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ORIGINAL PAPER

Identification and validation of a major QTL conferring crown rot resistance in hexaploid wheat

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Abstract Crown rot (CR), caused by various *Fusarium* species, is a chronic wheat disease in Australia. As part of our objective of improving the efficiency of breeding CR resistant wheat varieties, we have been searching for novel sources of resistance. This paper reports on the genetic control of one of these newly identified resistant genotypes, 'CSCR6'. A population derived from a cross between CSCR6 and an Australian variety 'Lang' was analyzed using two *Fusarium* isolates belonging to two different species, one *Fusarium pseudograminearum* and the other *Fusarium graminearum*. The two isolates detected QTL with the same chromosomal locations and comparable magnitudes, indicating that CR resistance is not species-specific. The resistant allele of one of the QTL was derived from 'CSCR6'. This QTL, designated as *Qcrs.cpi-3B*, was located on the long arm of chromosome 3B and explains up to 48.8% of the phenotypic variance based on interval mapping analysis. Another QTL, with resistant allele from the variety 'Lang', was located on chromosome 4B. This QTL explained up to 22.8% of the phenotypic variance. A strong interaction between *Qcsr.cpi-3B* and *Qcsr.cpi-4B* was detected, reducing the maximum effect of *Qcrs.cpi-3B* to 43.1%. The effects of *Qcrs.cpi-3B* were further validated in four additional populations and the presence of this single QTL reduced CR severity by up to 42.1%. The fact that significant effects of *Qcrs.cpi-3B* were detected across all trials with different genetic backgrounds and with the use of isolates belonging to two different *Fusarium* species make it an ideal target for breeding programs as well as for further characterization of the gene(s) involved in its resistance.

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Introduction

Crown rot (CR) is a cereal disease caused by various *Fusarium* species. It has been a serious and chronic disease in Australia for many years. The disease incidence has increased in many other cereal growing regions worldwide in recent years (reviewed by Chakraborty et al. 2006), due most likely to the wider adoption of the minimum tillage as CR pathogens survive in crop residues (Burgess 2005). A recent study by Daniel and Simpfendorfer (2008) found an average of 25% yield loss in bread wheat and 58% loss in durum wheat across a wide range of environments in Australia due to CR disease. A survey in the Pacific

Northwest of the USA found that CR could reduce yield of winter wheat by 35% in commercial fields. In addition to yield losses, CR infected plants also accumulate mycotoxins in grain as well as other tissues (Mudge et al. 2006).

It is believed that the physical contact of wheat stem base with infested stubble of the preceding years facilitate the initial infection process of CR (Burgess 2005). Thus, reducing the inoculum load has been the focus of management practices such as crop rotation and stubble burning (Kirkegaard et al. 2004). However, stubble burning results in the loss of the valuable soil moisture and the practice is also an environmental concern. Crop rotation also has its limitations economically as CR pathogens can survive several years in stubble (Burgess 2005). Growing resistant wheat varieties has long been recognized as the most effective way to minimize CR damage and there have been efforts in Australia in breeding CR resistant varieties. The first report on differential varietal reactions to CR infection was reported as early as 1966 (Purss 1966). However, varieties with high levels of resistance are not yet available. One of the keys for successful breeding is the availability of high quality sources of resistance. Such sources, however, seem to be rare in wheat. Till date, genetic studies have been reported on only three sources with partial resistance. The first one was on the variety 'Kukri'. The adult plant resistance of this variety was assessed by growing plants in open-ended tubes placed in outdoor terraces. A locus conferring CR resistance was identified on chromosome 4B near the semi-dwarfing gene *Rht1* (Wallwork et al. 2004). The second study was on the breeding line '2-49' based on a seedling assay. Two QTL conferring CR resistance were detected in this genotype. One of them, located on chromosome arm 1DL, explained up to 21% of phenotypic variance. The other, located on chromosome arm 1AL, explained up to 10% of the variance (Collard et al. 2005). The third study was on the genotype 'W21MMT20', again based on a seedling assay (Bovill et al. 2006). This study identified several putative QTL conferring CR resistance but none of them reached significant levels in all of the three assays reported. The most significant QTL was located on 5D. This QTL was significant in two of the three assays and explained 28.0 and 14% of the phenotypic variance, respectively. The other QTL, located on 2D, was significant only in one of the three assays and it explained 10.2% of the variance. These variable results are not surprising as accurate CR assessment is notoriously difficult (Collard et al. 2005).

Our capacity in breeding resistant varieties could be dramatically enhanced if a few well-characterized sources with high levels of resistance were available. In an effort to identify novel sources of resistance, we carried out a screening of hexaploid wheats and their close relatives. One of the novel sources of resistance identified from this screening was investigated by QTL mapping and the effects of the detected QTL were further assessed in several different genetic backgrounds. Results obtained from these investigations are reported in this paper.

Materials and methods

Plant materials

Based on a survey of over 2,200 genotypes (CJL unpublished), the genotype 'CSCR6' was selected for this study. This genotype, belonging to the taxon *T. spelta*, was used to generate five populations for this study. These populations were all generated in the glasshouses at the Queensland Bioscience Precinct in Brisbane, Australia. One of the populations, consisting of F8 recombinant inbred lines (RIL), was derived from a cross between the Australian variety 'Lang' and 'CSCR6'. Ninety-two of these RILs were used in this study. The other four populations were developed for validating the effects of QTL identified from the mapping population. They included:

a) 'Aus13832'/'CSCR6' F5
b) 'Janz'/'CSCR6' F5
c) 'Janz'*2/'CSCR6' F4, and
d) 'Drysdale'//'Janz'/'CSCR6' F4.

Phenotyping for CR reaction

Two *Fusarium* isolates, one *F. pseudograminearum* (CS3096) and the other *F. graminearum* (CS3005), were used in this study for assessing CR reactions. These isolates were collected in northern New South Wales, Australia and maintained in the CSIRO collection (Akinsanmi et al. 2004). The procedures used for inoculum preparation, inoculation and CR assessment were based on that described by Li et al. (2008). Specifically, inoculum was prepared using plates of 1/2 strength potato dextrose agar. Incubated plates were kept for 12 days at room temperature (about 22°C constant) before the mycelium in the plates were scraped. The plates were then incubated for a further 5–7 days under a combination of cool white and black fluorescent lights with 12-h photoperiod. The spores were then harvested and the concentration of spore suspension was adjusted to 1×10^6 spores/ml. Tween 20 was added (0.1% v/v) to the spore suspension prior to use.

Seeds were germinated in Petri dishes on three layers of filter paper saturated with water. The germinated seedlings were immersed in the spore suspension for 1 min and sown in a 5 cm square punnet (Rite Grow Kwik Pots, Garden City Plastics, Australia) containing autoclaved potting mix. The punnets were arranged in a randomized block design in

either a glasshouse or a controlled environment facility (CEF). Settings for the glasshouses were: 25/18 (±5) °C day/night temperature and 65/80 (±5)% day/night relative humidity, with natural sunlight levels and variable photoperiod depending on the time of year. The settings for the CEF were: 25/15(±1) °C day/night temperature and 65/85 (±5)% day/night relative humidity, and a 14-h photoperiod with 500 μmol m^{-2} s^{-1} photon flux density at the level of the plant canopy. To promote CR development, water-stress was applied during the CR assessment. Inoculated seedlings were watered only when wilt symptoms appeared.

Four replicated trials were carried out using the isolates CS3005 (designated as CR05-1 to CR05-4, respectively) and two replicated trials were carried out using CS3096 (designated as CR96-1 and CR96-2, respectively). Each trial contains two replicates, each with ten seedlings. CR severity was assessed 35 days after inoculation, using 0 (no obvious symptom) to 5 (whole plant severely to completely nectrotic) scale as described by Li et al. (2008). A disease index (DI) was then calculated for each line following the formula of $DI = (\Sigma_{nX}/5N) \times 100$, where X is the scale value of each plant, n is the number of plants in the category, and N is the total number of plants assessed for each line.

Genotyping, map construction and QTL analysis

Two marker systems, DArT and SSR, were used for linkage map construction with 92 RILs of the 'Lang'/'CSCR6' population. DArT genotyping of the parents and the mapping population was carried out by the Triticarte Pty. Ltd. (http://www.tritcarte.com.au). A wheat DArT array (Version 2.3) consisting of 3,200 random markers and another array containing 2,700 markers from purified chromosome 3B clones were used. Procedures of hybridization of genomic DNA to the DArT array, image analysis and polymorphism scoring were as described by Akbari et al. (2006). In addition, 54 SSR markers polymorphic between two parents of the mapping population were also used for the linkage map construction. PCR reactions for the SSR analyses were carried out using [α-^{33}P]dCTP following manufacturer's protocol (Multiplex-Ready Marker User Handbook, version 2.0), and amplified samples were separated on 4% polyacrylamide gel containing 7 M urea.

Segregation ratios of markers were tested by Chi-square goodness-of-fit to a 1:1 ratio at the significant level of $p = 0.01$. Linkage analysis was carried out using the computer package JoinMap (version 4.0, Van Ooijen 2006). LOD thresholds from 3 to 10 were tested, until a threshold with the optimum number of markers in linkage groups maintaining linkage order and distance was obtained. The Kosambi mapping function was used to convert recombination ratios to map distances. Known chromosomal locations of some of the SSR and DArT markers were used to assign linkage groups to specific chromosomes.

MapQTL® 5.0 (Van Ooijen 2004) was used for QTL analysis. The Kruskal–Wallis test was used in a preliminary testing of associations between markers and CR reaction. Interval mapping (IM) was then used to identify major QTL. Automatic cofactor selection was used to fit the multiple QTL model (MQM) and to select significantly associated markers as cofactors. For each trial, a test of 1,000 permutations was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5% ($P < 0.05$). Based on the permutation test, a threshold LOD value was used to declare the presence of a QTL. A linkage map showing the QTL positions was drawn using MAPCHART (Voorrips 2002).

For QTL validation, 60 lines randomly selected from each of the four validation populations were assessed for CR reaction twice, one in the CEF and the other in the glasshouses. Similar to the QTL mapping exercises, two replicates, each consisting of ten individual plants, were used in each of the validation trials. Based on the presence or absence of marker alleles from the resistant parent 'CSCR6', the 60 lines from each of the four populations were grouped into two classes. The difference in CR severity between the two groups within each of the populations was used for measuring the QTL effects.

Results

Linkage map constructed for the population of 'Lang'/'CSCR6'

The 3,200 random DArT markers and the 2,700 DArT markers from purified chromosome 3B clones generated 635 and 332 polymorphic markers, respectively. These 967 polymorphic DArT markers and 54 polymorphic SSR markers were used for the linkage map construction in the 'Lang'/'CSCR6' population. Of these, 776 were assigned to 18 of the 21 wheat chromosomes and they covered a total genetic distance of 912.9 cM. The overall average distance between markers across all linkage groups was about 1.2 cM (Supplementary Table 1). None of the SSR or DArt markers with known chromosome locations were mapped to chromosomes 4D, 5D or 6D. As expected, as many as 198 loci were mapped on the single chromosome 3B. The average distance between a pair of the chromosome 3B markers was 0.6 cM (Supplementary Table 1 and Fig. 1).

Of the 332 polymorphic markers derived from the purified chromosome 3B clones, 144 were mapped to

chromosome 3B. Another 78 were mapped to 15 different chromosomes and the others were either mapped to linkage groups which could not be allocated to any particular chromosomes or formed singletons showing no linkage with other markers.

Of the 776 loci assigned to the linkage map, 251 (30.9%) showed skewed segregation. Among them, 145 (57.8%) were skewed favouring alleles from the male parent 'CSCR6' and the remaining favouring alleles from the female parent ''Lang'. A large proportion of these skewed loci were clustered on chromosomes 2B, 3B, 7D and 6B, respectively. The 59 skewed loci on chromosome 2B all favoured alleles from 'Lang' with an average ratio between alleles of the two parents being 1:2.4. The 51 skewed loci on chromosome 3B all favoured alleles from 'CSCR6' with a ratio of 1:0.4. The 15 skewed markers on chromosome 6B all favoured alleles from 'Lang' with an average ratio of 1:2.1 and the 28 skewed loci on chromosome 7D all favoured alleles from 'CSCR6', with a ratio of 1:0.2 (Fig. 1).

QTL conferring resistance to CR

A total of six replicated trials were conducted for assessing CR reaction of the RIL population. Two of the trials (designated as CR96-1 and CR96-2, respectively) were carried out using the *F. pseudograminearum* isolate CS3096 and the other four (designated as CR05-1, CR05-2, CR05-3 and CR05-4, respectively) using the *F. graminearum* isolate CS3005. Transgressive segregations were observed in each of the trials (Table 1). The correlation coefficients between CR05-1 and all the others were only intermediate with correlation coefficients (R) ranging between 0.50 and 0.53. Correlations among the other five trials were all strong, with R ranging from 0.82 to 0.99 (Table 2).

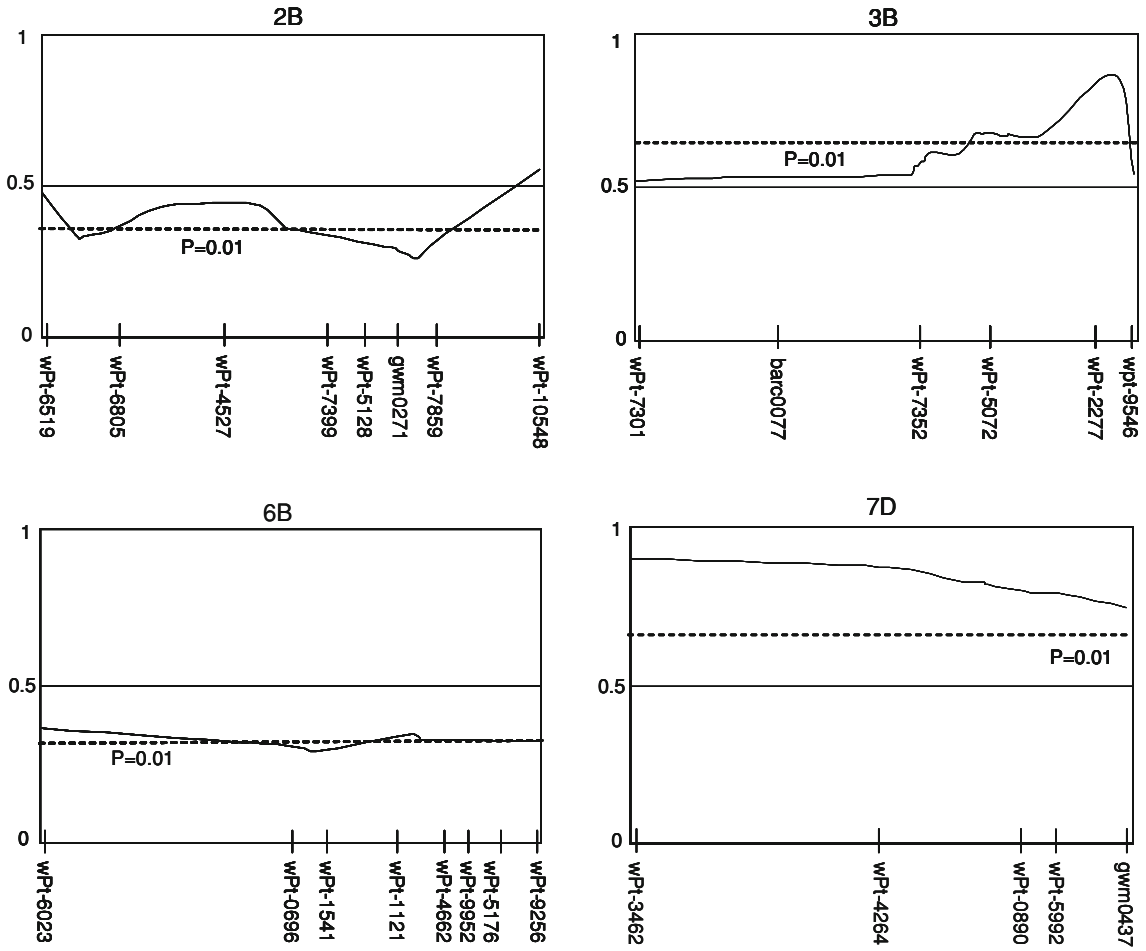

Fig. 1 Framework maps show the distributions of skewed loci on four linkage groups. The segregation ratios were calculated by the formula of $r = a/(a + b)$. Thus, $r < 0.5$ indicates segregation favouring alleles from 'Lang' and $r > 0.5$ favouring alleles from 'CSCR6'. Markers with segregation ratios fallen between the *dotted line* marked by $P = 0.01$ and $r = 1$ (for chromosomes 3B and 7D) or $r = 0$ (for chromosomes 2B and 6B) were highly significantly skewed favouring alleles from 'CSCR6' and 'Lang', respectively

Table 1 Distribution of crown rot severity in the population of 'Lang'/'CSCR6'

Trial	Parent		Population			
	Lang	CSCR6	Min	Max	Mean	SD
CR96-1	40.98	28.39	25.10	64.90	41.37	7.83
CR96-2	41.32	28.66	22.79	59.34	38.26	7.67
CR05-1	40.05	24.65	12.92	51.42	31.23	7.47
CR05-2	40.74	26.13	22.97	58.33	37.10	7.19
CR05-3	40.98	27.27	23.54	62.13	37.85	7.31
CR05-4	41.55	30.00	22.01	71.12	39.09	8.30

The two trials carried out using the *F. pseudograminearum* isolate CS3096 were designated as CR96-1 and CR96-2, respectively, and the four trials conducted using the *F. graminearum* isolate CS3005 were designated as CR05-1, CR05-2, CR05-3 and CR05-4, respectively

Table 2 Correlation coefficients among CR reactions assessed using two *Fusarium* isolates in six replicated trials in the population of 'Lang'/'CSCR6'

Trial	CR96-1	CR96-2	CR05-1	CR05-2	CR05-3	CR05-4
CR96-1	1	–	–	–	–	–
CR96-2	0.89	1	–	–	–	–
CR05-1	0.51	0.51	1	–	–	–
CR05-2	0.93	0.94	0.52	1	–	–
CR05-3	0.95	0.95	0.53	0.99	1	–
CR05-4	0.82	0.82	0.50	0.83	0.83	1

The two trials carried out using the *F. pseudograminearum* isolate CS3096 were designated as CR96-1 and CR96-2, respectively and the four trials conducted using the *F. graminearum* isolate CS3005 were designated as CR05-1, CR05-2, CR05-3 and CR05-4, respectively

Permutation tests found that LOD value of 3.2 was the threshold for the two trials conducted with the *F. pseudograminearum* isolate CS3096. Based on this threshold, interval mapping (IM) analyses identified two QTL conferring CR resistance. One of them was located on the long arm of chromosome 3B with its resistant allele derived from 'CSCR6'. Following convention, this QTL was designated as *Qcrs.cpi-3B*, where 'crs' stands for 'crown rot severity' and cpi, 'CSIRO Plant Industry'. It was detected in both trials and explained 47.0 and 41.7% of the phenotypic variance, respectively (Table 3). This QTL was located in one of the regions in which loci were significantly skewed favouring alleles from 'CSCR6' (Fig. 2). A second QTL was detected on chromosome 4B with resistant allele derived from the variety 'Lang'. This QTL, designated as *Qcrs.cpi-4B*, was detected in only one of the two trials using this isolate and it explained 14.6% (CR96-1) of the phenotypic variance. IM analysis of the combined data from the two CR96 trials detected only *Qcrs.cpi-3B* explaining 44.0% of the phenotypic variance (Table 3).

Permutation tests found that LOD values of 3.0, 3.2, 3.2 and 3.0 were the thresholds for the four trials conducted with the *F. graminearum* isolate CS3005. Based on these threshold values, IM analyses identified the same two QTL from the four trials. *Qcrs.cpi-3B* was detected in each of the four trials and it explained between 29.7 and 48.8% of the phenotypic variance (Fig. 3). *Qcrs.cpi-4B* was detected in two of the four trials and it explained 15.6% (CR05-2) and 22.8% (CR05-4) of the phenotypic variance, respectively (Table 3). IM analysis of the combined data from the four CR05 trials detected the same two QTL, with *Qcrs.cpi-3B* becoming 44.8% and *Qcrs.cpi-4B* 18.1% (Table 3).

MQM analysis found a strong interaction between *Qcrs.cip-3B* and *Qcrs.cpi-4B*, which reduced the maximum magnitudes of the former to 40.8% for CS3096 (CS96-2) and 43.1% for CS3005 (CR05-3), respectively. The effects of *Qcrs.cpi-4B* became detectable in only one of the six trials (CR05-4) and it explained 13.5% of the phenotypic variance (Table 3).

Effects of Qcsr.cpi-3B in different genetic backgrounds

Four populations were developed and used for evaluating the effects of *Qcrs.cpi-3B* in different genetic backgrounds. Sixty randomly selected lines from each of the four populations were assessed in two different environments, one in a glasshouse and the other in the CEF. The average DI under the two environments was used for further analysis.

As the majority of the markers linked closely with *Qcrs.cpi-3B* are DArT markers which are inapplicable to follow specific loci, the most closely linked SSR marker, gwm0181, was used for validating the effect of this QTL. This SSR marker was located about 2.2 cM away from *Qcrs.cpi-3B* (Fig. 2). The segregation ratios of this marker fit the expected ratio of 1:1 in three of the four validation populations. The exception is the F4 population of 'Drysdale'//'Janz'/'CSCR6'. The average allelic ratio for this SSR marker in this population was 1:2.0, favouring 'CSCR6'.

Based on the presence or absence of the allele from the resistant parent 'CSCR6', the 60 randomly selected lines from each of the four validation populations were placed into two classes. The average CR severity for each of the four populations showed variation, with the population of 'Janz'/'CSCR6' F5 giving the lowest DI for both of the classes (19.6 for RR and 27.5 for rr, Table 4). The differences of CR severity between these two classes of lines were used to measure the effect of *Qcrs.cpi-3B*, which varied from 28.8 to 42.1% with an average of 33.2% among the four populations (Table 4).

Table 3 QTL for crown rot severity identified in the RIL population of 'Lang'/'CSCR6' with the use of two different *Fusarium* isolates

Trial	Analysis	QTL	Interval	Flanking markers	LOD	R^2 (%)	Origin
CR96-1	IM	Qcrs.cpi-3B	32.8–39.8	wPt10505 and wPt2277	9.8	47.0	CSCR6
		Qcrs.cpi-4B	0.0–16.3	wPt7569 and wPt4918	3.1	14.6	Lang
	MQM	Qcrs.cpi-3B	34.2–40.8	wPt10505 and wPt2277	10.3	39.9	CSCR6
CR96-2	IM	Qcrs.cpi-3B	32.8–39.8	wPt10505 and wPt2277	9.7	41.7	CSCR6
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	10.3	40.8	CSCR6
CR96 Combined	IM	Qcrs.cpi-3B	32.8–39.8	wPt10505 and wPt2277	10.2	44.0	CSCR6
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	11.1	41.6	CSCR6
CR05-1	IM	Qcrs.cpi-3B	30.7–32.8	wPt8438 and wPt8513	7.0	29.7	CSCR6
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	6.4	29.6	CSCR6
CR05-2	IM	Qcrs.cpi-3B	32.8–39.8	wPt10505 and wPt2277	9.8	43.0	CSCR6
		Qcrs.cpi-4B	0.0–16.3	wPt5334 and wPt4918	3.1	15.6	Lang
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	10.6	39.4	CSCR6
CR05-3	IM	Qcrs.cpi-3B	32.8–39.8	wPt10505 and wPt2277	10.8	48.8	CSCR6
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	11.5	43.1	CSCR6
CR05-4	IM	Qcrs.cpi-3B	32.8–38.8	wPt11143 and wPt2277	8.0	32.9	CSCR6
		Qcrs.cpi-4B	0.0–11.2	wPt5334 and wPt4918	4.7	22.8	Lang
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	8.0	30.0	CSCR6
		Qcrs.cpi-4B	0–5.6	wPt5334 and wPt4918	4.3	13.5	Lang
CR05 Combined	IM	Qcrs.cpi-3B	32.8–38.8	wPt11143 and wPt2277	11.9	44.8	CSCR6
		Qcrs.cpi-4B	0–11.8	barc0199 and gwm0495	4.0	18.1	Lang
	MQM	Qcrs.cpi-3B	34.2–39.8	wPt10505 and wPt2277	12.6	42.2	CSCR6
		Qcrs.cpi-4B	0–5.6	wmc0048 and wPt5334	3.7	9.7	Lang

The two trials carried out using the *F. pseudograminearum* isolate CS3096 were designated as CR96-1 and CR96-2, respectively and the four trials carried out using the *F. graminearum* isolate CS3005 were designated as CR05-1, CR05-2, CR05-3 and CR05-4, respectively

IM Interval mapping; *MQM* analysis conducted using multiple QTL model

Discussion

The source of resistance investigated in this study, 'CSCR6', belongs to *T. spelta*. There were two main considerations in the selection of this genotype. First, it is one of the genotypes which showed the best resistance among the over 2,200 genotypes screened (CJL unpublished). Second, all of the three genetic mapping studies on CR resistance in wheat used genotypes of *T. aestivum* (Wallwork et al. 2004; Collard et al. 2005; Bovill et al. 2006) thus the *T. spelta* genotype is more likely to provide novel resistance genes. The chromosomal location of the major QTL *Qcrs.cpi-3B* identified from this genotype on 3BL represents a new locus and the magnitude of this QTL explaining up to 48.8% of the phenotypic variance (Table 2) is also unprecedented.

The taxon *T. spelta* is cultivated wheat sharing the same AABBDD genome structure with the bread wheat (Miller 1987). There are no known barriers in generating fully fertile progeny and in transferring genes between these two different taxa. The principal differences between them are due to two major genes, q (or the speltoid gene and its dominant allele Q confers free-threshing grain and a tough rachis) located on chromosome 5A and c (and its dominant compact-ear producing allele C) on chromosome 2D (Miller 1987). Thus, any resistance genes identified from *T. spelta* should be easily incorporated into breeding programs of bread wheat. In fact, lines showing high level of CR resistance but without characteristics of *T. spelta* have been isolated from the QTL mapping population as well as from each of the four validation populations used in this study. These lines would be highly valuable for breeding CR-resistant wheat varieties.

Different from resistance to biotrophic pathogens which are frequently controlled by the action of large effect resistance genes that follow classic Mendelian inheritance, plant defences against necrotrophic pathogens are often found to be controlled by multiple loci each with a small effect (Zhao and Meng 2003; Rowe and Kliebenstein 2008). Judging from the three available reports (Wallwork et al. 2004; Collard et al. 2005; Bovill et al. 2006), quantitative inheritance also seems to be the case for CR resistance in wheat. However, the magnitude of the *Qcrs.cpi-3B* identified in this study, explaining up to 48.8% of phenotypic variance in the mapping population and reducing CR severity by up to 42.1% in the validation

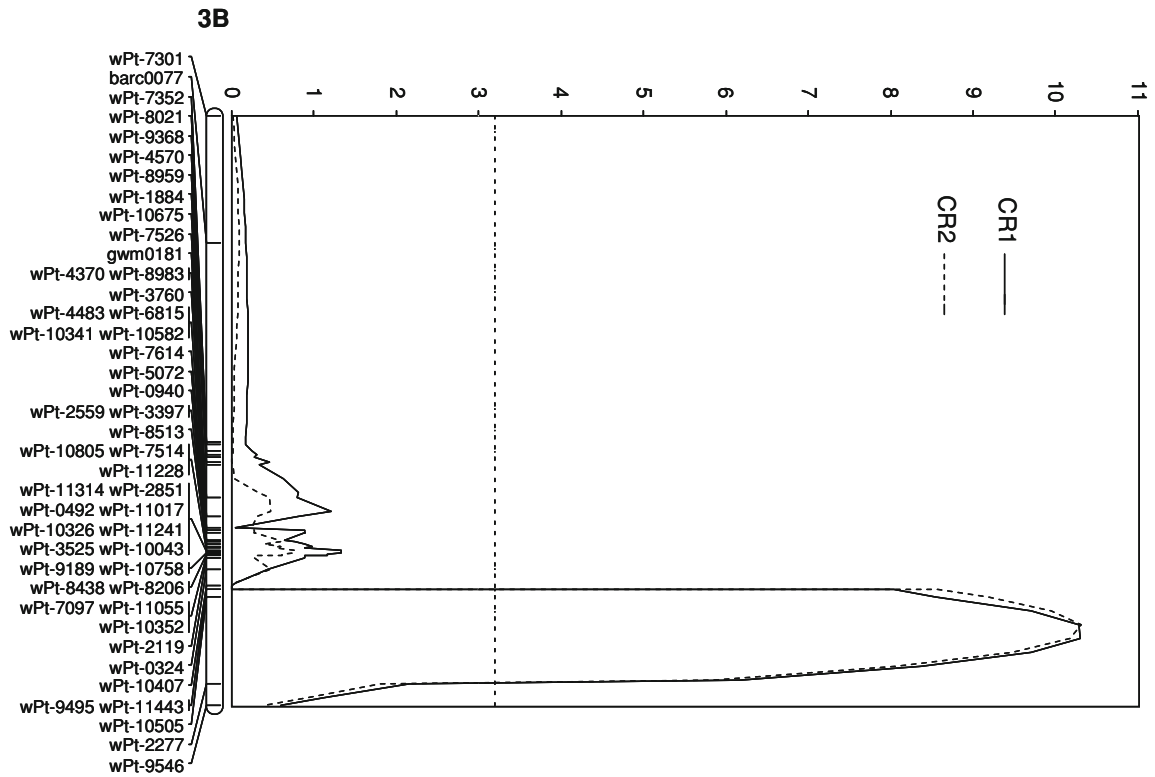

Fig. 2 QTL conferring crown rot resistance detected in two replicated trials using *Fusarium pseudograminearum* (CS3096) in the wheat population 'Lang'/'CSCR6'. The LOD values from each centimorgan of the chromosome were plotted against the chromosome, and the threshold LOD value (3.2) based on permutation test for declaring the presence of a QTL was indicated by a *dotted line*

populations, indicates the presence of major genes conferring resistances to necrotrophic pathogens as well. The large effect of this QTL must have contributed to its consistent detection across all of the trials conducted in this study with the use of populations with different genetic backgrounds. Further, different from some other CR QTL reported in wheat (Wallwork et al. 2004) or barley (Li et al. 2009), no known dwarfing genes are located in close proximity with *Qcrs.cpi-3B* on chromosome arm 3BL, making it even more attractive for breeding programs.

Segregations of all markers around *Qcrs.cpi-3B* in the mapping population were skewed (Fig. 1), and the effect of segregation distortion on the result of QTL mapping is of interest. As the mapping population consisted of F8 RILs, it would not be unreasonable to assume that any QTL detected would be primarily with additive effect. A recent study showed that, although ignoring segregation distortion in QTL analysis could result in slight power loss, the loss is negligible for QTL with additive effect and this is especially the case when a genome is covered by densely distributed markers (Xu 2008). The linkage map used in this study has a high marker density with an average distance of only 1.2 cM between any pair of markers (Fig. 2). Thus, the reliability of the QTL detected should be high. In addition, the presence of *Qcrs.cpi-3B* reduced CR severity by an average of 33.2% among the four validation populations again supporting the reliability of this QTL. Further, the normal segregations of this locus in three of the four validation populations suggested that the *Qcrs.cpi-3B* locus is unlikely to be associated with a gene(s) conferring preferential transmission as found in some other species (Miller et al. 1982; Konduri et al. 2000).

Prior to this report, QTL mapping studies on CR resistance have been reported for three partially resistant genotypes: a single locus on chromosome 4B was reported for 'Kukri' (Wallwork et al. 2004), two loci (one on chromosome arm 1DL and the other on 1AL) were reported for '2-49' (Collard et al. 2005), and two further loci, one on chromosome 5D and the other on chromosome 2D, were reported for the genotype 'W21MMT20' (Bovill et al. 2006). The major QTL derived from 'CSCR6' on chromosome 3B represents a new locus conferring CR resistance. The fact that the six QTL identified from the four different CR resistance sources located on six different chromosomes suggests that a large numbers of resistance loci for CR resistance may exist in wheat. This has been shown to be the case for resistance to FHB which is caused by the same pathogens as CR. Loci conferring resistance to FHB have been detected on 20 of the possible 21 bread wheat chromosomes (Buerstmayr et al. 2009).

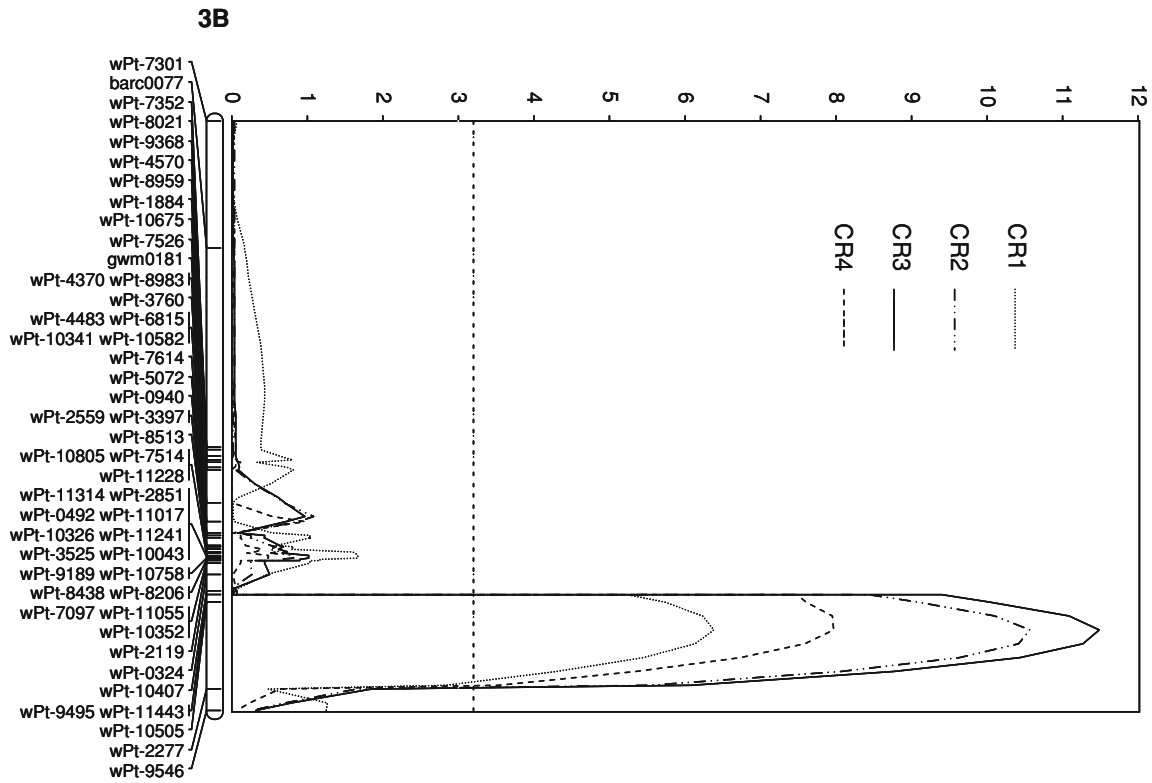

Fig. 3 QTL conferring crown rot resistance detected in four replicated trials using *Fusarium graminearum* (CS3005) in the wheat population 'Lang'/'CSCR6'. The LOD values from each centimorgan of the chromosome were plotted against the chromosome, and the threshold LOD value (3.2) based on permutation test for declaring the presence of a QTL was indicated by a *dotted line*

Table 4 Effects of Qcrs.cpi-3B in four validation populations

Population	RR	rr	Difference (%)	*P* value
Janz/CSCR6 F5	19.6	27.5	28.8	<0.05
Aus13832/CSCR6 F5	19.8	28.9	31.5	<0.01
Janz*2/CSCR6 F4	25.5	36.7	30.6	<0.01
Drysdale//Janz/CSCR6 F4	21.5	37.1	42.1	<0.01

RR represents homozygous alleles from 'CSCR6', and rr homozygous alleles from non-'CSCR6' parents

The possible homoeologous relationship between *Qcrs.cpi-3B* and the major QTL conferring CR resistance on chromosome 3H in barley (Li et al. 2009) warrants further investigation. Both of the 3B and 3H QTL have large effects on CR reaction and both are located on the long arms of the homoeologous group 3 chromosomes. Relative rearrangements between 3H and 3B chromosomes are unknown (Devos 2005). A possible difference between the two loci is that their relative genetic distances from the centromeres seem to be different. The 3B QTL seems to be more distally located. However, genetic distance can be affected by many factors including the use of different populations (Liu et al. 1996), thus the differences in genetic distances between the two different genera may have only limited value.

The second QTL identified in this study, *Qcrs.cpi-4B*, was derived from 'Lang' which is one of the Australian varieties showing partial resistance to CR (http://www.awb.com.au/NR/rdonlyres/40F06360-D0F8-42CC-8847-9E75D654AB92/0/Lang_Factsheet__Feb_2008.pdf). The location and magnitude of this QTL indicated that it is likely to be the same as the one detected from another Australian variety 'Kukri' (Wallwork et al. 2004). Results from Collard et al. (2005) suggested that a third Australian genotype '2-49' may also contain the same locus. These results seem to indicate that *Qcrs.cpi-4B* is common in Australian wheat varieties.

The two *Fusarium* isolates used in this study belong to two different species, one *F. pseudograminearum* and the other *F. graminearum*. They were well characterized (Akinsanmi et al. 2004) and have been routinely used in different aspects of CR and head blight research (Xie et al. 2007; Stephens et al. 2008, Li et al. 2009). In this study, these two isolates detected the same two QTL. The same two *Fusarium* isolates also detected the same QTL from another source of CR resistance (HB Li et al. unpublished). These results suggested that CR resistance in wheat is unlikely to be species-specific.

The linkage map constructed in this study based on 776 markers has a total distance of about 912.9 cM. This map is

much shorter than linkage maps constructed using the same marker systems, even taking into consideration that a large number of the markers used in this study were generated from purified chromosome 3B clones. For example, the four linkage maps described by Francki et al. (2009) contain only 275, 385, 468, and 575 DArT and SSR markers, respectively, but the lengths of them are longer, being 2,198, 3,013, 3,058, and 2,825 cM, respectively. As reported earlier, the shorter map distance obtained in this study could be due to many factors including effects of major genes (Temin and Marthas 1984), the overall genome homoeology (Liu et al. 1996) or local rearrangements between the parental genotypes (Liu et al. 1994).

With the inclusion of a large number of markers derived from purified chromosome 3B clones, it was not unexpected that 198 markers were mapped to this single chromosome. With an average distance of less than 0.6 cM between markers, the 3B map represents one of the most densely covered chromosomes ever reported in wheat. Surprisingly, however, the large number of the 3B markers failed to form one single linkage group corresponding to this chromosome. Rather, they formed two linkage groups (Supplementary Fig. 1), suggesting the existence of at least one large gap in the current linkage map of this chromosome. As there are already 198 markers mapped on this single chromosome, adding additional random markers is unlikely to be effective in joining the two linkage groups together. An alternative approach is to exploit the physical map of chromosome 3B (Paux et al. 2008), which should facilitate the identification of polymorphic markers for any targeted region on this chromosome.

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