The Homoeologous Regions on Long Arms of Group 3 Chromosomes in Wheat and Barley Harbour Major Crown Rot Resistance Loci

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Abstract: Crown rot (CR), caused by various *Fusarium* species, has become an important cereal disease worldwide and growing resistant varieties is an essential strategy to reduce the $A80 mil annual loss from CR in Australia. To facilitate the breeding of resistant varieties, we have screened 2514 wheat and 1059 barley genotypes and identified several lines with high levels of CR resistance in each crop. Initially focused on two wheat and one barley resistance sources, we have identified major QTL with unprecedented magnitudes. Two wheat QTL explain between 35% (LOD 7.6) and 49% (LOD 10.8) and the barley QTL explains up to 63% (LOD 14.8) of the phenotypic variance. One of the wheat QTL has been further assessed in four validation populations, and the presence of this QTL alone reduces CR severity by 33% on average. Surprisingly, all of the three major CR QTL are located in similar regions on the long arms of the homoeologous group 3 chromosomes, the two wheat QTL on 3BL and the barley QTL on 3HL. The possible homoeologous relationship between the 3BL wheat QTL and the 3HL barley QTL warrants further investigation. Relative rearrangements between 3H and 3B chromosomes are unknown, although the relative distances between the different QTL and the centromeres seem to be different. Compared with the barley QTL, the 3BL wheat QTL seems to be more distally located. However genetic distance can be affected by many factors including the use of different populations, thus the differences in genetic distances between the two different genera may have only limited value. The physical map of wheat chromosome 3B, which was recently made available as the first such resources for wheat, would make such a study much easier. Results will be presented on the detection, genetic analysis and mapping of these new sources of CR resistance.

Keywords: Fusarium diseases; genome mapping; germplasm screening; quantitative trait loci

Crown rot (CR) is a serious disease of wheat and barley caused predominantly by *Fusarium pseudograminearum* and *F. culmorum* in most wheat growing countries. These pathogens survive in residues of infected cereals and grass hosts and CR has increased in many cereal growing regions worldwide in recent years (Chakraborty *et al.* 2006), due most likely to the wider adoption of minimum tillage. In Australia, CR is widespread and chronic in the 11 million hectare wheat belts.

According to recent estimates, CR costs nearly $80 million each year in lost production and quality (Murray & Brennan 2009). A survey in the Pacific Northwest of the USA found that CR could reduce yield of winter wheat by 35% in commercial fields and further addition of pathogen inoculum can increase this to 61%. (Smiley *et al.* 2005). These and other *Fusarium* species also cause head blight where trichothecene mycotoxins contaminate grains (Mudge *et al.* 2006) and these mycotoxins are harmful to human and animal health. With no resistant varieties, stubble management, crop rotation and partially resistant varieties are used to manage CR.

Coordinated by the Australian Grains Research and Development Corporation, there has been a concerted effort to improve understanding of CR so as to develop effective management. A team at CSIRO Plant Industry has focused on various aspects of crown rot pathology, epidemiology and host resistance for the last eight years. New knowledge has emerged of pathogenicity, toxigenicity, and phylogenetics of *F. pseudograminearum*, *F. graminearum* and *F. culmorum*; genomic regions controlling CR resistance and pathogenicity; mechanisms of host invasion in CR, including the role of mycotoxins and the identification of novel sources of CR using high throughput bioassays. This paper gives a brief overview of our host resistance research including the identification of novel sources of resistance in both wheat and barley and the genetic understanding on three of the novel resistant sources identified.

MATERIALS AND METHODS

Plant materials

To identify sources of resistance in wheat, we have assessed a total of 2514 genotypes. These consist of more than 30 taxa representing hexaploid, tetraploid and diploid wheats (Table 1). Many of the genotypes in the unknown category are varieties or landraces collected from various parts of the world and the majority of them likely belong to common wheat (*Triticum aestivum* L.).

Two of the most resistant genotypes identified from this screening were used for generating a large numbers of populations for introducing the resistance into local varieties and for genetic studies. One of these new sources of resistance is Ernie, a soft red winter wheat from the US and the other resistant source is CSCR6, a genotype belonging to the taxon *T. spelt* L. A microspore-derived doubled haploid (DH) population consisting of 153 lines were generated from a cross between Ernie and a CR susceptible commercial variety Batavia and the population was used to detect QTL conferring CR resistance in Ernie. A population of recombinant inbred lines (RIL, F8) was developed from a cross between CSCR6 and the commercial variety Lang. Ninety two of these RILs were used for mapping CR resistance in CSCR6. Another four populations were used for validating the effects of QTL identified from CSCR6. These populations are:

(a) Aus13832/CSCR6 F_5
(b) Janz/CSCR6 F_5
(c) Janz × 2/CSCR6 F_4, and
(d) Drysdale//Janz/CSCR6 F_4.

Using a similar approach, 1059 barley genotypes from at least five different *Hordeum* taxa (Table 2) were screened to identify CR resistance. The genetic mapping of CR resistance commenced

Table 1. *Triticum* genotypes assessed for crown rot resistance

Taxon	No.	Taxon	No.
T. aestivum	1301	*T. ovatum*	1
T. bicorne	2	*T. polonicum*	4
T. boeoticum	6	*T. pseudo-boeoticum*	1
T. carthlicum	4	*T. sinskajae*	1
T. comosum	2	*T. spelta*	29
T. compactum	7	*T. speltoides*	2
T. crassum	1	*T. sphaerococcum*	5
T. cylindicum	2	*T. thaoudar*	3
T. dicoccoides	376	*T. timopheevi*	7
T. dicoccum	4	*T. triunciale*	1
T. durum	161	*T. umbellulatum*	3
T. juvenale	1	*T. uniaristatum*	2
T. longissimum	2	*T. urartu*	3
T. macha	2	*T. vavilovii*	4
T. militinae	1	*T. ventricosum*	2
T. monococcum	4	*T. zhukovskyi*	3
Unknown	563	*T. tauschii*	3
Total		2514	

Table 2. Barley genotypes assessed for crown rot resistance

Taxon	No. of genotypes
H. vulgare L.	370
H. spontaneum K. Koch	24
H. agriocrithon A. E. Åberg	14
H. chilense Roem. et Schult.	1
H. bulbosum L.	1
Unknown	649
Total	1059

following the identification of TX9425, a Chinese landrace, as a source of CR resistance. CR resistance of TX9425 was better than 53 commercial varieties tested (Li *et al.* 2009). A DH population was derived from TX9425 and Franklin, an elite Australian variety highly susceptible to CR.

More than half of the genotypes screened have not been classified into species. Similar to the situation in wheat, these unclassified barley genotypes are predominantly varieties and landraces collected from various parts of the world. Their morphology suggests that most of them belong to *H. vulgare* L. with a small number of *H. spontaneum* K. Koch (CJL, unpublished).

Phenotyping for crown rot reaction

Two Fusarium isolates, one each from *F. pseudograminearum* (CS3096) and *F. graminearum* (CS3005), were used for assessing CR resistance. 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).

Genotyping, map construction and QTL analysis

Two marker systems, DArT and SSR, were used for linkage map construction. DArT genotyping of the parents and the mapping populations was carried out by the Triticarte Pty. Ltd. (http://www.tritcarte.com.au). Procedures for hybridization of genomic DNA to the DArT array, image analysis and polymorphism scoring were as described by Akbari *et al.* (2006). In addition, SSR markers polymorphic between the two parents for each of the mapping populations 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 7M urea. Known chromosomal locations of some of the SSR and DArT markers were used to assign linkage groups to specific chromosomes.

RESULTS

Table 3 lists the top 10 wheat lines identified from a screening of 2514 genotypes. Three were either landraces or varieties and the others included three varieties, two breeding lines and a wild collection from *T. spelta*. All of the top ten genotypes are late maturing in Brisbane (27°24'S, 153°09'E), Australia and at least seven of them are winter type.

Populations have been generated from crosses between most of these top ten wheat genotypes and several Australian varieties. Several of these populations were used to investigate the genetics of CR resistance in two of these top ten genotypes. Transgressive segregations in CR resistance were detected in each of the populations analyzed (Figure 1) and the top lines from some of these populations have been released to breeding companies and collaborators.

Populations derived from two of the resistance sources have been used for QTL mapping studies. One of these sources is the *T. spelta* accession CSCR6. QTL mapping showed that its resistance was controlled by a major locus on the long arm of chromosome 3B. This QTL, designated as *Qcrs.cpi-3B*, was flanked by two DArT markers (wPt10505 & wPt2277). The same QTL was detected using either the *F. pseudograminearum* or the *F. graminearum* isolates (data not shown). This QTL explains up to 49% (LOD 10.8) of the phenotypic variance based on interval mapping analysis and it does not co-locate with any gene known to affect plant height. However, a strong interaction between *Qcrs.cpi-3B* and the reduced height locus *Rht1* on chromosome arm 4BS was

Table 3. Origins of the top ten wheat and top ten barley genotypes identified[#]

Series No.	Wheat			Barley		
	origin	taxon	type	origin	taxon	type
1	China	*T. aestivum*	collection	Iran	*H. spontaneum*	wild barley
2	India	*T. aestivum*	collection	Japan	*H. vulgare*	landrace
3	India	*T. aestivum*	collection	Japan	*H. vulgare*	landrace
4	India	*T. aestivum*	collection	Russia	*H. spontaneum*	wild barley
5	Morocco	*T. aestivum*	collection	South Korea	*H. vulgare*	landrace
6	USA	*T. aestivum*	line	South Korea	*H. vulgare*	landrace
7	USA	*T. aestivum*	variety	South Korea	*H. vulgare*	landrace
8	USA	*T. aestivum*	variety	?	*H. vulgare*?	?
9	?	*T. aestivum*	collection	?	*H. vulgare*?	?
10	?	*T. spelta*	wild	?	*H. vulgare*?	?

[#]line = breeding line, collection = not clear if belonging to landrace or variety; ? = unknown or not sure

detected, where shorter plants tended to give better CR resistance. QTL analysis using the *Rht1* locus as a cofactor reduced the maximum effect of *Qcrs.cpi-3B* to 43%. The most closely linked SSR marker, gwm0181, located about 2.2 cM away from the CR locus was used for validating the effect of this QTL in four additional populations. This analysis showed that the presence of this single QTL reduced CR severity between 29 and 42% with an average of 33%.

The other source of resistance investigated is the winter wheat variety Ernie from the USA. QTL mapping showed that the resistance of this 2nd source of resistance was also controlled by a major locus on the long arm of chromosome 3B. Again, the same QTL was detected using either the *F. pseudograminearum* or the *F. graminearum* isolates and it explains up to 35% (LOD 7.6) of the phenotypic variance.

Our barley work on CR resistance started with the genetic mapping of CR resistance using an existing DH population derived from TX9425 and Franklin. TX9425 was identified from a very limited survey of 53 commercial varieties (Li *et al*. 2009). The CR resistance of TX9425 was conditioned by a major QTL, designated *Qcrs.cpi-3H*, was mapped near the centromere on the long arm of chromosome 3H. This QTL explained 34% (LOD 5.2), 45% (LOD 8.1) and 60% (LOD 9.8) of the phenotypic variation respectively in three separate trials. The location of *Qcrs.cpi-3H* was coincident with a major QTL conferring plant height (PH) and the effect of PH on CR reaction was also highly significant. When the effect of PH was accounted for by covariance analysis, the *Qcrs.cpi-3H* QTL remained highly significant, accounting for over 40% of the phenotypic variation.

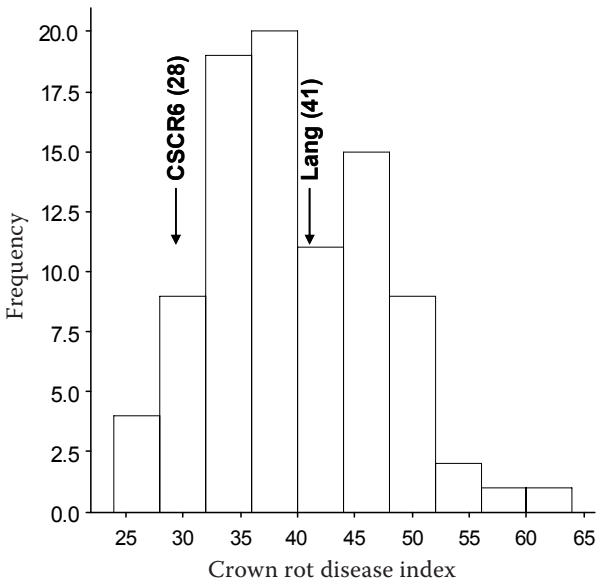

Figure 1. Showing transgressive segregation of crown rot disease index in the F_8 RIL population inoculated with the *F. pseudograminearium* isolate CS3096; the positions of disease index (DI) of the two parental genotypes (numbers in brackets) in the population are indicated

Following the successful QTL mapping, we have assessed CR resistance of 1 059 barley genotypes from at least five different *Hordeum* taxa (Table 2). The top ten barley lines identified from this screening include two *H. spontaneum* genotypes and the other eight most likely belong to *H. vulgare* (Table 3). At least five of the top ten barley genotypes are winter type.

DISCUSSION

To facilitate the breeding of varieties with enhanced resistance to CR infection, we have screened 2514 wheat genotypes and 1059 barley genotypes. These screenings showed that the difference in CR resistance between the best barley genotypes identified and those of the commercial barley varieties is huge. All of the available varieties showed very severe CR symptom but a few of the best lines identified from the screening showed very limited symptom. However, this is not the case in wheat. Although the best wheat lines identified also have drastically improved CR resistance, the magnitude of difference in CR resistance between these and existing wheat varieties does not match that seen with barley.

CR resistance of the two novel sources in wheat we analyzed was each controlled by a major 3BL locus (Li *et al.* 2010; Ma *et al.* 2010). The segregating populations used so far do not allow us to clarify if the same gene is involved in these two resistance sources. The answer to this question may have to wait until the fine mapping work is completed. Similarly, the possible homoeologous relationship between *Qcrs.cpi-3B* (Ma *et al*. 2010) and the major QTL conferring CR resistance on chromosome 3H in barley (Li *et al*. 2009) also warrants further investigation. Both 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. Near isogenic lines for these major CR loci and large populations segregating at only the targeted chromosome regions are being developed. These populations will be used for fine mapping these loci and the identification of polymorphic markers for this homoeologous region would be facilitated by exploiting the physical map of chromosome 3B which, as the first such resources in hexaploid wheat, is now available (Paux *et al*. 2008).

For effectively exploitation of a resistance gene/locus, it is essential to clarify what other traits of agronomic importance it may affect and when it functions. We have initiated such studies and demonstrated for the first time that different genes are responsible for CR resistances at different stages of plant developments (Yang *et al.* 2010), and that plant height per se affects CR severity (Liu *et al.* 2010). Many other issues such as why water stress promotes CR severity need urgent attentions.

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