

Molecular Mapping of Stem Rust Resistance in HD2009/WL711 Recombinant Inbred Line Population

Jagdeep Kaur^{1,2} • Urmil K. Bansal¹ • Renu Khanna³ • Ram G. Saini³ • Harbans S. Bariana^{1*}

¹ University of Sydney Plant Breeding Institute-Cobbitty, Faculty of Agriculture, Food and Natural Resources, PMB11, Camden, NSW 2570, Australia

² Donald Danforth Plant Science Centre, 975 Warson Road, Saint Louis, 63132, Missouri, USA

³ Department of Plant Breeding, Genetics & Biotechnology, Punjab Agricultural University, Ludhiana-141004, India

Corresponding author: * harbansb@camden.usyd.edu.au

ABSTRACT

The Indian wheat cultivar 'HD2009' exhibited moderate level of stem rust resistance both under Indian and Australian conditions since its release in 1976. Ninety two 'HD2009'/'WL711'-derived recombinant inbred lines (RILs) were tested in the field for three years against *Puccinia graminis* f. sp. *tritici*. Genetic analyses indicated the presence of three to four genes for resistance across all sites over three years, except at the Lansdowne site during 2004, where resistance was controlled by two to three genes. A linkage map using 46 SSR and 295 DArT markers was generated and used for molecular mapping of stem rust resistance carried by 'HD2009'. Three consistent QTLs; *QSr.sun-3BS*, *QSr.sun-5DL* and *QSr.sun-7A* were identified. *QSr.sun-3BS* explained 9-15% phenotypic variation and was concluded to be *Sr2*. The most significant QTL, *QSr.sun-5DL*, corresponded to the previously reported location of *Sr30* and explained 20-44% phenotypic variation in adult plant stem rust response. The contribution of *QSr.sun-7A* was almost equal to *QSr.sun-3BS* (7-13%). Additional inconsistent QTLs, *QSr.sun-1D*, *QSr.sun-2B*, *QSr.sun-4B* and *QSr.sun-5B* were also detected. All QTLs, except *QSr.sun-4B*, were contributed by 'HD2009'. *QSr.sun-4B* was contributed by 'WL711'. The physiological trait, pseudo-black chaff (PBC), which is reported to be linked with adult plant stem rust resistance gene *Sr2*, was controlled by QTLs, *QPbc.sun-3BS*, *QPbc.sun-5DL* and *QPbc.sun-7DS*. *QPbc.sun-5DL* and *QPbc.sun-7DS* corresponded to the locations of *Sr30* and *Lr34*, respectively.

Keywords: adult plant resistance, durable resistance, molecular mapping, *Puccinia graminis* f. sp. *tritici*, stem rust, wheat

INTRODUCTION

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), is one of the most important diseases of wheat (*Triticum aestivum* L.) and is found in various wheat growing regions of the world. The historical estimates of losses caused by stem rust in Australia are summarised in Park (2007). The Pgt pathotype TMB (race 15B) caused severe epidemics during 1950-1954 in the USA (Kolmer 2001). The 1973 epidemic of stem rust in south eastern Australia caused severe losses and resulted in the adoption of a national approach to stem rust control. The whole world appeared to have recovered from the menace of stem rust epidemics through the use of rust resistant cultivars by the 1980s. This resulted in the decline of research effort on stem rust resistance in wheat (Singh *et al.* 2006). However, the nationally co-ordinated approach to achieve sustained control of stem rust in Australia continued and resulted in the development of genetically diverse germplasm (Brown 1994; Bariana *et al.* 1996).

Resistance to stem rust can be conferred by both seedling and adult plant resistance (APR) genes. Forty five stem rust resistance genes have been designated in wheat (McIntosh *et al.* 2008). *Sr2* is the only named APR source of stem rust and was transferred from a tetraploid wheat, Yaroslav emmer, into common wheat variety Hope (McFadden 1930). *Sr2* on its own provides a low level of resistance but in combinations with other genes it has provided moderate to high levels of stem rust resistance (Knott 1989; Singh *et al.* 2006; Bariana *et al.* 2007a). The so-called '*Sr2*-complex' has been the back bone of stem rust resistance in wheat germplasm developed at CIMMYT, Mexico.

Seedling resistance genes *Sr5*, *Sr6*, *Sr8a*, *Sr12*, *Sr9g*, *Sr17*, *Sr24*, *Sr30*, *Sr31*, *Sr36*, *Sr38* and APR gene *Sr2* are

commonly detected among hexaploid wheat germplasm. Unfortunately, Pgt pathotypes with virulence against these genes singly or in combinations have been detected in many parts of the world (Pretorius *et al.* 2000; Kolmer *et al.* 2007; Park 2007). The wheat cultivar 'HD2009' was released in India in 1976 and remained resistant to the predominant Pgt pathotypes in Australia (H.S. Bariana unpublished results) and India (Kaushal *et al.* 1982). The present investigation was conducted to understand the genetics of stem rust resistance in 'HD2009' and to determine genomic regions contributing to stem rust resistance through molecular mapping.

MATERIALS AND METHODS

Plant material

Cultivar 'HD2009' (Lerma Rojo 64A/Nainari 60) was crossed with cultivar 'WL711' (S308/Chris//Kalyansona) and a recombinant inbred line (RIL) population of 92 lines was developed by selecting a single head per line at each generation. RILs were bulk harvested at the F₆ generation.

Adult plant stem rust response tests

Ninety two RILs from 'HD2009'/'WL711' population along with parents were sown as 60 cm rows in field at the Plant Breeding Institute (PBI), Cobbitty during the 2004, 2005 and 2006 crop seasons. Each block of 50 rows was surrounded by a susceptible infector row to establish uniform disease development. During the crop season 2004, the RIL population was tested at two sites; Karalee (K) and Lansdowne (L) and in the 2005 and 2006 crop seasons, it was tested only at the Lansdowne site. Experimental area was irrigated to provide favourable conditions for rust deve-

lopment. Urediniospores of Pgt pathotype 98-1,2,(3),5,6 (PBI accession no. 279) suspended in light mineral oil (Shellsol T[®]) were misted over susceptible spreader and experimental rows using an ultra low volume applicator (Microfit[®], Micron Sprayer Ltd.). Inoculations were performed three times. Stem rust susceptible spreader rows served as secondary source of inoculum for epidemic development. Adult plant stem rust response assessments were made on a 1-9 scale described in Bariana *et al.* (2007b).

Pseudo-black chaff

Pseudo-black chaff (PBC) is a dark pigmentation developed around stem internodes and glumes and is completely linked with the durable stem rust resistance gene *Sr2* (Hare and McIntosh 1979). The 'HD2009'/'WL711' RIL population was scored on a 1-5 scale for PBC (1 = no pigmentation, 2 = slight pigmentation, 3 = medium pigmentation, 4 = high pigmentation and 5 = very high pigmentation) at the Richmond and Lansdowne sites during the 2005 and 2006 crop seasons, respectively.

Greenhouse testing

Ten seeds of each RIL and parents were sown in 9 cm plastic pots filled with pine bark and coarse sand (4:1) mixture. Potting mix filled pots were fertilized with Aquasol[®] at 30 g/10 L of water for 200 pots. An additional nitrogen treatment (Nitram[®] at 30 g/10 L of water for 200 pots) was applied to 7-10 days old seedlings. Seedlings were raised in a rust-free microclimate room maintained at 20°C prior to inoculation.

The Pgt pathotype 98-1,2,(3),5,6 was used for greenhouse tests. Seedling stem rust inoculations were performed at the two-leaf stage. Urediniospores suspended in light mineral oil (Shellsol T[®]) were atomized over seedlings using a hydrocarbon propellant pressure pack. Inoculated seedlings were placed under polythene hoods in water-filled steel trays and were incubated under natural light at 18-20°C for 48 h. They were finally moved to temperature and irrigation-controlled microclimate room set at 25°C. Seedling responses were recorded 14 days after inoculation. Infection types were recorded on a 0-4 scale described in McIntosh *et al.* (1995).

Chi-squared analyses

The RIL population was classified into resistant and susceptible groups based on the phenotypic scores. The high response group included RILs that produced stem rust responses equivalent to or higher than the parent 'WL711'. Chi-squared (χ^2) analyses were performed to check the goodness-of-fit of observed segregations with the expected genetic ratios.

Molecular mapping

SSR markers

Genomic DNA from 10 days old seedlings of parents and RILs was isolated using CTAB method described by Doyle and Doyle (1990). A total of 240 SSR markers (Röder *et al.* 1998) were tested on parents 'HD2009' and 'WL711' to identify parental polymorphisms. Polymorphic SSR markers were screened across the 'HD2009'/'WL711' RIL population. A 20 μ L polymerase chain reaction (PCR) mixture contained 50-100 ng genomic DNA, 0.2 mM dNTPs, 0.5 μ M of each primer (forward and reverse), 1.5 mM MgCl₂, 1xPCR buffer and 1U Taq DNA polymerase. Amplifications were performed by using the touch down profile (Don *et al.* 1991). PCR products were separated on 6% (19: 1 acrylamide: bis acrylamide) denatured polyacrylamide gels containing 8 M urea.

Gels were silver-stained using the protocol described by Basam *et al.* (1991).

Diversity array technology (DArT) marker analysis

For DArT assays, 500-1000 ng of restriction grade DNA, suspended in TE at a concentration of 50-100 ng/ μ L of each RIL (n = 92) and the parents ('HD2009' and 'WL711') was sent to Triticarte Pty. Ltd., Canberra, Australia (<http://www.triticarte.com.au>) for whole genome profiling (Wenzl *et al.* 2004; Huttner *et al.* 2006). Two hundred and ninety five loci were scored as present (1) or absent (0). DArT markers names had the prefix 'wPT' and the number corresponding to the particular clone in the genomic representation, where w stands for wheat, P for *Pst*I (primary restriction enzyme) and T for *Taq*I (secondary restriction enzyme).

'HD2009'/'WL711' linkage map construction and QTL mapping

Polymorphic loci (SSR and DArTs) were scored on the RIL population. A partial genetic linkage map was constructed using MapManager QTXb20 (Manly *et al.* 2001). The Kosambi mapping function (Kosambi 1944; Lander *et al.* 1987) was used with the threshold value of P = 0.01. QTL detection was performed using the composite interval mapping (CIM) with QTL Cartographer v2.5 (Wang *et al.* 2006). Map Chart v2.2 (www.biometris.nl) was used to depict QTLs in different linkage groups.

RESULTS

Inheritance

1. Adult stem rust resistance

Adult plant stem rust response of 'HD2009' across sites and years varied from 3 to 4, whereas 'WL711' produced stem rust responses ranging from 7 to 9. Of 92 families tested, 84 were resistant and eight were susceptible at the Karalee site. This segregation conformed to three to four gene model (Table 1). In contrast, an increased number of families showed susceptible responses at the Lansdowne site in 2004. Chi-squared analysis of data from the Lansdowne site indicated the involvement of two to three resistance genes. Stem rust responses during 2005 and 2006 at the Lansdowne site produced results similar to those achieved during 2004 at the Karalee site indicating segregation at three to four loci. The stem rust response variations among the 'HD2009'/'WL711' RIL population during different years and sites are shown in Fig. 1. In the 2004 season, distribution was skewed towards resistance at the Karalee site, whereas it was skewed towards susceptibility at Lansdowne site. The parent 'WL711' was also scored higher at this site. Some RILs displayed stem rust responses beyond the parental scores in all data sets (Fig. 1). Significant to highly significant correlation co-efficient (r = 0.22 to 0.58 at P = 0.05 and 90 d.f.) were observed among different stem rust response data sets.

2. Seedling stem rust resistance

Parents 'HD2009' and 'WL711' produced infection types (IT) 2 and IT3+, respectively when tested against Pgt pathotype 98-1,2,(3),5,6 under greenhouse conditions. Of 92 RILs, 55 produced IT2 to 3 and 37 RILs produced IT3+. Chi-squared analysis of data indicated monogenic inheri-

Table 1 Genetic analyses of adult plant stem rust response variation in HD2009/WL711 RIL population.

Site	Year	Stem rust response			χ^2	№ of genes	
		Resistant	Susceptible	3:1			
Karalee	2004	84	8	-	1.21	0.93	3-4
Lansdowne	2004	77	15	3.71	1.21	-	2-3
Lansdowne	2005*	81	6	-	2.50	0.06	3-4
Lansdowne	2006*	82	7	-	1.74	0.39	3-4

Table value of χ^2 at P = 0.05 and 1 d.f. is 3.84, * 5 and 3 RILs did not germinate during 2005 and 2006, respectively. - denotes significant values

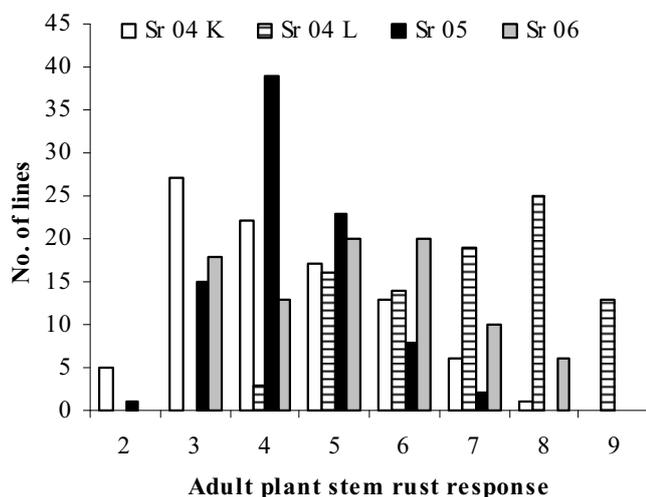


Fig. 1 Stem rust response variation among HD2009/WL711 RILs.

tance of seedling stem rust resistance ($\chi^2 = 3.52$ non-significant at $P = 0.5$ and 1 *d.f.*). This seedling stem rust resistance locus was temporarily designated as *SrHD*.

3. Pseudo-black chaff (PBC)

Parents 'HD2009' and 'WL711' showed PBC score of 3 and 1, respectively. Data were pooled for the families showing scores of 2, 3 and 4 as positive for PBC. No RIL displayed PBC score of 5. Of 87 RILs tested, 70 expressed PBC (at varying levels) in 2005, and in 2006, 69 RILs expressed PBC (Table 2). Seventeen and 18 RILs did not express PBC in 2005 and 2006, respectively. Statistical analysis of data indicated the digenic inheritance of PBC. Some RILs expressed higher PBC pigmentation than the parent 'HD2009' presumably due to phenological differences (Fig. 2).

Linkage map construction

Forty six SSR markers were polymorphic between the parents 'HD2009' and 'WL711', and of these 12 belonged to the A genome, 19 to the B genome and 15 to the D

genome. Two hundred and ninety five DArT markers were polymorphic among parents. Overall, 341 markers were placed into 19 linkage groups. *SrHD* was incorporated into the linkage map and it mapped to the long arm of chromosome 5D, 25.1cM distal to *Xgwm182*. The total length of the map was 1680.5 cM.

QTL analysis

1. Stem rust

The QTL Cartographer-based CIM analysis was performed separately for each of the four data sets scored over three years. Three QTLs with additive effects were detected consistently across all data sets (Table 3). *Qsr.sun-3BS* was located on the short arm of chromosome 3B in the marker interval wPT-8093 and gwm566. It explained 9-15% (R^2) of phenotypic variation across different experiments. Marker gwm389 mapped closest to *Qsr.sun-3BS* (Fig. 3a). The second highly significant QTL, *Qsr.sun-5D*, located on the long arm of chromosome 5D, explained phenotypic variation ranging from 20-44% (Table 3). *Qsr.sun-5DL* peaked at the seedling stem rust resistance gene *SrHD* (Fig. 3b). *Qsr.sun-7A* mapped closer to the DArT marker wPT-4515. This QTL explained 7-13% phenotypic variation over years and across sites (Fig. 3c).

In addition to the consistent QTLs, CIM analysis also detected inconsistent QTLs which varied with the environment. *Qsr.sun-2B* mapped between the markers wPT-3378 and wPT 4892 and was detected at these sites in the 2004 crop season (Fig. 3d). This QTL contributed 8% and 13% towards phenotypic variation at the Karalee and Lansdowne sites, respectively. *Qsr.sun-5B* (Fig. 3e) explained 11-15% stem rust response variation. These QTLs were contributed by 'HD2009'. The 4B located *Qsr.sun-4B* mapped closer to the DArT marker wPT1046 and was contributed by the parent 'WL711'. It was observed only at the Karalee site in the crop season 2004 and it explained 9% of total phenotypic variation (Fig. 3f). The QTL *Qsr.sun-1D* observed only in the season 2005 at the Lansdowne site accounted for 12% of phenotypic variation and was mapped closer to wPT4687 (Table 3). Involvement of the chromosome 7DS located marker gwm295 in explaining stem rust response variation (5-9%) was detected at only suggestive levels of significance. The total phenotypic variance explained by

Table 2 Genetic analysis of PBC variation in HD2009/WL711 RIL population.

Location/Years	PBC		χ^2		№ of genes
	Present	Absent	3:1	7:1	
Richmond 2005	70	17	1.38	3.94*	2
Lansdowne 2006	69	18	0.86	5.33*	2

Table value of χ^2 at $P = 0.05$ and 1 *d.f.* is 3.84

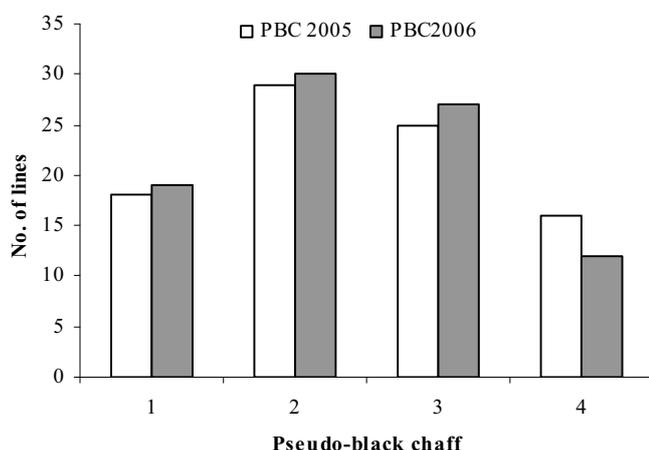


Fig. 2 Phenotypic variation in PBC expression in the HD2009/WL711 RIL population.

Table 3 Summary of QTLs explaining stem rust response variation among the HD2009/WL711 RIL population.

Year/Site/QTL	Chromosome	Closest locus	LOD score	R^2	Source of resistance
2004 Karalee					
<i>Qsr.sun-2B</i>	2B	wPT-4892	3.8	8.0	HD2009
<i>Qsr.sun-3BS</i>	3BS	gwm389	4.1	12.0	HD2009
<i>Qsr.sun-4B</i>	4B	wPT-1046	3.4	9.0	WL711
<i>Qsr.sun-5B</i>	5B	wPT-2586	5.2	11.0	HD2009
<i>Qsr.sun-5DL</i>	5DL	<i>SrHD</i> (<i>Sr30</i>)	9.7	30.0	HD2009
<i>Qsr.sun-7A</i>	7A	wPT-4515	3.0	7.0	HD2009
2004 Lansdowne					
<i>Qsr.sun-2B</i>	2B	wPT-3378	4.7	13.0	HD2009
<i>Qsr.sun-3BS</i>	3BS	gwm389	4.5	12.0	HD2009
<i>Qsr.sun-5B</i>	5B	wPT-2586	3.5	12.0	HD2009
<i>Qsr.sun-5DL</i>	5DL	<i>SrHD</i> (<i>Sr30</i>)	8.1	20.0	HD2009
<i>Qsr.sun-7A</i>	7A	wPT-4515	3.8	11.0	HD2009
2005 Lansdowne					
<i>Qsr.sun-1D</i>	1D	wPT-4687	4.9	12.0	HD2009
<i>Qsr.sun-3BS</i>	3BS	gwm389	3.1	9.0	HD2009
<i>Qsr.sun-5DL</i>	5DL	<i>SrHD</i> (<i>Sr30</i>)	14.0	44.0	HD2009
<i>Qsr.sun-7A</i>	7A	wPT-4515	3.1	13.0	HD2009
2006 Lansdowne					
<i>Qsr.sun-3BS</i>	3BS	gwm389	4.7	11.0	HD2009
<i>Qsr.sun-5B</i>	5B	wPT-2586	5.4	15.0	HD2009
<i>Qsr.sun-5DL</i>	5DL	<i>SrHD</i> (<i>Sr30</i>)	10.3	32.0	HD2009
<i>Qsr.sun-7A</i>	7A	wPT-4515	3.3	8.0	HD2009

R^2 = % phenotypic variation explained

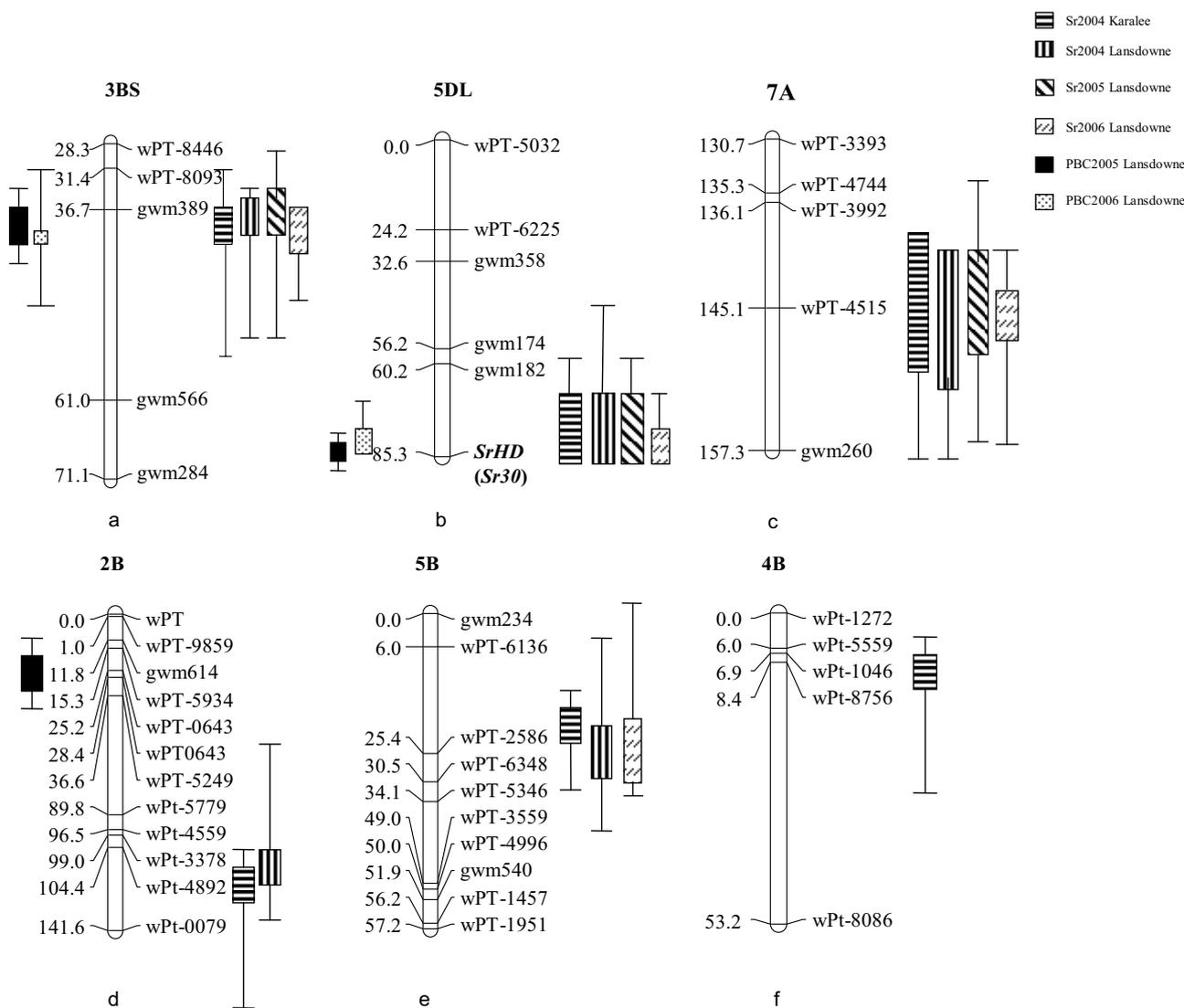


Fig. 3 Genomic locations of stem rust resistance and PBC controlling QTLs in HD2009/WL711 RIL population. QTLs for stem rust resistance are presented on the right hand side of the map (*Qsr.sun-3BS*, *Qsr.sun-5DL*, *Qsr.sun-7A*, *Qsr.sun-2B*, *Qsr.sun-5B* and *Qsr.sun-4B*) and QTLs for PBC are on the left hand side of the map (*QPbc.sun-3BS*, *QPbc.sun-5DL*, *QPbc.sun-7A*, *QPbc.sun-2B*).

various QTLs varied from 66-78% depending on the year and site.

2. Pseudo-black chaff

The quantitative analysis of PBC identified a QTL, *QPbc.sun-3BS*, in the chromosome arm 3BS and explained 12-17% phenotypic variation (Table 4, Fig. 3a). Additional genomic regions affecting PBC expression were identified in chromosomes 5DL and 7DS and were named as *QPbc.sun-5DL* (Fig. 3b) and *QPbc.sun-7DS*, respectively. The *QPbc.sun-5DL* explained 9-11% phenotypic variation and it peaked at the seedling resistance locus *SrHD* present in 'HD2009' (Fig. 3b). The SSR marker gwm295 exhibited close genetic relationship with the PBC QTL, *QPbc.sun-7DS*, and explained 12-13% phenotypic variation. The QTL contours for *QPbc.sun-7DS* could not be produced due to a low number of marker loci mapped on this chromosome. All PBC enhancing alleles were contributed by the parent 'HD2009'. One inconsistent QTL *QPbc.sun-2B* was observed only in the 2005 crop season and explained 7% phenotypic variation in PBC (Fig. 3d).

Table 4 Summary of the QTLs controlling PBC variation among the HD2009/WL711 RIL population.

Year/Site/QTL	Chromosome	Closest locus	LOD score	R ²	Source of resistance
2005 Richmond					
<i>QPbc.sun-2B</i>	2B	gwm614	4.5	7.0	HD2009
<i>QPbc.sun-3BS</i>	3BS	gwm389	4.2	12.0	HD2009
<i>QPbc.sun-5DL</i>	5DL	<i>SrHD</i> (<i>Sr30</i>)	4.0	9.0	HD2009
<i>QPbc.sun-7DS</i>	7DS	gwm295	2.6	12.0	HD2009
2006 Lansdowne					
<i>QPbc.sun-3BS</i>	3BS	gwm389	4.8	17.0	HD2009
<i>QPbc.sun-5DL</i>	5DL	<i>SrHD</i> (<i>Sr30</i>)	4.1	11.0	HD2009
<i>QPbc.sun-7DS</i>	7DS	gwm295	2.5	13.0	HD2009

DISCUSSION

Stem rust resistance in cultivar 'HD2009' was conditioned by three to four genes across different years and sites, except at the Lansdowne site in the year 2004, where a low stem rust response was controlled by two to three genes. The involvement of two dominant complementary genes and one recessive gene controlling stem rust resistance in

'HD2009' was reported by Kaushal *et al.* (1982). Their study was based on F₂ analysis and results were inconclusive. Tests on F₃ or more advanced populations would have provided these workers with a better genetic interpretation of stem rust resistance in 'HD2009'. Transgressive segregation in both directions in 'HD2009'/'WL711' RIL population over years indicated additive nature of genes controlling low stem rust response (Fig. 1).

A seedling stem rust resistance gene detected in 'HD2009'/'WL711' RIL population was temporarily designated *SrHD*. Infection type expressions among resistant RILs were similar to that expressed by the stem rust resistance gene *Sr30*. Knott and McIntosh (1978) located *Sr30* in the long arm of chromosome 5D. Comparative tests using *Sr30*-avirulent (98-1,2,3,5,6) and *Sr30*-virulent (34-1,2,3,6,8,9) pathotypes (data not presented) and mapping of *SrHD* to the long arm of chromosome 5D confirmed that *SrHD* was *Sr30*. In this study *Sr30* mapped 25.1cM from the *Xgwm182* in chromosome 5DL. Bariana *et al.* (2001a) also mapped *Sr30* on 5DL in a Cranbrook/Halberd-derived doubled haploid population. *Sr30* explained 20-44% of phenotypic variation in adult plant stem rust response. Bariana *et al.* (2001b) reported variable responses of *Sr30* carrying genotypes. These authors divided *Sr30* (only) carrying genotypes (based on seedling tests) into two groups. For example, Australian cultivars Frame and Yitpi both carry *Sr30* based on the seedling tests using *Sr30*-avirulent and *Sr30*-virulent pathotypes and these cultivars produced different responses under field conditions. Yitpi showed a susceptible response when tested against *Sr30*-avirulent and *Sr30*-virulent pathotypes under field conditions, whereas Frame showed a uniform moderately resistant to moderately susceptible response against both *Sr30*-avirulent and *Sr30*-virulent pathotypes. This study indicated that *Sr30* contributed significantly to stem rust resistance in cultivar 'HD2009' similar to Frame.

Molecular mapping identified three consistent QTLs (*Qsr.sun-3BS*, *Qsr.sun-5DL* and *Qsr.sun-7A*) in 'HD2009'/'WL711' RIL population for adult plant stem rust resistance. *Qsr.sun-5DL* explained high proportion of phenotypic variation (20 to 44%) in stem rust response in all the years and was associated with the stem rust resistance gene *Sr30*. Second consistent QTL *Qsr.sun-3BS* mapped close to *Xgwm389*. Spielmeier *et al.* (2003) reported flanking of *Sr2* by marker loci *Xgwm389* (2.7 cM) and *Xgwm533* (1.6 cM). Overlapping of *Qsr.sun-3BS* with the *Sr2*-linked PBC controlling locus, *QPbc.sun-3BS*, confirmed the presence of *Sr2* in 'HD2009'. The presence of *Sr2* and *Sr30* in 'HD2009' can be further substantiated from the pedigree analysis of 'HD2009'. One of the parents Lerma Rojo 64A (CIMMYT wheat variety) which appears in parentage of 'HD2009', carries *Sr2* (Luig 1983) and *Sr30* (<http://genebank.vurv.cz/wheat/pedigree/>). Based on these reports, it was concluded that 'HD2009' carried *Sr2* and *Sr30*. The third consistent QTL *Qsr.sun-7A* accounted for 7-13% phenotypic variation. *Qsr.sun-1D*, *Qsr.sun-2B*, *Qsr.sun-4B* and *Qsr.sun-5B* and *Qsr.sun-7A*, have not yet been reported. All QTLs, except *Qsr.sun-4B*, were contributed by the parent 'HD2009'. *Qsr.sun-4B* was contributed by 'WL711' and may have been derived from cv. 'Chris'.

The QTL for PBC, *QPbc.sun-3BS*, mapped to the *Sr2* region in chromosome 3BS (*Qsr.sun-3BS*). Genomic regions on chromosomes 5DL and 7DS also contributed towards the expression of PBC in the year 2005 and in the year 2006. The chromosome 5DL located PBC enhancer corresponded to the *Sr30* region. The chromosome 7DS located marker *gwm295* was closely associated with enhanced expression of PBC. The marker *gwm295* has been reported to be associated with the leaf rust resistance gene *Lr34* (Suenaga *et al.* 2003). Attempts to transfer *Sr2* to *Lr34* carrying WW15-derivatives showed high levels of PBC in the eastern Australian wheat germplasm (Brown and Bariana, pers. obs.). In contrast, these workers observed the suppression of PBC when crosses were made with the Western Australian germplasm. Genotyping of Western Australian cultivars with the

Lr34-linked marker *csLV34* (Lagudah *et al.* 2006) indicated the absence of *Lr34* in these cultivars. This could be due to the accumulation of low PBC conditioning alleles in the Western Australian germplasm.

Only a few studies on QTL mapping of stem rust resistance have been conducted in wheat and none of them reported the involvement of chromosomes 1D, 4B, 5B and 7A in reducing stem rust severity. Involvement of *Qsr.sun-7DS* which corresponded to the *Lr34* carrying genomic region was detected only at the suggestive levels of significance. Dyck (1987) reported an increase in stem rust resistance carried by the Canadian wheat cultivar Thatcher with the transfer of *Lr34*. Recently, Bansal *et al.* (2008) also observed the contribution of *Lr34* in stem rust reduction in winter wheat Forno. Based on the *Lr34*-linked marker, *csLV34*, 'HD2009' was found to carry *Lr34* (Bansal and Bariana unpublished). It is likely that a lower but positive contribution may have been made by *Lr34* in 'HD2009'.

Genetic analysis coupled with QTL mapping identified genomic regions previously not reported to be associated with stem rust resistance. The consistent detection of genomic regions on chromosomes 3BS, 5DL and 7A and low levels of contribution from regions on chromosomes 1D, 2B, 4B, 5B and 7DS supported the existence of minor adult plant resistance genes for stem rust resistance.

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