



Research Report

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Development and Validation of Markers Closely Linked to Crown Rot Resistance in Wheat and Barley

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Abstract Crown rot (CR), caused by various *Fusarium* species, is a chronic cereal disease in Australia and many other parts of the world. As part of our objective of improving the efficiency of breeding CR-resistant wheat and barley varieties, we have been searching for novel sources of resistance and identifying new genes conferring CR resistance in the two crops. This paper reports on the development of more closely linked markers and validation of the effects of the previously identified wheat 3B CR quantitative trait loci (QTL) and the barley 3H major CR locus using four wheat and three barley validation populations. With an effect varied from 36.7 to 59.4% in wheat and 29.4 to 55.2% in barley, the validation experiments demonstrated that both of the 3B and 3H CR locus have a stable and significant effect in reducing CR severity at different genetic backgrounds. The huge effect of a single QTL as the CR loci detected in this study warrant a significant value of incorporating these two genes in wheat and barley breeding. The possible homoeologous relationship between these two major CR loci is preliminarily investigated by comparative mapping the two genomic regions and it warrants further investigation.

Keywords Wheat; Barley; Crown rot; Validation; Comparative mapping

Background

Crown rot (CR) is a severe and chronic disease of cereals found in many parts of the world (Chakraborty et al., 2006). Both barley and wheat can be seriously affected by CR, which has recently become more prevalent due to the widespread adoption of minimum tillage (reviewed by Chakraborty et al., 2006) as CR pathogens are carried over in residues (Wildermuth et al., 1997; Wallwork et al., 2004). A recent study by Daniel and Simpfendorfer (2008) found that, on average, CR caused 25% yield loss in bread wheat, 58% in durum, and 20% in barley across a wide range of environments in Australia. A survey in the Pacific Northwest of the USA found that CR could reduce yield of winter wheat by 35% and barley by 13% in commercial fields (Smiley et al., 2005). In addition to yield loss, CR infected plants in glasshouse assays have been shown to contain mycotoxins (Mudge et al., 2006), which may potentially have adverse health effects if

high levels are present in food and feed products.

Growing resistant varieties has long been recognised as the most effective way to minimize CR damage, and there have been efforts in breeding CR resistance in Australia. However, varieties with high levels of resistance are not yet available. Studies on CR have mainly considered disease incidence and severity with very few reports on yield loss. It was reported that effects of CR are slightly different for wheat and barley (Smiley et al., 2005; Daniel and Simpfendorfer, 2008). These studies support the notion that, although barley gets more severe CR symptoms than bread wheat (Wildermuth and Purs, 1971; Burgess et al., 1987; Klein et al., 1989), they are more tolerant to CR infection and suffer less yield loss than wheat.

A few of the wheat genotypes with moderate CR resistance have been identified and used for molecular mapping studies. The first study reported the CR



resistance in the variety ‘Kukri’. The resistance of adult plants of this variety was assessed by growing plants in open-ended tubes placed in outdoor terraces. Bulk segregant analysis identified a locus on chromosome 4B near the semi-dwarfing gene *Rht1* (Wallwork et al., 2004). The second genotype investigated was the breeding line ‘2-49’. Based on a seedling assay, two different quantitative trait loci (QTL) conferring CR resistance were detected from 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 report was on the genotype ‘W21MMT20’, again based on a seedling assay (Bovill et al., 2006). These authors reported several putative QTL conferring CR resistances. However, none of them reached significant levels in all of the three assays reported. The most significant QTL was located on 5D. It reached significant levels in two of the three trials and explained up to 28.0% of the phenotypic variance. The other QTL, located on 2D, reached significant level in only one of the three assays conducted and it explained 10.2% of the phenotypic variance. These variable results are not surprising as accurate CR assessment is notoriously difficult (Collard et al., 2005). It is of note that none of the five loci derived from the three different resistant genotypes co-locate. They were found on five different chromosomes. However, recent studies have identified a major QTL on the long arm of chromosome 3B for CR resistance in three different wheat genotypes (Li et al., 2010a; Ma et al., 2010; Bovill et al., 2010). The magnitude of the 3B CR QTL identified in these genotypes explained up to 34.6%, 40.2%, and 46.2% of the phenotypic variance with detection in each of the trials in the three independent studies. Although the three genotypes are totally different, one is a modern wheat variety from USA, another is an Australian breeding line, and the third is an old landrace from India; a major QTL was identified in each of these resources and they were mapped to a very similar position on 3BL (Li et al., 2010a; Ma et al., 2010; Bovill et al., 2010).

Compared to bread wheat, genetic studies on CR

resistance in barley is lag behind. However, a major QTL for CR resistance in barley has recently been identified from the Chinese landrace ‘TX9425’ which showed a moderate resistance to CR, markers linked to this QTL on the long arm of chromosome 3H count for over 60% of the phenotypic variation in resistance (Li et al., 2009). The discovery of promising QTLs for a trait of interest is an important, but primary step in developing a marker-assisted selection program for genetic improvement. Ideally, the identified QTLs should be validated in additional genetic backgrounds and environments and should not have undesirable effects on other important traits. Moreover, because the chromosome 3H in barley has a possible co-linearity with the group 3 chromosomes in wheat (Devos and Gale, 1993). It is interesting to know whether the 3B CR QTL in wheat and the 3H CR QTL in barley is homoeoalleles having a common origin. If a homoeoallelic relationships between the two loci exist, it would be much easier to characterise and isolate this gene in barley because barley is a diploid, self-pollinating species for which an experimental population can be developed with relative ease, and because barley has relatively few chromosomes, which can avoid any confounding effects from other homologous groups as it is in wheat.

The present study was carried out (1) to develop locus-specific molecular markers closely linked to the wheat 3B and barley 3H CR locus; (2) to validate the effect of the two loci in different genetic backgrounds in wheat and barley respectively for the purpose of using these major genes in breeding commercial wheat and barley varieties in the future; and to understand the possible homoeoallelic relationship between the wheat 3B and barley 3H CR loci.

1 Results

1.1 Development of more closely linked locus-specific markers in the regions conferring CR resistance on chromosomes 3B and 3H

The wheat 3B CR QTL was previously identified between two DArT markers, which were 9.4 cM apart (Li et al., 2010a). In order to more precisely determine the position of the QTL, and identify more closely linked locus-specific markers that can be used for validation this QTL in different genetic backgrounds



and in breeding for CR resistance in wheat. A total of 28 publicly available wheat PCR-based primers from this region or nearby (Somer et al., 2006; Paux et al., 2008) were screened for their polymorphisms in the population of Ernie/Batavia. Among these wheat primers, only 32% showed polymorphism between the two parents Ernie and Batavia and about 11% have more than one polymorphic PCR products (S-Table 1). A high-resolution linkage map for the 3B CR QTL region was established by insertion of the nine polymorphic simple sequence repeat (SSR) markers into this region (Figure 1). The QTL was precisely located to a 7 cM region flanked by co-dominant SSR markers *gwm0181* and *wmc0471* (Figure 2).

The barley 3H CR QTL was originally located to an interval between the DArT markers *bPb-4747* and *bPb-6765*, which are 12.1 cM apart (Li et al., 2009). Four PCR-based primers were designed from the DNA sequences representing the four DArT markers, which are associated with the barley 3H CR QTL (Li et al., 2009). Three of the four newly developed PCR-based markers showed polymorphism and only one (*CAPS-0079*) was mapped to the same position as the original DArT markers (*bpb-0079*). A total of 30 publicly available PCR-based primers from barley 3H (Ramsay et al., 2000) were also screened for their polymorphism between the two parents TX9425 and Franklin for the purpose of identification of locus-specific markers, which can be used in barley CR breeding. About 68% of these primers showed polymorphism and about 24% have more than one polymorphic PCR products (S-Table 1). However, only 11 of the polymorphic markers were inserted into the barley 3H CR region make the resolution of this region reached to an average of 1.1 cM between the two markers (Figure 1). The two closely linked co-dominant SSR markers *Bmac0209* and *HVM 33*, with 1.8 cM apart, were identified flanking this QTL (Figure 3).

In order to test the transferability of PCR based markers between wheat and barley and comparative mapping the barley 3H CR QTL and the wheat 3B CR QTL, the same list of barley primers was applied to

screen polymorphism between the wheat parents Ernie and Batavia, while the wheat primers were applied to screen between the barley parents TX9425 and Franklin. The polymorphism study showed that around 54% of the wheat primer showed polymorphism in barley and 56% of the barley primer showed polymorphism in wheat. However, the specificity of these primers was reduced when they were applied to the opposite crop, for example, 32% of the wheat primers have more than one polymorphic PCR products in barley and 15% of the barley primers have more than one polymorphic PCR products in wheat (S-Table 1). Comparative mapping of the 3H and 3B CR regions showed that none of 11 barley markers linked to 3H CR resistance can be mapped to wheat chromosome 3B, and only two of the nine wheat SSR markers linked to 3B CR resistance locus were mapped to an interval on barley chromosome 3H. However, it is interesting finding that the two wheat CR-associated SSR markers were mapped to a region overlapping the barley 3H CR QTL region (Figure 4).

1.2 Validation of 3B QTL

The phenotypic data for the four wheat validation populations all showed a continuous distribution (Table 1). The microsatellite markers closely linked with the wheat 3B major CR resistance locus, *gwm0181* and *wmc0471*, were used to identify homozygous individuals with (RR) or without (rr) the resistant locus on 3BL from the resistant parent Ernie. The segregation ratios of the two markers fit the expected ratio of 1:1 in the related validation populations. The average scores of CR severity differed among the four populations, with the F3 population of 10903/Ernie giving the lowest scores for both of the RR (0.87) and rr (2.31) classes (Table 2), and the F5 population of Kenedy/Ernie giving the highest scores (1.38 for RR and 3.56 for rr, respectively). The effects of the 3B locus, based on the differences in the average severities between the homozygous RR and rr individuals, varied from 36.7 to 61.2% with an average of 47.6% among the four validation populations (Table 2).

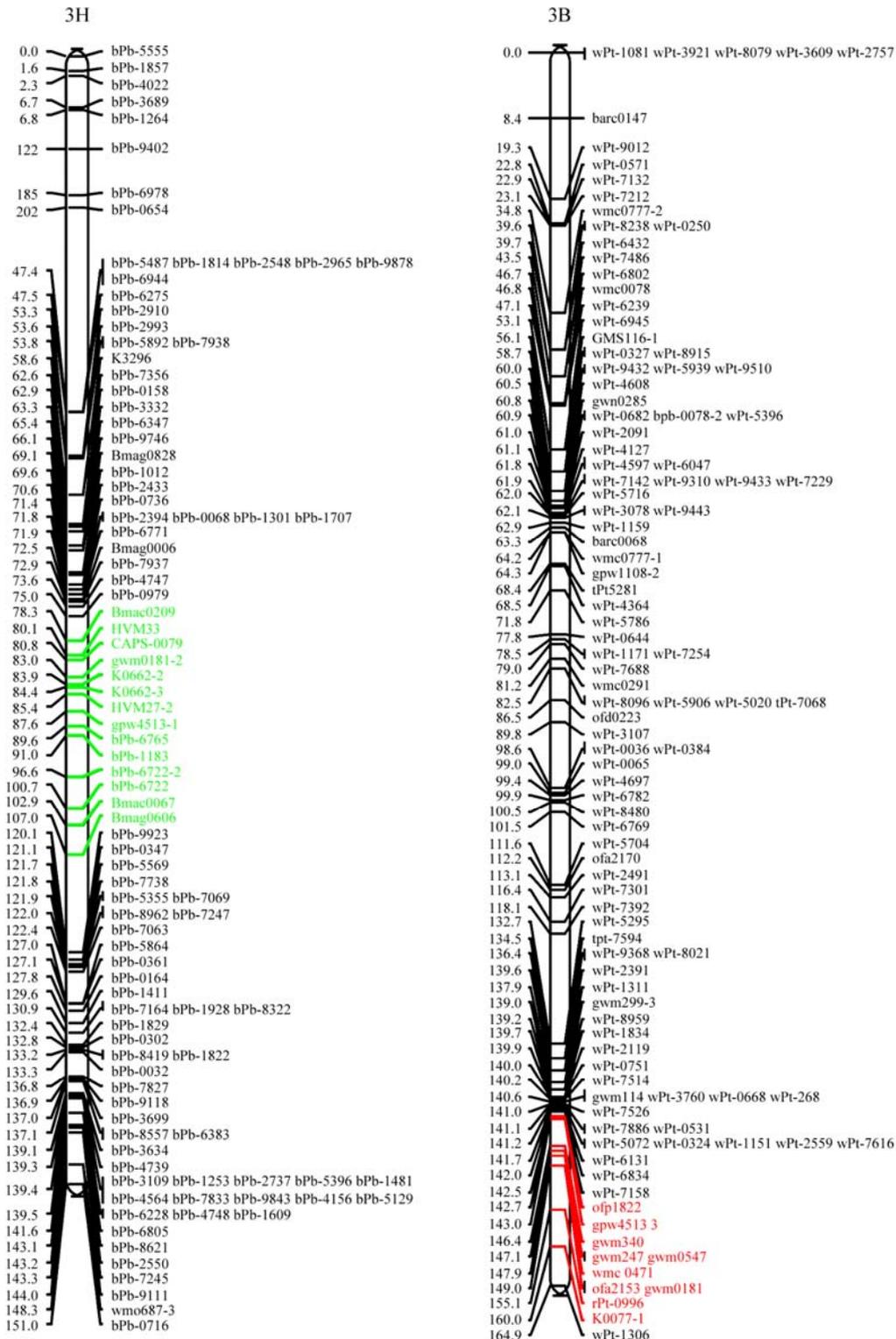


Figure 1 Saturation of the regions conferring crown rot resistance on chromosomes 3H and 3B, determined using more PCR-based markers

Note: Among the 34 available barley markers, 23 were polymorphic in the population of TX9425/Franklin and 11 of these polymorphic markers (in green) were filled in the 3H QTL region. A total of 28 publicly available PCR-based markers were used in this study and nine polymorphic ones (in red) were filled into the region where the 3B QTL harboured

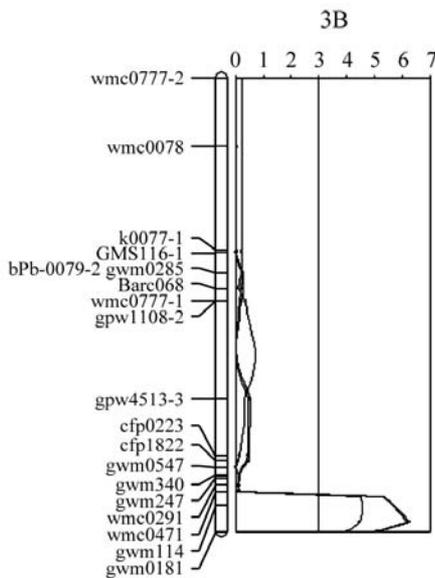


Figure 2 The wheat 3B crown rot QTL identified in the resistant variety Ernie (Li et al., 2010) was remapped using a linkage map constructed using only PCR-based markers. The CR-associated markers are applied in validation study and have the potential value to be used in breeding for CR resistance in wheat

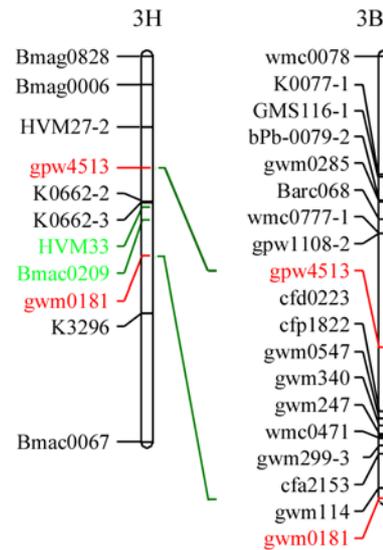


Figure 4 Comparative mapping of the chromosomal regions conferring crown rot resistance on barley 3H and wheat 3B, determined using only SSR markers

Note: The two flanking markers (in red) of the 3B CR QTL were mapped in a chromosomal region overlapping the barley 3H CR QTL flanked by two SSR markers (in green); However, the markers (in green) flanking the 3H QTL were not polymorphic in wheat Ernie/Botavia population

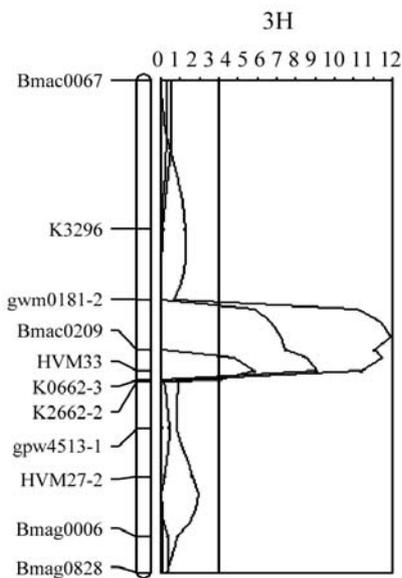


Figure 3 The barley 3H crown rot QTL identified in the resistant variety TX9425 (Li et al., 2009) was remapped using a linkage map constructed using only PCR-based markers. The CR-associated markers are applied in validation study and have the potentiality being used in breeding for CR resistance in barley

1.3 Validation of 3H QTL

The phenotypic data for the three barley validation populations all showed a continuous distribution (Table 3). The microsatellite markers closely linked with the major barley 3H CR resistance locus, *Bmac0209* and *HVM33*, were used to identify homozygous individuals with (RR) or without (rr) the resistant locus from the resistant parent TX9425. The segregation ratios of the two markers fit the expected ratio of 1:1 in all the three validation populations. The average scores of CR severity differed among the three populations, with the F3 population of TX9425/DYSYH giving the lowest scores for both of the RR (1.20) and rr (2.71) classes (Table 4), and the F5 population of TX9425/Naso Nijo giving the highest scores (1.74 for RR and 3.21 for rr, respectively). The effects of the 3H locus, based on the differences in the average severities between the homozygous RR and rr individuals, varied from 41.7 to 55.4%, with an average of 46.7% among the three validation populations (Table 4).

Table 1 Distribution of crown rot severity in the four wheat validation populations

Crosses	Populations	No. of lines	Parents		Populations			
			R-parent	S-parent	Min.	Max.	Mean	SD
27868/Ernie	F4/F5	67	1.23	3.20	0.83	3.79	2.25	0.68
13832/Ernie	F4/F5	120	1.17	3.45	1.05	3.57	2.17	0.47
Kennedy/Ernie	DH	55	1.44	3.78	1.27	4.15	2.80	0.52
10903/Ernie	F2/F3	120	0.95	2.97	0.78	3.34	2.10	0.70

Table 2 Effects of the 3B crown rot QTL in four wheat validation populations

Crosses	Associated markers	Validation of the effects of the crown rot-associated markers			
		RR	rr	Effects (%)	P value
27868/Ernie	gwm0181	1.15	2.1	45.2	<0.01
13832/Ernie	gwm0181	1.27	2.5	47.3	<0.01
Kennedy/Ernie	gwm0181	1.38	3.56	61.2	<0.05
10903/Ernie	wmc0471	2.09	3.31	36.7	<0.01

Table 3 Distribution of crown rot severity in the three barley validation populations

Crosses	Populations	No. of lines	Parents		Populations			
			R-parent	S-parent	Min.	Max.	Mean	SD
TX9425/Naso Nijo	DH	74	1.76	3.71	1.51	4.03	2.40	0.6
TX9425/Gairdner	F3/F4	120	1.19	3.23	0.97	3.95	2.23	0.58
TX9425/DYSYH	F3/F4	119	1.53	2.97	1.24	3.78	2.70	0.7

Table 4 Effects of the 3H crown rot QTL in three barley validation populations

Crosses	Associated markers	Validation of the effects of the crown rot-associated markers			
		RR	rr	Effects (%)	P value
TX9425/Naso Nijo	Bmac0209	1.74	3.21	45.8	<0.01
TX9425/Gairdner	HVM33	1.36	2.40	41.7	<0.01
TX9425/DYSYH	HVM33	1.20	2.71	55.4	<0.01

2 Discussion

2.1 The potentiality of using the CR loci in wheat and barley breeding practice

Both wheat and barley can be seriously affected by CR disease (Wildermuth and Purss, 1971). Extensive genetic studies on CR have been carried out in bread wheat and several loci conferring CR resistance have been reported for bread wheat, and were located on chromosomes 4B (Wallwork et al., 2004), 1A and 1D (Collard et al., 2005), and 2B, 2D, and 5D (Bovill et al., 2006). However, the effect of these reported QTL was not stable and none of them reached significant levels in all of the trials conducted in the related study. This makes it very hard for breeders to incorporate

CR resistance from these reported sources into commercial varieties and to track down the resistance genes in the progenies derived from these sources.

Fortunately, recent studies have identified a major QTL on the long arm of chromosome 3B conferring CR resistance in three different wheat genotypes, two of them belong to *T. aestivum* (Li et al., 2010a; Bovill et al., 2010), another belong to *T. spelta* (Ma et al., 2010). The magnitude of the 3B CR QTL identified in the wheat genotypes ‘Ernie’ and ‘CSCR6’ explained up to 34.6% and 46.2% of the phenotypic variance with detection in each of the trials with the use of two different *F. pseudograminearum* and *F. graminearum*



isolates. Although these two genotypes are totally different, one is a modern wheat variety from USA, the other is an old landrace from India, only one QTL was identified in each of these two resources and they were mapped to a very similar position on 3BL (Li et al., 2010a; Ma et al., 2010). The major QTL identified in the third resource ‘W21MMT20’ (an Australian breeding line) was also located to a position close to the distal end of chromosome 3B, although the author of this study failed to detect the QTL in their previous study using this same resistant resource (Bovill et al., 2006). However, the 3B major CR locus identified in the three recent studies indicates that this gene could be a dawn for wheat breeders in their effort of attacking crown rot disease and breeding resistant wheat varieties. Compared to wheat, genetic studies on CR resistance in barley are lacking. However, a major QTL for CR resistance in barley has recently been identified from the cultivar ‘TX9425’, and markers linked to this QTL on the long arm of chromosome 3H account for over 60% of the phenotypic variation in resistance (Li et al., 2009).

Because a significant proportion of the markers used in our previous QTL study (Li et al., 2009; 2010a) were DArT markers, it is important to develop closely linked PCR-based markers, which can be used to validate the effect of the 3B and 3H CR locus and for use in breeding programs. Four different wheat populations and three barley populations were used in this study to validate the effects of the 3B and 3H CR locus. With an effect varied from 36.7 to 59.4% in wheat and 29.4 to 55.2% in barley, the validation experiments in this study demonstrated that both the 3B and 3H CR locus have a stable and significant effect in reducing CR severity at different genetic backgrounds. The huge effect of a single gene as the CR locus detected in this study warrant a significant value of incorporating these two genes in wheat and barley breeding.

2.2 The possible homoeologous relationships between the 3B and 3H CR QTL

Wheat and barley are two important grass species that may have a common gene pool and share a significant level of synteny (Ahn et al., 1993; Morre et al., 1995).

Findings from these comparative mapping studies between grass families is of considerable values to plant breeders, since it means that similar genes in different plants can be treated as essentially allelic variants, and the different alleles can be transferred, more or less at will, from one species to another to improve or alter the performance of a crop.

The major CR QTL identified in the wheat variety Ernie and the barley genotype TX9425 have large effects on enhancing CR resistance, and they are both located on the long arms of the homoeologous group 3 chromosomes, with the barley CR locus proximal to the centromere of chromosome 3H (Li et al., 2009) and the one in wheat close to the distal end of wheat chromosome 3BL (Li et al., 2010). The possible homoeologous relationship between these two major CR loci may exist because chromosome 3H in barley has been reported to show a virtual collinearity with wheat chromosome 3B (Devos and Gale, 1993). However, recent studies investigating microcollinearity in the grass family has revealed numerous small rearrangements of gene content, order, and orientation between different grass genomes (Bennetzen and Ramakrishna, 2002); therefore, the arms of barley chromosomes may not be a completely identical to the genetically equivalent of the arms of wheat chromosome.

The low transferability of PCR markers between wheat and barley as showed in this study make the comparative mapping between the two crops very labour extensive and less efficient. However, the comparative mapping attempt in this study indicated that the barley 3H CR locus may be a homologous allele of the 3B CR resistance locus because the flanking markers of the wheat 3B CR locus were mapped to a barley genomic region, which co-locate with the barley 3H CR locus. Moreover, three markers from a region harbouring a CR major QTL on barley chromosome 3H were mapped to a region of chromosome 3B in wheat, which is only 15 cM away from the CR major locus identified on this chromosome. A small distance between the wheat CR locus and the region harbouring barley SSR marker on chromosome 3B results in a reasonable inference that the two regions could overlap if more markers were used in comparative mapping of the regions associated

with CR resistance in wheat and barley. If the homoeologous allelic relationship is true between the barley 3H CR QTL and the wheat 3B CR Locus, it could be relatively easier to characterise the functional expression of the candidate genes underlie the homologous locus and to isolate this major gene for CR resistance in barley than in wheat, considering the difference in chromosome sets and genome size between the two crops.

3 Materials and Methods

3.1 Wheat and barley populations used in this study

A wheat doubled haploid (DH) population consisting of 153 lines derived from a cross between two bread wheat varieties Batavia and Ernie was used for mapping QTL conferring CR resistance and for comparative mapping; a linkage map was constructed for this population by using 104 SSR and 852 DArT markers (Li et al., 2010a). Ernie is an American soft red winter wheat variety with high-level resistance to CR, and Batavia is an Australian variety highly susceptible to this disease. Four additional populations were developed for validating the effect of the 3B major QTL derived from the resistant variety Ernie (Li et al., 2010a). The four validation populations include 67 F4/F5 lines from a cross of 27868/Ernie, 120 F4/F5 lines from a cross of 13832/Ernie, 55 DH lines from a cross of Kennedy/Ernie, and 120 F2/F3 lines from a cross of 10903/Ernie.

A barley DH population of 92 lines was used to identify QTL conferring CR resistance and for comparative mapping. The DH population was derived from TX9425 (a Chinese landrace resistant to CR) and Franklin (an Australian variety highly susceptible to CR). A linkage map for this population was constructed using 412 DArT, 80 AFLP and 28 microsatellite markers (Li et al., 2008; 2010b). Three additional populations were developed for validating the effect of the 3H QTL derived from TX9425. The three validation populations used in this study include 74 DH lines from a cross of TX9425/Naso Nijo, 120 F3/F4 lines from a cross of TX9425/Gairdner, and 119 F3/F4 lines from a cross of TX9425/DYSYH.

3.2 Marker genotyping

A total of 34 barley primers and 28 Wheat SSR

primers (S-Table 1) from the related genomic regions associated with CR resistance in wheat and barley were screened for polymorphism between parents of the wheat and barley DH populations. Fourteen barley SSR markers showed polymorphism between TX9425 and Franklin, and 11 wheat SSR markers are polymorphic between Ernie and Batavia. These SSRs were amplified using fluorescent dUTPs (Molecular Probes, Eugene, Oregon, USA). Amplification reactions were performed in a total volume of 12.5 μ L containing 1 \times Buffer, 1.5 mM of MgCl₂, 0.2 mM of dNTPs, 0.2 μ M of unlabeled primer, 0.6 μ M fluorescent dUTPs, 0.5 U of Taq polymerase and 20 ng of template DNA. SSR name and amplification conditions were those published in the Genetics supplemental data site: <http://www.genetics.org/cgi/content/full/156/4/1997/DC1>. Gel electrophoresis was performed on an automated Gel scanner (Gel-Scan 2000, Corbett Research). Samples were electrophoresed on an 18-cm long 4% polyacrylamide gel containing 7M urea. Allele sizes were calculated by comparison with a 350 (TAMRA) size standard.

3.3 CR screening

A highly aggressive isolate of *Fusarium pseudograminearum* (Fp3096) collected from Northern New South Wales, Australia (Akinsanmi et al., 2004) was used in this study. The procedures used for inoculum preparation were based on that described by Mitter et al (2006). Specifically, plates of 1/4 strength PDA (potato dextrose agar) inoculated with Fp3096 were incubated for a week at room temperature. The mycelium was scraped and plates were incubated for a further week under a combination of cool white and black (UVA) fluorescent light with a 12-h photoperiod. Macroconidia were harvested, suspended in sterile distilled water after straining through layers of cheesecloth and spore concentration was adjusted to 1 \times 10⁶ spores/mL. Tween 20 was added (0.1% v/v) to the spore suspension prior to use.

CR assay was based on procedures described by Li et al (2008). Seeds were germinated in petridishes on 3-layers of filter paper saturated with water. Newly germinated seedlings were immersed in the conidial suspension (1 \times 10⁶ m/L) for 1 min. Three treated seedlings were sown in a 5 cm \times 5 cm square punnet



(Rite grow kwik pots, www.Gardencityplastics.com, Australia) containing autoclaved potting mix. Three replicates, each containing 15 seedlings in five punnets arranged in a randomised block design, were used. Thirty punnets were placed in each plastic seedling tray for easy handling. After planting, the seedling trays were incubated for 24 h in a humid chamber and then transferred to a glasshouse with 25/15 (± 5)°C day/night temperature and 60/80 (± 10)% day/night relative humidity (RH). The seedlings were watered only when wilt symptoms appeared. CR severity was assessed at 35 days after inoculation with a 0–5 scale, according to Li et al (2008).

3.4 Linkage and data analysis

Linkage analysis was conducted using JoinMap 4.0 as described by Li et al (2008). A new linkage map were generated for both of the 3H and 3B QTL region, and in order to relocate the CR QTLs based on new linkage maps, a QTL analysis based on same phenotypic data as in the previous studies (Li et al., 2009; 2010) was applied in both of the wheat and barley DH populations using MapQTL 5.0 as described by Li et al (2009). For QTL validation, various numbers of progeny lines were randomly selected from each of the four wheat and three barley validation populations (Table 1; Table 2), and were assessed for CR reaction twice at same condition, one in the controlled environment facility (CEF) and the other in the glasshouses. For each validation population, two replicates, each consisting of 10 individual plants for each line, were used in each screening trial. The average values of CR severity of the 10 plants in each replicate were used in further statistical analyses.

All statistical analyses were performed using GenStat for Windows, 12th edition (copyright Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Homogeneity of variance was tested using Bartlett's test to determine whether the data could be combined across replicates for further analyses. An analysis of variance was used to detect significant genetic effects for CR severities. Within each trial, the following mixed-effects model was used: $Y_{ij} = \mu + r_i + g_j + w_{ij}$. Where: Y_{ij} =observation on the j th genotype in the i th replication; μ =general mean; r_i =effect due to i th replication; g_j =effect due to the j th genotype;

w_{ij} =error or genotype by replication interaction, where genotype was treated as a fixed effect and that of replicates as random. A general mean across the two trials was calculated for each line and used to analyse the effect of related CR QTL in different populations. Based on the presence or absence of marker alleles from the resistant parents Ernie and TX9425, the lines from each of the 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.

Authors' Contributions

HBL is the executor of experimental research in this study and took responsibility to make the experimental design, data analysis, paper writing and revising; MXZ and CJL are supervisors of the project and provide the necessary facilities for the research to be performed.

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S-Table 1 Barley and wheat primers used for polymorphism markers analysis. The number of polymorphic scorable PCR products is listed for each primer. The primers with more than one polymorphic product in the barley TX9425/Franklin population are in red colour, while the primers having more than one polymorphic product in the wheat Ernie/Batavia population are highlighted yellow

Barley primers	Polymorphic in TX9425/Franklin	Polymorphic in Ernie/Batavia	Forward primer	Reverse primer	References
Bmac0067	1	*	AACGTACGAGCTCTTTTCTA	ATGCCAACTGCTTGTTTAG	Ramsay et al. 2000
Bmac0209	1	*	CTAGCAACTTCCCAACCGAC	ATGCCTGTGTGTGGACCAT	Ramsay et al. 2000
Bmag 0225	*	2	AACACACCAAAAATATTACATCA	CGAGTAGTTCCTCATGTGAC	Ramsay et al. 2000
Bmag0482	*	2	TATATGTCGGGAGAGATCAAG	ATAGTTTAGCCCTCCACTAGC	Ramsay et al. 2000
Bmag0122	*	1	AGGGAGGATTAATCACGG	AAGATGTGATGATCATTGTATTG	Ramsay et al. 2000
Bmag0877	1	*	AAAGCTCATGGTAGATCAAGA	TAGTTTTCCCAAAAGCTTCTA	Ramsay et al. 2000
Bmag0013	1	*	AAGGGGAATCAAAAATGGGAG	TCGAATAGGTCTCCGAAGAAA	Ramsay et al. 2000
HVM27	3	1	GGTCGGTCCCGGTAGTG	TCCTGATCCAGAGCCACC	Ramsay et al. 2000
HVM33	1	1	ATATTA AAAAAGGTGGAAAGCC	CACGCCCTCTCCCTAGAT	Ramsay et al. 2000
HVM44	1	1	AAATCTCAGGTTTCGTGGGCA	CCACGGAGACCACCTCACTT	Ramsay et al. 2000
HVM60	*	1	CAATGATGCGGTGAACTTTG	CCTCGGATCTATGGGTCCTT	Ramsay et al. 2000
bPb-4747	*	*	GGGAACGACATTTACGGAGA	TAAGGTTGAGGCAGTTGCAC	Developed in this study
bPb-0079	2	4	AGGCGGTGAATAACTGGAAC	GCGGTGAACCTTGGAAACAGAT	Developed in this study
bPb-1183	2	1	TCTGCTCAGTAGGGCATCCT	GTTGAACGGCATGGAAGTTT	Developed in this study
bPb-6722	2	*	GCAGGCTGTGTCTTCTCTTC	GCATCGGCATGTTTCTTCTT	Developed in this study
K03692	*	*	AACTCGAATCAGATCAGCCC	CTCTCCCTCCCACTCCTTTC	Sato et al. 2009
K03336	*	*	CGTCTTGTGTCGTGTCTCGT	AGCGGCAGTAGCTTGGTAAA	Sato et al. 2009
K03504	*	1	GGAGAGACGGCAGATTTGAG	GTGTACACCGTCTTCTCCGC	Sato et al. 2009
K00892	*	*	CCGCTAGTGTGGCTGAATTT	CAAAAGCTTGGTGAAGGAGC	Sato et al. 2009
K03426	*	*	GGAGACTGGATGCGTATGAGA	GATGCACAGGGAACGAAAGTT	Sato et al. 2009
K02692	*	*	CTCTGCTGTTTCGTGGATCA	TGATGACATTATCGCCCAGA	Sato et al. 2009
K03164	*	*	TGACACCGAAAACCATTTGA	TCAGGGAGCTGACGAAGACT	Sato et al. 2009
K03296	1	*	ATGTTACCTGACGAAAGCC	CAGCTCTTACTGCCCAAAG	Sato et al. 2009
K02534	2	1	GAGGCAAGCAGCAATACACA	TTTGTGACGGCTTACACCAC	Sato et al. 2009
K00354	2	1	TATACCAGCGCTGCACTTTG	ACCCAAACGCAAACAGACTC	Sato et al. 2009
K00933	1	1	AAATGCTCCAGTTGACAGGC	CGGCACTGAACATTCTGCTA	Sato et al. 2009
K00662	3	1	TAGCCTGGCAGCTTTCTGTT	CTACTTCCCCCGTTTCGAC	Sato et al. 2009
K00077	1	1	ACCAGTTCGCATCATCACA	GTCCCTGACGTCAACCAGAT	Sato et al. 2009
K02538	1	2	GGCATCGTGATGACGTACAG	TCCGCTGTGAGTCTTCTT	Sato et al. 2009

Barley primers	Polymorphic in TX9425/Franklin	Polymorphic in Ernie/Batavia	Forward primer	Reverse primer	References
K00688	3	*	TTTGTGGTGCATTTCTGA	CGACTTTGTGAGCACCGATA	Sato et al. 2009
K00088	1	1	ACACGGTCCATGGAAGAAAC	CATAGATGGGCCCTTGAAGA	Sato et al. 2009
GMS116	*	2	GAAAGACTGACAGGCGGAAG	TTTCTTTGTTGTGTGTGCAGTG	Ramsay et al. 2000
gwm0181	2	*	TCATTGGTAATGAGGAGAGA	GAACCATTTCATGTGCATGTC	Somers et al. 2004
gwm0547	*	*	GTTGTCCCTATGAGAAGGAACG	TTCTGTCTGCTGTTTTTCATTTAC	Somers et al. 2004
gwm114	1	1	ACAAACAGAAAATCAAACCCG	ATCCATCGCCATTGGAGTG	Somers et al. 2004
gwm155	2	1	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	Somers et al. 2004
gwm340	2	1	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG	Somers et al. 2004
gwm247	1	1	GCAATCTTTTTTCTGACCACG	ATGTGCATGTCGGACGC	Somers et al. 2004
gwm299	1	3	ACTACTTAGGCCTCCCCGC	TGACCCACTTGCAATTCATC	Somers et al. 2004
gwm285	4	*	ATGACCCTTCTGCCAAACAC	ATCGACCGGGATCTAGCC	Somers et al. 2004
wmc687	3	*	AGGACGCCTGAATCCGAG	GGGAGCGTAGGAGGACTAACA	Somers et al. 2004
wmc0471	1	*	GGCAATAATAGTGCAAGGAATG	GCCGATAATGGGCAATATAAGT	Somers et al. 2004
wmc261	*	*	GATGTGCATGTGAATCTCAAAGTA	AAAGAGGGTCACAGAATAACCTAAA	Somers et al. 2004
wmc274	2	*	AAGCAAGCAGCAAACTATCAA	GAATGAATGAATGAATCGAGGC	Somers et al. 2004
wmc0291	*	*	TACCACGGGAAAAGGAAACATCT	CACGTTGAAACACGGTGACTAT	Somers et al. 2004
wmc0777	*	*	GCCATCAAGCGGATCAACT	GTAGCGCCCTGTTTCACCTC	Somers et al. 2004
wmc0078	*	*	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	Somers et al. 2004
cfp 1822	1	1	TCTCCCTGCCTAGCCCC	CACCTCCCATCCCCTCTTATCAC	Somers et al. 2004
cfp 2012	*	*	GCGTCAAACCACTAATGTCGCTG	GGAAGAGAGGAGGAAGAAGAGGG	Somers et al. 2004
cfp 3019	*	*	GGAGGAGGAGGTGGTTGTAGG	CCGGGACTAATGGGCTGGAC	Somers et al. 2004
cfa 2153	*	*	TGCCTAAATCTAAATGCCCG	GGATAATGTGCATGTTCCACCG	Somers et al. 2004
cfid0223	*	*	AAGAGCTACAATGACCAGCAGA	GCAGTGTATGTCAGGAGAAGCA	Somers et al. 2004
cfp1037	2	*	ACCACCGTCGTGCTCTAAAAAG	CCAACCCATGCTACTGCTAACC	Paux et al. 2008
Barc0068	2	1	CGATGCCAACACACTGAGGT	AGCCGCATGAAGAGATAGGTAGAGAT	Somers et al. 2004
Bmac0035	*	*	GCGGTGTGCATGCTTGTCTGTAGGAGT	GCGTAGTGTAGTATGTGGCCCCGATTATT	Somers et al. 2004
gpw1108	*	2	TGCAAGTGCTCTTAGAATCTGAAC	TCTTGAACCTGAACCTTGC	Somers et al. 2004
gpw4513	2	3	GCGAGGAGGAGTCCGATT	TCTGTCTTGTATCTTCCCTCC	Somers et al. 2004
gpw7088	*	*	GCGAGAGCGTTAGCACACAA	ACATGAAGCTGGCGTGGTT	Somers et al. 2004
gpw8238	*	*	GGAGAGGTTGGGGAAGACTC	CATGGGCTGTAGGGATGTG	Somers et al. 2004