

# QTL mapping of multiple disease resistance traits in a synthetic hexaploid x bread wheat population

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## INTRODUCTION

The most cost effective and environmentally safe means by which wheat diseases can be controlled is through the use of genetic resistance in commercially grown cultivars. The identification and genetic characterisation of new sources of disease resistance and their transfer to the adapted genetic backgrounds is of great importance for breeding for disease resistance. The development of molecular markers closely linked to resistance genes could enable genes to be effectively pyramided. Synthetic hexaploid wheat lines offer diverse sources of unique alleles for wheat improvement contributed by cultivated tetraploid and wild diploid relatives of common bread wheat.

QTLs for resistance to root-lesion nematodes (RLN) were previously mapped in the synthetic hexaploid x bread wheat doubled haploid (DH) population CPI133872/Janz using a framework map of SSR and AFLP markers<sup>1</sup>. Here we report the integration of DArT markers into the genetic linkage map of CPI133872/Janz and QTL analysis of additional disease resistance traits of economic importance. In total, six disease resistance traits (two species of root-lesion nematode, *Pratylenchus thornei* and *P. neglectus*), yellow leaf spot or tan spot (*Pyrenophora tritici-repentis*), stripe rust (*Puccinia striiformis* f.sp. *tritici*), leaf rust (*Puccinia triticina*) and stem rust (*Puccinia graminis* f.sp. *tritici*) were phenotyped in this population over multiple years and/or locations. The relationship of QTLs with previously characterised resistance genes is discussed.

## MATERIALS AND METHODS

### Plant material

An F1-derived doubled haploid (DH) population, consisting of 111 lines, was created from a cross between wheat accession CPI133872 and the Australian wheat cultivar Janz using wheat-by-maize hybridisation. CPI133872, a synthetic hexaploid created from a cross between *Triticum turgidum* var. *durum*, accession CPI133821, and *Aegilops tauschii* var. *typica*, accession AUS24199, possesses a high level of resistance to root-lesion nematodes (*P. thornei* and *P. neglectus*) and yellow leaf spot, and is highly susceptible to leaf rust. Janz, a highly adapted and good quality bread wheat, is highly susceptible to root-lesion nematodes and yellow

leaf spot and is known to carry resistance to stripe rust, leaf rust and stem rust.

### Disease resistance testing

#### Root-lesion nematodes

The DH population was evaluated in replicated glasshouse trials for resistance to *P. thornei* (Pt2001, Pt2002) and *P. neglectus* (Pn2001, Pn2002) as described previously<sup>1</sup>.

#### Yellow leaf spot

Resistance reaction to an Australian isolate of *P. tritici-repentis* (accession no. BRIP 28204 a) was evaluated in seedling spray-inoculation tests conducted in pots (YS2001) or using a hydroponic growth method (YS2004 and YS2007) developed at the Leslie Research Centre, Toowoomba. The seedlings were rated nine (YS2001) or four (YS2004 and YS2007) days after inoculation using a qualitative scale from 1 (susceptible) to 9 (resistant), to provide a combined chlorosis/necrosis score based on lesion formation.

#### Rust diseases

Phenotyping of the DH population for resistance to all three rust diseases was performed at the University of Sydney Plant Breeding Institute - Cobbitty, following standardised procedures of the Cereal Rust Laboratory. Adult plant rust response variation was evaluated in four field stripe rust trials (YR2003, YR2006\_K, YR2006\_L, YR2007), three leaf rust trials (LR2006\_K, LR2006\_L, YR2007) and two stem rust trials (SR2004, SR2007). Trials were conducted at two locations in 2006: Karalee (K) and Lansdowne (L). Rust severity was evaluated on a scale of 1 (resistant) to 9 (susceptible)<sup>2</sup>. In addition, a seedling trial was conducted using an *Lr24*-avirulent pathotype of leaf rust to score the presence/absence of leaf rust resistance gene *Lr24*.

#### Molecular analysis

Diversity Array Technology (DArT) marker assays were performed by Triticarte Pty. Ltd. (Australia) using an array generated from the *PstI*-*TaqI* complexity reduction method<sup>3</sup>. DArT markers were merged with an existing mapping data set consisting of 183 SSR and AFLP markers<sup>1</sup> to provide additional genome coverage. Based on previous mapping results, additional SSR markers in target QTL regions on chromosome 2BS and 6D were incorporated into the marker data set. SSR markers tightly linked to the tan spot necrosis insensitivity gene,

*tsn1*<sup>4</sup>, listed on the Marker Assisted Selection in Wheat homepage<sup>5</sup> were screened for polymorphism in the DH population. *Xfcp2*, the only marker that revealed polymorphism, was incorporated into the genetic linkage map.

Genetic linkage mapping was performed using JoinMap v. 3.0. Chi-square test was applied to all markers to test for segregation distortion. Linkage groups were initially determined using LOD scores ranging from 3.0 to 10.0. The order of the markers along each chromosome was determined using a stringent LOD linkage threshold of 3.0 and recombination threshold of 0.45. Map distances (cM) were calculated using the Kosambi mapping function.

### QTL analysis

A separate QTL analysis was performed for each trait using MapQTL v. 4.0. Putative QTLs were initially identified using interval mapping. A genome-wide LOD significance threshold ( $P < 0.05$ ) was calculated for each set of phenotypic data using 1000 permutations. The marker closest to each of the QTL peaks was then selected as a cofactor and used in a multiple-QTL model implemented in the MQM mapping procedure of MapQTL. The set of cofactors was adjusted if the most likely position of the QTL differed from that identified in the cofactor selection round and subsequent rounds of MQM mapping were performed. Markers were removed as a cofactor if their LOD value dropped below the significance threshold.

## RESULTS AND DISCUSSION

### Linkage mapping

Among the total 335 DArT marker loci that were scored in the CPI13872/Janz DH population, 242 DArT markers were integrated together with 125 SSR and 17 AFLP markers to form the final genetic linkage map, which covered 1521 cM. In addition, the leaf rust resistance gene *Lr24* was positioned on the genetic linkage map. Seven percent of the markers on the genetic linkage map exhibited significant segregation distortion. DArT markers mapped to all 21 wheat chromosomes, except chromosome 5D. The total number of mapped loci per chromosome ranged from 7 on chromosome 5A to 28 on chromosome 6D. The genetic linkage map had an overall average marker density of 1 marker every 4.0 cM, with an average of 1 DArT marker every 6.3 cM. The largest interval between two consecutive markers was 21 cM on chromosome 3B.

### QTL analysis

Results of multiple QTL mapping for the various phenotypic trials for the disease resistance traits are shown in Table 1. A genome-wide LOD significance threshold of 3.1 was applied for all traits. However, QTLs with a LOD < 3.1 are reported when they were detected consistently in the same position across different experiments. Comparison of common markers with the wheat consensus map<sup>6</sup> and the literature

revealed that some of the detected QTLs are likely to co-localize with known resistance genes, while others appear to be new resistance loci.

### Resistance to root-lesion nematodes

Stable QTLs for *P. thornei* resistance were detected on chromosomes 2BS, 6DS and 6DL, and stable QTLs for *P. neglectus* resistance were detected on 2BS and 6DS. Implementation of the multiple-QTL model revealed that the location of the QTLs for both species of RLN were different on 2BS, while those on 6DS were coincident. Increasing the marker density through the addition of DArT markers has allowed a more accurate estimation of the location of RLN QTLs previously identified. Flanking markers were identified for all QTLs associated with RLN resistance within a genetic distance of 10 cM.

### Resistance to yellow leaf spot

The recessive gene conferring insensitivity to necrosis causing toxins produced by *Pyrenophora tritici-repentis*, *tsn1*, was detected as a major QTL peak associated with the marker *Xfcp2* in all three years of phenotypic trials. This QTL on chromosome 5BS explained between 29 to 40% of the phenotypic variation across years. Additional QTLs were detected in two of the three years, or in a single phenotypic trial only. The experimental procedure used to evaluate the reaction to yellow leaf spot varied in subsequent years as methods were improved and this may have contributed to the detection of QTLs in a single year only. All three QTLs detected in the YS2001 trial (located on chromosomes 5BS, 3DS and 3AL) are in regions that are expected to be the same as known genes for insensitivity to toxin necrosis (*tsn1*<sup>4</sup>, *tsn3*<sup>7</sup> and *tsn4*<sup>8</sup> respectively). The QTL on chromosome 2BS, detected in the YS2007 trial, is likely to be the same as a major QTL, *tsc2*<sup>9</sup>, conditioning insensitivity to chlorosis. New QTLs for resistance to yellow leaf spot, all inherited from the synthetic hexaploid parent, were detected on chromosomes 2DL, 4BS and 5AS. These QTLs explained 8.6%, 12.9% and 10.7% of the phenotypic variation, respectively.

### Resistance to rust diseases

Both parents contributed to stripe rust resistance, whereas only QTLs inherited from Janz were detected for leaf rust and stem rust resistance. QTLs for both stripe rust and leaf rust mapped to the same location on 7DS and corresponded to the durable, non-specific adult plant resistance gene combination *Lr34/Yr18*<sup>10</sup>. This QTL was stable over all stripe rust phenotypic trials and two of the three leaf rust trials. *Lr34* was not detected above the significance threshold in 2007 since leaf rust gene *Lr24* located on 3DL was effective in this year. The QTL for stem rust resistance detected on chromosome arm 3DL co-localized with the leaf rust gene *Lr24* and thus corresponds to the linked gene combination *Sr24/Lr24*<sup>10</sup>. Additional QTLs for stripe rust resistance were detected on 1BL and 4BL in three of the four phenotyping trials. Although the 1BL QTL did not account for variation in leaf rust response, this QTL appeared to be located in the *Yr29/Lr46* region<sup>11</sup>. Failure

**Table 1. QTLs detected in each of the phenotypic trials using a multiple-QTL model.**

Disease	Resistance Trait	Chr	Marker interval <sup>a</sup>	LOD score (% variance explained)		Reported QTL/gene	Contributing Parent
<i>P. thornei</i>				Pt2001	Pt2002		
	2BS	wPt-22410 (1.1)	wPt-6706 (1.4) wPt-6311 (4.7) wPt872	4.3 (12.7)	7.9 (21.9)	<i>QRtm.lrc-2B.1</i>	CPI133872
	6DS	<i>Xcfd49</i> (2.4)	<i>Xbarc183</i> (8.4) <i>Xcfd135</i>	6.1 (18.4)	10.1 (26.8)	<i>QRtm.lrc-6D.1</i>	CPI133872
	6DL	<i>Xgdm098</i> (7.3)	<i>Xgdm95010</i> (7.0) <i>Xbarc021</i>	5.1 (14.5)	2.4 (5.5)	<i>QRtm.lrc-6D.2</i>	CPI133872
<i>P. neglectus</i>				Pn2001	Pn2002		
	2BS	wPt-4527 (13.0)	wPt-2410 (1.9) wPt-6706	2.7 (11.2)	6 (16.0)	<i>QRtm.lrc-2B.1</i>	CPI133872
	4BL	<i>Xymc047</i> (3.4)	<i>Xgwm166a</i> (1.6) <i>Xgwm368</i>	ns	5.5 (12.8)	<i>QRtm.lrc-4B.1</i>	CPI133872
	3DS	<i>Xgwm314</i> (2.8)	<i>Xgwm664</i> (1.3) <i>Xgwm002</i>	ns	3.4 (7.5)	<i>QRtm.lrc-3D.1</i>	CPI133872
	4DL	<i>Xymc048</i> (6.3)	wPt-5809	ns	5.3 (13.6)	<i>QRtm.lrc-4D.1</i>	Janz
	6DS	<i>Xbarc183</i> (8.4)	<i>Xcfd135</i>	2.3 (9.4)	3.9 (8.9)	<i>QRtm.lrc-6D.1</i>	Janz
Yellow leaf spot				YS2001	YS2004	YS2007	
	2BS	wPt-5027 (3.0)	<i>Xymc025b</i> (4.7) <i>Xcfd238</i>	ns	ns	4.7 (14.7)	<i>tsc2</i>
	2DL	<i>Xbarc168</i> (2.0)	<i>Xgwm484</i>	ns	4.0 (8.6)	ns	CPI133872
	3AL	wPt-2698 (16.8)	<i>Xymc322b</i>	3.0 (6.5)	ns	ns	<i>tsn4</i>
	3DS	<i>Xgwm114c</i> (1.8)	<i>P45M53d</i> (1.6) <i>Xymc169b</i>	5.8 (12.9)	ns	2.6 (5.4)	<i>tsn3</i>
	4BS	<i>Xymc047</i> (3.4)	<i>Xgwm166a</i>	ns	5.6 (12.0)	ns	CPI133872
	5AS	wPt-1370 (9.5)	<i>Xgdm132b</i> (13.7) <i>Xgwm443</i>	ns	6 (12.9)	4.4 (10.7)	CPI133872
5BS	<i>Xgwm499</i> (11.5)	<i>Xcgp2</i> (11.6) <i>Xgwm408</i>	14 (40.0)	12.9 (33.0)	10 (28.9)	<i>tsn1</i>	
Stripe rust				YR2003	YR2006 K <sup>b</sup>	YR2006 L <sup>b</sup>	YR2007
	1BL	wPt-4532 (15.1)	P37M54c	4.8 (17.1)	3.5 (12.7)	2.8 (10.0)	ns
	1DS	<i>Xymc147</i> (3.2)	P45M53a	3.4 (13.3)	ns	ns	CPI133872
	4BL	<i>Xgwm365</i> (1.0)	<i>Xymc238</i> (2.7) wPt-8543	ns	4.5 (14.3)	5.2 (16.8)	3.8 (9.4)
	7AL	wPt-7214 (8.8)	wPt-4345 (3.2) wPt-4877	ns	ns	ns	4.4 (10.6)
	7DS	wPt-2863 (8.8)	wPt-3727	6.3 (23.9)	4.6 (14.7)	5.9 (19.8)	12.2 (41.6)
						<i>Lr34/Yr18</i>	
						Janz	
Leaf rust				LR2006 K <sup>b</sup>	LR2006 L <sup>b</sup>	LR2007	
	7DS	wPt-2863 (8.8)	wPt-3727	9.3 (39.9)	9.4 (41.1)	2.1 (16.2)	<i>Lr34/Yr18</i>
Stem rust				SR2004	SR2007		
	3DL	<i>Xymc169b</i> (0.9)	Lr24 (0.4) P37M54e	2.3 (11.8)	5.3 (21.7)		<i>Sr24</i>
						Janz	

<sup>a</sup> Distance between markers associated with QTL peak is shown in parenthesis

<sup>b</sup> Stripe and stem rust experiments were conducted in two locations in 2006: K, Karalee and L, Lamsdowne  
ns = non-significant

to detect QTL for leaf rust on 1BL may be attributed to poor expression of *Lr46* in our experiments or alternatively, this stripe rust QTL may be different from *Yr29*. The 4BL stripe rust QTL was detected previously in other mapping populations<sup>11</sup>. QTLs on 1DS and 7AL were detected in a single year only and need confirmation. All QTLs detected in 2006 were consistent across two locations and were confirmed by at least one other phenotypic trial conducted in a different year.

## CONCLUSIONS

The recombinants combining resistance to root-lesion nematode, yellow leaf spot and rust diseases from parents CPI133872 and Janz would serve as donors for improvement of these biotic stresses. Markers flanking the QTL peaks were located within 1 to 15 cM of all major QTL detected and are candidates for tagging these resistance genes in MAS.

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