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Simple sequence repeat markers associated with three quantitative trait loci for black point resistance can be used to enrich selection populations in bread wheat

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Abstract. Black point in wheat has the potential to cost the Australian industry $A30.4 million a year. It is difficult and expensive to screen for resistance, so the aim of this study was to validate 3 previously identified quantitative trait loci (QTLs) for black point resistance on chromosomes 2B, 4A, and 3D of the wheat variety Sunco. Black point resistance data and simple sequence repeat (SSR) markers, linked to the resistance QTLs and suited to high-throughput assay, were analysed in the doubled haploid population, Batavia (susceptible) × Pelsart (resistant). Sunco and Pelsart both have Cook in their pedigree and both have the *Triticum timopheevii* translocation on 2B. SSR markers identified for the 3 genetic regions were *gwm319* (2B, *T. timopheevii* translocation), *wmc048* (4AS), and *gwm341* (3DS). *Gwm319* and *wmc048* were associated with black point resistance in the validation population. *Gwm341* may have an epistatic influence on the trait because when resistance alleles were present at both *gwm319* and *wmc048*, the Batavia-derived allele at *gwm341* was associated with a higher proportion of resistant lines. Data are presented showing the level of enrichment achieved for black point resistance, using 1, 2, or 3 of these molecular markers, and the number of associated discarded resistant lines. The level of population enrichment was found to be 1.83-fold with 6 of 17 resistant lines discarded when *gwm319* and *wmc048* were both used for selection. Interactions among the 3 QTLs appear complex and other genetic and epigenetic factors influence susceptibility to black point. Polymorphism was assessed for these markers within potential breeding material. This indicated that alternative markers to *wmc048* may be required for some parental combinations. Based on these results, marker-assisted selection for the major black point resistance QTLs can increase the rate of genetic gain by improving the selection efficiency and may facilitate stacking of black point resistances from different sources.

Additional keywords: *Triticum aestivum*, SSR markers, validation.

Introduction

Black point is a common grain defect, particularly in areas of the world where grain development and ripening are prematurely prolonged by humid conditions (Machacek and Greaney 1938). In wheat (*Triticum aestivum* L.), black point results in a dark melanisation at the germ end of otherwise healthy grain. Black point results in down-grading of grain to a lower quality class, with considerable financial loss for growers. For example, in Australia the prescribed limit is <5% and annual losses through downgrading have the potential to be as high as A$30.4 million (Brennan and Murray 1998). In the USA, only 2% is permitted in wheat graded as US no. 1, and 4% in US no. 2 (Davis and Jackson 2003).

Black point is a physiological response triggered by humidity during the later stages of grain filling (Williamson 1997). Black point is widely thought to be the result of saprophytic fungal infection (Davis and Jackson 2003), but the often-reported association between symptoms and the most common grain coloniser, *Alternaria alternata*, is weak and several studies have been unable to confirm any link (Williamson 1997; Ellis 1999).

Wheat varieties have been found to vary in their resistance to black point (Conner and Thomas 1985), which suggests a level of genetic control. Phenotypic testing for resistance is both expensive and imprecise (Williamson 1997). Genetic markers would provide a tool to help breeders more effectively select for resistance to black point.

Genetic regions have been identified that are associated with resistance to black point (Lehmensiek *et al*. 2004) in a population of 180 F_1-derived doubled haploid (DH) lines from a Sunco × Tasman cross. Sunco is derived from the variety Cook and both have an interstitial *Triticum timopheevii* translocation on 2B. Three QTLs were identified for black point resistance contributed by Sunco (resistant parent) and another 3 suggestive QTLs were contributed by Tasman (susceptible parent). They showed that the marker-assisted selection would have significantly enriched the population for black point resistance. Another line, Cascades, was found to have

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different sources of resistance (Lehmensiek *et al*. 2004). These genetically different resistances could potentially be pyramided using markers to provide a higher level of resistance.

It is important to validate the genetic regions identified as associated with black point resistance in mapping studies, so that marker-assisted selection for the trait can be implemented in selection programs. Several steps are involved in the validation process. Firstly, if the markers used in the mapping population are not polymorphic between parents of the validation population, new markers need to be identified. Polymerase chain reaction (PCR)-based markers, especially simple sequence repeats (SSRs), are preferred for high-throughput application to a selection program because of their robustness, co-dominance, and low cost. Secondly, the markers should be tested in a related, structured, phenotyped population to confirm the marker–trait association and linkage relationships. Thirdly, potential recipient parents should be tested for polymorphism of the markers of interest. It is useful to develop a suite of markers in regions of interest, so that polymorphic markers linked to the trait are available for important parental combinations. The ultimate goal is to integrate the marker into the selection program in a manner most likely to improve rate of genetic gain, enhance expression, or improve robustness of a desirable trait.

Numerous quantitative trait loci (QTLs) for important traits have been identified in wheat (Gupta *et al*. 1999; Langridge *et al*. 2001). However, only a limited number of studies to validate QTLs have been conducted (Jefferies *et al*. 2000; Harjit-Singh *et al*. 2001; Sharp *et al*. 2001; Prasad *et al*. 2003; Zhou *et al*. 2003; Schnurbusch *et al*. 2004; Torada *et al*. 2005; Chen *et al*. 2006).

Our objective in this study was to validate markers for selection of resistance to black point in wheat populations that derive their resistance from the variety Cook. The effects of the 3 black point resistance QTLs from Sunco in a related population were verified by evaluating the predictive value of SSR markers linked to the QTLs and by assessing the polymorphism of these SSR markers among potential parents.

Materials and methods

Plant material

The validation population studied was an F_1-derived DH population, made from the cross Batavia (susceptible) × Pelsart (resistant, Cook-derived). The population of 85 individuals was produced using the maize pollination method (Laurie and Bennett 1986). Pelsart carries the 2B translocation from *T. timopheevii* derived from Cook (Friebe *et al*. 1996), and was used as the black point resistant parent. The pedigree of Pelsart is Potam 70/4*Cook (QDPI 1994). Batavia (Brennan *et al*. 1994) was used as the susceptible parent. Both are hard white spring wheat varieties from the Queensland Department of Primary Industries and Fisheries Wheat Improvement Program, at the Leslie Research Centre, Toowoomba, Queensland, Australia.

Ninety-three additional lines were selected by plant breeders as potential recipients of crossing for black point resistance. Some of these lines already express good levels of resistance to black point.

Black point screening

Lines were screened for black point resistance 4 times: twice in 1998 in the humidified glasshouse, once with heads bagged, once not bagged; and in twice-replicated plots in the field in 2000. The black point score is the maximum black point rating from the 4 tests, each of which was the mean percentage of symptomatic seed from counts of 3 lots of 100 seeds. The maximum score was used because 'escapes' from symptoms are common, but high ratings are only achieved by susceptible lines (P. M. Williamson, unpublished).

DNA isolation

Three seeds from each DH line and parents were germinated on filter paper soaked with sterile water in Petri dishes. Leaf was sampled between 7 and 10 days for DNA isolation. DNA was isolated using the sarkosyl extraction method (Song and Henry 1995; Garland *et. al.* 2000). Briefly, tissue was manually broken down under liquid nitrogen to a fine powder and re-suspended in extraction buffer (2.5% N-laurylsarcosine; 0.1 M TRIS-HCl, pH 8.0; 0.01 M EDTA, pH 8.0) and 25 : 24 : 1 phenol : chloroform : isoamyl alcohol (Amresco, Solon, OH, USA). Plant matter and protein were further eliminated by a series of chloroform : isoamyl alcohol extractions. DNA was precipitated in cold isopropanol and 3 M sodium acetate, washed in 70% ethanol, and re-suspended in 1 × TE Buffer. DNA samples were diluted to a concentration of 50 ng/μL.

Markers and PCR reaction

Three SSR markers specific for the 3 identified QTLs were screened for linkage to black point resistance. The primer sequences, annealing temperatures, and observed allele sizes for Batavia and Pelsart were: *gwm319* (2B) forward-GGTTGCTGTACAAGTGTTCACG and reverse-CGGGTGC TGTGTGTAATGAC, 55°C, Batavia 180 bp, Pelsart 170 bp; *wmc048* (4A) forward-GAGGGTTCTGAAATGTTTTGCC and reverse-ACGTGCTAGGGAGGTATCTTGC, 70–60°C touchdown, Batavia 126 bp, Pelsart 138 bp; and *gwm341* (3D) forward-TTCAGTGGTAGCGGTCGAG and reverse-CCGACATCTCATGGATCCAC, 70–60°C touchdown, Batavia 141 bp, Pelsart 137 bp (Röder *et al.* 1998; Dubcovsky 2003).

Each PCR reaction mixture contained 1 × PCR buffer, 0.5 units of Qiagen Taq-polymerase, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, either 0.25 μM (*gwm319*) or 0.2 μM each of left- and right-flanking primers, and 50 ng of DNA template in a final reaction volume of 10 μL.

PCR reactions were performed using a PTC-100™ Programmable Thermal Controller (MJ Research, Watertown, MA, USA). Touchdown programs decreased at either 1.0°C from 70°C to 60°C (*wmc048*) or 0.5°C from 65°C to 55°C (*gwm341*) per cycle. Touchdowns were followed by 35 cycles of 94°C for 30 s, 60°C (*wmc048*), or 55°C *(gwm341*) for 30 s and 72°C for 30 s. The *gwm319* program consisted of 45 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. All thermal cycles finished with a final 5–10 min 72°C extension step.

Products were detected on an 8% polyacrylamide gel electrophoresis run at a constant 55 W for 1 h 45 min. PCR product was visualised by silver staining (Bassam and Caetano-Annollés 1993).

Statistical analyses

The single-point regression analysis in MapManager QTXb19 (Manly *et al*. 2001) was used to test associations between

markers and the trait data, including percentage of phenotypic variation attributable to each marker.

Significant differences between mean black point scores in classes defined by markers were calculated using a Mann-Whitney test (Weir 1996). 'Enrichment' in Table 1 was calculated using the following formula:

$$\text{Enrichment} = (\text{no. of resistant selected lines}/\text{no. of lines selected using marker})/(\text{total no. of resistant lines/total no. of lines})$$

Polymorphism information content (PIC) was calculated according to the following formula:

$$\text{PIC}_i = 1 - \sum \text{p}_{ij}{}^{2\text{j}}$$

where p_{ij} is the frequency of the *j*th allele for marker *i*, and summation extends over *n* alleles (Anderson *et al*. 1993).

Results

Validation of the QTLs in the Batavia × Pelsart population

The frequency distribution of black point score as maximum percentage of affected grain in an F_1-derived DH Batavia × Pelsart population ($n = 85$) was continuous and spanned the range of black point scores from 1 to 30% (Fig. 1). Pelsart had a score of 3%, whereas Batavia had a score of 10%. It shows transgressive segregation and some evidence of a trough at 4% (Fig. 1).

To validate the effect of the 3 QTLs on black point resistance, DH individuals were genotyped with 3 SSR markers, *gwm319* (2B), *wmc048* (4A), and *gwm341* (3D). SSR markers were chosen that were genetically close to markers identified as linked to QTLs conferring resistance to black point, by Lehmensiek *et al*. (2004) using a consensus map (Appels 2004), and were polymorphic between Batavia and Pelsart.

Two genotypes were identified for each marker among the DH population, one allele associated with Batavia another with Pelsart. For *gwm319* the mean black point score for lines carrying the Batavia allele was 9.47 and for the Pelsart allele 6.87 ($P < 0.01$). For *wmc048* the mean black point score for lines carrying the Batavia allele was 8.58 and for the Pelsart allele 7.14 ($P < 0.05$). For *gwm341* the mean black point score for lines carrying the Batavia allele was 7.98 and for the Pelsart allele 7.77 (not significantly different).

The frequency distributions of the 2 genotypes for markers *gwm319*, *wmc048*, and *gwm341* for black point score were overlapping, continuous, and had different means. Some bimodality is suggested within genotypes in some distributions (Fig. 2). The frequency distribution for lines carrying the Pelsart (B) alleles at both *gwm319* and *wmc048* loci show a distinct shift in the distribution towards lower black point scores when compared with lines carrying the Pelsart allele at either one or neither locus (Fig. 2*d*). The mean of the distribution is 6.5 for BB lines and 8.6 for not BB lines.

The percentage of total variation explained by the markers associated with the reported QTLs and likelihood ratio statistic (LRS) scores was relatively low when the combined phenotypic data were considered. The phenotypic variance in black point scores explained was 7% (LRS 6.2) by *gwm319*, 2% (LRS 1.9) by *wmc048*, and 0% (LRS 0) by *gwm341*. The alleles associated with resistance for *gwm319* and *wmc048* were derived from Pelsart; the allele most often associated with resistance for *gwm341* was derived from Batavia. However, when the maximum percentage black point scores are considered for the year 2000 alone, percentage variation explained increases to 17% (LRS 15.6) for *gwm319* and 4% (LRS 3.2) for *wmc048*. In comparison, the Lehmensiek *et al*. (2004) study identified a highly significant QTL on chromosome 2B linked with the marker *ABC356* (LOD 4, 15% phenotypic variance explained), another significant QTL on 4AS linked with the marker *germin* (LOD 2.9, 8% variance explained), and a site on 3DS linked with *wmc375* (LOD 2.5, 5% variance explained) was suggestive of a further QTL.

Application to selection

Lines were divided into a resistant class, where percent black point score was ≤4%, and a susceptible class of scores >4%. This was based on a trough in the frequency distribution at this point (Fig. 1), and on the criterion used for penalising growers.

Chi-square analysis was performed to determine whether black point score and marker genotype were independent (Table 1). Eighty-five DH lines were screened using *gwm319* and *wmc048*, and 84 for *gwm341*. Markers *gwm319* and *wmc048* were not independent from black point scores ($P > 0.05$), whereas marker *gwm341* was independent. Combined, the markers were not independent of black point scores at $P = 0.01$.

The segregation ratio for resistant to susceptible fit a ratio of 1 : 3 (Table 1). Individual markers were expected to segregate in a 1 : 1 ratio for A and B alleles,

Table 1. Chi-square values showing a significant relationship between black point resistance and the markers for all combinations except *gwm341* alone

Segregation for resistance to black point fits a 1 : 3 segregation ratio for resistant *v.* susceptible. Segregation of alleles for *wmc048* and *gwm341* fit the expected 1 : 1, 1 : 1 : 1 : 1 ratios separately and in combination. *Gwm319* showed segregation distortion. The greatest number of resistant lines would be selected by applying *gwm319* alone, with a moderate level of enrichment and only 2 resistant lines being discarded. For each chi-square column, degrees of freedom are shown in parentheses and n.s. indicates non-significance; $*P < 0.05$; $**P < 0.01$

SSR markers	$\chi^2_{\text{ind.}}$	$\chi^2_{\text{bp 1 : 3}}$	$\chi^2_{\text{m 1 : 1}}$	No. of lines selected	No. of selected lines resistant	Enrichment (fold)	No. of lines discarded
gwm319	4.76 (1) *	0.88 (1) n.s.	4.71 (1) *	53	15	1.41	2/17
wmc048	4.94 (1) *	0.88 (1) n.s.	0.00 (1) n.s.	42	13	1.55	4/17
gwm341 (Batavia)	0.05 (1) n.s.	1.16 (1) n.s.	0.30 (1) n.s.	45	10	1.10	7/17
gwm319 & *wmc048*	18.22 (3) **	0.88 (1) n.s.	7.85 (3) *	30	11	1.83	6/17
gwm319 & *wmc048* & *gwm341*	26.48 (7) **	1.16 (7) n.s.	10.67 (7) n.s.	16	8	2.47	9/17

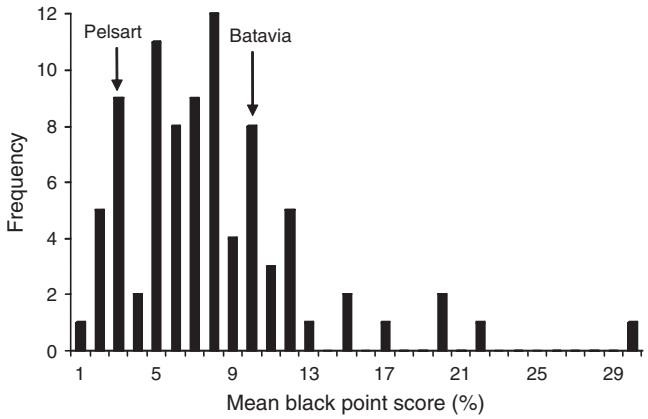

Fig. 1. The frequency distribution of black point scores as maximum percentage of affected grain in the doubled haploid population Batavia × Pelsart ($n = 85$) showing transgressive segregation and some evidence of a trough at 4%.

in a 1 : 1 : 1 : 1 ratio (AA : AB : BA : BB) for 2 markers, and a 1 : 1 : 1 : 1 : 1 : 1 : 1 : 1 segregation ratio (AAA : AAB : ABA : ABB : BAA : BAB : BBA : BBB) for 3 markers combined (Table 1). This prediction was based on an assumption of no segregation distortion of the inheritance of the chromosome segment carrying the QTL, and on the 3 markers being inherited independently. The expected ratio was observed in all cases except for *gwm319* alone, and for *gwm319* and *wmc048* combined.

Phenotypically, 17 of the 85 lines were resistant to black point. For each marker and marker combination, this ratio of 17 : 85 was compared with the ratio of resistant lines to the total number of lines if phenotypic screening was performed after marker-based selection (Table 1). For example, for *gwm319*, 53 lines had the marker allele associated with resistance to black point. Of those 53, 15 were shown to be resistant to black point. 'Enrichment' refers to an increase in proportion of resistant lines, achieved by the application of marker-assisted selection. In this case, if lines were selected for the *gwm319* allele associated with resistance before phenotypic testing, a 1.4-fold enrichment for resistance to black point would have resulted, but 2 resistant lines would have been discarded. Thus, 88% of resistant lines were selected and 38 out of the 53 lines selected were false positives. This compares with 87% resistant lines selected and 67 out of 141 lines selected being false positives when the chromosome 2B marker was used for selection in the study by Lehmensiek *et al*. (2004) Similarly, for *wmc048*, of the 42 lines selected using the molecular marker, 13 would be resistant and 29 susceptible to black point. Four resistant lines would have been discarded. If both *gwm319* and *wmc048* were applied, 30 lines would be selected, 11 of which would be resistant of the total 17 resistant lines (65%), and 19 susceptible (false positives) and 6 resistant lines would have been discarded. In the Sunco/Tasman study, when the 2B and 4A markers were combined, 72% of lines would have been chosen

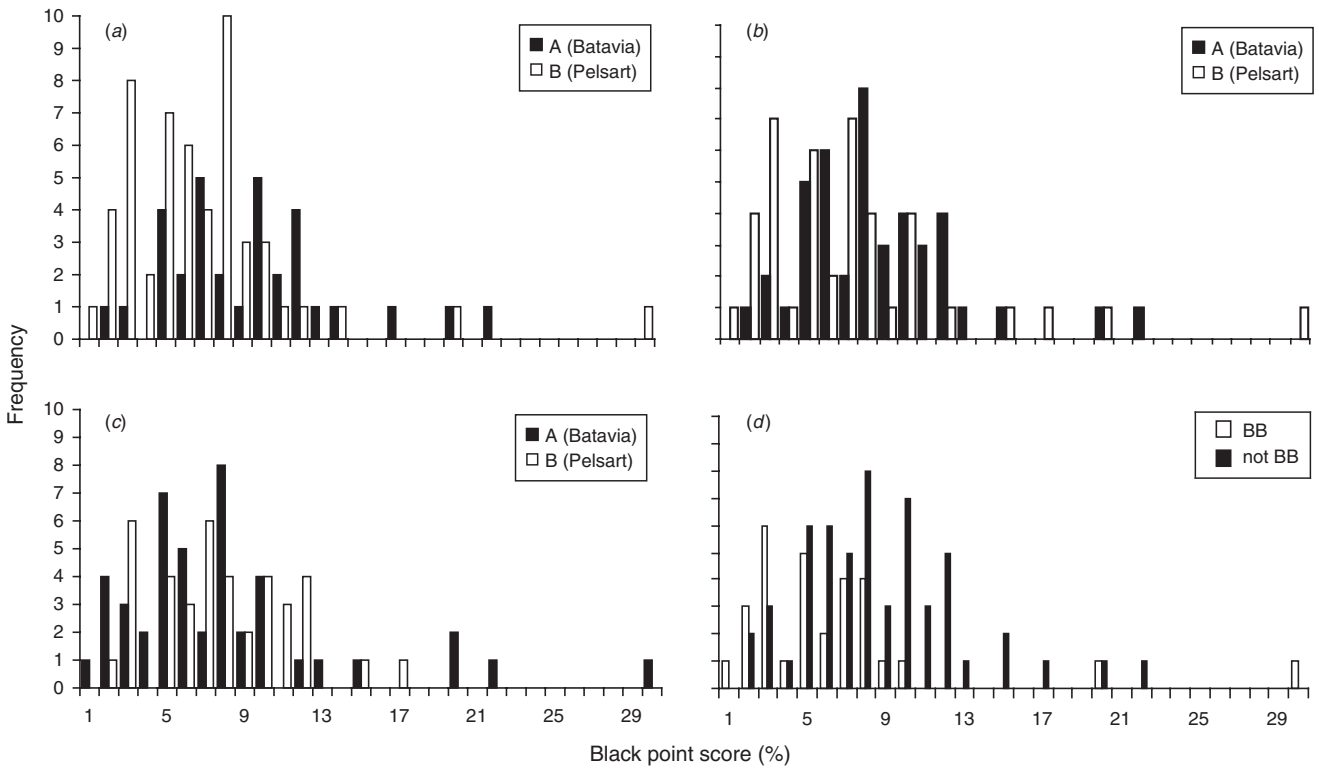

Fig. 2. Frequency distributions of percentage black point scores for A and B alleles for markers (*a*) *gwm319*, (*b*) *wmc048*, and (*c*) *gwm341* of the doubled haploid population Batavia × Pelsart, showing overlapping, continuous distributions with different means for each genotype and some evidence of bimodality within genotypes. (*d*) The frequency distribution for lines carrying the Pelsart (B) alleles at both *gwm319* and *wmc048* loci (mean 6.50) showing a shift in the distribution towards lower black point scores when compared with lines carrying the Pelsart allele at either one or neither locus (mean 8.62).

with 24 false positives. By including *gwm341* with the other 2 markers the highest level of enrichment was achieved, but 9 of the 17 resistant lines would not be captured. Eight out of a total of 17 resistant lines (47%) would have been selected compared with 53% in the Sunco/Tasman study. In this case the Batavia allele would be chosen at this locus.

In summary, the level of enrichment achieved would have been increased with the application of molecular markers in the order *gwm341* < *gwm319* < *wmc048* < *gwm319* & *wmc048* < *gwm319* & *wmc048* & *gwm341*. However, with this enrichment, the number of resistant lines that would be discarded by applying marker-based selection would have increased from 2 to 9 in 17.

Interactions

Of the 2 resistant DH lines not carrying the allele associated with resistance from *gwm319*, both carried the allele associated with resistance from *wmc048*. Thirty-eight of the total 85 lines carried the *gwm319* allele from Pelsart but were susceptible to black point, i.e. were false positives. In fact, the line with the greatest black point score of 30% carried the markers associated with black point resistance at all 3 loci.

Of the 4 resistant DH lines not carrying the allele associated with resistance from *wmc048*, all carried the allele associated with resistance from *gwm319*. Twenty-nine of the 85 lines were false positives. Of the 7 resistant DH lines not carrying the allele associated with resistance from *gwm341*, all carried the allele associated with resistance from either *gwm319*, *wmc048*, or both. Thirty-five of the 84 lines were false positives.

Polymorphism of markers linked to the QTLs associated with black point resistance

The size of PCR products generated from *gwm319*, *wmc048*, and *gwm341* was determined for a set of 95 potential-recipient wheat lines from the prime hard wheat breeding program of Enterprise Grains Australia, including Batavia and Pelsart (see Supplementary Table in Accessory Publication). Ninety-one percent of the 94 potential recipients differed from the Pelsart allele for *gwm319* (PIC 0.15), 71% differed from the Pelsart allele for *wmc048* (PIC 0.68), and 90% differed from the Batavia allele for *gwm341* (PIC 0.61). The PIC of *gwm319* on the 2B translocated segment is low because only 2 product patterns were observed, indicating either presence or absence of the translocated segment. Pelsart has the second most common genotype for *wmc048*, with the PCR product size shared with 27 of the 94 recipient lines. Potential alternative markers for the 4A region include *gwm610*, *wmc491*, *wmc089*, and *wmc420*, which are all thought to lie within ~10 cM of *wmc048* (Appels 2004). One band from the marker *wmc435* was found to co-segregate with *wmc048* in the Batavia × Pelsart DH lines (data not shown). Batavia shares its allele for *gwm314* with only 9 of the 94 recipient lines.

Discussion

At least 2 markers for QTLs conferring black point resistance can be useful for improving selection efficiency by enriching populations for resistance (Table 1). If lines were chosen for phenotyping with the allele associated with resistance for *gwm319*, a 1.41-fold enrichment for resistance would be achieved in the population. If lines were chosen with the allele associated with resistance for *wmc048*, a 1.55-fold enrichment for resistance would be achieved. By using these 2 markers together an enrichment of 1.83-fold could be achieved. The improved population black point scores can be seen in Fig. 2*d*. So, by applying these markers, 30 from the total 85 lines would be selected for phenotyping. Of the total 17 resistant lines in the population, 11 of those would be in that set of 30. Six of the resistant lines would have been discarded. In a breeding context this would mean that over 2.8 times the lines could be screened, using the same resources for phenotyping, resulting in ~1.8 times more resistant lines being selected. The *gwm341* locus may have a modifying effect on the phenotype because it had little effect if used alone but enhanced enrichment when used in conjunction with the other 2 markers. This enrichment, however, came at the expense of a high proportion or resistant lines being discarded. Without further evidence it is unlikely that this marker would be used for marker-assisted selection.

The percentage of selected lines expressing resistance is in close agreement with the Lehmensiek *et al*. (2004) study, but in the current study more lines not expressing resistance were selected based on markers. The most likely explanation for this is that the Sunco/Tasman population did not express the preferential transmission of the 2B *H. timopheevii* segment that was seen in the Batavia/Pelsart study. Thus a smaller proportion of lines in total was selected by the 2B marker in the Sunco/Tasman study (101/180 *v.* 53/85). So it seems that the higher transmission of the marker in the Batavia/Pelsart study does not mean a higher transmission of black point resistance. This suggests that the marker *abg356* used in the Sunco/Tasman study is closer to the QTL than *gwm319*, but this argument is difficult to accommodate if we believe that recombination between the translocated segment and wheat DNA is very low. In the 2 studies, 22% and 20% of lines were classified as resistant, so despite the slightly different separation point for resistant and susceptible lines (5% and 4%, respectively), it seems unlikely that differences in phenotyping are contributing to this observation. In fact, one might expect fewer, rather than more, false positives with the more stringent classification.

The inheritance of resistance to black point is clearly complex. The transgressive segregation observed suggests interactions between several genes in determining the genetic component of black point resistance (Fig. 1). The segregation for resistant to susceptible to black point was consistent with a ratio of 1 : 3 (Table 1). This predicted ratio is based on a model for 2 major, recessive genes controlling resistance to black point. This fit is dependent upon where the division is made between resistant and susceptible. In fact, if a 10% cut-off is applied to distinguish between resistant and susceptible lines, *gwm319* is not shown to be associated with black point resistance at all. Also, using this criterion, the segregation for resistant to susceptible to black point changes from 1 : 3 to 3 : 1, thus suggesting a different dominance relationship for different levels of resistance (Christopher *et al*. 2003).

The suggestive bimodality in several the frequency distributions for individual alleles (Fig. 2) implies involvement of at least one other major QTL in determining resistance to black point. Variation within a genotypic class results from the genetic effects of other QTLs, environmental variation, interactions

between QTLs, and variability in the phenotypic evaluations (experimental error).

The markers *wmc048* and *gwm341* showed no distortion from the expected 1 : 1 segregation, but *gwm319* showed considerable preferential transmission (Table 1). The *gwm319* allele associated with black point resistance is located on a chromosome segment translocated from *T. timopheevii* (Allard and Shands 1954). It has previously been observed that the chromosome segment translocated from *T. timopheevii* is transmitted preferentially (Nyquist 1962; McIntosh and Luig 1973). This translocation occurs in descendents of the Australian varieties Timvera, Mendos, Timgalen, Cook, and Songlen, including Sunco and Pelsart. It carries *Sr36* and *Pm6*, and is thought to span the centromere of chromosome 2B (Friebe *et al*. 1996). The QTL for black point resistance on chromosome 2B resides on this translocation (Lehmensiek *et al*. 2004).

The interaction between these 3 QTLs appears to be complex. The 3 alleles associated with resistance in the mapping study were contributed by Sunco (Lehmensiek *et al*. 2004). In this study, the alleles associated with resistance came from the Cook-derived Pelsart for *gwm319*, *wmc048*, and from Batavia for *gwm341*. All resistant lines carried one or both alleles associated with resistance to black point at either the *gwm319* or the *wmc048* loci. In no case did the 'resistance' allele from *gwm341* confer resistance on its own. When resistance alleles were present at both *gwm319* and *wmc048*, the Batavia-derived allele at *gwm341* was associated with a higher proportion of resistant lines, although the chi-square value was not significant ($\chi^2_1 = 1.54$ n.s.). On the other hand, many lines carrying 1, 2, or 3 of the alleles thought to be associated with resistance were susceptible to damage from black point. Several factors may be contributing to this. Firstly, genes other than those identified at these 3 QTLs may be contributing to resistance to black point. Some of these may have pleiotropic effects, such as effects on maturity or head morphology. Also, 'susceptibility' loci may exist within the genome, which override the resistance effects conferred by the identified QTLs. Secondly, *wmc048* and *gwm341* are not 'perfect' markers, in that recombination will occur between the marker and genes conferring the effect in a percentage of the population. *Gwm319* is likely to be a near-perfect marker, since recombination between the *T. timopheevii* and wheat segment on 2B is expected to be greatly reduced due to lesser homology than within wheat (Friebe *et al*. 1996). Thirdly, complex epistatic effects may be occurring among QTLs. Finally, the criteria of a 4% cut off between resistant and susceptible lines may be distorting the application of genetic models.

The percentage of total phenotypic variation explained by these markers seems small, and is smaller than that explained by the markers used in the mapping study. Several factors may explain this. Firstly, almost certainly the large environmental effect and genotype × environment interactions involved in determining this trait have contributed to the small percentage of total variation explained by genetic variation, and variation in these factors will have contributed to the difference between the 2 studies. Secondly, this difference may be due to weaker linkage between the marker and the QTL. A third factor contributing to the different percentage of total variation explained by these QTLs is the random components involved in sampling populations for detection of QTLs (Beavis 1994). Despite the low overall percentage of phenotypic variation explained in this study, significant differences are observed between the black point score of groups of lines with different marker alleles, for *gwm319* and *wmc048*, and these markers can be useful in enrichment of populations for resistance to black point (Table 1).

The purpose in determining the level of polymorphism for these markers in potential mates is to determine in what proportion of potential parental backgrounds these markers will be useful. When markers are not polymorphic between potential parent pairs, alternative polymorphic markers will be required. In the set of genetic material studied, *gwm319* shows 2 basic patterns, associated with either presence or absence of the translocation. Since crossing over is thought to occur at a greatly reduced rate between foreign chromosome segments and wheat (Sears 1993), any marker located on this segment can be considered a 'near-perfect' marker, in that its inheritance is highly correlated with the inheritance of the QTL. The Pelsart-derived allele for *wmc048* is shared with a high proportion of recipient lines. It is not known to what extent this shared allele indicates the presence of the black point resistance QTL in these lines. The group of lines sharing this allele includes both black point resistant and black point susceptible lines, but is dominated by lines derived from Cook. Alternative polymorphic markers will be required for these parent combinations. For *gwm341* the Batavia allele is associated with resistance. This allele is relatively rare in the potential parent set, so there will be few instances when alternate markers are required. It is desirable to have a suite of markers associated with a QTL, so that polymorphic markers are available for most potential parent combinations.

The markers identified in this study can be usefully applied to a selection program. Markers could be applied to enrich a selection population for one or more alleles associated with QTLs conferring black point resistance. The result will be that a higher proportion of lines subjected to phenotypic testing for black point resistance will be resistant. In many cases, however, there may be little or no benefit in deploying *gwm341*. Although the QTL associated with *gwm319* has the greatest influence on resistance to black point, this QTL is associated with a translocation from *T. timopheevii*. This segment is linked in repulsion to some desirable traits, and therefore will not always be a suitable source of black point resistance. In some instances, breeders may wish to select against the Pelsart allele from *gwm319* but select for the black point resistance associated allele from Pelsart associated with *wmc048*.

Given the inaccuracy and expense of phenotypic screening, in many cases it will be acceptable to sacrifice a small number of resistant lines, to enrich the population to be phenotyped, for alleles which are associated with resistance. Thus, for an equal amount of phenotypic screening a greater genetic gain can be achieved. In addition, these markers can be used, in combination with those associated with other sources of black point resistance, to combine different genetic sources of resistance. Depending upon epistatic effects, this may lead to higher levels of resistance to black point.

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