Theor Appl Genet (2015) 128:377–385
DOI 10.1007/s00122-014-2437-1

ORIGINAL PAPER

Markers for seedling and adult plant crown rot resistance in four partially resistant bread wheat sources

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Received: 31 August 2014 / Accepted: 21 November 2014 / Published online: 4 December 2014

Abstract

Key message **QTL identified for seedling and adult plant crown rot resistance in four partially resistant hexaploid wheat sources. PCR-based markers identified for use in marker-assisted selection.**

Abstract Crown rot, caused by *Fusarium pseudograminearum*, is an important disease of wheat in many wheat-growing regions globally. Complete resistance to infection by *F. pseudograminearum* has not been observed in a wheat host, but germplasm with partial resistance to this pathogen has been identified. The partially resistant wheat hexaploid germplasm sources 2-49, Sunco, IRN497 and CPI133817 were investigated in both seedling and adult plant field trials to identify markers associated with the resistance which could be used in marker-assisted selection programs. Thirteen different quantitative trait loci (QTL) conditioning crown rot resistance were identified in the four different sources. Some QTL were only observed in seedling trials whereas others appeared to be adult plant specific. For example while the QTL on chromosomes 1AS, 1BS, and 4BS contributed by 2-49 and on 2BS contributed by Sunco were detected in both seedling and field trials, the QTL on 1DL present in 2-49 and the QTL on 3BL in IRN497 were only detected in seedling trials. Genetic correlations between field trials of the same population were strong, as were correlations between seedling trials of the same population. Low to moderate correlations were observed between seedling and field trials. Flanking markers, most of which are less than 10 cM apart, have now been identified for each of the regions associated with crown rot resistance.

Communicated by Xianchun Xia.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2437-1) contains supplementary material, which is available to authorized users.

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Introduction

In Australia and elsewhere, stubble-borne diseases have become important constraints to winter cereal production, particularly in farming systems which incorporate minimum tillage practices (Backhouse et al. 2004; Smiley et al. 2005). Under Australian conditions, crown rot, primarily due to infection by *Fusarium pseudograminearum*, is one of the most significant of these stubble-borne diseases. Stubble management is critical for the control of crown rot because the incidence and severity of infection is directly related to inoculum buildup and persistence, particularly in unincorporated straw which survives the summer fallow (Burgess et al. 2001).

Complete resistance to infection by *F. pseudograminearum* has not been observed in a wheat host. However, potentially useful differences in resistance have been

demonstrated in a number of studies (Li et al. 2010; Wallwork et al. 2004), and some of the germplasm identified has the potential to reduce yield losses caused by crown rot. Unfortunately, assessment of suitable material is time consuming and expensive (Percy et al. 2012; Poole et al. 2012). Furthermore, as the expression of disease severity is strongly dependent on levels of in-crop rainfall and the degree of moisture stress late in the growing season, variation between years is problematic (Dodman and Wildermuth 1987).

As a result of the environmental variability and the labour costs of phenotyping large field trials, methods that involve the inoculation and evaluation of seedlings, with the anticipation that the results of such assays are directly applicable to the field situation, have been developed. For example, Wildermuth and McNamara (1994) developed a seedling test for assessing resistance to crown rot by examining parameters such as temperature and inoculation method. They found that growing the seedlings at 25 °C minimised the time needed for assessment of seedling response, and that a banded inoculum increased the likelihood of contact with an emerging seedling and closely modelled the field situation. This test depends on scoring the level of leaf sheath discoloration (tissue browning) and can be completed in a 3-week period. A relatively high correlation ($r = 0.6$) was shown between seedling reaction and field reaction within the 28 genotypes examined (Wildermuth and McNamara 1994). Recent seedling studies based on quantitative PCR of fungal DNA (Knight et al. 2012), fungal re-isolation studies (Percy et al. 2012) and fluorescence microscopy of fungal invasion of wheat (Knight and Sutherland 2013) indicate close correspondence between the presence of the fungus and visible tissue browning.

Increasing the genetic resistance of commercial wheat varieties, in conjunction with the adoption of appropriate crop management practices, is seen as critical to the development of effective control measures for this economically important disease. Conventional breeding to increase resistance has proven particularly difficult due to the multigenic and quantitative nature of the partial resistances currently available, the significant genotype/environment interactions and the labour costs of accurately phenotyping field trials (Percy et al. 2012; Poole et al. 2012). Consequently there has been a concerted effort to identify molecular markers closely linked to quantitative trait loci (QTL) contributing to disease resistance (Bovill et al. 2006, 2010; Collard et al. 2005, 2006; Li et al. 2010; Ma et al. 2010; Poole et al. 2012; Wallwork et al. 2004). Such markers can be used by breeding programs to increase the probability of having resistance selected in resistant germplasm.

Our group has previously published results on the identification of QTL linked to partial crown rot resistance in wheat sources 2-49, Sunco and W21MMT70 (Bovill et al. 2006, 2010; Collard et al. 2005). These studies were based upon phenotypic data obtained from seedling trials. Since this work was published additional markers have been added to the genetic maps, particularly in previously identified QTL regions, with the result that more closely linked flanking markers have been identified. Hence these seedling trials have been re-analysed. To use markers for marker-assisted selection in breeding programs we also need to identify markers for QTL expressed under field conditions. In this study we report on crown rot resistance QTL identified in field trials with the wheat sources, Sunco, 2-49, IRN497 and CPI133814 and compare the performance of QTL detected in seedling trials with their contribution to resistance in adult plants under field conditions.

Materials and methods

Plant material

Table 1 lists the doubled haploid (DH) populations used in this study. Line 2-49 (Gala/Gluyas Early) is a hexaploid white spring wheat with partial seedling and field resistance and is considered as one of the most robust sources of crown rot resistance identified to date. Sunco (Cook3*/3/WW15//4*SUN9E-27/3Ag14) is an Australian commercial spring wheat variety which has a useful level of adult field resistance to crown rot but is moderately susceptible to crown rot infection in seedling trials (Wildermuth and Morgan 2004; Wildermuth et al. 2001). IRN497 with pedigree ND137/Conley is a line from the 1965 International Rust Nursery and has partial seedling and adult plant resistance. ND137 has the pedigree ND81/Lee while the pedigree for Conley is Thatcher/4/McMurachy/Exchange/3/2*Redman/5/Lee. CPI133814 is a synthetic hexaploid wheat with partial seedling and adult field resistance. CPI133814 is a cross between CIMMYT durum line No.7 and *Aegilops tauschii* parent AUS-24201 alias KU2157 from Iran. The Australian cultivar Janz (3Ag3/4*Condor//Cook) is susceptible to crown rot in seedling and field trials.

Table 1 Details of DH populations and genetic linkage maps used in this study

Population	Number of lines	Total no. of markers	Average distance between markers (cM)	Total map length (cM)
2-49/Janz	153	158	11.0	1,749.4
Sunco/2-49	134	521	3.4	1,770.2
IRN497/Janz	122	879	4.1	3,622.0
CPI133814/Janz	110	598	5.3	3,147.1

Table 2 Descriptive statistics for crown rot seedling (SDL) and field (FLD) trials for the different DH populations used in this study

DH population	Trial	♀ score	♂ score	Mean	Min	Max	Std error	Skewness	*H*
2-49/Janz									
SDL	2001[a]	39.3	68.1	50.8	18.0	91.8	1.1	−0.8	0.83
	2006	38.3	51.3	40.8	4.2	87.8	1.3	0.2	0.63
FLD	2004	19.3	67.0	49.5	24.2	91.0	1.5	0.8	0.80
	2005	38.1	64.6	50.4	24.0	97.5	1.1	0.8	0.76
	2006	45.7	99.7	61.5	37.0	91.7	0.9	0.4	0.80
Sunco/2-49									
SDL	2004[a]	57.2	40.3	52.0	20.5	78.0	0.9	−0.2	0.73
	2007[a]	88.4	26.7	71.0	18.3	141.3	1.7	0.2	0.60
	2008[a]	68.9	40.6	57.0	32.0	79.5	0.9	−0.0	0.71
	2009	68.2	46.9	53.9	25.5	75.4	1.1	−0.6	0.73
FLD	2009	74.9	51.7	65.5	35.5	98.5	1.4	0.3	0.76
	2010	116.1	87.5	95.7	67.1	152.1	1.6	1.0	0.83
IRN497/Janz									
SDL	2003	42.3	74.4	64.2	26.7	100.2	1.4	−0.1	0.80
FLD	2006	43.4	70.9	65.7	39.1	115.8	1.2	0.8	0.72
	2007	27.0	56.1	40.0	24.0	73.9	0.8	0.9	0.76
CPI133814/Janz									
SDL	2007	52.8	115.5	47.8	0	131.4	2.2	0.5	0.56
	2008	77.3	84.4	73.6	45.1	114.8	1.2	0.3	0.57
FLD	2011	89.4	104.2	86.2	64.9	110.5	0.9	0.0	0.63
	2012	66.5	76.3	40.9	18.1	68.6	0.9	0.4	0.86

The crown rot disease severity scores for the female (♀ score) and male (♂ score) parent of each cross are listed together with the minimum (Min), maximum (Max), standard error (Std error), skewness and heritability (*H*) values. Disease severity is expressed as a percentage of the susceptible check cultivar Puseas

[a] Data previously published (Bovill et al. 2010; Collard et al. 2005)

Crown rot seedling trial

Seedling data collected for the various populations are listed in Table 2. Populations were phenotyped as per the method of Wildermuth and McNamara (1994), as described in more detail by Bovill et al. (2010) and Percy et al. (2012). Entries were replicated in a randomised block design. The susceptible check cultivar Puseas was included in each trial.

Crown rot field trials

At least two field trials were conducted on each DH population at the Wellcamp Research Station in southern Qld (Table 2). From 2004 to 2007 each inoculated trial was planted in replicated, randomised block design in June according to moisture availability as per methods described by Wildermuth et al. (2001). From 2009 to 2012 field experiments were planted using a Hege 90 cassette system to deliver 5 g of seed per 3 m row with colonised millet inoculum delivered into the furrow above the seed via a Micro Band Granular Applicator. Inoculum preparation was the same as described in Wildermuth et al. (2001). Each year inoculum consisted of a mixture of up to six aggressive isolates of *F. pseudograminearum* and was delivered at a rate of 2.2 g/m. Isolates were selected after being tested for aggressiveness in a seedling trial using a range of genotypes. Plots were hand harvested at maturity.

Phenotypic data analysis

Data from the seedling trials are based on a score out of 12, where three leaf sheaths are rated using the 0–4 scale and summed (Wildermuth and McNamara 1994). All plants in a plot are collected from the field and each bundle is stored at room temperature until rating. Individual plants are separated and up to 35 plants are randomly sampled from each bundle. Individual tillers were assessed by rating the degree of discolouration on the stem internodes and number of white heads produced, using the scale: 0 = no lesions, 1 = first tiller internode partly lesioned, 2 = first internode fully or first and second internode partially lesioned, 3 = greater than two internodes lesioned, and 4 = dead head due to crown rot. Field trial data were based on percent disease severity. Disease severity is calculated from the number of tillers categorised into each level of the rating scale using the formula below:

$$\left(\frac{1.5 \times \text{no. tillers in category 1} + 3.5 \times \text{no. tillers in category 2} + 6 \times \text{no. tillers in category 3} + 9 \times \text{no. tillers in category 4}}{\text{Total no. tillers} \times 9}\right) \times 100$$

The weighting factors were added to reflect the increase in yield loss associated with severe stem browning and white head formation. Data from each line were indexed with respect to the highly susceptible cultivar Puseas, and converted to a % Puseas scale.

A separate multi-environment trial (MET) analysis was conducted on the disease severity data for each of the four populations. The MET analysis uses a linear mixed model which partitions the data into three main parts for each trial (environment): the genetic effects; the experimental design effects; and extraneous error. Genotypes were fitted as random effects to estimate the variance–covariance of the genetic effects within and between trials. For the seedling trials in any 1 year each population was assessed across several smaller more manageable internally replicated experiments. As a result there were only three genotypes in common between seedling trials within each of the 2-49/Janz 2001, Sunco/2-49 2004 and IRN497/Janz 2003 experiments. Combining across the seedling trials within these years improved the genotype concurrence; therefore, the estimation of genetic effects was modelled across years rather than individual trials. The modelling of the experimental design effects and extraneous error, however, was applied to each trial. A term for replicate was included to account for the randomised complete block structure of the trials. In addition, spatial variation was modelled through a separable autoregressive structure for the rows and columns of each trial. Any extraneous field trend due to rows and columns was also included, following the method of Gilmour et al. (1997).

The genetic variance–covariance of the IRN497/Janz and the CPI133814/Janz data sets were estimated using an unstructured variance structure. For the 2-49/Janz and Sunco/2-49 data sets the factor analytic (FA) approach (Smith et al. 2001) provided the most parsimonious variance structure using two and three factor loadings, respectively. In addition to genetic correlations, broad sense heritabilities were calculated from these models using the method described in Cullis et al. (2006). The residual maximum likelihood (REML) (Patterson and Thompson 1971) algorithm was used to provide estimates of the variance components. Data were analysed with ASReml-R (Butler et al. 2009) using R software (R Development CoreTeam 2013).

Genetic mapping and QTL analysis

Genetic map construction of the 2-49/Janz and Sunco/2-49 populations has been previously described (Bovill et al. 2010; Collard et al. 2005). Primer sequences for additional microsatellite markers were obtained from GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) and a standard protocol was used to amplify the markers. The reaction mixture consisted of 20 ng DNA, 5 μM of each primer, 100 μM of each dNTP, 1.5 mM $MgCl_2$, 1 × buffer (Bioline Pty Ltd., Australia) and 0.1 U Immolase™ DNA polymerase (Bioline Pty Ltd., Australia) in a total volume of 10 μl. The following polymerase chain reaction (PCR) cycle profile was used: 7 min at 95 °C, followed by 35 cycles at 94 °C for 30 s, 50–60 °C (depending on annealing temperature) for 30 s and 72 °C for 30 s and one cycle at 72 °C for 10 min. The amplified products were visualised using a Gel-Scan 2000™ (Corbett Life Sciences, Sydney, Australia). Both the IRN497/Janz and CPI133814/Janz linkage maps consist of microsatellite and DArT (Diversity Arrays Technology; http://www.diversityarrays.com) markers and were constructed using the method described in Bovill et al. (2010). The number of markers present for each linkage map is given in Table 1. A number of DArT markers were successfully converted to polymerase chain reaction (PCR)-based markers and the abbreviation "PCR" was added to the name of the original DArT marker to indicate this. Sequences for the DArT markers were obtained from DArT Pty Ltd, Canberra (http://www.diversityarrays.com/). Primer3 version 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/input.htm) was used to design primers for the DArT markers (Table 4). The PCR-based DArT markers were amplified using a standard PCR as described above using an annealing temperature of 55 °C.

IciMapping v 3.2 (http://www.isbreeding.net) was used to identify QTL for seedling and field resistance to crown rot. Composite interval mapping was conducted using 1 cM steps and a probability in stepwise regression of 0.001. Permutation tests of 1,000 were used to identify significant QTL. MapChart Map version 2.1 (Voorrips 2002) was used to produce figures.

Results

Phenotypic analysis

Descriptive statistics for each trial are presented in Table 2. Parental scores varied across trials but in general 2-49 had the lowest crown rot scores followed by IRN497 in both seedling and field trials. The 2-49/Sunco population exhibited high crown rot scores in the 2010 field season due to extreme weather conditions with very high rainfall (a total of 766 mm during the season compared to an average of 350 mm in other years (Bureau of Meteorology, Australian Government, 2014).

For each trial, progeny with crown rot scores lower and higher than the parental scores were observed indicating transgressive segregation. Heritability estimates were above 0.6 for most trials with the exception of the

Table 3 Correlation values between all measured phenotypes of the 2-49/Janz (A), Sunco/2-49 (B), IRN497/Janz (C) and CPI133814/Janz (D) populations

		SDL 2006	FLD 2004	FLD 2005	FLD 2006
(*A*)					
SDL	2001	0.87	0.48	0.43	0.54
SDL	2006		0.57	0.51	0.63
FLD	2004			0.71	0.98
FLD	2005				0.72

		SDL 2007	SDL 2008	SDL 2009	FLD 2009	FLD 2010
(*B*)						
SDL	2004	0.76	0.57	0.70	0.59	0.24
SDL	2007		0.76	0.81	0.57	0.34
SDL	2008			0.98	0.41	0.29
SDL	2009				0.42	0.20
FLD	2009					0.77

		FLD 2006	FLD 2007
(*C*)			
SDL	2003	0.39	0.45
FLD	2006		0.80

		SDL 2008	FLD 2011	FLD 2012
(*D*)				
SDL	2007	0.91	0.17	0.23
SDL	2008		0.40	0.41
FLD	2011			0.72

seedling trials conducted on the CPI133814/Janz population (Table 2). Correlations were conducted between all trials of a population (Table 3). Moderate to strong correlations were observed between seedling trials ranging from 0.57 to 0.98 whereas strong correlations were observed between field trials ranging from 0.71 to 0.98. Correlations between seedling and field trials were low to moderate for most of the populations with the highest correlations observed in the 2-49/Janz population ranging from 0.43 to 0.63.

Genetic analysis

Four different populations were used in the crown rot resistance QTL analyses. Linkage map details for these populations are presented in Table 1. In two of these populations the partially resistant line 2-49 was one of the parents. The other three sources of crown rot resistance, Sunco, IRN497 and CPI133814, were only present in one population each. Total map distance ranged from 1,749 to 3,622 with the average distance between adjacent markers ranging from 3.4 to 11.0 cM.

DArT fragment sequences were available for some of the DArT markers and these were obtained from DArT Pty Ltd for 20 DArT markers previously mapped to regions linked to crown rot resistance QTL. Primers were designed for these markers and 9 out of the 20 markers were polymorphic in at least one of the crown rot populations (Table 4). Seven out of the nine polymorphic markers mapped to the expected region with the exception of wPt-4808 and wPt-3030 (Table 4). Six of the PCR-based DArT markers were dominant with only three being co-dominant.

QTL results for all populations and trials are summarised in Table 5 and Supplementary Figure 1. QTL were listed if (1) they had a significant LOD score of 2.8 or greater; (2) the LOD score was only suggestive but the QTL occurred in more than one trial involving the same population; (3) LOD score was only suggestive but the QTL was located in the same region as a QTL located in another population. For the 2-49 resistance, five QTL were detected on chromosomes 1AS, 1BS, 1DL, 3BS and 4BS of which four, the QTL on chromosomes 1AS, 1BS, 1DL and 4BS had significant LOD scores in both the Sunco/2-49 and 2-49/Janz populations. For the 2-49 resistance most QTL were detected in both seedling and field trials with the exception of QTL 1DL which was only detected in seedling trials. The QTL on 1BS was only detected in field trials in the 2-49/Janz population but was only significant in one seedling trial in the Sunco/2-49 population.

Significant QTL were detected on chromosomes 2BS and 2DS for the Sunco resistance in the Sunco/2-49 population. The 2BS QTL was significant in one seedling (2009) and one field (2009) trial, whereas the 2DS QTL was only detected in one field trial (2009). A QTL in the same region as the Sunco QTL on 2BS was also detected in the 2-49/Janz population contributed by Janz. This QTL, however, was not observed in the other Janz populations (IRN497/Janz and CPI133814/Janz).

From the IRN497/Janz population, a significant seedling QTL was identified on chromosome 3BL (LOD score 5.8) and two adult plant QTL were identified on chromosomes 2AL and 4BS. A QTL on 6DL that was suggestive in one seedling and two field trials was also identified.

A QTL in the same region as the significant 3BL QTL detected in the IRN497 parent was also observed in CPI133814, however, the LOD score was only significant for one (2008) of the two seedling trials in this population. Another significant seedling QTL was detected on chromosome 3AL contributed by parent CPI133814. A QTL on 6DL contributed by CPI133814 was significant in one field trial and this QTL was in a similar location to the QTL identified in IRN497. Furthermore a field QTL contributed

Table 4 Primer sequences for DArT markers converted to PCR-based markers and mapped in crown rot populations 2-49/Janz, Sunco/2-49, IRN497/Janz and CPI133814/Janz

Chromosome	Marker name	Primer sequence (5′-F; 5′-R)	Size (bp)	Polymorphism in population	Marker type
1D	wPt-9380PCR	GTAATGAAGGGCCCAACTGA; CTCAACTTTCGTGCCATCAA	206	Sunco/2-49	Co-dominant
2B	wPt-3561PCR	AGGCACGTGCGTTCTACTTT; CGTGAGGAATGCAGAGACAA	214	Sunco/2-49	Dominant (2-49)
2B	wPt-1992PCR	CAGTGCCAAACACTCCCTTT; CCAATTTCCAAATCCCCTTT	158	Sunco/2-49	Dominant (2-49)
3B	wPt-9310PCR	TGAAATGGCATCAGGGAGAT; TGAGCCAAAGAACAATGTCG	243	Sunco/2-49	Dominant (2-49)
3B	wPt-0365PCR	GCAAAGTGGGATCCTCAAGA; AGGGCTTGAACAGAATCACC	151	IRN497/Janz	Dominant (IRN497)
3B[a]	wPt-4808PCR	GTTCACCACAAACGACAAGC; TGCAGATAGGGTGTGTCAGG	155	CPI133814/Janz IRN497/Janz	Dominant (CPI133814, IRN497)
3B	wPt-0021PCR	ATTGCCTCGTGTGAGCTTCT; TGGTCATCAGAGCCTCTTCC	196	CPI133814/Janz	Co-dominant
4B	wPt-7569PCR	ATGCAACCAGCATCATCAAA; GCGAGTTCATACGGATTGGT	211	Sunco/2-49 2-49/Janz	Dominant (2-49)
5B[b]	wPt-3030PCR	GCGAGCAGGGAGTCTAATTG; GTCCCTCCAACGGTAGATGA	206	Sunco/2-49	Co-dominant

The parent in brackets behind the marker type indicates the parent in which the fragment amplifies

[a] PCR-converted DArT marker mapped to chromosome 3A

[b] PCR-converted DArT marker mapped to chromosome 4B

by Janz was identified on 2DS in the same region as the QTL contributed by Sunco.

Flanking markers for all QTL are listed in Table 5 and most of these have a distance of <10 cM between flanking markers. Flanking markers for the 4BS QTL of the IRN497/Janz population are furthest distance apart at 25.0 cM. Even though a large number of SSR markers were tested, markers polymorphic in the two parents could not be identified for this region. The flanking markers listed are all PCR-based markers which are easily amplified and visualised without the need for sophisticated equipment.

Discussion

This study reports QTL for crown rot resistance identified in seedling and field trials involving populations derived from the partially resistant lines 2-49, Sunco, IRN497 and CPI133814. Seedling QTL have previously been published by our group for a replicated seedling trial of the 2-49/Janz population (Collard et al. 2005) and three seedling trials of the Sunco/2-49 population (Bovill et al. 2010). Following enhancement of the mapping densities around identified QTL, the seedling data sets for these two populations have been included to allow comparisons with the more recently conducted field trials reported in this study for the first time. As the Sunco/2-49 map consisted mainly of DArT markers, PCR-based flanking markers were not available for most of the QTL regions. Since that study, 60 microsatellite markers have been added to the Sunco/2-49 map and a selection of DArT markers for which sequences were available were successfully converted to PCR-based markers. This resulted in the identification of PCR-based flanking markers for all QTL regions with the distance between most pairs of flanking markers being less than 10 cM.

Similar to other studies (Bovill et al. 2006, 2010; Collard et al. 2005; Li et al. 2010; Poole et al. 2012), this study has also observed transgressive segregation in all populations confirming that both parents contribute to resistance in the progeny. Strong correlations greater than 0.70 were observed between field trials of the same population, whereas seedling trials varied between 0.57 and 0.98. Correlations between field and seedling trials were generally low to moderate, with the highest correlation observed being 0.63. Seedling trials are suitable as either initial or supplementary tests for rating lines and to exponentially increase material carried forward from one generation to the next while also increasing the proportion of lines with favourable alleles in each generation. However, field trial disease scores are the most likely to best predict field performance.

In this study 13 different crown rot resistance QTL were identified from four different sources. Some QTL were only observed in seedling trials such as the QTL on

Table 5 QTL identified for crown rot seedling (a) and field (b) resistance in four different DH populations

Chr[a]	Trial[b]	LOD[c]	PVE[d]	*P*[e]	Flanking markers[f]	Dist[g]
(*a*)						
2-49/Janz						
1AS	2001	***6.0***	12.8	2	barc148; gwm164	5.0
	2006	1.8	5.2			
1DL	2001	***8.0***	17.4	2	cfd19; wmc216	3.0
	2006	***4.8***	13.6			
2BS	2001	***4.2***	8.8	J	gdm86; cfa2278	5.7
Sunco/2-49						
1AS	2007	1.6	3.3	2	barc148; gwm164	6.0
1BS	2004	1.5	4.4	2	gwm11; cfd65	4.7
	2007	***3.3***	7.2			
1DL	2008	2.5	6.6	2	wmc216; barc162	6.4
	2009	***3.3***	12.6			
2BS	2006	2.0	6.1	S	gwm630; cfa2278	0[#]
	2007	***3.2***	7.3			
3BS	2009	1.8	6.1	2	gwm131; wPt-9310PCR	8.0
4BS	2007	***3.5***	7.5	2	wpt-7569PCR; wmc467	
	2008	***7.1***	20.4			
	2009	***2.6***	9.9			
IRN497/Janz						
3BL	2003	***5.8***	18.8	I	wmc236; wPt-0365PCR	13.0
6DL	2003	2.6	7.8	I	cfd188; cfd47	6.2
CPI133814/Janz						
3AL	2007	1.8	6.6	C	cfa2134; cfa2262	3.0
	2008	***3.2***	11.9			
3BL	2007	1.5	6.9	C	wPt-0021PCR; gwm299	12.2
	2008	***3.4***	12.1			
(*b*)						
2-49/Janz						
1AS	2004	***3.2***	16.4	2	barc148; gwm164	5.0
	2006	***5.1***	11.3			
1BS	2004	1.8	6.9	2		
	2005	***5.0***	12.6		cfd65; gwm11	1.7
	2006	2.5	5.2			
4BS	2004	***3.1***	13.8	2	wmc467; gwm165	4.2
	2005	***4.4***	11.0			
	2006	***8.2***	18.3			
Sunco/2-49						
1AS	2009	***3.4***	8.5	2	barc148; gwm164	6.0
	2010	1.6	7.2		gwm630; cfa2278	0[#]
2BS	2009	***4.7***	12.2	S		
	2010	2.0	8.5			
2DS	2009	***3.4***	8.6	S	gwm484; gwm102	13.0
3BS	2009	***4.2***	11.1	2	gwm131; wPt-9310PCR	8.0
4BS	2009	1.8	4.0	2	wPt-7569PCR; wmc657	9.5
	2010	2.5	11.8			
IRN497/Janz						
2AL	2006	***5.6***	19.7	I	gwm95; cfa2043	5.0
4BS	2007	***2.8***	8.6	I	barc193; wmc349	25.0
6DL	2006	2.3	7.1	I	cfd188; cfd47	6.2
	2007	1.8	5.1			
CPI133814/Janz						
2DS	2011	1.7	6.0	J	gwm484; gwm102	8.6
	2012	***3.1***	12.1			
6DL	2011	***4.6***	18.6	C	barc196; barc273	6.2

Significant LOD scores are indicated in italic and bold

[a] Chromosome arm location of the QTL; [b] Year in which the trial was conducted; [c] Logarithm (base 10) of odds; [d] Percent phenotypic variance explained; [e] Parent contributing the favourable allele; [f] Markers flanking the QTL region; [g] Distance between flanking markers; [#] QTL located on *Triticum timopheevi* introgression

1DL and 3BL in 2-49 and IRN497, respectively, whereas other QTL seem to be adult plant specific such as the 2DS and 2AL QTL identified in Sunco and IRN497, respectively. The variation in effectiveness of some QTL with the stage of plant development explains the lower correlation between seedling and field screens compared to the correlation between trials of these host populations at the same stage of development. The variable nature of this disease was evident in the inconsistent results for minor QTL detected across different trials. However, a number of QTL, including 1AS, 1BS, 1DL and 4BS contributed by 2-49 and 2BS contributed by Sunco were regularly detected in a number of trials and, in the case of the 2-49 resistance, in two different populations. Furthermore the seedling QTL on chromosome 1DL contributed by 2-49 has been previously identified in a 2-49/W21MMT70 population (Bovill et al. 2010).

A resistance locus on chromosome 4B approximately 20 cM away from the height gene *Rht-B1b* (Supplementary Figure 1, 4B_2-49/Janz) has been reported in a number of other studies (Collard et al. 2005; Li et al. 2010; Poole et al. 2012; Wallwork et al. 2004). Collard et al. (2005) reported that taller plants in the 2-49/Janz population were more resistant and considered that the linkage in repulsion between *Rht-B1b* and crown rot resistance could be broken. Other studies did not find an association between plant height and crown rot resistance (Poole et al. 2012) or found that plant height negatively correlated with crown rot resistance, with shorter plants giving the same or better crown rot resistance (Li et al. 2010).

A major seedling QTL for crown rot resistance has been identified on the long arm of chromosome 3B in four

other studies. Ma et al. (2010) identified this QTL in line CSCR6 in which it explained up to 48.8 % of the phenotypic variance in seedlings and was flanked by markers wPt-10505 and wPt-2277 and Li et al. (2010) identified the same seedling QTL in variety Ernie in which it explained up to 34.6 % of the phenotypic variance, flanked by markers wmc471 and wPt-1834. Marker wPt-1834 is also at the peak of the 3BL QTL identified in IRN497 in our study. This QTL was also the main source of resistance identified by Poole et al. (2012). In their study the QTL was contributed by lines Macon and Otis and marker gwm299 was located 1.2 cM away from the peak of the QTL. Up to 36 % of the phenotypic variance was explained by this QTL in the seedling trials. In their study the 3BL QTL was also observed in field trials but it was not significant. Marker gwm299 is one of the flanking markers for the QTL on 3BL identified in our study and the other flanking marker wPt-0365 was also flanking the QTL in the Poole et al. (2012) study. Furthermore this QTL was identified in the same location in line W21MMT70, a Western Australian breeding line with pedigree HPSP#7/2*Cranbrook, in which it explained up to 40.5 % of the phenotypic variance (Bovill et al. 2010). The QTL on 3BL identified in CPI133817 was also in the same location but was only significant in one of the two seedling trials. Despite being identified in numerous studies and inherited from seemingly diverse germplasm, this locus was not linked to disease resistance in field trials in the current study, and, therefore, the value of this QTL for crown rot resistance in adult plants needs to be further evaluated.

A significant QTL contributed by Sunco was identified on chromosome 2BS in both seedling and field trials. This QTL is located on a *Triticum timophevii* introgression, which carries the stem rust-resistance gene *Sr36* and has also been associated with crown rot resistance in Mendos which similarly carries this introgression (Bovill et al. 2006). Poole et al. (2012) included Sunco as a parent in two mapping populations and also identified a QTL on chromosome 2B.

Two significant QTL were identified in the susceptible parent Janz, located on chromosomes 2BS and 2DS. The 2DS QTL, detected in field trials of CPI133814/Janz was in the same region as the QTL contributed by Sunco in the Sunco/2-49 population which was also only observed in field trials. A QTL on 6DL identified in both seedling and field trials in the IRN497/Janz population was also detected in a similar location in the CPI133814/Janz population.

This study summarises genomic regions associated with *F. pseudograminearum*-crown rot resistance in seedling and adult wheat plants. Results confirm that this is a quantitative trait involving mostly minor genes whose expression can be strongly influenced by genotype by environment interactions. However, several QTL are consistently identified in multiple trials, in several populations and from different sources of crown rot resistance. Useful and closely linked PCR-based markers are now available for these loci and these can be trialled as tools for marker-assisted selection in commercial breeding programs.

Author contribution statement AM and WDB wrote the manuscript and performed the QTL mapping analyses. CDP and DH conducted and phenotyped the seedling and field trials. SF and AK provided the statistical support for the trial designs and phenotypic data analyses. SMN and MWS were involved in the initiation of the study and contributed to the manuscript.

Acknowledgments This research was funded by the Grains Research and Development Corporation.

Conflict of interest The authors declare that we have no conflict of interest.

Ethical standards The authors declare that all experiments complied with the ethical standards in the country in which they were performed.

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