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ORIGINAL PAPER

Pyramiding QTL increases seedling resistance to crown rot (*Fusarium pseudograminearum*) of wheat (*Triticum aestivum*)

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Abstract Crown rot of wheat (*Triticum aestivum*), predominantly caused by the fungus *Fusarium pseudograminearum*, has become an increasingly important disease constraint in many winter cereal production regions in Australia. Our group has previously identified a range of quantitative trait loci (QTL) for partial resistance to crown rot in various bread wheat sources. Here, we report on work that has assessed the effectiveness of pyramiding QTL to improve resistance to crown rot. Two doubled haploid populations were analysed—one from a cross between two previously characterised sources of partial seedling resistance (2-49 and W21MMT70; $n = 208$) and one from a cross between 2-49 and the commercial variety Sunco, a source of adult field resistance ($n = 134$). Both populations were phenotyped for seedling resistance to crown rot. Microsatellite and DArT markers were used to construct whole genome linkage maps for use in composite interval mapping (CIM) to identify QTL. Three QTL were detected in both trials conducted on the 2-49/W21MMT70 population. These were located on chromosomes 1D (*QCr.usq-1D.1*), 3B (*QCr.usq-3B.1*) and 7A. *QCr.usq-1D.1* and the previously undetected 7A QTL were inherited from 2-49. *QCr.usq-3B.1,* inherited from W21MMT70, was the most significant of the QTL, explaining up to 40.5% of the phenotypic variance. Three QTL were identified in multiple trials of the Sunco/2-49 population. These were located on chromosomes 1D (*QCr.usq-1D.1*), 2B (*QCr.usq-2B.2*) and 4B (*QCr.usq-4B.1*). Only *QCr.usq-2B.2* was inherited from Sunco. *QCr.usq-4B.1* was the most significant of these QTL, explaining up to 19.1% of the phenotypic variance. In the 2-49/W21MMT70 population, several DH lines performed significantly better than either parent, with the best recording an average disease severity rating of only 3.8% of that scored by the susceptible check cultivar Puseas. These lines represent a new level of seedling crown rot resistance in wheat.

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Introduction

Crown rot of wheat is predominantly caused by *Fusarium pseudograminearum* (Aoki & O'Donnell) in the Northern grain growing region of Australia. In cooler, south-eastern regions, *F. culmorum* (WG Smith) Sacc. is also associated with this disease while other species [such as *F. avenaceum* (Corda:Fr) Sacc., *F. crookwellense* Burgess, Nelson & Toussoun and *F. graminearum* Schw.] are isolated infrequently (Backhouse et al. 2004). The disease has become more prevalent in the last two decades due to widespread adoption of conservation farming techniques which retain infected standing stubble between seasons. A shift to these practices in the Pacific Northwest of the United States has also resulted in an increase in disease incidence in this region (Smiley et al. 2005). Yield losses due to crown rot in Australia can be as high as 89% (Klein et al. 1991) and

annual losses have been estimated to cost the Australian wheat and barley industries an average of $79 and $18 million Australian dollars, respectively (Brennan and Murray 2009).

Partial resistance to crown rot in wheat is a quantitatively inherited trait showing a continuous distribution in all of the segregating populations that have been examined thus far (Wallwork et al. 2004; Collard et al. 2005, 2006; Bovill et al. 2006). Quantitative traits are not easily grouped into distinct categories, as the range of appearance of one genotype often overlaps that of others, creating the appearance of a continuous distribution (Kearsey and Pooni 1996). Such quantitative inheritance of complex traits results from a combination of: (1) multiple genes with main effects; (2) their interaction with other loci; and (3) their interaction with environments that affect their expression (Wade et al. 2001). Thus, in complex physiological systems, interactions between quantitative trait loci (QTL) or between QTL and the particular genetic and environmental background in which they are expressed can be expected to make a substantial contribution to the phenotypic variation of quantitative traits (Carlborg and Haley 2004; Cheverud and Routman 1995).

To date, most QTL mapping studies have focused on QTL discovery and estimations of the contribution made to phenotypic variation by each QTL within a population. This important first step is often undertaken with the goal of identifying QTL that can be combined with others, in order to achieve a desired phenotype. Indeed, pyramiding of QTL for increased disease resistance has been seen as perhaps the most valuable use of molecular markers linked to QTL (Dekkers and Hospital 2002). However, only relatively few studies have examined the outcomes of combining QTL from characterised sources of resistance. The outcomes are not always as expected. For example, Miedaner et al. (2006) combined three QTL (one each on chromosomes 3B and 5A, inherited from CM82036, and one on chromosome 3A, inherited from Frontana) for Fusarium head blight (FHB) resistance into an elite European spring wheat background. They found that individually, each QTL reduced deoxynivalenol (DON) concentration but that the effect of the 3A QTL on disease rating, either alone or when in combination with the 3B and 5A QTL, was not significant.

To investigate the effects of combining QTL for partial CR resistance from different sources, we have assessed two doubled haploid populations (2-49/W21MMT70 and Sunco/2-49), involving two previously characterised sources (2-49 and W21MMT70) of crown rot resistance in seedlings (Collard et al. 2005; Bovill et al. 2006) and the Sunco source of adult field resistance. Line 2-49 in particular has rated consistently well in many seedling and field trials for crown rot resistance (Wildermuth and McNamara 1994) and is widely considered as the current benchmark in the search for a more robust resistance, despite poor agronomic characteristics. The commercial variety Sunco is moderately susceptible to crown rot infection in seedling trials (Wildermuth et al. 2001), but is one of the few commercial varieties available in Australia with a useful level of adult field resistance to crown rot (Wildermuth and Morgan 2004). QTL for resistance to crown rot from the Sunco source have not been previously reported. Our results provide evidence for the benefit of pyramiding sources of quantitative resistance, while also demonstrating the challenges of combining QTL whose expression may alter with genetic background.

Materials and methods

Plant materials

Two wheat × maize induced doubled haploid populations were prepared from crosses between 2-49 (Gluyas Early/Gala) and W21MMT70 (Western Australian breeding line of unknown pedigree) and between Sunco (SUN9E27*4/3AG14//WW15/3/3*COOK) and 2-49. The 2-49/W21MMT70 cross produced 208 DH lines, while the Sunco/2-49 cross produced 134 DH lines.

Seedling disease assessment

Two replicated seedling trials were conducted on the 2-49/W21MMT70 population in glasshouses at the Leslie Research Centre in 2006 and 2007. Three replicated seedling trials were conducted on the Sunco/2-49 population in 2004, 2007, and 2008. Phenotyping was conducted according to the method of Wildermuth and McNamara (1994). Briefly, 13 seeds of each genotype were sown in pots containing partially sterilised soil inoculated with *Fusarium pseudograminearum*. After 21 days, each of the first three leaf sheaths from 10 seedlings per pot were rated for disease severity using a five point scale whereby: 0 = no infection; 1 = 0–25%; 2 = 25–50%; 3 = 50–75%; and 4 = 75–100%. The values obtained for each leaf sheath were added to give an overall score out of 12 for each seedling. All trials included the susceptible check cultivar 'Puseas', and the mean disease severity ratings per plant of the doubled haploid lines were converted to a percent (%) 'Puseas' scale.

DNA extraction

DNA was extracted from 5 to 7-day old etiolated seedlings that were grown in 24-well culture plates in a 25°C incubator. DNA was extracted using a Wizard genomic DNA purification kit (Promega), as per the manufacturer's instructions. DNA was diluted to a concentration of 10 ng/μL

prior to use in PCR. PCR conditions for microsatellite analysis were those detailed in Bovill et al. 2006.

Molecular mapping

We initially constructed partial linkage maps of the Sunco/2-49 and 2-49/W21MMT70 populations, using microsatellite (SSR) markers which flanked QTL previously identified in the 2-49/Janz (Collard et al. 2005) and the W21MMT70/Mendos (Bovill et al. 2006) DH populations. Subsequently, genotypic assays were outsourced to Triticarte Pty Ltd (http://www.triticarte.com.au) for Diversity Arrays Technology (DArT) analysis, leading to construction of whole genome linkage maps of both populations incorporating co-dominant SSR and dominant DArT markers. This exercise was necessary to discover QTL that may not have been segregating in the original populations. Microsatellite and DArT markers were ordered using RECORD (Van Os et al. 2005) and placed into linkage groups using MapManager QTX b20 (Manly et al. 2001). A P value of 0.001 was used to define linkage groups, and linkage groups were not forced into chromosomes if this condition was not met. Each map was curated as recommended by Lehmensiek et al. (2005). The 2-49/W21MMT70 linkage map is composed of 462 markers, of which 288 were deemed not redundant for QTL mapping, and covers 1940.1 cM. The Sunco/2-49 linkage map is composed of 460 markers, of which 252 were deemed not redundant for QTL mapping, and covers 1,536.4 cM.

QTL detection

QTL detection by composite interval mapping (CIM) was performed using Windows QTL Cartographer version 2.5 (Wang et al. 2007). One thousand (1,000) permutation tests at 2 cM intervals were carried out to determine likelihood ratio statistic (LRS; equal to LOD ×4.61) significance thresholds for QTL detection for all trials. MapChart 2.2 (Voorrips 2002) was used for graphical presentation of linkage groups and QTL. QTL were named as per the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), only if the locus had been previously detected in an independent population (for example, *QCr-usq-1A.1*; where *Q* refers to QTL, *Cr* to crown rot, *usq* to the University of Southern Queensland, and 1A.1 to chromosome location; 1A.2 would indicate a second QTL on chromosome 1A).

Results

The histograms of mean seedling response to infection for each of the populations show a continuous distribution (Fig. 1). Line 2-49 displayed greater resistance to crown rot than the alternate parent (W21MMT70 or Sunco) in each of the crosses. Based upon the mean rating for each line in two seedling trials, 28.8% of the individuals in the 2-49/W21MMT70 population scored lower disease ratings than 2-49, reflecting the transgressive segregation expected in a population in which both parents possessed independent, additive seedling resistance.

Descriptive statistics for the individual trials of each population are shown in Tables 1, 2. A significant correlation was evident between the 2006 and 2007 seedling trials of the 2-49/W21MMT70 population (Table 1; $r = 0.61$). The best performing individuals recorded disease severity ratings of only 2.2 and 2.4% Puseas in 2006 and 2007, respectively. In comparison, the best performing individuals in the Sunco/2-49 population displayed a disease severity rating of 20.5% Puseas in the 2004 trial, 18.3% Puseas in the 2007 seedling trial, and 32.0% Puseas in the 2008 trial. As was the case with the 2-49/W21MMT70 trials, two-way correlations between all three Sunco/2-49 trials were significant (Table 2).

In the 2-49/W21MMT70 population, a total of six QTL (located on chromosomes 1D, 2B, 3B, 5B, 6B, and 7A)

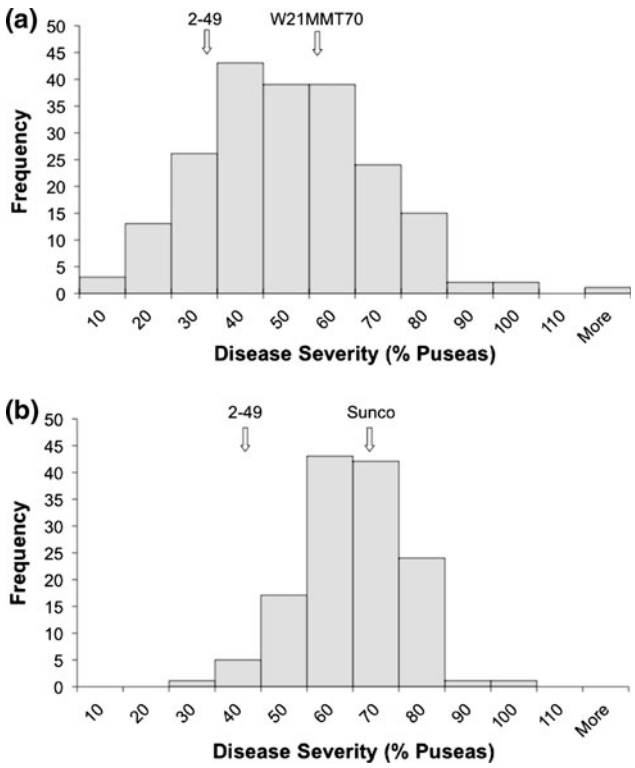

Fig. 1 Histograms of mean crown rot severity ratings for (**a**) the 2-49/W21MMT70 population and (**b**) the Sunco/2-49 population. Disease severity is expressed as a percentage of the susceptible check cultivar Puseas. Parental means are indicated by *arrows*

Table 1 Descriptive statistics of response to infection with *Fusarium pseudograminearum* in each trial of the 2-49/W21MMT70 populations at the seedling stage

Year	2-49	W21MMT70	*n*	Population mean	Population range	*r* (2007)
2006	28.0	46.9	208	43.0	2.2–143.3	0.61***
2007	31.8	58.5	208	48.2	2.4–114.5	

Values represent percentage infection relative to the susceptible check cultivar Puseas. The correlation co-efficient (*r*) between each of the trials is shown

*** Significant at the 0.1% level

were detected in at least one of the individual trials (Table 3). Of these QTL, three (on chromosomes 1D, 3B, and 7A) were detected in both trials. The 1D QTL (*QCr.usq-1D.1*), inherited from 2-49, has also been detected in a 2-49/Janz population (Collard et al. 2005) and a Gluyas Early/Janz population (Collard et al. 2006). The 3B QTL (*QCr.usq-3B.1*), inherited from W21MMT70, was originally identified in a W21MMT70/Mendos population (Bovill et al. 2006). The 7A QTL, inherited from 2-49, has not been detected previously in either parent. *QCr.usq-3B.1* was the most significant QTL, explaining 20.4% of the phenotypic variance in 2006, and 40.5% of the phenotypic variance in 2007. *QCr.usq-1D.1* was also highly significant, explaining up to 10.4% of the phenotypic variance based upon the 2007 trial, while the 7A QTL explained 3.8% of the phenotypic variance and was rated as suggestive in both the 2006 and 2007 trials (Table 3). LRS plots for the consistent QTL identified in the 2-49/W21MMT70 population are shown in Fig. 2.

In the Sunco/2-49 population, a total of ten QTL (located on chromosomes 1B, 1D, 2B, 3B [three QTL], 4B, 5B, 5D, and 6B) were detected in at least one of the three seedling trials (Table 4). Of these ten QTL, only three (on chromosomes 1D, 2B, and 4B) were detected in at least two of the three seedling trials. The 1D and 4B QTL were inherited from 2-49, whereas the 2B QTL was inherited from Sunco. The 1D QTL (*QCr.usq-1D.1*) has now been detected in four independent populations (see above). The 4B QTL *(QCr.usq-4B.1*) has been previously detected in a 2-49/Janz population (Collard et al. 2005) while the 2B QTL (*QCr.usq-2B.2*) is located on the same introgression

Table 2 Descriptive statistics of response to infection with *Fusarium pseudograminearum* in each trial of the Sunco/2-49 populations at the seedling stage

Year	Sunco	2-49	*n*	Population mean	Population range	*r* (2007)	*r* (2008)
2004	57.2	40.3	134	52.0	20.5–78.0	0.49***	0.28**
2007	88.4	26.7	134	71.0	18.3–141.3		0.48***
2008	68.9	40.6	134	57.0	32.0–79.5		

Values represent percentage infection relative to the susceptible check cultivar Puseas. The correlation co-efficient (*r*) between each of the trials is shown

** Significant at the 1% level, *** at the 0.1% level

Table 3 QTL identified in the 2-49/W21MMT70 population in 2006 and 2007

Chr.[a]	Flanking Markers	2006			2007			Parent[e]	Validated?[f]
		LRS[b]	VE[c]	SL[d]	LRS	VE	SL		
1D	wPt-3738–cfd19	21.6	6.8	HS	30.9	10.4	HS	2-49	Y (*QCr.usq-1D.1*)
2B	wPt-5680–wPt-0615	22.6	7.2	HS	5.3	1.4	NS	W21MMT70	N
3B	wPt-7301–wPt-0365	59.6	20.4	HS	118.4	40.5	HS	W21MMT70	Y (*QCr.usq-3B.1)*
5B	wPt-3569–wPt-0921	8.8	2.9	Sg	2.3	0.6	NS	W21MMT70	N
6B	wPt-8268–wPt-5270	12.5	4.3	Sg	3.2	0.9	NS	W21MMT70	N
7A	wPt-4748–wPt-8418	12.2	3.8	Sg	13.3	3.8	Sg	2-49	N

[a] The chromosome (Chr.) location of the QTL

[b] Likelihood ratio statistic (LRS)

[c] Percent phenotypic variance explained (VE)

[d] Significance level (SL; based upon 1,000 permutations at 2 cM intervals: *HS* highly significant, *S* significant, *Sg* suggestive.)

[e] The parent contributing the favourable allele (Parent)

[f] Whether the detected QTL have confirmed those in the original studies (Validated? *Y* yes, *N* no) are shown

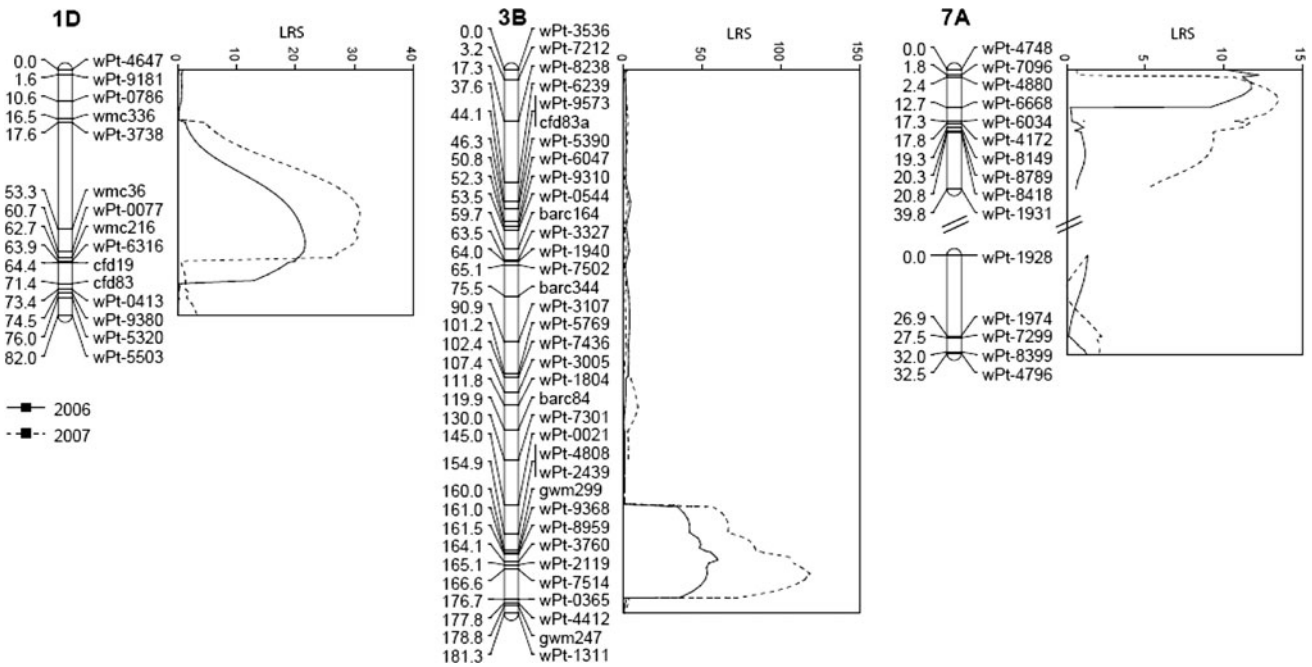

Fig. 2 LRS plots of the three consistent QTL detected in the 2-49/W21MMT70 population

Table 4 QTL identified in the Sunco/2-49 population in 2004, 2007 and 2008

Chr.[a]	Flanking markers	2004			2007			2008			Parent[e]	Validated?[f]
		LRS[b]	VE[c]	SL[d]	LRS	VE	SL	LRS	VE	SL		
1B	wPt-1399–wPt-8168	5.0	2.9	NS	5.4	3.2	NS	2.1	1.0	NS	2-49	N
1D	wPt-9380–cfd19	3.7	2.1	NS	7.8	3.7	Sg	17.6	8.6	S	2-49	Y (*QCr.usq-1D.1*)
2B	wPt-5374–wPt-0434	10.5	6.4	Sg	16.4	8.4	S	5.3	3.3	NS	Sunco	Y (*QCr.usq-2B.2*)
3B	wPt-1804–wPt-2458	5.5	3.4	NS	12.6	6.7	Sg	3.0	1.8	NS	Sunco	N
3B	wPt-2458–wPt-4209	5.5	3.3	NS	41.2	25.1	HS	2.4	1.3	NS	2-49	N
3B	wPt-8238–wPt-7212	0.4	0.3	NS	1.5	0.8	NS	9.7	5.5	Sg	2-49	N
4B	wPt-4535–gwm251	2.0	1.2	NS	20.7	10.0	HS	35.3	19.1	HS	2-49	Y (*QCr.usq-4B.1*)
5B	wPt-1482–wPt-3661	1.6	0.9	NS	2.6	1.3	NS	7.9	4.6	Sg	2-49	N
5D	cfd8–wPt-3931	1.9	1.2	NS	9.7	5.6	Sg	0.6	0.3	NS	2-49	N
6B	wPt-2424–wPt-8814	12.8	9.3	Sg	4.4	2.1	NS	2.8	1.5	NS	Sunco	N

[a] The chromosome (Chr.) location of the QTL

[b] Likelihood ratio statistic (LRS)

[c] Percent phenotypic variance explained (VE)

[d] Significance level (SL; based upon 1,000 permutations at 2 cM intervals: *HS* highly significant, *S* significant, *Sg* suggestive)

[e] The parent contributing the favourable allele (Parent)

[f] Whether the detected QTL have confirmed those in the original studies (Validated? *Y* yes, *N* no) are shown

on which a QTL was detected in a W21MMT70/Mendos population (Bovill et al. 2006). Consequently both *QCr.usq-4B.1* and *QCr.usq-2B.2* have now been validated in this current study. LRS plots of the QTL identified in the Sunco/2-49 population are shown in Fig. 3.

To assess the effectiveness of the pyramiding strategy to increase resistance to crown rot, we compared the resistance level of individuals with varying combinations of QTL from each of the donor parents (Fig. 4). In the 2-49/W21MMT70 population (Fig. 4a), lines bearing all three of the *QCr.usq-1D.1*, *Qcr.usq-3B.1*, and the 7A QTL were significantly more resistant to crown rot than lines possessing none of the QTL. Combining *QCr.usq-1D.1* and *Qcr.usq-3B.1* led to a 51.2% decrease in crown

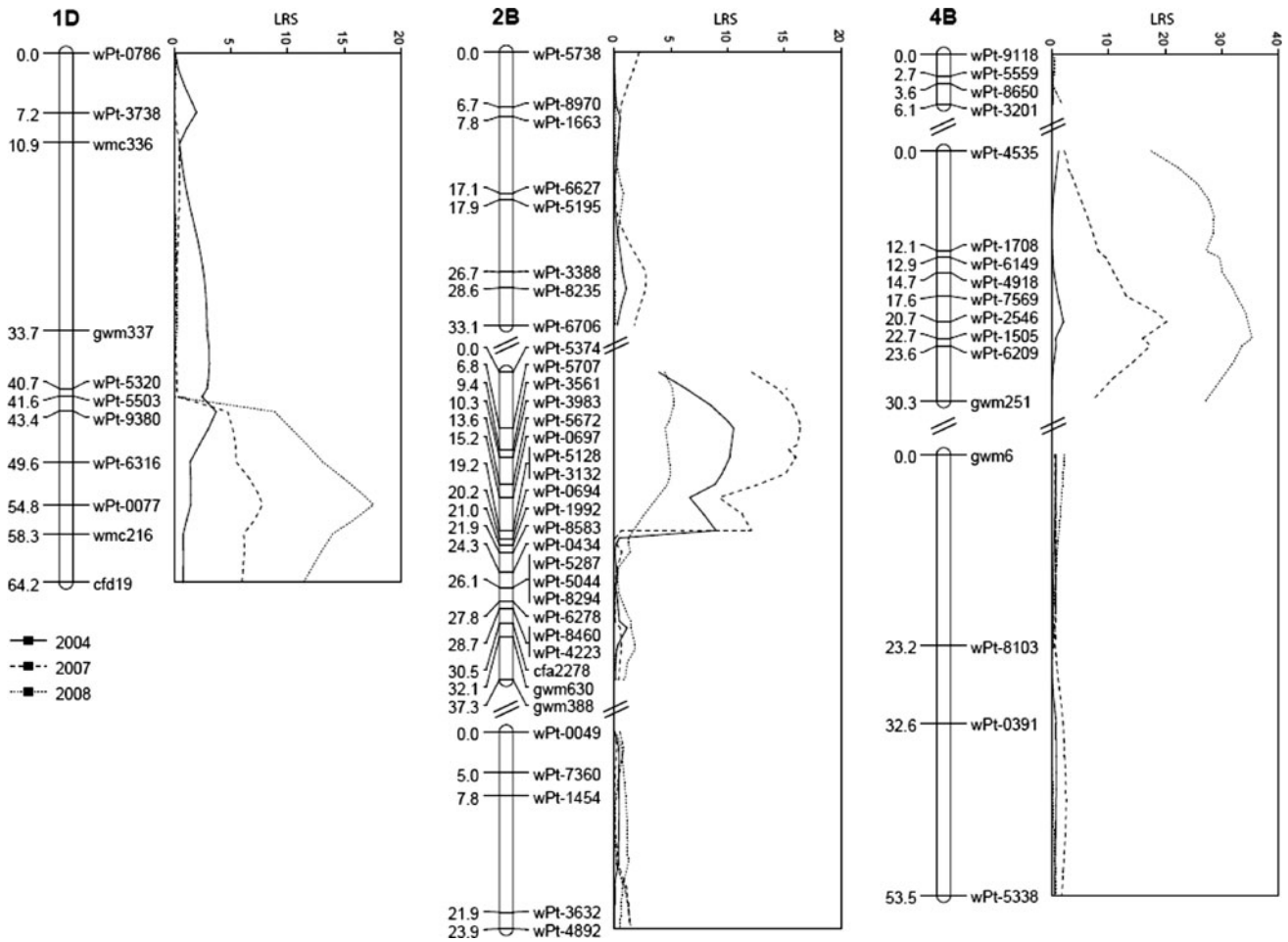

Fig. 3 LRS plots of the three consistent QTL detected in the Sunco/2-49 population

severity compared to the no QTL class; on average, individuals with these QTL were not significantly different to individuals with all three QTL. Lines with neither of these QTL (i.e. only the 7A QTL) gave a mean disease severity rating 15% lower than the no QTL class. Interestingly, the lines with *Qcr.usq-3B.1* alone were not significantly different to lines with a combination of *QCr.usq-3B.1* and the 7A QTL, or *QCr.usq-1D.1* and the 7A QTL.

Only a few individuals were represented in some QTL classes in the Sunco/2-49 population (Fig. 4b). Each of these classes with low frequency contain individuals that do not possess *QCr.usq-2B.2*. This is a result of the location of this QTL on a *Triticum timopheevi* introgression present in Sunco. Segregation distortion in favour of retention of this introgression has been reported previously (Bovill et al. 2006; Kammholz et al. 2001). Individuals which possessed all three QTL were significantly more resistant to crown rot compared to all other QTL combinations (28.3% lower than individuals with no QTL), with the exception of individuals which possess *QCr.usq-4B.1* alone. However, numbers of lines in this latter class are very low due to the segregation distortion discussed above.

Discussion

The goal of this study was to examine the level of crown rot resistance which results from pyramiding resistance QTL from different sources. QTL from 2-49 (in a 2-49/Janz population) and QTL from W21MMT70 (in a W21MMT70/Mendos population) have been previously described (Collard et al. 2005; Bovill et al. 2006). The breeding lines 2-49 and W21MMT70 possess both seedling and field resistance to crown rot (Wildermuth and McNamara 1994; Bovill et al. 2006; Wildermuth unpublished results). While Sunco possesses partial resistance to crown rot in field trials of adult plants (Wildermuth et al. 2001), this variety shows moderate susceptibility in seedling trials (Wildermuth and McNamara 1994).

Based upon the mean relative scores across all trials, a number of doubled haploid lines performed better than 2-49 in each population (Fig. 1). This effect was most obvious in the 2-49/W21MMT70 population, where almost 30% of individuals returned lower disease ratings than 2-49, indicating the additive nature of the contributing QTL. Line 2-49 is itself an early exercise in gene pyramiding,

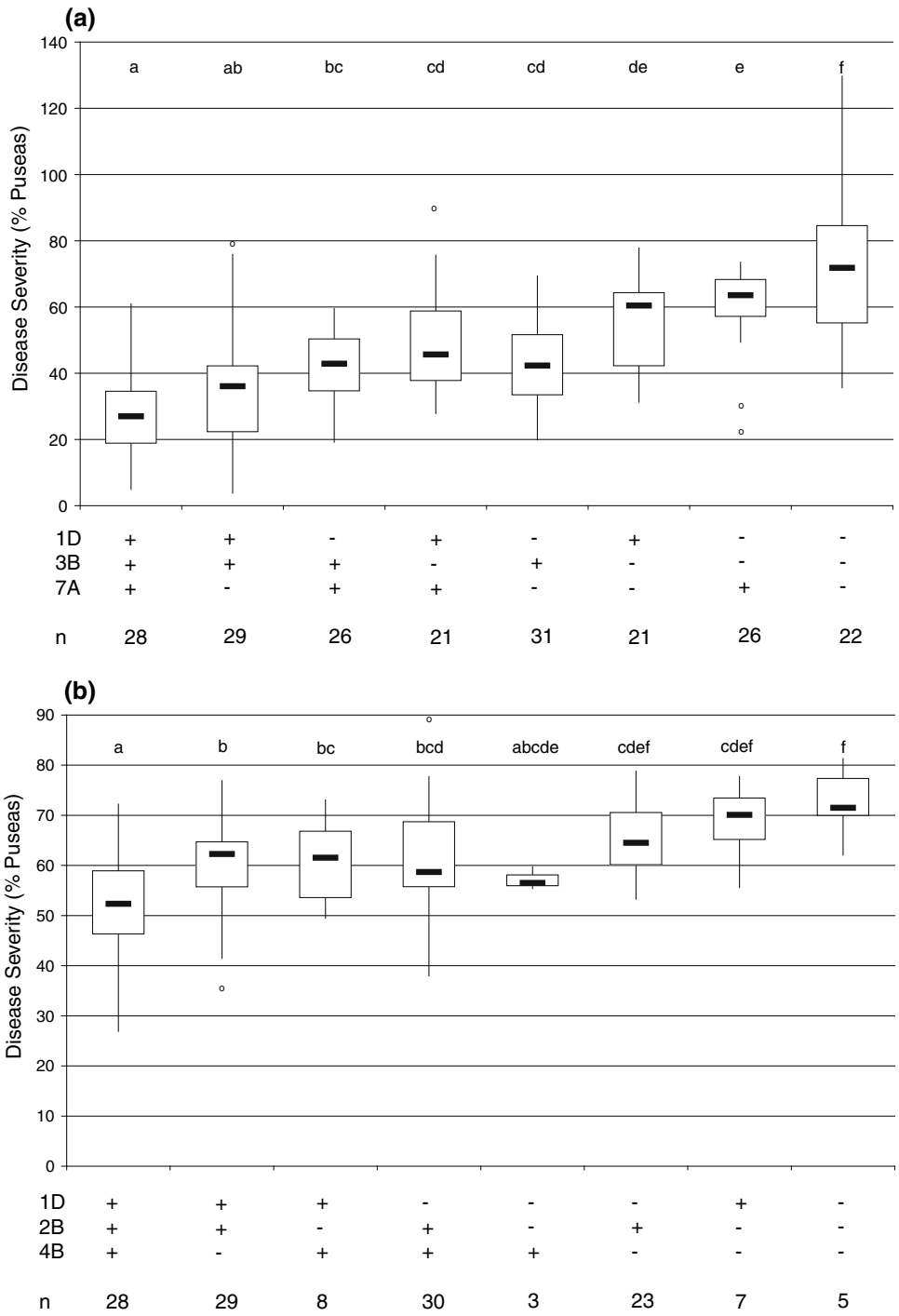

Fig. 4 *Box* plot distributions of disease severity ratings (% Puseas) of lines possessing various QTL combinations based upon the mean of the trials, in the 2-49/W21MMT70 population (**a**) and the Sunco/2-49 population (**b**). *Boxes* indicate the 25 and 75 percentiles; the median is indicated by the *solid horizontal line*. *Vertical lines* represent the range; outliers are indicated by *circles*. *n* represents the number of individuals per QTL class. *Boxes* which share the *same letter* are not significantly different (LSD, $P > 0.05$)

being derived from a cross between Gluyas Early and Gala (Dodman et al. 1980), which were among the most resistant lines available at that time. Subsequent analysis has demonstrated that resistance QTL from both parents were donated to 2-49 (Collard et al. 2006). For some time 2-49 has been recognised as the bench mark for resistance to crown rot, and the identification in this current study of lines showing significantly lower disease scores is extremely promising.

The correlations between trials of the same population were significant in all cases (Tables 1, 2). This highlights the reproducibility of the Wildermuth and McNamara (1994) method used for assessing plant responses to seedling infection with *F. pseudograminearum*. A number of

alternative methods for phenotyping seedlings infected with *F. pseudograminearum* have recently been reported (Mitter et al. 2006; Li et al. 2008). These approaches use conidial suspensions placed on the stem rather than a subsurface band of ground, colonised grain as an inoculum. These alternative tests are slower to complete (assessed 5 weeks after planting rather than the 3 weeks required by the Wildermuth and McNamara (1994, method) and their correlation with other seedling trial methods and field screening of adult plants, is yet to be demonstrated.

In the 2-49/W21MMT70 population, two of seven QTL identified in the parents in independent mapping populations were detected (Table 3). Collard et al. (2006) have previously validated the 1D QTL (*QCr.usq-1D.1*) in a Gluyas Early × Janz doubled haploid population. The detection of *QCr.usq-1D.1* in two further populations in this current study indicates the consistent seedling expression of this QTL across a range of backgrounds. The other QTL previously detected in line 2-49 on chromosomes 1A, 4B, and 7B (Collard et al. 2005) and originally derived from Gala (Collard et al. 2006), were not detected in the 2-49/W21MMT70 population. This suggests that these minor QTL undergo significant interaction with the genetic background into which they are crossed. A novel and minor QTL on chromosome 7A from 2-49 was identified in the 2-49/W21MMT70 population, but was not detected in the 2-49/Janz mapping population (Collard et al. 2005), perhaps again the result of a genetic background effect or alternatively a lack of polymorphism at this locus in the 2-49/Janz population.

Of the three W21MMT70-derived QTL identified in the W21MMT70/Mendos mapping population (located on chromosomes 2D, 3B, and 5D; Bovill et al. 2006) only one (*QCr.usq-3B.1*) was shown to have an effect in the 2-49/W21MMT70 population. There are a number of reasons that may explain the inability to detect the 2D and 5D QTL. In the source W21MMT70/Mendos population, the 5D QTL was shown to have a highly significant effect in the 2001 growth cabinet trial, but significant and suggestive effects in the 2003 and 2005 glasshouse trials. The more recent phenotyping of the 2-49/W21MMT70 population was conducted in glasshouse trials. It may be possible that this QTL could have been detected if the trial was conducted in a growth cabinet environment where superior temperature control is achieved. It is also possible that less than optimal numbers of individuals for genotyping and phenotyping in the W21MMT70/Mendos population ($n = 95$) may have led to the identification of false positive QTL whose effects were overestimated (Beavis 1994). Alternatively, their lack of detection may also indicate that their expression is significantly dependent upon the genetic background into which they are introgressed (as is the case with *QCr.usq-4B.1*; see below). *QCr.usq-3B.1* had the greatest effect (LRS 112.3, explaining 34.5% of the phenotypic variance based upon the mean disease severity rating) of any of the QTL detected in the 2-49/W21MMT70 population. In the W21MMT70/Mendos population, this QTL was suggestive in two of the three seedling trials (2003 and 2005). The strong effect of this QTL in the 2-49/W21MMT70 population was unexpected.

In contrast to the 2-49/W21MMT70 population, only a few individuals (less than 2% of the population) performed better than 2-49 in the Sunco/2-49 population. Sunco is relatively susceptible at the seedling stage, and the lack of transgressive segregation towards resistance observed was not unexpected. Nevertheless, a minor seedling resistance QTL was found on chromosome 2BS in Sunco. Although QTL from Sunco have not been reported previously, Sunco possesses an introgression from *Triticum timopheevi* in this region of chromosome 2B, originally introduced because it carries the stem rust resistance gene Sr36. This introgression, which is also present in Mendos, has been shown to contribute to resistance in the W21MMT70/Mendos population (Bovill et al. 2006).

Of five QTL previously identified (the four mentioned above from 2-49, and the likely effect of the 2B *T. timopheevii* introgression in Sunco), three were detected in the Sunco/2-49 population. These include *QCr.usq-1D.1*, *QCr.usq-2B.2*, and *QCr.usq-4B.1* (Table 4; Fig. 3). Thus, *QCr.usq-1D.1* has now been confirmed in four populations (Collard et al. 2006; and this study) while *QCr.usq-2B.2*, originally identified in the W21MMT70/Mendos population, has now been validated in the Sunco/2-49 population.

QCr.usq-4B.1, which was not detected in the 2-49/W21MMT70 population, was shown to have an effect in the Sunco/2-49 population. In the original analysis of the 2-49/Janz population (Collard et al. 2005) this QTL was found to be linked in repulsion to the dwarfing gene allele *Rht1*. Wallwork et al. (2004) identified a QTL in a similar region in a Kukri/Janz population, which explained up to 48% of the relatively narrow phenotypic variance observed. In the Sunco/2-49 population, this QTL explained up to 19.1% of a much larger phenotypic variance. Given the relatively loose linkage to the *Rht1* dwarfing gene (19.8 cM, Collard et al. 2005), the selection of semi-dwarf individuals possessing *QCr.usq-4B.1* should be achievable. While these results demonstrate the potential value of this QTL in conferring resistance to crown rot, the lack of significance for *QCr.usq-4B.1* in the 2-49/W21MMT70 population indicates that expression of this QTL may be strongly influenced by genetic background.

To ascertain the effectiveness of pyramiding QTL from different resistance sources, we compared means of individuals with varying combinations of QTL (Fig. 4). In the 2-49/W21MMT70 population (Fig. 4a), the combination of all three QTL (*QCr.usq-1D.1*, *QCr.usq-3B.1*, and the 7A

QTL) significantly reduced crown rot disease severity. Interestingly, the combination of *QCr.usq-1D.1* and *QCr.usq-3B.1* was not significantly different in effect to the combination of all three QTL, indicating that the effect of the 7A QTL is relatively minor. One individual in the 2-49/W21MMT70 population recorded an average disease severity rating (over the two independent trials) of only 3.8% Puseas—a level of resistance that would be highly sought after in a commercial variety. Thus, pyramiding the 2-49 and W21MMT70 sources of resistance was effective in significantly reducing crown rot severity.

In the Sunco/2-49 population, individuals with all three QTL (*QCr.usq-1D.1*, *QCr.usq-2B.2*, and *QCr.usq-4B.1*; Fig. 4b) performed significantly better than all other QTL classes, with the exception of the *QCr.usq-4B.1* alone class. As the numbers of individuals in the QTL classes without *QCr.usq-2B.2* are low (as a result of segregation distortion in favour of the *Triticum timopheevi* introgression), we are cautious in our interpretation of these results. However, combining *QCr.usq-1D.1* and *QCr.usq-2B.2* did result in the production of individuals which performed better than individuals with either of these QTL in isolation. In comparison to the 2-49/W21MMT70 population, in which the average disease severity of individuals which possessed all three QTL was 59.7% lower than the no QTL class, in the Sunco/2-49 population (Fig. 4b) individuals which possessed all three QTL scored an average disease severity rating that was only 28.3% lower than the no QTL class. Thus, in this population, the results of pyramiding QTL for seedling resistance do not appear to be as effective as in the 2-49/W21MMT70 population. This difference between the two populations was not unexpected, as both parents possess seedling resistance in the 2-49/W21MMT70 population, whereas only 2-49 possesses seedling resistance in the Sunco/2-49 population. Much more significant benefits from combining the Sunco and 2-49 sources of resistance are likely to be realised in field trials.

This study has successfully pyramided QTL for CR seedling resistance in a significant proportion of individuals in the 2-49/W21MMT70 population, providing resistant semi-dwarf lines for future crossing into elite backgrounds. Field trials to further assess the materials in this study are in progress. We note that while expression of these QTL is largely additive, several show a degree of background dependence. Finally, we emphasise the necessity of developing very tightly linked flanking markers for routine selection of these QTL in breeding programs.

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References

Backhouse D, Abubakar AA, Burgess LW, Dennis JI, Hollaway GJ, Wildermuth GB, Wallwork H, Henry FJ (2004) Survey of *Fusarium* species associated with crown rot of wheat and barley in eastern Australia. Australas Plant Pathol 33:255–261

Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In: Proceedings of the Forty-ninth Sorghum Industry Research Conference. American Seed Trade Association, Washington, DC

Bovill WD, Ma W, Ritter K, Collard BCY, Davis M, Wildermuth GB, Sutherland MW (2006) Identification of novel QTL for resistance to crown rot in the doubled haploid wheat population 'W21MMT70' × 'Mendos'. Plant Breed 125:538–543

Brennan JP, Murray GM (2009) Current and potential costs from diseases of wheat in Australia. GRDC Report. ISBN 978-1-875477-92-0

Carlborg O, Haley CS (2004) Epistasis: too often neglected in complex trait studies? Nat Rev Genet 5:618–625

Cheverud JM, Routman EJ (1995) Epistasis and its contribution to genetic variance components. Genetics 139:1455–1461

Collard BCY, Grams RA, Bovill WD, Percy CD, Jolley R, Lehmensiek A, Wildermuth GB, Sutherland MW (2005) Development of molecular markers for crown rot resistance in wheat: mapping of QTLs for seedling resistance in a 2–49 x Janz population. Plant Breed 124:1–6

Collard BCY, Jolley R, Bovill WD, Grams RA, Wildermuth GB, Sutherland MW (2006) Confirmation of QTL mapping and marker validation for partial seedling resistance to crown rot in wheat line '2-49'. Aust J Agric Res 57:967–973

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nat Rev Genet 3:22–32

Dodman RL, Wildermuth GB, Cameron HF, Lavers JRW (1980) Plant pathology and soil microbiology: research on fungal diseases. Biennial report 1978–1980. Queensland Department of Primary Industries, Toowoomba

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

Kearsey MJ, Pooni HS (1996) The genetical analysis of quantitative traits. Stanley Thornes, Birmingham

Klein TA, Burgess LW, Ellison FW (1991) The incidence and spatial patterns of wheat plants infected by *Fusarium graminearum* Group 1 and the effect of crown rot on yield. Aust J Agric Res 42:399–407

Lehmensiek A, Eckermann PJ, Verbyla AP, Appels R, Sutherland MW, Daggard GE (2005) Curation of wheat maps to improve map accuracy and QTL detection. Aust J Agric Res 56:1347–1354

Li X, Liu C, Chakraborty S, Manners JM, Kazan K (2008) A simple method for the assessment of crown rot disease severity in wheat seedlings inoculated with *Fusarium pseudograminearum*. J Phytopath 156:751–754

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

Miedaner T, Wilde F, Steiner B, Buerstmayr H, Korzun V, Ebmeyer E (2006) Stacking quantitative trait loci (QTL) for *Fusarium* head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effect of deoxynivalenol (DON) content and disease severity. Theor Appl Genet 112:562–569

Mitter V, Zhang MC, Liu CJ, Ghosh R, Ghosh M, Chakraborty S (2006) A high-throughput glasshouse bioassay to detect crown rot resistance in wheat germplasm. Plant Pathol 55:433–442

Smiley RW, Gourlie JA, Easley SA, Patterson LM, Whittaker RG (2005) Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. Plant Dis 89:595–604

Van Os H, Stam P, Visser RGF, Van Eck HJ (2005) RECORD: a novel method for ordering loci on a genetic linkage map. Theor Appl Genet 112:30–40

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTL. Heredity 93:77–78

Wade MJ, Winther RG, Agrawal A, Goodnight CJ (2001) Alternative definitions of epistasis: dependence and interaction. Trends Ecol Evolut 16:498–504

Wallwork H, Butt M, Cheong JPE, Williams KJ (2004) Resistance to crown rot in wheat identified through an improved method for screening adult plants. Australas Plant Pathol 33:1–7

Wang S, Basten CJ, Zeng Z-B (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wildermuth GB, McNamara RB (1994) Testing wheat seedlings for resistance to crown rot caused by *Fusarium graminearum* Group 1. Plant Dis 78:949–953

Wildermuth GB, Morgan JM (2004) Genotypic differences in partial resistance to crown rot caused by *Fusarium pseudograminearum* in relation to an osmoregulation gene in wheat. Australas Plant Pathol 33:121–123

Wildermuth GB, McNamara RB, Quick JM (2001) Crown depth and susceptibility to crown rot in wheat. Euphytica 122:397–405