management of field trials in an effort to maximise expression of genetic differences in dormancy and minimise or avoid differential effects within experiments due to environmental factors. These included: provision of adequate fertiliser pre-sowing and post-anthesis, use of preventative fungicide sprays for foliar diseases and insecticides for aphids likely to transmit BYDV; avoidance of moisture stress by use of irrigation as required; and avoidance of sites prone to black point and crown rot. During the later stages of ripening, from just prior to physiological maturity until harvest was completed, the experiments were covered with translucent, white plastic to avoid confounding effects of rain (Mares 1989; Trethowan 1995). From approximately 35–40 days after anthesis, 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. Spikes were harvested from the primary tillers in each plot when all green colour had just disappeared from the leaves and stems of the plants and stored under cover at ambient temperature for 4 days, during which time the grain was recovered by gentle hand-threshing. Grain drying was completed by placing samples in a forced-air dryer for 24 h at 35°C to ensure that the final moisture content was below 12% (harvest-ripeness). At 5 days after harvest, the grain was transferred to −20°C to preserve dormancy (Mares 1983) until all plots had been

Table 1 Origin and characteristics of germplasm

Cultivar	Origin	Dormancy phenotype	Grain colour	GI ± SE	Heightphenotype
Janz	Australia	Non-dormant	White	0.72 ± 0.025	SD
Cunningham	Australia	Non-dormant	White	0.80 ± 0.020	SD
Cascades	Australia	Non-dormant	White	0.65 ± 0.030	SD
AUS1408	South Africa	Dormant	White	0.12 ± 0.015	Tall
SW95-50213	China	Dormant	White	0.06 ± 0.017	Tall
AUS1490	Australia	Dormant	Red	0.05 ± 0.012	Tall
SUN325 Sister lines	Australia	Dormant	White	0.08 ± 0.016	SD
QT7475	Australia	Intermediate	White	0.36 ± 0.029	SD
AUS1490-EMS mutant	Australia	Intermediate	White	0.50 ± 0.032	Tall

(SD = semi-dwarf). Mean germination indices (GI) and standard errors (SE) for samples grown in 2002

Table 2 Population details and numbers of doubled haploids

Population	No. of lines
Janz×AUS1408	74
Cascades×AUS1408	106
SW95-50213×Cunningham	172
AUS1408×SW95-50213	95
Janz×AUS1490	99

harvested and all the seed required for germination testing was available.

Germination test

Replicate samples of 50 sound, well-filled grains free from obvious defects, were incubated on moist filter paper in petri dishes at 20°C. Germinated grains were counted at daily intervals and expressed as a cumulative percentage germination curve or as a weighted germination index (Walker-Simmons 1988). This index gives maximum weight to grains that germinate rapidly and is calculated from the following formula:

Germination Index (GI) = $(7 \times N1 + 6 \times N2 + \dot{s}..1 \times N7)/$ total days of test×total grains, where $N1, N2, N3, \dot{s}N7$ are the number of grains that had germinated on day 1, day 2, $\dot{s}$ day 7. 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.

DNA Analysis

DNA was extracted from the mutant, the inbred lines, the parents and each of the lines from the Janz×AUS1408, Cascades×AUS1408 and SW95-50213×Cunningham populations, and from two sets of ten lines representing the dormant and non-dormant extremes from the Janz×AUS1490 population using a mini-prep method adapted from Rogowsky et al. (1991). Simple sequence repeat (SSR or microsatellite) primers specific for sequences on chromosome 4A, a previously identified residence of QTLs controlling grain dormancy in white wheats (Mares and Mrva 2001; Anderson et al. 1993) and red-grained wheats (Kato et al. 2001; Flintham et al. 2002), were selected for screening the DNA samples. Primers labelled gwm and wmc were selected according to microsatellite mapping studies of Röder et al. (1998) and of the International Wheat Consortium, respectively.

Candidate gene primers were derived from gene sequences contained in the Genbank database. The Aquaporin primers (forward, 5′-CCCACCTCCATTTT-TTCC-3′ and reverse, 5′-CCGACACATAATAACAC-GACC-3′) were derived from *T. aestivum* mRNA (accession#AF139814). A 879 bp product of the Aquaporin primers was polymorphic between the parents, Cunningham and SW950213 and dormant vs non-dormant bulked DNA samples, and was cloned and sequenced. Primers (5′-GAGTAGTGTAGATGGTA-GTTGG-3′ and 5′-GACACATAATAACACGACCA-CC-3′) were designed from the cloned sequence, labelled Aqua and used for linkage and interval mapping. Primers for PDI-SmII (protein disulphide isomerase) (forward, 5′-CAACATCCTTACCTCTTGAAATA-3′ and reverse 5′-GCCTGATCCTCTTTCAGGCCA-3′) were supplied by Dr Mehmet Cakir (Murdoch University, WA, Australia) and derive from a report by Johnson et al. (2001). Bmag337 is a barley chromosome 5H microsatellite marker (Ramsay et al. 2000) selected from the syntenic relationship between barley and wheat. Microsatellite markers were amplified using a touchdown PCR protocol (Williams et al. 2002), in 10 μl reaction mixes containing 20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.0 mM dNTP, 1.875 mM MgCl$_2$, 15 ng of each primer, 0.5 U of Taq DNA polymerase (Life Technologies) and 16 ng template DNA.

Molecular Marker Analysis

Molecular and trait data for each of the lines were entered into Map Manager QTXb19 (Manly et al. 2001) for linkage and interval analyses.

Results

Germination characteristics of parents, inbred lines and doubled haploid lines

Breeding lines and parental genotypes used to generate the doubled haploid populations maintained non-dormant (Janz, Cunningham, Cascades) or dormant (AUS1408, AUS1490, SW95-50213) phenotypes in all experiments consistent with previous experience (Fig. 1) (Table 1). QT7475 and AUS1490-EMS mutant had intermediate dormant phenotypes. Frequency distributions of GI for both Janz×AUS1408 and SW95-50213×Cunningham were similar for both years (data from 2002 only presented), covered the range from dormant to non-dormant, and approximated to normal distributions that were centred on population means of 0.39 and 0.36 respectively (Figs. 2a,b). There was no evidence of significant transgressive segregation.

Simple correlation regression coefficients (r) for the replicate analyses in 2002 and comparisons of these replicates with the 2001 data were 0.72, 0.74 and 0.71 for Janz×AUS1408 and 0.68, 0.53 and 0.68 for SW95-50213×Cunningham.

Since the dormancy phenotype distribution was continuous between the extremes represented by the parents, it was difficult to calculate the proportion of dormant or non-dormant lines. When lines were classified according to whether the GI was equal to, or within 1 standard deviation of, the mean of either parent, 29% of progeny of SW95-50213×Cunningham were dormant and 23% non-dormant. Similar calculations for Janz×AUS1408 gave 15% dormant and non-dormant lines, respectively. Genotypes selected from either end of the distribution maintained a dormant or non-dormant phenotype in subsequent seasons. Importantly, many genotypes from the central part of the distri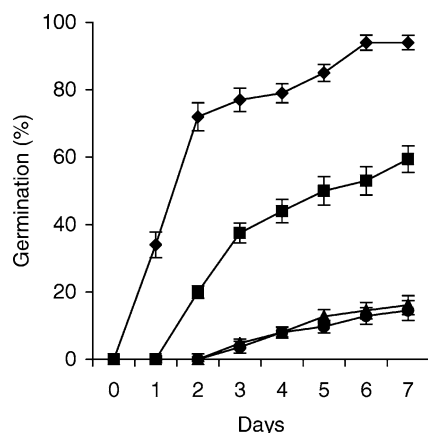bution maintained an intermediate phenotype that was significantly different from either parent and similar to the breeding line QT7475 and the white-grained AUS1490-EMS mutant.

The population mean for the dormant×dormant cross, AUS1408×SW95-50213, was 0.11 and individuals ranged from GI = 0 to GI = 0.405. Lines from the lower (mean GI = 0.016 ± 0.002, 12–15% of lines) and the upper (mean GI = 0.352 ± 0.01, 12–15% of lines) extremes of the distribution in 2001 (Fig. 3) generally maintained a similar dormancy phenotype in 2002 and 2003 (mean GI values of 0.09 ± 0.012 and 0.05 ± 0.013 for the lower group and 0.33 ± 0.045 and 0.35 ± 0.040 for the upper group in 2002 and 2003, respectively).

Red-grained lines from Janz×AUS1490 ranged in dormancy phenotype from dormant to non-dormant, whereas, the white-grained progeny varied between intermediate and non-dormant (Fig. 4). Within the red-grained sub-population the distribution appeared to be bi-modal.

Genetic Analysis

DNA samples from the parents and doubled haploid lines were screened by PCR with SSR and STS (sequence tagged site) primer pairs for polymorphic DNA products corresponding with dormant and non-dormant parental samples. Primers that amplified consistent polymorphic products from the individual DNA samples were subsequently evaluated for marker linkages. Analysis of individual marker genotypes suggested that nine SSR markers – wmc513, wmc048, gwm397, gwm269, barc170, wmc468, gwm258, wmc161, wmc262 - and the Aqua markers were linked within a 110 cM genetic distance in SW95-50213×Cunningham (Fig. 5a), whilst 10 SSR – gwm610, wmc048, gwm397, gwm269, barc170, wmc468, wmc258, wmc161, barc347, wmc262 - together with Aqua and PDI_SmII markers, were linked within a 105 cM genetic distance in Janz×AUS1408 (Fig. 5b).

Interval analysis for QTLs indicated that markers gwm397, gwm269 and barc170 flanked a highly significant QTL (likelihood ratio statistic (LRS) = 22.2 – 41.1) in SW95-50213×Cunningham. The map position of gwm269 explained up to 25% of the trait variation ($R^2 = 0.25$). The same 3 markers flanked a highly significant QTL defined by a LRS peak of 26.9 – 35.3 in Janz×AUS1408 and map position of gwm269 explained 30 to 38 % of the trait variation ($R^2 = 0.30$ and 0.38), respectively. Selection within the Janz×AUS1408 population based on whether the genotype had AUS1408 alleles at $Xgwm397$, $Xgwm269$ and $Xbarc170$ or Janz alleles at these loci, resulted in a substantial separation of phenotypes based on the means of 2001 and 2002 (Fig. 6). Importantly however, genotypes with AUS1408 alleles at the flanking marker loci were never associated with a non-dormant phenotype or conversely Janz alleles with a fully dormant phenotype. With few exceptions, genotypes with an intermediate dormancy

Fig. 1 Cumulative germination curves for non-dormant, Janz (*filled diamond*), intermediate, QT7475 (*filled square*), and dormant, AUS1408 (*filled triangle*) and SW95-50213 (*filled circle*), genotypes from 2002. Standard errors represented by *bars*

Fig. 2 Frequency distributions for germination index in the Janz×AUS1408 (**a**) and SW95-50213 (**b**) doubled haploid populations from 2002. Mean GI values for parents and the intermediate dormant genotype, QT7475, marked by *arrows*
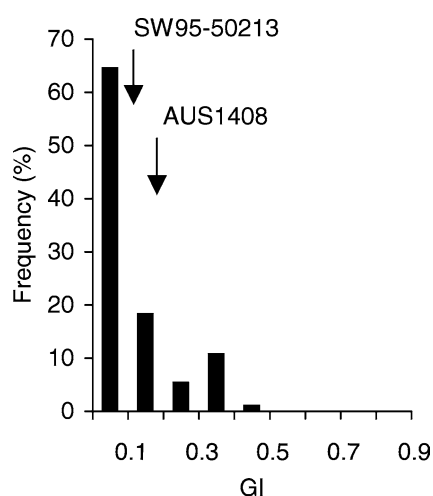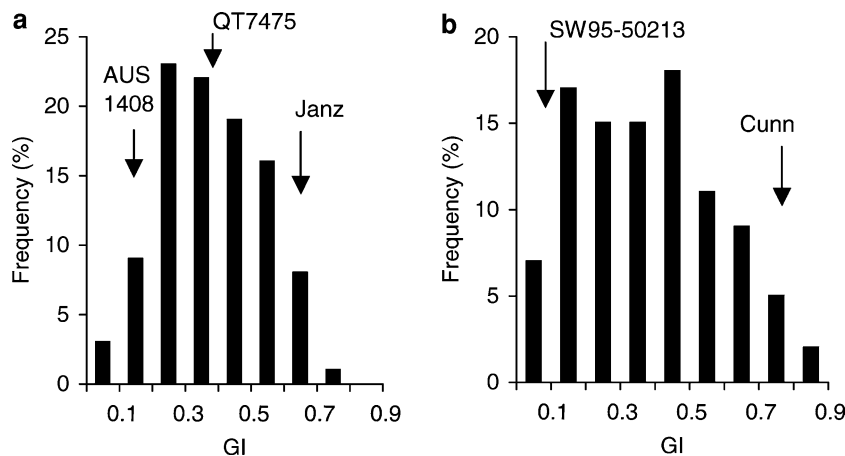

Fig. 3 Frequency distribution for germination index in the AUS1408×SW95-50213 doubled haploid population from 2001. Mean GI of parents marked by *arrows*
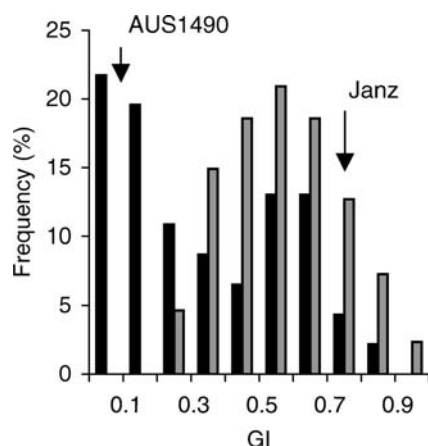

Fig. 4 Frequency distributions for germination index in the Janz×AUS1490 doubled haploid population. Red-grained (*filled rectangle*) and white-grained genotypes (*shaded rectangle*). Mean GI of parents marked by *arrows*

phenotype comparable to QT7475 (AUS1408 derivative) and AUS1490-EMS mutant, carried the dormant parent alleles at the 4A QTL.

Analysis of lines from the extremes in the distribution for GI in Janz (white non-dormant)×AUS1490 (red-grained dormant) indicated that there was also a strong association between the AUS1490 *Xgwm397, Xgwm269 and Xbarc170* alleles and dormancy phenotype. Eight out of ten red-grained, dormant doubled haploids contained the AUS1490 alleles whilst the other two genotypes were recombinants. At the other extreme (eight red- and two white-grained lines), non-dormant genotypes contained the Janz alleles at the flanking marker loci or were recombinants.

Validation of markers gwm397, gwm269 and barc170 in Cascades×AUS1408

With the exception of gwm468, all the 4A markers were polymorphic in Cascades×AUS1408. The highest LRS value of 23.7 was associated with gwm269 and was flanked on either side by gwm397 and barc170 as in the other populations used in this study.

Discussion

The frequency of dormant and non-dormant individuals, defined here as lines with a GI within one standard deviation of the respective parent, ranged from 15 to 29% and was substantially lower than the 50% expected for a single gene in a doubled haploid population. Given the continuous distribution for dormancy phenotype, separation into sub-classes for statistical analysis was not considered appropriate. Segregation patterns in each of the dormant×non-dormant populations were consistent with a two or three gene models for genetic control of dormancy in white-grained wheat genotypes and support previous models of segregation for dormancy phenotype in F_2 plant populations (Mares 1993, 1996).

Fig. 5 Partial interval map of QTL for dormancy on chromosome 4A from **a** SW95-50213×Cunningham and **b** Janz×AUS1408. *Vertical lines* indicate suggestive ($P=0.63$), significant ($P=0.05$), and highly significant ($P=0.001$) thresholds for the likelihood ratio statistic (LRS, Manley et al. 2001). The histogram under the LRS trace indicates the confidence interval of the QTL, as determined by bootstrap re-sampling. *cM* centimorgan
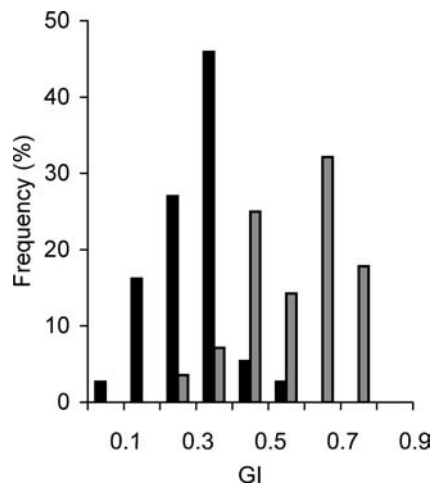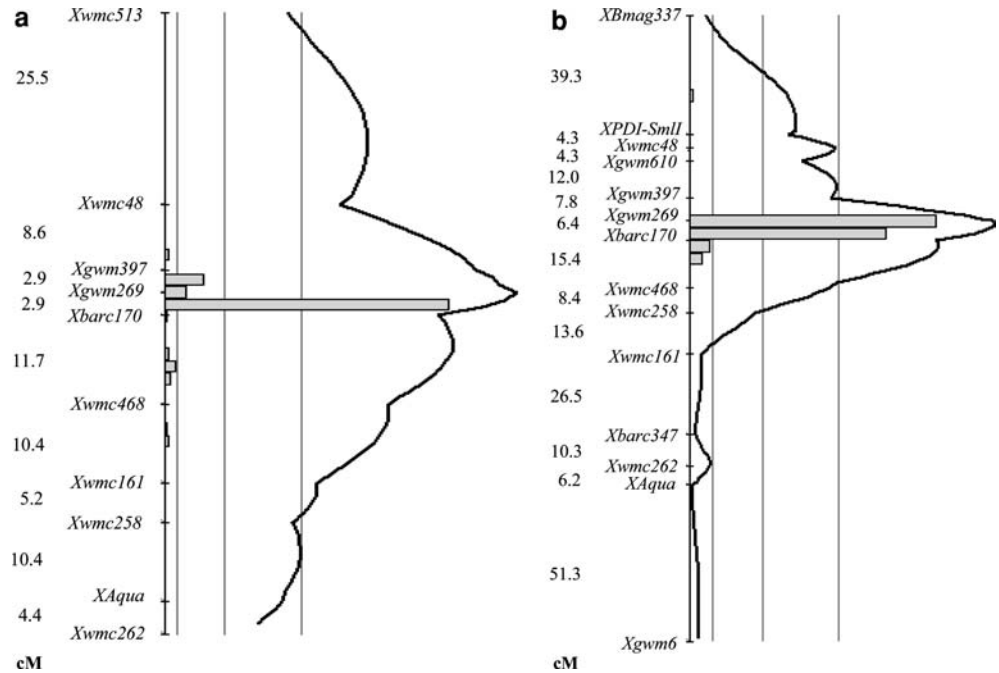

Fig. 6 Frequency distribution for germination index in subpopulations selected from Janz×AUS1408 on the basis of genotype at the flanking marker loci. AUS1408 alleles at Xgwm397, Xgwm269 and Xwbarc170 (*filled rectangle*) compared with Janz alleles at these loci (*shaded rectangle*). Data is the mean of 2001 and 2002

Genetic analysis identified a common, highly significant QTL located on chromosome 4A in both sources of dormancy, despite their seemingly diverse origin, that was flanked by SSR markers gwm397, gwm269 and barc170. Chromosome 4A (Anderson et al. 1993) and the 4A QTL (Mares and Mrva 2001; Kato et al. 2001; Flintham et al. 2002) have been previously shown to be associated with dormancy in white-grained wheats with intermediate dormancy and dormant red-grained wheats. Recently, Noda et al. (2002) have suggested that sensitivity of wheat embryos to germination inhibition by abscisic acid (ABA) is controlled primarily by a

gene(s) located on the long arm of chromosome 4A. This trait has been reported to exercise an important role in the expression of dormancy (Walker-Simmons 1987; Morris et al. 1989; Mares 1996; Kawakami et al. 1997), and to decline in parallel with the time dependent loss of dormancy in intact caryopses. The work reported in this present study clearly indicates, however, that presence of the putative dormancy QTL on chromosome 4A, on its own, is not sufficient to produce a dormancy phenotype typical of the dormant parents. A substantial proportion of the doubled haploid lines that contained the 4A QTL allele from the dormant parent only expressed an intermediate level of dormancy similar to the breeding line QT7475 (AUS1408 derivative) whose intermediate phenotype has been confirmed during several years of screening in a range of field and controlled environments. One possible model that would be consistent with these results has the 4A QTL (sensitivity of the embryo to ABA) as a critical component of dormancy, on its own giving an intermediate phenotype similar to QT7475, which interacts with one or two additional genes to give the dormant phenotype typical of AUS1408 and SW95-50213. In the absence of a 4A QTL allele from a dormant genotype, these additional genes have either a limited effect or no effect at all. This model also appeared to apply to the single gene red-grained wheat, AUS1490, and in this instance the additional gene appears to be pleiotrophic with the *R* gene controlling seed coat pigment. Non-dormant red-grained wheat cultivars are common and non-dormant, red-grained genotypes were readily identified in both the Janz×AUS1408 AUS1490 doubled haploid population and in a previous study of breeders lines (Mares 1999). Conversely, mutation of AUS1490, *R-A1b,B1a,D1a*, (Mares 1999) and Chinese Spring, *R-A1a,B1a,D1b*,

Fig. 7 Comparison of chromosome 4A markers and dormancy (**a–d**), or PHS (preharvest sprouting) (**e**), QTL locations in five bread wheat populations: Janz×AUS1408 (**a**), SW95-50213×Cunningham (**b**), populations derived from Japanese red-grained wheat cultivars (**c**) (Torada 2004 pers comm.), Cranbrook×Halberd (**d**) (Mares and Mrva 2001), and Boxer×Soleil, Chinese Spring×Synthetic (**e**) (Flintham et al. 2002)
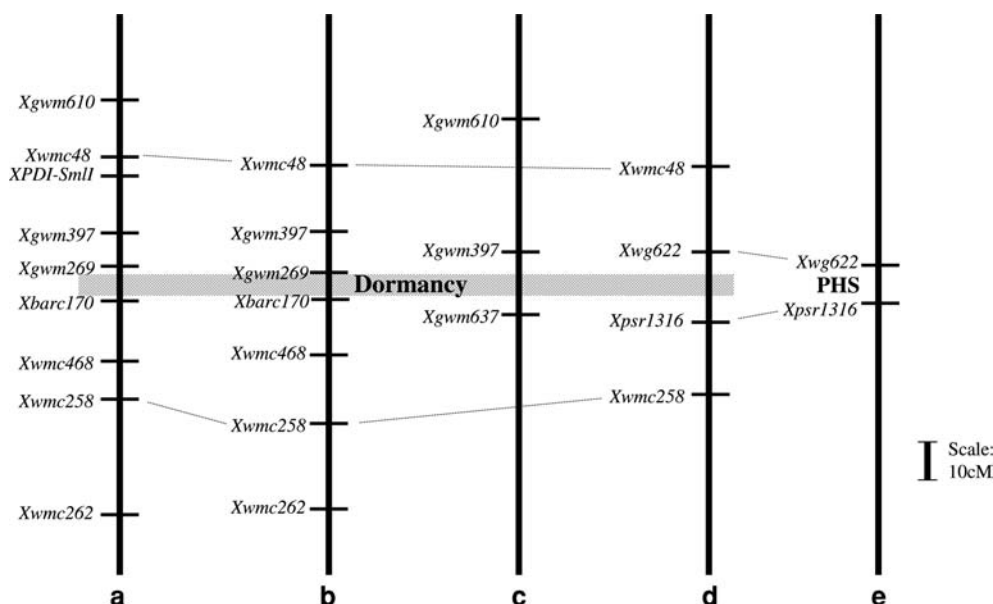
(Warner 2000) from red- to white-grained types resulted in a significant reduction in dormancy, whilst the best white-grained doubled haploids from Janz×AUS1490 only achieved an intermediate level of dormancy. Recently Himi and Noda (personal communication. 2004) have found that AUS1490-EMS mutant contains a point mutation in *Tamyb10-A1* that they believe corresponds to the *R* gene on chromosome 3A. The proposed model is consistent with results reported for Boxer (non-dormant)×Soleil (dormant), both red wheats with *R* genes on each of the group 3 chromosomes, which suggested that dormancy was associated with a single gene, initially located on chromosome 7D, but later relocated to 4A (Flintham 2002).

In the absence of the putative dormancy 4A QTL, doubled haploid lines were either comparable to the non-dormant parent or were distributed between the non-dormant parent and QT7475. Further work is required to resolve whether the observed variation for GI in lines lacking the putative 4A dormancy QTL is due to the effects of additional genes on their own or rather a reflection of physiological and environmental effects. A number of factors including: maturity, tiller synchrony, moisture stress, temperature, and plant health appear to have significant effects on the dormancy phenotype.

Inter-crossing the two white-grained sources of dormancy resulted in limited transgressive segregation on both sides of the dormant parents but no non-dormant recombinants. Since both parents contained the 4A dormancy QTL, this observation suggests that the sources differ in the location of the one or two other genes involved in determining the dormant phenotype. A search within dormant×non-dormant populations for QTL representing the additional dormancy genes has revealed only weak and inconsistent association with QTL on the group 3 chromosomes (data not presented).

This is perhaps not unexpected if the additional genes have limited effect on their own. Current work is focussed on doubled haploid populations derived by crossing dormant×intermediate genotypes, both of which are homozygous for the 4A dormancy QTL, on the assumption that variation in dormancy phenotype will then be due to the additional genes.

Presence of the AUS1408 or SW95-50213 alleles of flanking markers gwm397, gwm269 and barc170 were associated with variation in dormancy phenotype from dormant to intermediate dormant in Janz×AUS1408 and SW95-50213×Cunningham, respectively. Similarly, selection based on AUS1408 alleles at *Xgwm397, Xgwm269* and *Xbarc170* in Cascades×AUS1408 resulted in a sub-population that was highly enriched for dormant and intermediate dormant types. Identification of the truly dormant lines still requires traditional screening of these enriched sub-populations based on phenotype.

Surveys to identify molecular markers with tighter linkage are continuing with the aim of improving the efficiency of marker-assisted selection for grain dormancy.

References

Anderson JA, Sorrells ME, Tanksley SD (1993) RFLP analysis of genomic regions associated with resistance to preharvest sprouting in wheat. Crop Sci 33:453–459

Flintham JE, Gale MD (1996) Dormancy gene maps in homologous cereal genomes. In: Noda K, Mares DJ (eds) Preharvest Sprouting in Cereals 1995. Centre for Academic Societies, Osaka, Japan, pp 143–149

Flintham JE, Adlam R, Gale MD (1999) Seedcoat and embryo dormancy in wheat. In: Weipert D (eds) Eighth International Symposium on Preharvest Sprouting in Cereals 1998. Association of Cereal Research, Federal Centre for Cereal Potato, and Lipid Research, Detmold, Germany, pp 67–76

Flintham J, Adlam R, Bassoi M, Holdsworth M, Gale M (2002) Mapping genes for resistance to sprouting damage in wheat. Euphytica 126:39–45

Johnson JC, Clarke BC, Bhave M (2001) Isolation and characterization of cDNSs encoding protein disulphide Isomerases and Cyclophilins in wheat. J Cereal Sci 34:159–171

Kammholtz SJ, Campbell AW, Sutherland MW, Hollamby GJ, Martin PJ, Eastwood RF, Barclay I, Wilson RE, Brennan PS, Sheppard J (2001) Establishment and characterisation of wheat genetic mapping populations. Aust J Agric Res 52:1079–1088

Kato K, Nakamura W, Tabiki T, Miura H, Sawada S (2001) Detection of loci controlling seed dormancy in group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. Theor Appl Genet 102:980–985

Kawakami N, Miyake Y, Noda K (1997) ABA insensitivity and low ABA levels during seed development of non-dormant wheat mutants. J Expt Bot 48:1415–1421

McIntosh RA, Hart GE, Devos KM, Gale MD (1995) Catalogue of gene symbols for wheat. 1995 supplement. Ann Wheat Newslett 41:330–335

Manly KF, Cudmore Jr RH, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mammal Genome 12:930–932

Mares DJ (1983) Preservation of dormancy in freshly harvested wheat grain. Aust J of Agric Res 34:33–38

Mares DJ (1984) Temperature dependence of germinability of wheat grain (Triticum aestivum L.) grain in relation to pre-harvest sprouting. Aust J of Agric Res 35:115–128

Mares DJ (1987) Pre-harvest sprouting tolerance in white-grained wheat. In: Mares SJ (ed) Fourth international symposium on preharvest sprouting in cereals, Westview Press, Boulder pp 64–74

Mares DJ (1989) In: Derera NF (ed) Preharvest sprouting damage and sprouting tolerance assay methods and instrumentation (Preharvest Field Sprouting in Cereals). CRC, Boca Raton, pp 130–166

Mares DJ (1993) Genetic studies of sprouting tolerance in red and white wheats. In: Walker-Simmons K, Reid JL (eds) Preharvest sprouting in Cereals 1992. AACC, St Paul, MN, USA, pp 21–9

Mares DJ (1996) Dormancy in white wheat : mechanism and location of genes. In: Noda K, Mares DJ (eds) Preharvest Sprouting in Cereals 1995'. Centre for Academic Societies, Osaka, Japan, pp 179–184

Mares DJ (1999) The seed coat and dormancy in wheat grains. In: Weiprt D (ed) Eighth International Symposium on Preharvest Sprouting in Cereals 1998'. Association of Cereal Research Federal Centre for Cereal Potato and Lipid Research. Detmold, Germany, pp 77–81

Mares DJ, Mrva K (2001) Mapping quantitative trait loci associated with variation in dormancy in Australian wheat. Aust J of Agric Res 52:1257–1266

Mori M, Uchino N, Chono M, Kato K, Miura H (2005) Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. Theor Appl Genet 110:1315–1323

Morris CF, Moffat JM, Sears RG, Paulsen GM (1989) Seed dormancy and responses of caryopses, embryos and calli to abscisic acid in wheat. Plant Physiol 90:643–647

Noda K, Matsuura T, Maekawa M, Taketa S (2002) Chromosomes responsible for sensitivity of embryo to abscisic acid and dormancy in wheat. Euphytica 123:203–209

Paterson AH, Sorrells ME (1990) Inheritance of grain dormancy in white-kernelled wheat. Crop Sci 30:25–30

Ramsay L, Macaulay M, Ivanissivich S degli, MacLean K, Cardle L, Fuller J, Edwards K, Tuvesson S, Morgante M, Massari A, Maesti E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Theor Appl Genet 156:1997–2005

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rogowsky PM, Guidet FLY, Langridge P, Shepherd KW, Koebner RMD (1991) Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat. Theor Appl Genet 82:537–544

Trethowan RM (1995) Evaluation and selection of bread wheat (Triticum aestivum L.) for preharvest sprouting tolerance. Aust J Agric Res 46:463–474

Walker-Simmonds MK (1987) ABA levels and sensitivity in developing embryos of sprouting resistant and susceptible cultivars. Plant Physiol 84:61–66

Walker-Simmons MK (1988) Enhancement of ABA responsiveness in wheat embryos at higher temperature. Plant Cell Environ 11:769–775

Warner RL, Kurdna DA, Spaeth SC, Jones SS (2000) Dormancy in white-grained mutants of Chinese Spring wheat (Triticum aestivum L.). Seed Sci Res 10:51–60

Williams KJ, Taylor SP, Bogacki P, Pallotta M, Bariana HS, Wallwork H (2002) Mapping of the root lesion nematode (Pratylenchus neglectus) resistance gene Rlnn1 in wheat. Theor Appl Genet 104:874–879