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ORIGINAL PAPER

Major QTL for Fusarium crown rot resistance in a barley landrace

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Abstract Fusarium crown rot (FCR) is a serious cereal disease in semi-arid regions worldwide. In assisting the effort of breeding cultivars with enhanced resistance, we identified several barley genotypes with high levels of FCR resistance. One of these genotypes, AWCS079 which is a barley landrace originating from Japan, was investigated by developing and assessing three populations of recombinant inbred lines. Two QTL, one located on the long arm of chromosome 1H (designated as *Qcrs.cpi-1H)* and the other on 3HL (designated as *Qcrs.cpi-3H*), were found to be responsible for the FCR resistance of this genotype. *Qcrs.cpi-1H* is novel as no other FCR loci have been reported on this chromosome arm. *Qcrs.cpi-3H* co-located with a reduced height (*Rht*) locus and the effectiveness of the former was significantly affected by the latter. The total phenotypic variance explained by these two QTL was over 60 %. Significant effects were detected for each of the QTL in each of the three populations assessed. The existence of these loci with major effects should not only facilitate breeding and exploitation of FCR-resistant barley cultivars but also their further characterization based on fine mapping and map-based gene cloning.

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Introduction

Fusarium crown rot (FCR) of wheat and barley, which can be caused by multiple species of *Fusarium* with *F. pseudograminearum* as the predominant pathogen in Australia, is a persistent problem in semiarid regions worldwide (Chakraborty et al. 2006; Hogg et al. 2010). The disease can inflict serious yield loss especially to crops affected by moisture-stress during grain filling, which is often referred to as a 'dry finish' in Australia. It reduced yields of malting barley cultivars by an average of 13 % in the Pacific Northwest of USA in a study reported by Smiley et al. (2005). Surveys in Australia found the disease in each of the three cereal growing regions (Chakraborty et al. 2006) and it causes an estimated annual yield loss of $97 million Australian dollars in wheat and barley combined (Murray and Brennan 2009, 2010). In addition, glasshouse-based assays showed that FCR-infected wheat plants could also produce mycotoxins in grains as well as other tissues (Mudge et al. 2006). The presence of these compounds in food and feeds can be a serious safety concern.

FCR pathogens are carried over in crop residues and they can survive for two or more seasons in the field (Smiley et al. 2005; Chakraborty et al. 2006; Hogg et al. 2010). It thus seems obvious that reducing crop stubble should reduce FCR severity. Several practices were assessed in managing FCR damage (Burgess et al. 1996; Kirkegaard et al. 2004) but they do not seem to be effective as

the incidence of FCR has increased in Australia as well as in many other cereal growing regions worldwide in recent years. The high intensity of cereals in cropping systems combined with the wider adoption of minimum tillage for moisture conservation are likely factors responsible for the increased FCR severity (Smiley et al. 2005; Chakraborty et al. 2006; Hogg et al. 2010).

It has long been recognized that growing resistant cultivars has to be an integral component in effectively managing this disease (Wildermuth and Purss 1971). However, barley cultivars with enhanced resistance to FCR are not available. Compared with those of wheat, barley cultivars seem to suffer less yield loss (Smiley et al. 2005) but accumulate much higher concentrations of *Fusarium* pathogens at every stage of FCR infection (Liu et al. 2012a). Thus, growing resistant barley cultivars would not only reduce yield loss in the barley crop itself but also the inoculum load to subsequent barley or other cereal crops. As part of our goal of assisting in the breeding of FCR-resistant cultivars, we have identified several genotypes with high levels of resistance from a systematic screening in barley (Liu et al. 2012b). As expected, those genotypes with the best resistance are predominantly landraces or wild barley accessions. The genetics of FCR resistance on one of the best sources of resistance, a wild barley accession belonging to *Hordeum spontaneum* (L.), had been investigated and a major QTL on chromosome arm 4HL detected responsible for its resistance (Chen et al. 2013). Naturally, breeding resistant cultivars would require more than one QTL. To maximize the chance of detecting FCR QTL different from the one in the wild barley genotype, a landrace originating from Japan was investigated and results obtained are reported in this paper.

Materials and methods

Plant materials

Three populations of recombinant inbred lines (RILs) between AWCS079 and three commercial cultivars were produced and used in this study. They included:

1. Baudin/AWCS079 $RILF_8$—125 lines
2. Gairdner/AWCS079 $RILF_7$—117 lines
3. Franklin/AWCS079 $RILF_7$—101 lines

AWCS079 is one of the most resistant genotypes identified from a systematic screening of genetic stocks representing different geographical origins and plant types (Liu et al. 2012b). These populations were generated in glasshouses at the Queensland Bioscience Precinct in Brisbane (QBP), Australia. The first population was used for QTL mapping and the other two were used for validating putative QTL identified from the mapping population.

FCR inoculations and assessments

The highly aggressive *F. pseudograminearum* (CS3096) obtained from a wheat field in northern New South Wales, Australia and maintained in the CSIRO collection (Akinsanmi et al. 2004) was used in this study. Inoculum preparation, inoculations, and FCR assessments were as described by Li et al. (2008). Briefly, inoculum was prepared using plates of ½ strength potato dextrose agar. Inoculated plates were kept for 12 days at room temperature before the mycelium in the plates were scraped and discarded. The plates were then incubated for a further 7–12 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. The spore suspensions were stored in minus 20 freezer and 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. Seedlings of 3-day-old were immersed in the spore suspension for 1 min and two seedlings were planted into a 5 cm square punnet (Rite Grow Kwik Pots, Garden City Plastics, Australia) containing sterilized University of California mix C (50 % sand and 50 % peat v/v). The punnets were arranged in a randomized block design in either a glasshouse or a controlled environment facility (CEF). Settings for the glasshouse were: 25/18 (±1) °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: 25/16(±1) °C day/night temperature and 65/85 % 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 FCR development, water-stress was applied during plant growth. Inoculated seedlings were watered only when wilt symptoms appeared.

For QTL mapping, three replicated trials were carried out using the mapping population (designated as FCR01, FCR02, FCR03, respectively). For QTL validation, two replicated trials were conducted using the two validation populations. Each of these trials contained two replicates, each replicate with ten seedlings. FCR severity was assessed 4 weeks after inoculation, using a 0 (no obvious symptom)–5 (whole plant severely to completely necrotic) scale as described by Li et al. (2008). A disease index (DI) was then calculated for each line following the formula of $DI = (\Sigma_{nX}/5\ N) \times 100$, 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.

Evaluation for plant height

To assess possible effects of plant height (PH) on FCR resistance, three trials (two field trials and one pot-based glasshouse trial) were conducted using the mapping population of Baudin/AWCS079. The two field trials were conducted at the CSIRO Research Station at Gatton, Queensland (27°34′S, 152°20′E), in 2011 (designated as PH01) and 2012 (designated as PH02). A randomized block design was used with three replicates for each of the field trials. For each replicate, 20 seeds for each of the $RILF_8$ lines were sown in a single 1.5 m row with a 25 cm row-spacing. PH was measured as the height from the soil surface to the tip of the spike (awns excluded). Six measurements were taken from the six tallest tillers in each row and the average from the six measurements was used for statistical analyses. The glasshouse trial was conducted at the QBP (designated as PH03) using two replicates. Three plants, each in a different pot of 2.0 L, were used in each of the replicates. PH measurements were obtained from the two tallest tillers for each plant and their averages were used for statistical analysis.

Molecular marker analysis

DArT markers were initially used for linkage map construction and QTL analyses using 92 lines from the Baudin/AWCS079 population. DArT genotyping of the parents and the mapping population was carried out by the Triticarte Pty. Ltd. (http://www.tritcarte.com.au). Procedures of hybridization of genomic DNA to the DArT array, image analysis and polymorphism scoring were as described by Wenzl et al. (2004). SSR markers were then identified for regions where putative QTL conferring FCR resistance was detected and polymorphic SSR markers were used to genotype the whole mapping population of 125 lines. Analyses of SSR markers were as described by Chen et al. (2012). The SSR markers were selected based on their locations in existing barley linkage maps (Ramsay et al. 2000; Varshney et al. 2007; Sato et al. 2009). PCR reactions for the amplification of the SSR markers were carried out in a total volume of 12 µl containing 25 ng genomic DNA, 0.2 µM of forward and reverse primer, 3 mM $MgCl_2$, 0.2 mM dNTPs, and 0.5 U *Taq* DNA polymerase. During PCR reactions, the marker products were labeled with α-[^{33}P]dCTP (3,000 ci/mmol). Reactions were run on a Gene Amp PCR System 2700 thermocycler (PE Applied Biosystems, Foster City, Calif.) programmed with the cycling conditions: one cycle of 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at the appropriate annealing temperature (ranging from 50 to 56 °C depending on the marker) and 1 min at 72 °C, with a final extension step of 5 min at 72 °C. The amplified products were mixed with an equal volume of loading dye, denatured at 95 °C for 5 min, and 3.8 µl samples was run on a denaturing 5 % polyacrylamide (20:1) gel at 90 W for 2 h. The gels were subsequently dried using a gel dryer for 30 min at 80 °C and exposed to Kodak X-Omat X-ray film for 4–6 days.

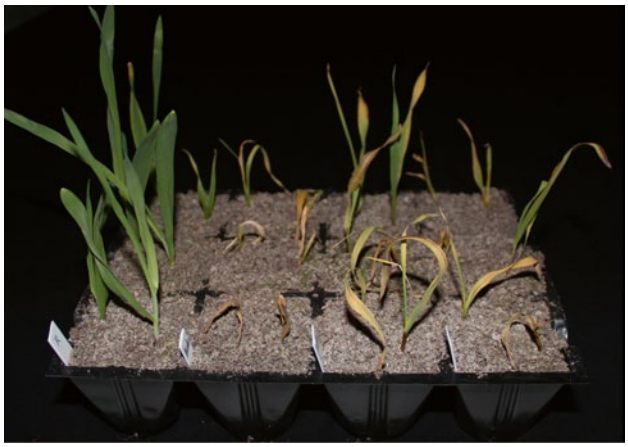

Fig. 1 Difference in resistance to Fusarium crown rot infection between the resistant genotype AWCS079 and the three commercial cultivars (Baudin, Franklin and Gairdner) used as parents in this study

Table 1 Disease index of FCR severity in the population of Baudin/AWCS079

Trial[a]	Parent		Population			
	Baudin	AWCS079	Min	Max	Mean	SD
FCR01	90.9	22.9	13.1	92.1	46.8	19.9
FCR02	87.1	16.9	16.5	90.5	49.4	18.5
FCR03	89.3	12.8	10.9	91.8	45.2	18.8

[a] The three trials conducted were designated as FCR01, FCR02 and FCR03, respectively

Table 2 Correlation coefficients of FCR severity and plant height in the population of Baudin/AWCS079

Trial[a]	FCR01	FCR02	FCR03	PH01	PH02	PH03
FCR01	1.00					
FCR02	0.79**	1.00				
FCR03	0.81**	0.80**	1.00			
PH01	0.33**	0.46**	0.40**	1.00		
PH02	0.31**	0.47**	0.38**	0.93**	1.00	
PH03	0.26*	0.42**	0.32**	0.76**	0.94**	1.00

**, * Indicate significant levels of $p < 0.01$ and $p < 0.05$, respectively

[a] For FCR severity, the three trials conducted were designated as FCR01, FCR02, and FCR03, respectively. For plant height, the two field trials conducted at the CSIRO Research Station at Gatton in Queensland in 2011 and 2012 were designated as PH01 and PH02, respectively, and the single glasshouse trial was designated as PH03

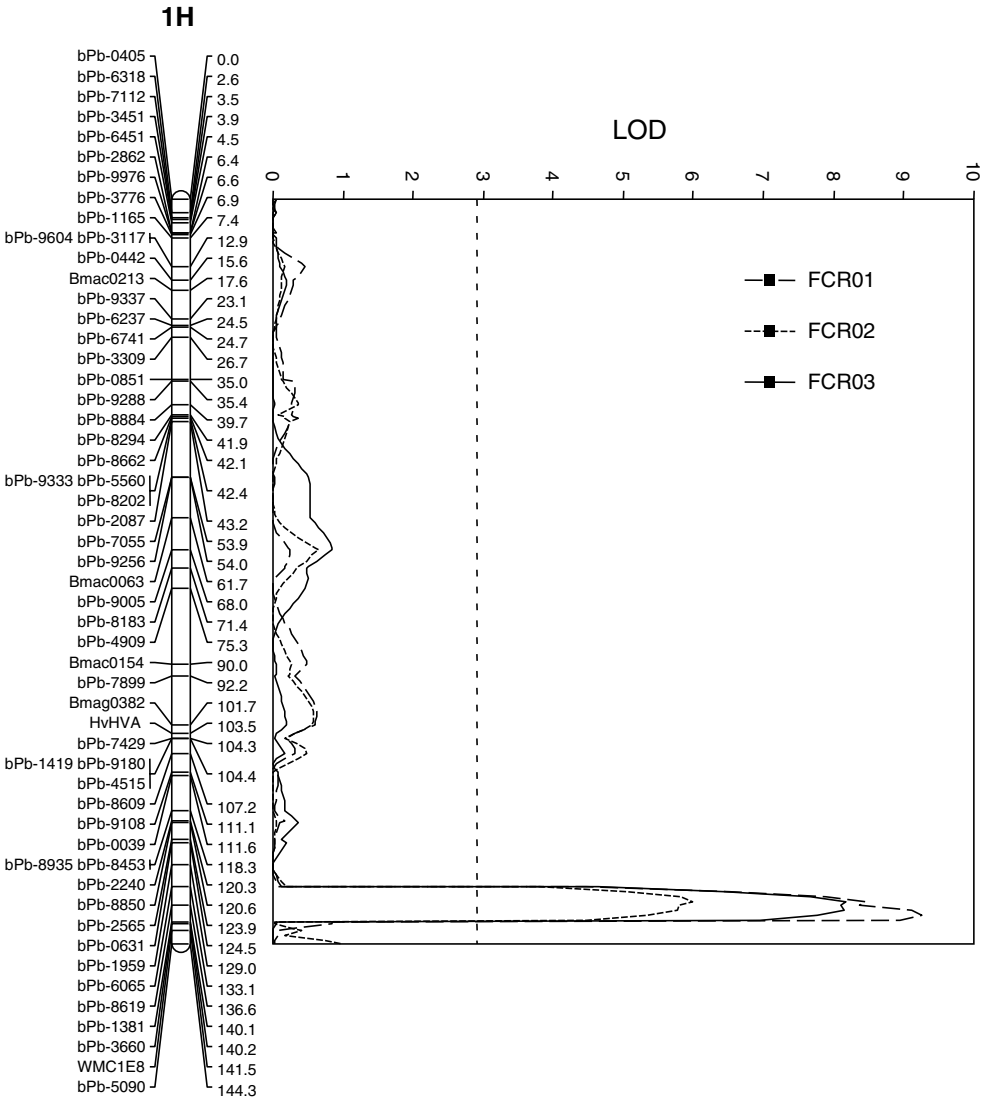

Fig. 2 QTL conferring FCR resistance detected on chromosome 1H in the population of Baudin/AWCS079. Marker positions are shown to the *left* of the linkage map and distances in centiMorgan (cM) between loci are shown to the *right*. The *vertical dotted line* indicates the average significance threshold (LOD = 2.9) derived from permutation tests

Data analysis and QTL mapping

Statistical analyses were performed using the GenStat, 13th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) and the SPSS statistics 17.0 for Windows statistical software package (SPSS Inc., Chicago, IL). For each trial, the following mixed-effect model was used: $Y_{ij} = \mu + r_i + g_j + w_{ij}$. Where: Y_{ij} = trait value on the jth genotype in the ith replication; μ = general mean; r_i = effect due to ith replication; g_j = effect due to the jth genotype; w_{ij} = error or genotype by replication interaction, where genotype was treated as a fixed effect and that of replicate as random. The effects of replicate and genotype for both FCR resistance and PH were determined using ANOVA. Pearson correlation coefficient was estimated between traits and trials.

Segregation ratios of the 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 JointMap® 4 (Van Ooijen 2006). 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 FCR 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

Table 3 QTL for FCR severity and plant height identified in the population of Baudin/AWCS079

Trial[a]	Analysis	QTL	Flanking markers	LOD	R^2 (%)	Origin
FCR01	IM	*Qcrs.cpi-1H*	bPb-0631 & bPb-5090	7.87	33.4	AWCS079
	MQM	*Qcrs.cpi-1H*	bPb-6065 & bPb-8619	9.52	32.2	AWCS079
	IM	*Qcrs.cpi-3H*	Bmac0209 & bPb-7872	4.12	28.1	AWCS079
	MQM	*Qcrs.cpi-3H*	bPb-7278 & bPb-0619	5.76	21.0	AWCS079
FCR02	IM	*Qcrs.cpi-1H*	bPb-6065 & bPb-5090	4.58	21.4	AWCS079
	MQM	*Qcrs.cpi-1H*	bPb-6065 & bPb-8619	6.16	21.1	AWCS079
	IM	*Qcrs.cpi-3H*	bPb-6347 & bPb-1183	8.61	39.2	AWCS079
	MQM	*Qcrs.cpi-3H*	bPb-7278 & bPb-0619	10.56	38.0	AWCS079
FCR03	IM	*Qcrs.cpi-1H*	bPb-0631 & bPb-5090	6.68	30.0	AWCS079
	MQM	*Qcrs.cpi-1H*	bPb-6065 & bPb-8619	8.46	29.8	AWCS079
	IM	*Qcrs.cpi-3H*	bPb-6347 & bPb-7872	5.19	31.4	AWCS079
	MQM	*Qcrs.cpi-3H*	bPb-7278 & bPb-0619	7.58	26.4	AWCS079
PH01	IM	*Qph.cpi-3H.1*	Bmac0209 & bPb-7872	7.19	39.3	AWCS079
	MQM	*Qph.cpi-3H.1*	bPb-7278 & HVM33	8.62	28.9	AWCS079
	IM	*Qph.cpi-3H.2*	bPb-9110 & bPb-4830	2.92	18.0	Baudin
	MQM	*Qph.cpi-3H.2*	bPb-9110 & bPb-4830	3.55	11.2	Baudin
	IM	*Qph.cpi-6H*	bPb-7179 & bPb-3722	4.24	20.3	Baudin
	MQM	*Qph.cpi-6H*	bPb-9839 & bPb-0934	3.20	10.3	Baudin
PH02	IM	*Qph.cpi-3H.1*	Bmac0209 & bPb-7872	6.93	39.0	AWCS079
	MQM	*Qph.cpi-3H.1*	bPb-7278 & HVM33	8.71	27.2	AWCS079
	IM	*Qph.cpi-3H.2*	bPb-9110 & bPb-4830	3.08	16.5	Baudin
	MQM	*Qph.cpi-3H.2*	bPb-9110 & bPb-4830	3.71	10.7	Baudin
	IM	*Qph.cpi-6H*	bPb-7179 & bPb-7181	5.55	25.8	Baudin
	MQM	*Qph.cpi-6H*	bPb-9839 & bPb-0934	4.60	13.8	Baudin
PH03	IM	*Qph.cpi-3H.1*	bPb-3320 & HVM33	5.08	31.9	AWCS079
	MQM	*Qph.cpi-3H.1*	bPb-7278 & HVM33	5.50	20.4	AWCS079
	IM	*Qph.cpi-6H*	bPb-7179 & bPb-0934	5.13	23.6	Baudin
	MQM	*Qph.cpi-6H*	bPb-9839 & bPb-0934	4.06	13.6	Baudin

IM analysis conducted using interval mapping, *MQM* analysis conducted using the multiple QTL model

[a] For FCR severity, the three trials conducted were designated as FCR01, FCR02, and FCR03, respectively. For plant height, the two field trials conducted at the CSIRO Research Station at Gatton in Queensland in 2011 and 2012 were designated as PH01 and PH02, respectively, and the single glasshouse trial was designated as PH03

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).

Results

Characterization of FCR resistance in the mapping population of Baudin/AWCS079

Highly significant differences in FCR resistance were detected between the three commercial cultivars and AWCS079 (Fig. 1). Among the three trials conducted against the mapping population and its two parents, DI values for AWCS079 ranged between 12.8 and 22.9 (average 17.5) and those for Baudin ranged between 87.1 and 90.9 (average 89.1). Transgressive segregation was observed in the mapping population (Table 1). DI values were significantly and positively correlated among the three trials, with correlation coefficients (R) ranging from 0.79 to 0.81 (Table 2).

Linkage map construction

Of the DArT markers analysed, 982 detected polymorphism between Baudin and AWCS079. These markers formed 458 segregation clusters (markers within each of them co-segregated). As co-segregating markers provide the same information as a single marker within any given cluster, a single marker from each of the clusters was used

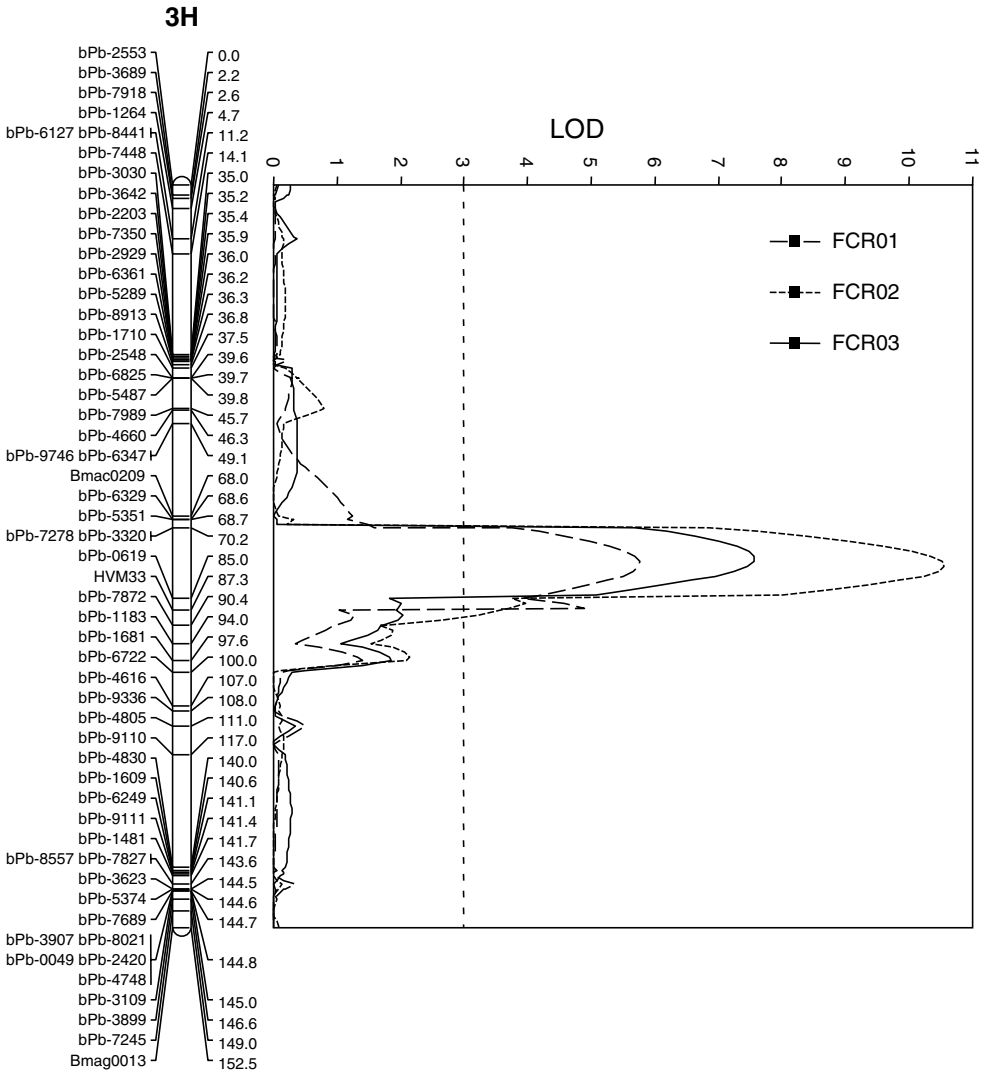

Fig. 3 QTL conferring FCR resistance detected on chromosome 3H in the population of Baudin/AWCS079. Marker positions are shown to the *left* of the linkage map and distances in centiMorgan (cM) between loci are shown to the *right*. The *vertical dotted line* indicates the average significance threshold (LOD = 3.0) derived from permutation tests

for linkage map construction. The linkage map also contained 11 SSR markers which were selectively used based on positions of putative QTL identified for FCR resistance. These 469 markers formed seven linkage groups and covered a total genetic distance of 1,101.3 cM with an average distance of 2.3 cM between loci (Fig. S1). Of the mapped markers, 115 (24.5 %) showed skewed segregation. Among them, 53 (46.1 %) were skewed favouring alleles from the male parent AWC079 and the remaining favouring alleles from the female parent Baudin.

Identification and validation of QTL for FCR resistance

Two QTL conferring FCR resistance were identified in the mapping population and resistant alleles for both were derived from the resistant parent AWCS079. One of the QTL was located on the long arm of chromosome 1H and segregation distortion was detected for none of the markers flanking the QTL (Fig. S1). This QTL was designated as *Qcrs.cpi-1H* following convention, where 'crs' stands for 'crown rot severity' and cpi, 'CSIRO Plant Industry'. Markers flanking *Qcrs.cpi-1H* were bPb-6065 and bPb-8619 with WMC1E8 as the most closely linked SSR marker to this QTL (Fig. 2). *Qcrs.cpi-1H* explained up to 33.4 % of the phenotypic variance (Table 3).

The second QTL was located on the long arm of chromosome 3H and it was designated as *Qcrs.cpi-3H*. Significant segregation distortion was detected for none of the markers flanking the QTL (Fig. S1). Markers flanking *Qcrs.cpi-3H* were bPb-7278 and bPb-0619 with Bmac0209 as the most closely linked SSR marker to this QTL (Fig. 3).

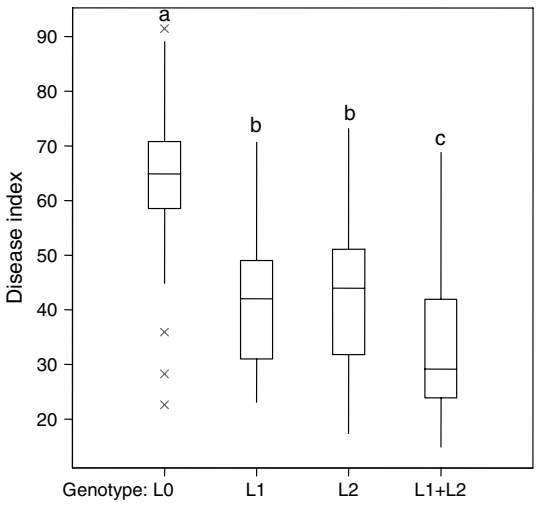

Fig. 4 Box plot distributions of disease index for FCR severity of lines possessing various QTL combinations. *Boxes* indicate the 25 and 75 percentiles and the medians are indicated by *solid horizontal lines*. *Vertical lines* represent ranges of variation and outliers are indicated by reticle. The *different letters* above the reticles or *bars* denote statistically significant differences at $p < 0.05$ with One-Way ANOVA Duncan's multiple range test. *L0* represent the individuals possessing none of the QTL, *L1* represent the individuals with only the *Qcrs.cpi-1H*, *L2* represent the individuals with only the *Qcrs.cpi-3H*, and *L1* + *L2* represent the individuals containing both of the *Qcrs.cpi-1H* and *Qcrs.cpi-3H*, respectively

Qcrs.cpi-3H explained 32.9 % of the phenotypic variance across the three trials (Table 3). The mean of DI values for lines with *Qcrs.cpi-1H* was not significantly different from that of those with *Qcrs.cpi-3H*. However, as expected, lines with both of the QTL gave significantly lower FCR severity on average than those with a single locus only (Fig. 4).

Possible effects of *Qcrs.cpi-1H* and *Qcrs.cpi-3H* were further assessed in the two validation populations. The SSR markers linked most closely to these two QTL (WMC1E8 and Bmac0209, respectively) were used to identify individual lines with or without alleles from the resistant parent AWCS079 in these populations. Significant effects were detected for each of these two QTL in both of the validation populations. The average difference between DI values of the two groups of lines (those with and those without the AWCS079 allele) for *Qcrs.cpi-1H* was 24.0 % in the population of Franklin/AWCS079 and 28.5 % in the population of Gairdner/AWCS079. The average difference for *Qcrs.cpi-3H* was 27.5 % in the population of Franklin/AWCS079 and 16.7 % in the population of Gairdner/AWCS079 (Table 4).

QTL for plant height and their effects on FCR resistance

Three QTL were detected for PH in the mapping population. Two of them, designated as *Qph.cpi-3H.1* and *Qph.cpi-3H.2,* were located on the long arm of chromosome 3H (Table 3). *Qph.cpi-3H.1* was derived from the parent AWCS079 and explained on average 36.7 % of the phenotypic variance across the three trials. *Qph.cpi-3H.2* was a minor QTL derived from the parent Baudin. This QTL was detected in two of the three trials and explained on average 11.6 % of the phenotypic variance across the two trials. The third QTL, designated as *Qph.cpi-6H*, was located on the long arm of chromosome 6H. It was derived from the parent Baudin and explained on average 23.2 % of the phenotypic variance across the three trials in the mapping population (Table 3).

The location of *Qph.cpi-3H.1* on chromosome arm 3HL was similar to that of *Qcrs.cpi-3H*. To quantify the possible effect of PH on FCR reaction, data from the three FCR trials were analysed against data from the three PH trials by a covariance analysis. This analysis showed that PH had little effect on *Qcrs.cpi-1H* but a significant effect on *Qcrs.cpi-3H*. Both LOD value and the magnitude of *Qcrs.cpi-3H* were reduced when the effect of height was accounted for by the covariance analysis. However, *Qcrs.cpi-3H* remained significant and its position was unchanged (Fig. 5).

Discussion

Two QTL, one on the long arm of chromosome 1H (*Qcrs.cpi-1H*) and the other (*Qcrs.cpi-3H*) on the long arm of chromosome 3H, were found responsible for FCR resistance in AWCS079 which is a barley landrace originating from Japan. Significant effects were detected for both QTL in each of the three populations analyzed. *Qcrs.cpi-1H* is novel as no other QTL conferring FCR resistance has ever been reported on this chromosome arm. The presence of *Qcrs.cpi-1H* reduced FCR severity by an average of 27.1 % across the three populations assessed and PH showed little effects on its resistance.

The presence of *Qcrs.cpi-3H* reduces FCR severity by an average of 26.7 % across the three populations assessed. However, this FCR QTL co-located with an *Rht* locus and the effectiveness of the former was drastically reduced when the latter was accounted for based on a covariance analysis. These results were similar to those obtained in an earlier study on a different genotype in which a FCR QTL was also co-located with an *Rht* locus in a similar chromosomal region (Li et al. 2009). Possible effects of PH on FCR resistance have also been reported in wheat using both segregating populations (Wallwork et al. 2004; Li et al. 2010) and near isogenic lines (NILs) for various *Rht* genes (Liu et al. 2010). Thus, understanding the relationship between loci controlling these two traits would be critical in determining the value of *Qcrs.cpi-3H*. As QTL mapping provides only limited resolution (Paterson et al. 1988), populations used for QTL studies may not be suitable for

Table 4 Effects of QTL conferring FCR resistance in two validation populations

QTL	Population	Trial[a]	RR	rr	Difference (%)	*p* value
Qcrs.cpi-1H	Franklin/AWCS079	FCRV01	34.3	49.4	30.4	<0.01
		FCRV02	42.9	52.0	17.6	<0.01
	Gairdner/AWCS079	FCRV03	39.1	54.3	27.9	<0.01
		FCRV04	31.2	43.9	29.0	<0.01
Qcrs.cpi-3H	Franklin/AWCS079	FCRV01	36.4	50.4	27.7	<0.01
		FCRV02	40.9	56.2	27.3	<0.01
	Gairdner/AWCS079	FCRV03	44.7	51.6	13.3	<0.01
		FCRV04	39.1	48.9	20.1	<0.01

RR represents homozygous alleles from the resistant parent AWCS079, *rr* those from the susceptible parents Franklin or Gairdner

[a] The two trials conducted using the population of Gairdner/AWCS079 were designated as FCRV01 and FCRV02, respectively, and the two trials conducted using the population of Franklin/AWCS079 were designated as FCRV03 and FCRV04, respectively

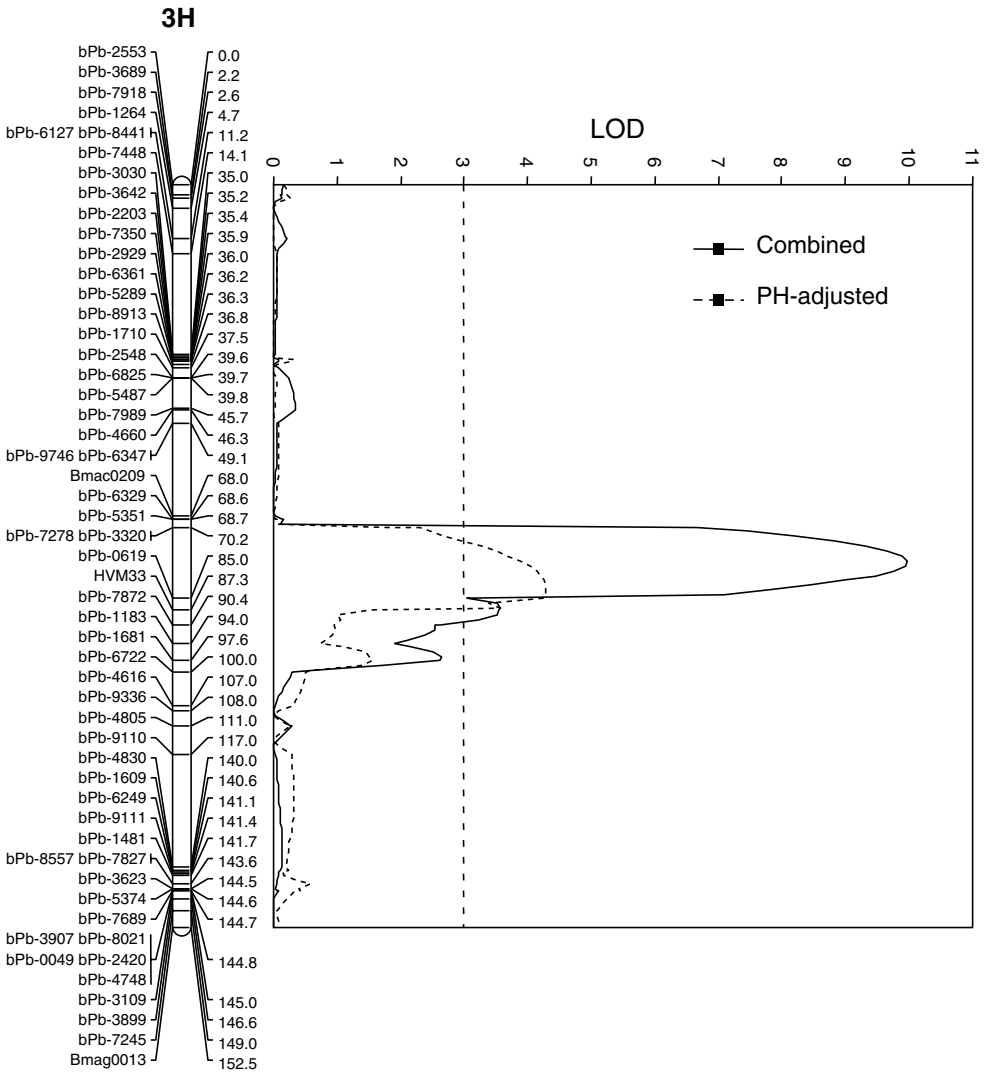

Fig. 5 LOD values of *Qcrs.cpi-3H* obtained from combined data of the three FCR trials pre- (combined) and post-adjustment by plant height (PH-adjusted)

determining if FCR and PH are controlled by closely linked genes in this chromosome region or by the same gene(s) with pleiotropic effects. Developing and exploiting NILs and NIL-derived populations, as carried out for traits related to spike morphology in barley (Chen et al. 2012), could be an option to further investigate the relationship between gene(s) controlling these two traits.

Fusarium pathogens are generally considered as necrotrophs although there is limited evidence showing that *F. graminearum* may not be a strict necrotroph (Goswami and Kistler 2004). As distinct from the gene-for-gene resistance shown for biotrophic pathogens, plant resistance to necrotrophic pathogens is often polygenic and not pathogen species-specific (Lai et al. 2011). Thus, it is not surprising that available results derived from studies on both barley (Li et al. 2009; Liu et al. 2012a, b) and wheat (Ma et al. 2010; Li et al. 2010) all show that FCR resistance is not pathogen species-specific. However, host resistance to FCR in barley does not seem to be polygenic. In addition to the one reported in this paper, two additional sources of resistance have been investigated and a single locus was found responsible for FCR resistance in each of these two genotypes (Li et al. 2009; Chen et al. 2013). Both the non-pathogen-species-specific feature of FCR resistance and the existence of loci with major effects should facilitate breeding and exploitation of resistant barley cultivars.

The existence of major loci for FCR resistance should also facilitate their further characterization based on fine mapping and map-based gene cloning. The capacity of such efforts has been dramatically enhanced recently with the release of a physical, genetic and functional sequence assembly of barley (The International Barley Genome Sequencing Consortium 2012). Considering the highly conserved synteny between the diploid barley and polyploid bread wheat (Mayer et al. 2011; Hernandez et al. 2012), fine mapping and cloning FCR resistance genes in the former should also enhance abilities of similar efforts in the latter.

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