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ORIGINAL PAPER

Molecular mapping of an adult plant stem rust resistance gene *Sr56* in winter wheat cultivar Arina

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Abstract

Key message **This article covers detailed characterization and naming of *QSr.sun-5BL* as *Sr56*. Molecular markers linked with adult plant stem rust resistance gene *Sr56* were identified and validated for marker-assisted selection.**

Abstract The identification of new sources of adult plant resistance (APR) and effective combinations of major and minor genes is well appreciated in breeding for durable rust resistance in wheat. A QTL, *QSr.sun-5BL*, contributed by winter wheat cultivar Arina providing 12–15 % reduction in stem rust severity, was reported in an Arina/Forno recombinant inbred line (RIL) population. Following the demonstration of monogenic segregation for APR in the Arina/Yitpi RIL population, the resistance locus was formally named *Sr56*. Saturation mapping of the *Sr56* region using STS (from EST and DArT clones), SNP (9 K) and SSR markers from wheat chromosome survey sequences that were ordered based on synteny with *Brachypodium distachyon* genes in chromosome 1 resulted in the flanking of *Sr56* by *sun209* (SSR) and *sun320* (STS) at 2.6 and 1.2 cM on the proximal and distal ends, respectively. Investigation of conservation of gene order between the *Sr56* region in wheat and *B. distachyon* showed that the syntenic region defined by SSR marker interval *sun209-sun215* corresponded to approximately 192 kb in *B. distachyon*, which contains five predicted genes. Conservation of gene order for the *Sr56* region between wheat and *Brachypodium*, except for two inversions, provides a starting point for future map-based cloning of *Sr56*. The Arina/Forno RILs carrying both *Sr56* and *Sr57* exhibited low disease severity compared to those RILs carrying these genes singly. Markers linked with *Sr56* would be useful for marker-assisted pyramiding of this gene with other major and APR genes for which closely linked markers are available.

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Introduction

Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), has remained one of the most important foliar diseases of wheat in Australia since 1906. The 1973 epidemic was considered as the most severe in the history of Australia (Watson and Butler 1984) and it gave rise to a national approach to control rust diseases. A majority of Australian wheat cultivars developed after 1973 carry more than one stem rust resistance genes and cultivars grown in the three geographically distinct wheat growing regions of Australia carry different combinations of genes (Bariana et al. 2007a).

Resistance to stem rust is controlled either by genes that remain effective against avirulent pathotypes throughout the life of a plant (seedling/major/all stage resistance; ASR) or by genes that condition resistance only at the post-seedling or adult plant stages (adult plant

resistance; APR) (Bariana 2003). A majority of the characterised genes belong to the ASR category. *Sr2*, transferred from Yaroslav Emmer wheat (McFadden 1930), belongs to the APR category. *Sr2* is present in many modern wheat cultivars and has remained effective since its initial transfer to common wheat in the last century. Cultivars carrying *Sr2* alone display stem rust severities ranging between 60 and 90 % indicating that it does not provide sufficient level of protection when deployed singly; however, it interacts with other genes that exhibit intermediate levels of resistance to condition satisfactory levels of resistance (Kaur et al. 2009; Singh et al. 2011; Yu et al. 2011). Hare (1997) described another APR gene in a durum landrace Glossy Huguenot. This gene has not yet been formally named.

Recent developments in DNA marker technologies have revolutionised the mapping of crop plant genomes. Microsatellites (SSRs) provided the first effective marker system for molecular mapping in wheat and were used to generate a consensus genetic map for the wheat genome (Sourdille et al. 2004; Somers et al. 2004; Xue et al. 2008). The recent development of high-density marker assays such as diversity array technology (DArT) and iSelect 9 K SNP bead chip (Cavanagh et al. 2013) have further increased the genome mapping efficiency. The availability of the whole genome sequences for several model crop species including *Brachypodium distachyon*, rice and sorghum, as well as assembled genome or chromosome-arm specific sequence information for wheat (Brenchley et al. 2013; http://www.wheatgenome.org/) provided an additional resource for detailed mapping of target genomic regions and gene isolation in wheat.

QSr.sun-5BL in an Arina/Forno RIL population was previously reported by Bansal et al. (2008). This study describes the detailed mapping of the *QSr.sun-5BL* region, formal naming of the locus, development of breeder-friendly marker(s) to track its inheritance in breeding populations and its interaction with APR gene *Sr57* (McIntosh et al. 2012).

Materials and methods

Plant materials

A Swiss winter wheat cultivar Arina was crossed with a stem rust-susceptible Australian wheat cultivar Yitpi and a recombinant inbred line (RIL) population F2:6 (178 RILs) was generated. In addition, Arina/Forno RIL population was used to saturate the chromosome 5BL map. A set of 32 Australian cultivars was used to validate markers closely linked with *Sr56* identified in this study.

Field evaluations

The Arina/Yitpi RIL population was sown at the Lansdowne and Karalee sites of the Plant Breeding Institute (PBI), Cobbitty, as 1-m rows in 2011 and 2012. Each block of 35 experimental rows was surrounded by a 30 cm row of susceptible infector mix to develop uniform infection. The infector mix included susceptible genotypes More, Cezanne, Craklin and W195. An artificial epidemic in each season was created by using Pgt pathotype 34-1,2,7 + *Sr38* (culture no. 565) that was virulent on the seedling resistance gene *Sr48* carried by Arina (Bansal et al. 2008). Rust response assessments were made at a weekly interval for 3 weeks on a 1–9 scale, where one is resistant and nine is susceptible (Bariana et al. 2007b).

SNP genotyping

SNPs associated with *Sr56* were identified using bulk segregant analysis (Michelmore et al. 1991). Resistant and susceptible bulks were prepared by pooling equal amounts of genomic DNA from 20 resistant and 20 susceptible RILs from the Arina/Yitpi population. An artificial F1 sample was prepared by combining an equal amount of DNA from each of the two bulks. The bulked DNA samples, artificial F1, Arina and Yitpi were genotyped using 9,000 gene-based SNPs using a custom Infinium iSelect bead chip assay (Cavanagh et al. 2013) on the iScan instrument following the manufacturer's instructions (Illumina Ltd). Genotypic analysis was performed using GenomeStudio v2011.1 software (Illumina Ltd). The SNPs were assessed for putative linkage by comparing the normalised theta values for each sample (Cavanagh et al. 2013). Polymorphism was considered to be putatively linked to *Sr56* when the normalised theta values for the resistant bulk and Arina, and the susceptible bulk and Yitpi, were similar, and when the normalised theta value for the artificial F1 samples was about half way between that of the other samples.

SNPs showing linkage with *Sr56* were converted to single-marker assays. PCR assays were performed using the CFX96 Touch™ real-time PCR detection system (Biorad, USA) using a pair of primers that were designed to span the SNP and amplify a fragment of 50–150 bp. Each assay contained 20 ng genomic DNA, 3.0 pmol of forward and reverse primers, 5 µl of SensiFast SYBR® and Flouorescein one step kit. PCR amplifications were carried out with an initial denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s followed by HRM ramping at 60–95 °C with fluorescence data acquisition at 0.05 °C increments. Analysis of HRM variants was based on differences in shape of the melting curves and in Tm values.

Fig. 1 Adult plant stem rust responses of **a** Arina, and **b** Yitpi

SSR and STS genotyping

PCR primers were designed using primer 3 (http://frodo.wi.mit.edu/primer3/) with annealing temperature 60 °C. The forward primer for SSR genotyping was designed with an M13 sequence at their 5′ end. For STS genotyping, primers were designed to span predicted exon–exon junctions within ESTs to amplify the intervening intronic sequence. Primer sequences for *Sr56*-linked markers are shown in Table 3.

For SSR genotyping, PCR assays were performed in a 10-µl reaction volume containing 30 ng genomic DNA, 50 nM forward primer with M13 tail, 100 nM reverse primer, 50 nM infared 700 or 800-labelled M13 primer, 125 nM dNTPs, 0.04 U/ul Immolase DNA polymerase (Bioline), and 1X Immolase PCR buffer containing 1.5 mM $MgCl_2$. PCR was performed in a T100™ Thermal Cycler (BIO-RAD USA) using a touchdown profile comprising initial denaturation of 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. PCR products were separated on 8 % polyacrylamide gel using Analyzer Gene ReadIR 4200, Li-COR sequencing system (Li-COR Biosciences, USA).

For STS genotyping, PCR profile comprised initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 40 s and extension at 72 °C for 50 s. PCR products were separated on 2 % agarose gel.

Table 1 Adult plant stem rust response distribution of Arina/Yitpi F6 RILs, when tested under field conditions against *Puccinia graminis* f. sp. *tritici* pathotype 34-1,2,7+Sr38

Phenotype	Number of F6 lines		$\chi^2_{1:1}$
	Observed	Expected	
Non-segregating resistant	97	89	0.72
Non-segregating susceptible	81	89	0.72
Total	178	178	1.44

Synteny analysis

To saturate the *Sr56* region, Illumina survey sequence contigs from flow-sorted wheat chromosome arms (International Wheat Genome Sequencing Consortium, www.wheatgenome.org) were used to identify orthologous genes in *B. distachyon* through reciprocal BLASTn analysis of the sequences of *Sr56*-linked markers. Gene-containing contigs greater than 5 kbp were ordered based on synteny to *B. distachyon*. These were then screened for di-, tri-, tetra-, penta- and hexa-nucleotide repeats using the SSR IT Tool (http://www.gramene.org/db/markers/ssrtool) and used to develop SSR genotyping assays.

Statistical analysis and genetic mapping

Chi squared analyses were performed to assess deviations of observed segregations from the expected genetic ratios. MapManager QTXb20 (Manly et al. 2001) was used to construct a genetic linkage map. Composite interval mapping (CIM) was performed with QTL Cartographer software version 2.5 (Wang et al. 2007). Linkage maps were drawn using Map Chart version 2.2 (Voorrips 2002).

Results

Rust response assessment

Cultivar Arina produced a stem rust response score of 6 (moderately susceptible) on a 1–9 scale with comparatively smaller pustules than the susceptible parent Yitpi (Fig. 1). RILs showing rust responses of six or less were categorised as resistant and RILs with rust responses higher than six were regarded susceptible. Chi squared analysis conformed to monogenic segregation for stem rust response among the Arina/Yitpi RIL population (Table 1).

Table 2 List of *Sr56*-linked SNP loci identified in 9 K Infinium iSelect bead chip assay based bulked segregant analysis

SNP	Name	SNP	Top blast hit		
			Brachypodium CDS	Rice CDS	*Sorghum* CDS
IWA2093	wsnp_Ex_c16045_24471413	[T/C]	*Bradi3g17630.1*	LOC_Os08g10030.2	Sb07g005630.1
IWA2931	wsnp_Ex_c2482_4636722	[T/C]	*Bradi1g01200.1*	LOC_Os03g63590.1	Sb01g000850.1
IWA3359	wsnp_Ex_c3175_5864335	[A/G]	*Bradi1g01830.1*	LOC_Os03g63040.1	Sb01g001380.1
IWA4329	wsnp_Ex_c57209_59016692	[A/G]	*Bradi3g59200.5*	LOC_Os02g18820.1	Sb06g009200.1
IWA5802	wsnp_JD_c12221_12509932	[T/G]	*Bradi3g59200.5*	LOC_Os02g18820.1	.
IWA6578	wsnp_Ku_c16116_24914991	[A/C]	.	.	.
IWA6580	wsnp_Ku_c16116_24916749	[T/G]	.	.	.
IWA6902	wsnp_Ku_c3151_5892200	[T/G]	*Bradi1g02517.1*	LOC_Os07g14580.1	Sb01g002070.1
IWA6994	wsnp_Ku_c38713_47298856	[T/C]	.	.	.

Molecular mapping

QSr.sun-5BL peaked at the most distal RFLP marker (*glk354*) on chromosome 5BL in an Arina/Forno RIL population (Bansal et al. 2008). We attempted to saturate the QTL region in the Arina/Forno RIL population with additional DNA markers, but the level of polymorphism among the parents was very low. Therefore, further work was performed on Arina/Yitpi RIL population developed in this study.

Screening of 30 SSR markers located in the 5BL distal region (Somers et al. 2004) and 16 STS markers derived from DArT markers also located in the same region (A Kilian pers. comm.) identified five SSR (*gwm118, barc59, gwm497, gpw3076, barc2*) and five STS markers [*wPt-484 (sun323), wPt-3922 (sun319), wPt-5118 (sun321), wPt-7665(sun320)* and *wPt-9116 (sun322)*] that were polymorphic between Arina and Yitpi. These markers were genotyped on the entire Arina/Yitpi RIL population and a genetic map was generated. Marker *sun320* mapped 1.2 cM distal to the gene, whereas all other markers were proximal. To confirm the deletion bin in which these markers were located, each marker was tested on DNA from deletion stocks 5BL-6, 5BL-5, 5BL-18, 5BL-9 and 5BL-16 with Chinese Spring as a control. Amplification of *gwm118, sun319* and *sun320* markers was observed only in 5BL-16 and Chinese Spring, confirming the location of *QSr.sun-5BL* in 5BL16-0.79-1.00. As no known adult plant stem rust resistance gene is located in chromosome 5BL, *QSr.sun-5BL* was formally named *Sr56*.

STS markers derived from ESTs located in the 5BL16-0.79-1.00 deletion bin were used to saturate the region. Of 40 EST-derived markers, five were polymorphic between Arina and Yitpi, but none of the markers showed linkage with *Sr56*. The iSelect 9 K bead chip-based BSA identified 9 SNPs linked with *Sr56* (Table 2). PCR primers designed to span the SNPs were used to genotype the Arina/Yitpi RIL population using high-resolution melt analysis (Table 3). SNP marker *IWA6902* (*sun469*) was integrated into the map (Fig. 2b).

To further saturate the *Sr56* region, genome sequence scaffolds corresponding to the mapped STS markers were identified and used to establish the syntenic region in *Brachypodium*; 115 sequence scaffolds were identified, which corresponded to *Bd1 g* (Fig. 2c). A total of 114 primers were developed for SSRs identified within 75 scaffolds and used to screen parents Arina and Yipti. Thirty polymorphic SSRs were genotyped on the Arina/Yitpi RIL population and integrated into the genetic map. *Sr56* was flanked by *sun209* at 2.6 cM proximally and *sun320* at 1.2 cM distally (Fig. 2b).

Colinearity within the Sr56 *region*

The genetic linkage map containing *Sr56* included 24 SSR markers (derived from wheat scaffolds), two STS markers (*sun319* and *sun320*), and one SNP marker (*sun469*). The flanking SSR markers *sun209* and *sun215* provided an ideal anchor point to investigate conservation of gene order between the *Sr56* region in wheat and *B. distachyon* (Fig. 2c). The *sun209-sun215* interval corresponded to 192.59 kbp of *Brad1g* that includes five predicted genes (Fig. 2d). Within the *Sr56* region, gene order was conserved between wheat and *B. distachyon*, except for two inversions (Fig. 2b, c).

Revised QTL analysis and interaction of Sr56 and Sr57

Markers closely linked with *Sr56* were tested on cultivars Arina and Forno. Three polymorphic markers *sun214*, *sun209* and *sun215* were genotyped on Arina/Forno RIL population and QTL analysis was carried out using QTL cartographer (Fig. 2a). Phenotypic variation explained by *QSr.sun-5BL* increased from 12–15 % to 14–27 % following incorporation of new markers.

Table 3 Sequence details of primers designed in this study using different genomic resources to saturate the *Sr56* region

Primer name[a]	Contig no	Forward primer	Reverse primer
sun218	Ta5BS-10919232-1	AAACCCAACATTTCAGTTTGCC	ATCATCCCAACATGCCATCC
sun221	Ta5BS-10919232 -2	TTCCTTAAGACATGACAACC	AATGGACTTCACTACTACGT
sun267	Ta5BS-10829097.1	GATGGTAGAAAGCGCGAGAC	TTTGACAAAGAGACGCATCG
sun263	Ta5BS-10919232.1	CTGGTAGGCAAAGGAATGGA	TACAAGGCCGGAATCACTCT
sun262	Ta5BS-5174313	AGGCCTCTGGCCAATATACA	AAACCACCGAACACAAGGTC
Sun469	IWA6902	TTGGCGGGAAGTAAAAGAAG	GCTGCCTCCGGTACAATAGA
sun280	Ta5BS-10910307.1	CGCTTTCTGGTTATTCCCTTC	CCGACTTGACACCGCTAGA
sun244	Ta5BS-10879225	CCCTTTGTTCCGTTGCTC	AGAGTGTCTGCGCATTTACC
sun222	Ta5BS-10875975	GACCATGTCCTCGAAGCTC	ACAACAATTAGTGAGCACCA
sun247	Ta5BS-10817323-2	CGTTATTGGGCTTGTTTCGG	CAGCATCAACACTGAATATCTG
sun224	Ta5BS-10863645	TGTTGTATCACCCTCTCCTC	ACTTTGTTGTGGAATGCCC
sun319	wPt-3922	CAGGGGTCACCTTTTCTCAA	CAACAAAATCCTTGGCATCC
sun214	Ta5BS-10881960	TACCTCTGAATCTCCATTGCT	AGAATTGTACCAGTGTTGTC
sun297	Ta5BS-10810016.1	GCCAAACGTGTTTGTCCCTA	TAAGGCAGTGACCCATCCTC
sun209	Ta5BS-10804384-1	CTGTAAGGTTCTTTCGGATTGG	CATGGTCTTCGACGACTTAGTG
sun320	wPt-7665	TAGCAAACGCAACAATTTGG	CATCAGTTTCTACGGCAGCA
sun215	Ta5BS-10892706	GCCAAGACAAGCTAGGACAG	TGATGTGAGCTGATGACATGG
sun303	Ta5BS-10924292.1	CGCGTGCACGAGTAATAAAT	CATTCCTGGATCTGGATGGA
sun207	Ta5BS-10786907-7	GCTAATTCTTGCTGGTGCTG	GTTGGTTGGTAGGTTGTTTGAG
sun307	Ta5BS-10886394	TTGGATCAGCATCAAGCAAG	ATACATTCATGGCCCGGATA
sun239	Ta5BS-10790252	GTTGATGAAATAAGTGGCAGCAG	TTGTCGCATTTGAAGGTCAC
sun251	Ta5BS-10905297	TGAAGTTTGGAGATGAGGTGG	ACTACAAGGAAATCAAGCGA
sun211	Ta5BS-10824599-1	CTCTTCTTTCCCTGTTCTATACTC	GTTTACGGATTTGGTCACCC
sun216	Ta5BS-10909279-1	TGACCTTTGTTTACCTTTGTCC	GGCAACTAGTTAAGCACTATCC
sun236	Ta5BS-10814941 -2	GAGACAAACGACCTTCCAC	ACTATGCAGCCTCACCGA
sun242	Ta5BS-10901139-2	GTTAGCCGCACTCTATCTTCC	AAGCAGTTCGTACACCACAC

[a] Sun stands for Sydney University

Forno carries the APR gene *Sr57/Lr34/Yr18* (*Sr57*). Arina/Forno RILs were grouped into four categories on the basis of *Sr57*-linked marker *csLV34* (Bansal et al. 2008) and *Sr56*-linked markers *sun209* and *sun215* (present study) and mean adult plant stem rust responses of these groups were compared (Table 4). The mean disease severity (averaged stem rust severity over replications and years) of RILs carrying both *Sr56* and *Sr57* (~22 %) was less than those RILs that carried *Sr56* (~40 %) or *Sr57* (~37 %) singly (Table 4). These results demonstrated the additive interaction of *Sr56* with *Sr57* to lower stem rust severity.

Validation of flanking markers

Markers *sun209* and *sun320* flanking *Sr56* were validated on a set of Australian cultivars (Table 5). Twenty-eight cultivars did not amplify *Sr56* linked product for both markers. Cultivars Derrimut, Emu Rock and Orion produced false positive for marker *sun209* (*Sr56*-linked 448 bp product) and Diamondbird was the only false positive for *sun320* (*Sr56*-linked 179 bp product).

Discussion

Development of rust-resistant cultivars is underpinned by the availability of diverse sources of resistance. Minor/slow rusting/APR genes are often non-race specific and are considered to be more durable compared to major/seedling resistance genes. Until 2012, *Sr2* was the only formally named APR stem rust resistance gene in wheat (McIntosh et al. 2012) and its deployment in many wheat cultivars grown over large acreage globally demonstrates its durability. More recently, *Sr55* and *Sr57* were added to the list of APR genes for stem rust (McIntosh et al. 2012). Cultivars carrying combinations of three or more APR genes are expected to be durable (Bariana and McIntosh 1995).

A high proportion of winter wheats show susceptible responses to stem rust, presumably due to the absence of selection for this disease. Monogenic segregation at the *QSr.sun-5BL* (Bansal et al. 2008) locus was observed in the Arina/Yitpi RIL population and the QTL was formally named *Sr56*. QTL for stem rust response have been reported in several chromosomes (1AL, 1BL, 2BS, 2BL, 2D, 3BS,

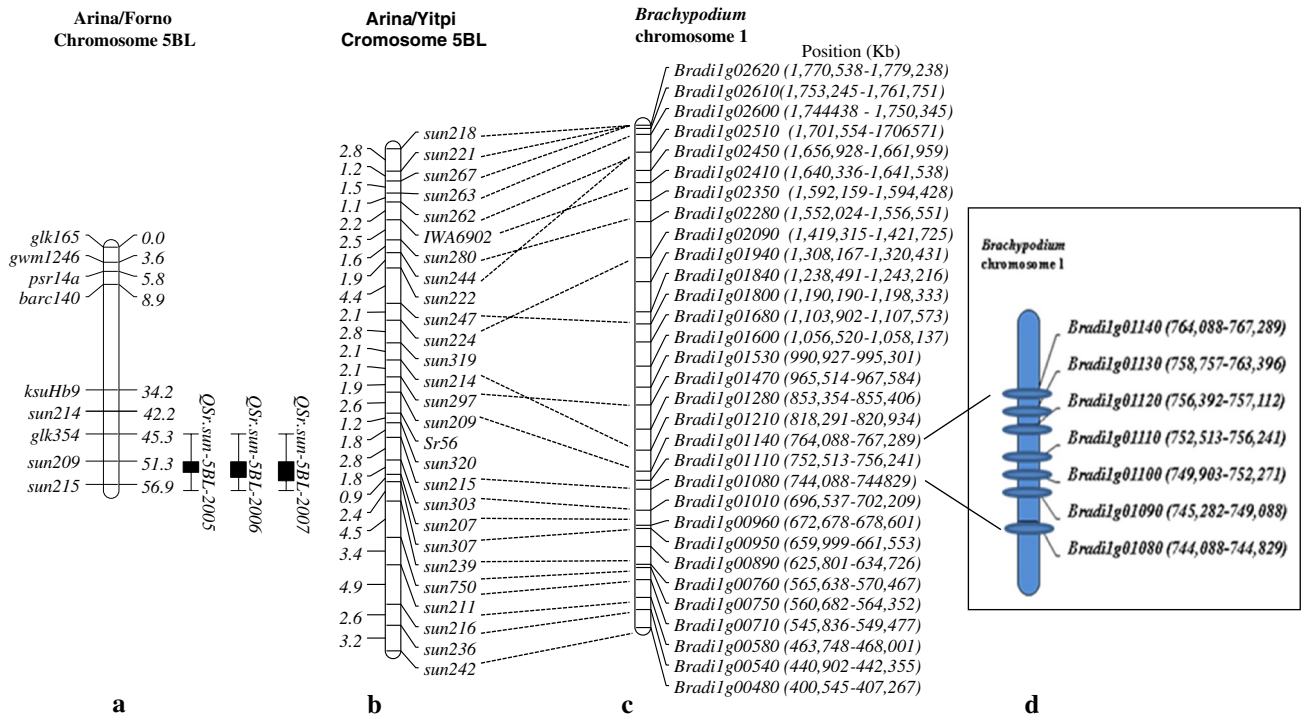

Fig. 2 Genetic linkage map of **a** Arina/Forno RIL chromosome 5BL in **b** Arina/Yitpi RIL chromosome 5BL **c** *B. distachyon* chromosome 1 marked with significant hits with wheat survey sequences within the *Sr56* region **d** *Brachypodium* genes contained within the *Sr56* region in wheat

Table 4 Mean responses of Arina/Forno RILs carrying *Sr56* and *Sr57* singly and in combination

Gene(s)	Disease severity		
	sun209	*sun215*	glk354
Sr56	39.7 (30)*	40.5 (30)	46.8 (29)
Sr57	37.2 (33)	37.6 (29)	34.4 (29)
Sr56 + *Sr57*	21.8 (40)	22.8 (43)	25.2 (40)
NIL	57.2 (37)	56.7 (39)	51.2 (27)

* Value in parentheses represents number of RILs

4AL, 4B, 5A, 5BL, 5DL, 6B, 7A, 7B, and 7DS) in different mapping populations (Bansal et al. 2008; Bhavani et al. 2011; Haile et al. 2012; Kaur et al. 2009; Singh et al. 2013a, b). These QTL explain 5–32 % of phenotypic variation in stem rust response. QTL on chromosome 5BL have been reported in cultivars Arina, AC Cadillac, HD2009, Kingbird, Kiritati, Juchi, Hurivis and Sebatal. All these cultivars were either CIMMYT wheats or derived from CIMMYT germplasm, except Arina (a winter wheat from Europe) and Sebatal (a durum wheat). A comparison of positions of QTL reported in chromosome 5BL from the various studies was made. Bhavani et al. (2011) reported a QTL on chromosome 5BL in PBW343/Kingbird (linked markers: *wPt-5896* and *wPt-1733*); PBW343/Kiritati (linked markers: *gwm371* and *barc109*); PBW343/Hurivis (linked markers: *gwm371* and *NW2012ND*) and PBW343/Juchi (linked markers: *wPt-0750* and *wPt-5896*). Linked markers *gwm371* in PBW343/Kiritati and PBW343/Hurivis (Bhavani et al. 2011) and *barc59* and *barc142* in AC Cadillac/Cranberry (Singh et al. 2013a) and Kristal/Sebatal populations (Haile et al. 2012) were located in the deletion bin 5BL1-0.55-0.75, which is proximal to the location of *Sr56* (5BL16-0.79-1.00). Marker *wPt-5896*, which was associated with the QTL in three populations (Bhavani et al. 2011; Singh et al. 2013b), was mapped at 93 cM, whereas the *Sr56*-linked marker *sun320* (*wPt-7665*) is located at 155 cM in the DArT consensus map (A Kilian pers. comm.). *QSr.sun-5BL* from Kaur et al. (2009) peaked at the marker *wPt-6348*, which is located at 37 cM in the DArT consensus map. These comparisons confirmed the uniqueness of *Sr56*.

SSR markers located in the distal region of the chromosome 5BL and markers derived from wheat ESTs mapped in the most distal deletion bin 5BL16-0.79-1.00 were used to saturate the *Sr56* region. All markers mapped proximal to *Sr56*, confirming its location in the distal region where no markers were previously mapped. Enrichment of a target region in wheat can be complicated due to its polyploid nature, low levels of polymorphism among cultivated wheat and the lack of genome sequence information (Feuillet et al. 2008; Paux et al. 2008). Consequently,

Table 5 Validation of *Sr56*-linked markers on a set of Australian cultivars

Cultivars	*sun209*	*sun320*
Braewood	−	−
Calingiri	−	−
Camm	−	−
Carinya	−	−
Carnamah	−	−
Derrimut	+	−
Diamondbird	−	+
EGA Bonnie Rock	−	−
EGA Gregory	−	−
Ellison	−	−
Emu Rock	+	−
Espada	−	−
Forrest	−	−
Frame	−	−
Giles	−	−
Gladius	−	−
Goldmark	−	−
H45	−	−
Kukri	−	−
Magenta	−	−
Merlin	−	−
Orion	+	−
Rubric	−	−
Scout	−	−
Spitfire	−	−
Sunlin	−	−
Sunsoft 98	−	−
Sunvale	−	−
Sunzell	−	−
Tatiara	−	−
Ventura	−	−
Wyalkatchem	−	−

high-resolution mapping in wheat requires considerable investment of time and resources (Keller et al. 2005).

As SSR markers detect more variability due to their multi-allelic nature (Röder et al. 1998) and are a useful marker system for marker-assisted selection (Koebner and Summers 2003), 114 new SSR markers derived from wheat sequence scaffolds orthologous to *B. distachyon* genes in chromosome 1 were used to identify markers closely linked with *Sr56*.

Sr56 was flanked by SSR markers *sun209* and *sun215* and was located in a syntenic interval defined by *Bradi1g01140* and *Bradi1g01080*. The syntenic interval in *B. distachyon* contained five genes, which code for a heat shock factor (HSF)-type and DNA binding protein (*Bradig01130*), unknown protein (*Bradi1g01120*), RNA recognition motive domain (*Bradi1g01110*), Exonuclease phosphorolytic domain (*Bradi1g01100*) and Arf GTPase activating protein (*Bradi1g01090*). While these genes are not obvious candidates for *Sr56*, the synteny observed between wheat and *B. distachyon* provides a useful starting point for positional cloning of *Sr56*.

Despite the poor level of polymorphism in Arina/Forno RIL population, we found three markers (*sun214, sun209* and *sun215*) polymorphic among parents and were used to saturate the *QSr.sun-5BL* region. Revised QTL analysis using map with additional markers explained relatively higher phenotypic variation than that reported by Bansal et al. (2008).

Sr2 interacts with other stem rust resistance genes which condition intermediate level of resistance individually to provide increased level of stem rust protection (Kaur et al. 2009; Singh et al. 2011; Yu et al. 2011). APR conditioned by *Sr2* significantly reduces the number of stem rust pustules per unit area and delays the onset of disease when compared with susceptible controls (Hare 1997). Similarly, we observed lower mean stem rust responses of RILs carrying both *Sr56* and *Sr57*, when compared to those that carried these genes singly. Closely linked markers *sun209* and *sun320* were negatively validated on a set of commercially released Australian cultivars to confirm the utility of these markers for marker–assisted breeding (Table 5). RILs carrying *Sr56* and *Sr57* in combination have been crossed with other APR sources (*Sr2/Lr27/Yr30* and *Lr67/Yr46/Sr55*) to select durable triple rust-resistant derivatives through marker-assisted selection.

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Conflict of interest All authors read the manuscript and do not have any conflict of interest.

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