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ORIGINAL ARTICLE

Fine mapping of the chromosome 5B region carrying closely linked rust resistance genes *Yr47* and *Lr52* in wheat

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Abstract

Key message **Fine mapping of *Yr47* and *Lr52* in chromosome arm 5BS of wheat identified close linkage of the marker *sun180* to both genes and its robustness for marker-assisted selection was demonstrated.**

Abstract The widely effective and genetically linked rust resistance genes *Yr47* and *Lr52* have previously been mapped in the short arm of chromosome 5B in two F_3 populations (Aus28183/Aus27229 and Aus28187/Aus27229). The Aus28183/Aus27229 F_3 population was advanced to generate an F_6 recombinant inbred line (RIL) population to identify markers closely linked with *Yr47* and *Lr52*. Diverse genomic resources including flow-sorted chromosome survey sequence contigs representing the orthologous region in *Brachypodium distachyon*, the physical map of chromosome arm 5BS, expressed sequence tags (ESTs) located in the 5BS6-0.81-1.00 deletion bin and resistance gene analog contigs of chromosome arm 5BS were used to develop markers to saturate the target region. Selective genotyping was also performed using the iSelect 90 K Infinium wheat SNP assay. A set of SSR, STS, gene-based and SNP markers were developed and genotyped on the Aus28183/Aus27229 RIL population. *Yr47* and *Lr52* are genetically distinct genes that mapped 0.4 cM apart in the RIL population. The SSR marker *sun180* co-segregated with *Lr52* and mapped 0.4 cM distal to *Yr47*. In a high resolution mapping population of 600 F_2 genotypes *Yr47* and *Lr52* mapped 0.2 cM apart and marker *sun180* was placed 0.4 cM distal to *Lr52*. The amplification of a different *sun180* amplicon (195 bp) than that linked with *Yr47* and *Lr52* (200 bp) in 204 diverse wheat genotypes demonstrated its robustness for marker-assisted selection of these genes.

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Introduction

Common wheat (*Triticum aestivum* L.) is among the most important cereal crops grown around the world for human consumption (Gustafson et al. 2009). Demand for wheat is expected to increase by 60% by 2050 (Rosegrant et al. 2008). Stripe rust and leaf rust lead the list of diseases that result in 14 to 27% yield losses in wheat every year in many countries (Kosina et al. 2007). These diseases have been estimated to cause annual losses of A$139 million in Australia alone (Murray and Brennan 2009).

Deployment of rust resistance genes in new cultivars is one of the key objectives of wheat breeding programs worldwide. Pyramiding of two or more genes in new cultivars has been suggested to be an effective disease

resistance breeding strategy (Bariana and McIntosh 1995; Singh et al. 2000). Combinations of all stage resistance (ASR) and adult plant resistance (APR) genes provide effective and long lasting resistance (Bariana et al. 2007; Kolmer 2013). Pyramiding of ASR and APR genes in a single genotype can be difficult to select phenotypically due to complete or near complete protection provided by single ASR genes. This limitation is slowly being overcome with advances in the identification of robust marker-trait associations (Bariana 2003; Bariana et al. 2007; Choudhary et al. 2008; http://maswheat.ucdavis.edu/; Yang et al. 2015), which allow direct selection for rust resistance genes without phenotyping. Molecular markers linked closely with several rust resistance genes have been identified in wheat (Bariana et al. 2007; http://maswheat.ucdavis.edu/) and are now being used to produce desired combinations of genes in future cultivars.

Donor sources that carry linked resistance to more than one disease have been exploited in wheat breeding programs. Triple rust resistance (*Sr31/Lr26/Yr9*) carried on the short arm of rye chromosome 1RS was commonly deployed in wheat cultivars released from the CIMMYT germplasm (Singh et al. 2006). Similarly, sources of linked rust resistance (*Sr38/Lr37/Yr17* and *Sr24/Lr24*) have been used intensively in Europe and Australia (H.S. Bariana unpublished results). Matching virulences for these genes have now been detected (Bariana et al. 2007; Jin et al. 2008; Pretorius et al. 2000; Wellings 2007; http://sydney.edu.au/agriculture/documents/pbi/cereal_rust_report_2014_vol_12_3.pdf).

Several sources of linked ASR genes for resistance to stripe rust and leaf rust including *Yr35/Lr53* (Marais et al. 2005), *Yr40/L57* (Kuraparthy et al. 2007), *Yr42/Lr62* (Marais et al. 2009), *Yr47/Lr52* (Bansal et al. 2011) and *Yr70/Lr76* (Bansal et al. 2015) have been identified in the last decade. All these genes, except *Yr47/Lr52* (Aus28183), are located on translocated segments from related species and may result in linkage drag of deleterious traits. Aus28183 is not expected to suffer from this phenomenon. *Yr47* and *Lr52* confer resistance against predominant Australian, Indian and Kenyan pathotypes (H.S. Bariana unpublished work). *Lr52* was shown to be effective against 29 Pt isolates and was located in the short arm of chromosome 5B by Hiebert et al. (2005). Bansal et al. (2011) mapped *Yr47* in the short arm of chromosome arm 5B in two F_3 populations (Aus28183/Aus27229 and Aus28187/Aus27229) and showed its genetic association with *Lr52*. These genes were flanked by markers *gwm234* and *cfb309* at about 10 cM distally and proximally, respectively. This study utilised available genomic resources to saturate the *Yr47* and *Lr52* carrying chromosome arm 5BS region to develop robust DNA markers that can be reliably used for marker-assisted selection of these genes in wheat breeding programs.

Materials and methods

Plant materials

A recombinant inbred line (RIL) F_6 population of 120 lines was developed from the Aus28183/Aus27229 F_3 population. A set of 84 Australian and 120 Nordic wheat genotypes was used to validate the linkage of DNA markers with *Yr47* and *Lr52*. Aus28183 was crossed with an Australian cultivar Ventura and F_1 plant was backcrossed (BC) with Ventura to generate BC_3F_1. BC_3F_1 plants were selfed to generate BC_3F_3 population which was phenotyped for rust resistance and six homozygous resistant families were selected. These six backcross derivatives carrying *Yr47* and *Lr52* were also tested with the linked markers.

Greenhouse studies

The Aus28183/Aus27229 RIL population and parents Aus28183 and Aus27229 were screened against *Puccinia striiformis* f. sp. *tritici* (Pst) and *P. triticina* (Pt) in the greenhouse. Nine centimeter diameter pots were filled with soil comprising a mixture of sand and pine bark in the ratio of 2:1. Water soluble fertilizer Aquasol (20 g/10L of tap water) was applied to all pots before sowing. Four lines per pot with eight seeds per line were sown and the pots were placed in a rust-free microclimate room at 20 °C. Seven days after sowing, urea was applied to the seedlings at the same rate as Aquasol. Seedlings were inoculated with the Pst pathotype 134 E16A+Yr17+Yr27+ (culture 617) and the Pt pathotype 104 1,(2),3,(6),(7),11,13 (culture 547) at the 2-leaf stage. Rust spores were suspended in the light mineral oil Isopar-L and atomized on seedlings using an aerosol pressure pack (McIntosh et al. 1995). Stripe rust inoculated seedlings were shifted to the incubation room on water filled steel trays and covered tightly with polythene hoods for 24 h at 9–12 °C, whereas leaf rust inoculated seedlings were shifted to a humidified chamber for 24 h. Stripe rust and leaf rust inoculated seedlings were then transferred to microclimate rooms set at 17 ± 2 and 25 ± 2 °C, respectively. Seedling rust response assessments were made 12–16 days after inoculation using the scales described in McIntosh et al. (1995).

DNA extraction and quantification

DNA was extracted from 10-day-old seedlings of the RIL population and parents Aus28183 and Aus27229 using the protocol described in Bansal et al. (2014a). DNA samples were quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies) and diluted to 30 ng/µl concentration.

Table 1 Markers used to saturate the *Yr47/Lr52* carrying region of wheat chromosome arm 5BS

Marker name	Marker type	Contig no/EST/BAC	Forward primer*	Reverse primer	Motif	Annealing temp	Homology
sun480	SSR	Ta5BS-2294725	gcatgcatttgctctagttat	cttccttcatccaaagagact	$(CTGCTT)^{6}$	55	Bradi4g00490
sun183	SSR	Ta5BS-2276504	tatatagcagcagcgagtcac	aaccaaccaaccattgtaata	$(CTCAT)^{5}$	55	Bradi4g00760
sun180	SSR	Ta5BS-2262954	ctttcgtctgtttgtcatttt	atccaaatccaaaaacaactc	$(CATCC)^{5}$	55	Bradi4g00550
fcp652	SSR	Ta5BS-2228786	tgtgttgagctcatgtacaaa	cagccttccttactgtgaaa	$(TCATC)^{4}$	60	90 K SNP
fcp657	SSR	Ta5BS-1635726	attcacggttggtacatattg	cttgtatcgcacaaacagaac	$(GCAGT)^{4}$	60	90 K SNP
icg16c004_2	SSR	Not known	atggcatgccgctagaag	cacccgaagttgtcataaattacc	$(ATG)^{25}$	60	5BS physical map
icg16c008	SSR	Not known	attgcagactcgagaaccatac	ccctgacctctcttgcattatc	$(AAT)^{32}$	60	5BS physical map
icg16c041	SSR	Not known	gaatgctagtcgtggttgatttg	ccaaacacaagggttgctatg	$(TTC)^{22}$	60	5BS physical map
icg16c041_2	SSR	Not known	ttccgggcattcaactgtag	gggctttgagaatcggatagg	$(CTA)^{27}(TAG)^{35}$	60	5BS physical map
TC252302	STS	Ta5BS-2291172	acatctacaatgccggtgct	tgctcttgcaggcatgttat		55	Rice
cfb306	SSR‡	BAC1793L02	taaagcggatgggtcttgtt	ataagattacctcgggtgaa			
cfb309	SSR‡	BAC1793L02	tagggcatatttccaacact	taagtccgcgtattagcatt			
gwm234	SSR¥		gagtcctgatgtgaagctgttg	ctcattggggtgtgtacgtg	(CT)16(CA)20	55	
mag705	SSR≠	BM134523	cgacgacaaagtatgaggggg	tcggtagcgatgatgtattcacg		55	

* Forward primer labeled with M13: CACGACGTTGTAAAACGAC; sequence taken from ‡ Alfares et al. (2009); ¥ Roders et al. (1998); ≠ Xue et al. (2008)

Marker development and saturation of the target 5BS region

Previously reported markers *cfb306*, *cfb309*, *gwm234* and *mag705* (Bansal et al. 2011) were genotyped on the entire RIL population. The sequences of these primers and their annealing temperatures are given in Table 1.

Various genomic resources were used to develop new markers in the target region based on the approach described in Bansal et al. (2014a, b). Flow-sorted chromosome survey sequence (CSS) 5BS contigs of variety Chinese Spring (CS) (International Wheat Genome Consortium, http://www.wheatgenome.org) were initially used as BlastN queries against the *Brachypodium distachyon* genome sequence to identify orthologous regions. Once identified, *B. distachyon* genes in the orthologous region were used to identify additional CSS contigs through reciprocal BlastN analysis. In all cases, the CSS query with the best hit was considered to be syntenic to *B. distachyon*. CSS contigs within the target region were then tested for nucleotide repeats using the SSR IT tool (http://www.gramene.org/db/markers/ssrtool) and SSR markers were developed. Primers were designed using Primer 3 software (http://frodo.wi.mit.edu/primer3/). In addition, SSR markers from the physical map (Nesterov et al. 2015) of chromosome arm 5BS were also used in mapping. An M13 sequence tail was added to the 5′ end of each forward primer (Table 1). The new primers were labeled as "sun" (Sydney University) and "icg" (Institute of Cytology and Genetics).

Selective genotyping was performed on DNA from eight homozygous resistant and eight homozygous susceptible RILs using the Illumina iSelect 90 K Infinium wheat array (Wang et al. 2014) to identify SNPs associated with *Yr47* and *Lr52*. Closely linked SNPs were converted into Kompetitive allele specific PCR (KASP) having two allele specific forward primers and one common reverse primer. Twenty expressed sequence-tags (ESTs), gene-specific, SSR and STS based markers developed by Shi et al. (2016) were also used to saturate the chromosome 5BS region.

Markers flanking *Yr47* and *Lr52* were also used to screen 600 F_2 plants from the Aus27729/Aus28183 cross. The recombinant genotypes were raised to F_3 generation for phenotypic evaluation against stripe rust and leaf rust.

PCR amplification and visualization

For all SSR, STS, EST and gene-based markers, PCR was carried out in a 10 µl reaction mixture containing 60 ng DNA, 1× Immolase buffer, 1.5 mM $MgCl_2$, 250 µM dNTPs, 100 nM M13 tailed forward primer, 200 nM reverse primer, 5 nM M13 dye with labeled fluorescence (IR-700 or IR-800), 0.04 U of Immolase DNA polymerase (Bioline). Thermocycling was performed with the touch down profile: initial denaturation at 95 °C for 10 min, followed by 92 °C for 30 s, 65 or 60 °C (with 1 °C drop down every cycle) for 30 s and 72 °C for 30 s for 5 cycles, and 35 cycles of 30 s at 92 °C, 30 s at 60 or 55 °C (depending upon the annealing temperature of primers) and 30 s at 72 °C and a final extension step of 72 °C for 10 min.

The amplified PCR products were visualized on 2% agarose gel stained with GelRedTM (Biotium) using a UV gel documentation unit (UVP-GelDoc-It). A 1 Kb DNA ladder (Fermentas) was run alongside the products to define allele sizes. To resolve small base pair differences, the amplified products were separated in 6.5% polyacrylamide gel on a LICOR 4300 DNA analyser using the protocol described in Randhawa et al. (2015).

KASP genotyping

KASP assays were performed in an 8 µl reaction mixture containing 3 µl of 30 ng/µl genomic DNA, 0.11 µl of primer mix having 12 µM each of the two allele specific forward primers and 30 µM of common reverse primer, 0.89 µl of double distilled water and 4 µl of KASP mix comprised of Taq polymerase, dNTPs, buffer with $MgCl_2$, universal FAM and HEX fluorescence resonance energy transmitted cassettes and ROX™ reference dye (KD Biosciences).

PCR was performed using the Bio-Rad, USA CFX96 Touch™ real time PCR detection system. The PCR profile included an initial denaturation step of 94 °C for 15 min followed by 9 touchdown cycles at 94 °C for 20 s and 61 °C for 60 s dropping 0.6 °C per cycle and 38 cycles at 94 °C for 20 s and 55 °C for 60 s. Allelic discrimination was achieved using the Bio-Rad CFX Manager software (Bio-Rad, USA).

Statistical analysis and molecular mapping

Chi-squared analysis was employed to observe the goodness of fit of the observed phenotypic segregations to the expected genetic ratios. The genetic linkage map was constructed using MapDisto v.1.8 software (Lorieux 2012) using the Kosambi mapping function (Kosambi 1943) and the linkage map was drawn using Map Chart version 2.2 (Voorrips 2002).

Results

Phenotypic evaluation

The resistant parent Aus28183 produced infection type (IT) ;C-1CN against Pst pathotype 134 E16A+Yr17+Yr27+ and IT0;-;1 against Pt pathotype 104-1,(2),3,(6),(7),11,13 (Fig. 1). The susceptible parent Aus27729 exhibited $IT3^+$ for both rust diseases (Fig. 1). The RIL population was classified as homozygous resistant (HR) and homozygous susceptible (HS) on the basis of rust responses in the greenhouse. Chi-squared analysis of rust response variation data for both leaf rust and stripe rust conformed to monogenic segregation among the Aus28183/Aus27729 RIL population (Table 2). Two recombinants between *Yr47* and *Lr52* were identified. One recombinant showed susceptibility to leaf rust but was resistant to stripe rust and the other recombinant exhibited opposite responses (Table 2).

Molecular mapping

Sixty SSR markers designed through the identification of orthologous genes in *Brachypodium distachyon* from the wheat genome survey sequences and 21 markers from the physical map (Nesterov et al. 2015) were used to saturate the *Yr47* and *Lr52* region. Each marker was tested for linkage to *Yr47* and *Lr52* using the parental lines and a pair of DNA bulks comprised of RILs with resistance and susceptibility to the two rust diseases. Twenty-five markers that showed linkage between bulks were genotyped on the entire RIL population and seven markers (*sun180*, *sun183*, *sun480*, *icg16c004_2*, *icg16c008*, *icg16c041* and *icg16c041_2*) were used for genetic map construction (Table 1).

Twenty SSR and STS markers developed from wheat ESTs located in the 5BS6-0.81-1.00 deletion bin (Shi et al. 2016) and resistance gene analog (RGA) contigs from chromosome arm 5BS were similarly tested for association with *Yr47* and *Lr52*. Four markers (*fcp652*, *fcp657*, *mag705* and *TC252302*) showing linkage were genotyped on the entire RIL population and used for linkage map construction (Table 1).

Selective genotyping performed using the Illumina iSelect 90 K Infinium wheat array identified 36 SNPs that showed linkage with *Yr47* and *Lr52*. Single-marker KASP assays designed for each linked SNP were tested on the parental lines. Sixteen KASP assays giving good SNP allele discrimination were genotyped on the entire RIL population. Eight KASP markers (*KASP_75279*, *KASP_12199*, *KASP_25183*, *KASP_1736*, *KASP_36048*, *KASP_8814*,

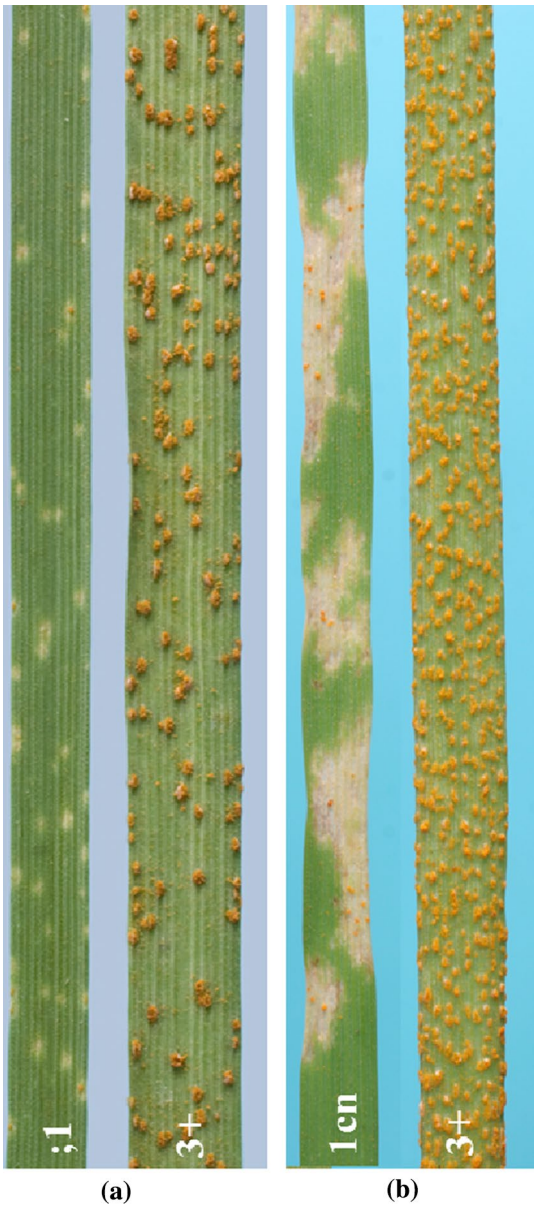

Fig. 1 Seedling infection types produced by Aus28183 and Aus27229 against **a** *Puccinia triticina* and **b** *Puccinia striiformis* f. sp. *tritici,* respectively

KASP_7565 and *KASP_6180*) were incorporated into the genetic map (Table 3).

A total of 22 unambiguous markers showing linkage with *Yr47* and *Lr52* were used to construct a genetic map for the RIL population (Fig. 2). The map included the previously reported SSR markers *gwm234*, *cfb306* and *cfb309* (Bansal et al. 2011), which were also genotyped on the entire RIL population. The total genetic distance of the map was 11.2 cM. *Yr47* and *Lr52* mapped 0.4 cM apart. SSR marker *sun180* co-segregated with *Lr52* and mapped

Table 2 Distribution of Aus28183/Aus27229 RIL population when tested against *Puccinia striiformis* f. sp. *tritici* pathotype 134 E16A+Yr17+Yr27+ and *P. triticina* pathotype 104-1,(2),3,(6),(7),11,13

Genotype/frequency	*Lr52Lr52*	*lr52lr52*	Total
Yr47Yr47	67	1	68
yr47yr47	1	51	52
Total	68	52	120

$\chi^2_{1:1(Lr52 \text{ vs } lr52)} = 2.13$(non-significant at $P = 0.05$ and $1df$)
$\chi^2_{1:1(Yr47 \text{ vs } yr47)} = 2.13$(non-significant at $P = 0.05$ and $1df$)
$\chi^2_{1:1:1:1(Lr52 \text{ vs } Yr47)} = 116.4$(significant at $P = 0.05$ and $3df$)

0.4 cM distal to *Yr47*. The SSR marker *sun180* corresponded to the *Brachypodium* gene *Bradi4g00550*.

Construction of a high-resolution linkage map

Marker *sun180* that co-segregated with *Lr52* and the flanking markers *sun480*, *fcp652*, *icg16c008* and *gwm234* spanning a 5.1 cM region were genotyped on 600 F_2 (Aus27729/Aus28183) plants to identify recombinants for the construction of a high resolution genetic map. Forty-five recombination events were identified from the 1200 gametes screened. Recombinant F_2 plants were selected and raised to generate F_3 generation. The F_3 generation was tested against Pst and Pt pathotypes. The recombinant F_2 progenies were scored as homozygous resistant, homozygous susceptible and segregating.

Marker *sun180*, which co-segregated with *Lr52* in the lower resolution map of the RIL population, mapped 0.4 and 0.6 cM to *Lr52* and *Yr47*, respectively. *Yr47* and *Lr52* mapped 0.2 cM apart (Fig. 2). Markers *sun480* and *fcp652* mapped 0.4 cM and 0.6 cM distal to *sun180*, respectively. The interval between *Yr47* and markers *gwm234* and *icg16c008* was reduced to 1.4 and 1.8 cM, respectively, in the high resolution map.

Evaluation of markers on Australian and Nordic wheats

Markers *sun180*, *sun480*, *mag705* and *fcp652*, which showed close linkage with *Yr47* and *Lr52* among the RIL population, were evaluated on 84 Australian and 120 Nordic wheat genotypes. Marker *sun180* amplified a 200 bp fragment in the resistant parent Aus28183 and a 195 bp product in the susceptible parent Aus27729. None of the cultivars contained the 200 bp allele associated *Yr47* and *Lr52* (Table 4; Fig. 3). Markers *sun480*, *mag705* and *fcp652* amplified 32 false positives in Australian and Nordic wheat cultivars indicating their loose genetic association with *Yr47* and *Lr52*. Thus, marker *sun180* proved to be diagnostic for the selection of

Table 3 KASP markers developed for SNP loci identified to be linked to *Yr47/Lr52* through selective genotyping using the iSelect 90 K wheat assay

KASP marker	iSelect 90 K SNP name	SNP	Top BLAST Hit			Primer sequence		
			Brachypodium CDS	Rice CDS	Sorghum CDS	Allele 1[a]	Allele 2[b]	Reverse
KASP_75279	tplb0060p09_735	[T/C]	*Bradi5g25610.1*	LOC_Os08g01520.1	.	cgcgagctcaccgagatcagt	gcgagctcaccgagatcagc	gggaagtagtcctccaggttgaat
KASP_12199	BS00102828_51	[T/G]				aatttcttcagaatgtgtctgttgttcatt	cttcagaatgtgtctgttgttcatg	ctatctgacgttggagtacggcat
KASP_25183	Excalibur_c32189_998	[A/G]	*Bradi3g23200.1*	LOC_Os11g28065.1	Sb01g032600.1_PACid_1953014	gaggtttctccaagaaggccgt	aggtttctccaagaaggccgc	cttaccatacatgtcgacctctgcat
KASP_1736	BobWhite_c23714_130	[A/G]	*Bradi2g27230.1*	.	Sb04g002060.1_PACid_1965108	gcccaccttgctgccctgt	cccaccttgctgccctgc	gggctttgcagagtgtcgcaatttt
KASP_8814	BS00060650_51	[A/G]	.	.	.	gtcttatttctaatttagcaatgttggtgt	cttatttctaatttagcaatgttggtgc	ccctaaaagagattagggtctgtttactt
KASP_7565	BS00024993_51	[T/C]	.	.	.	gtcaacacaagcgagtgatgtga	gtcaacacaagcgagtgatgtgg	ggaccataacatcgcccctatacta
KASP_36048	IACX5872	[A/G]	.	.	.	cgaaaaatattattagcattttcggtcacat	gaaaaatattattagcattttcggtcacac	cggtgggtataattggaatgggacat
KASP_6180	BS00010153_51	[A/G]	.	.	.	gatgctgccagttacaatttgtggat	atgctgccagttacaatttgtggac	tcagcacttgatccttcacagatttcata

[a] A1 primer labeled with FAM: GAAGGTGACCAAGTTCATGCT

[b] A2 primer labeled with HEX: GAAGGTCGGAGTCAACGGATT

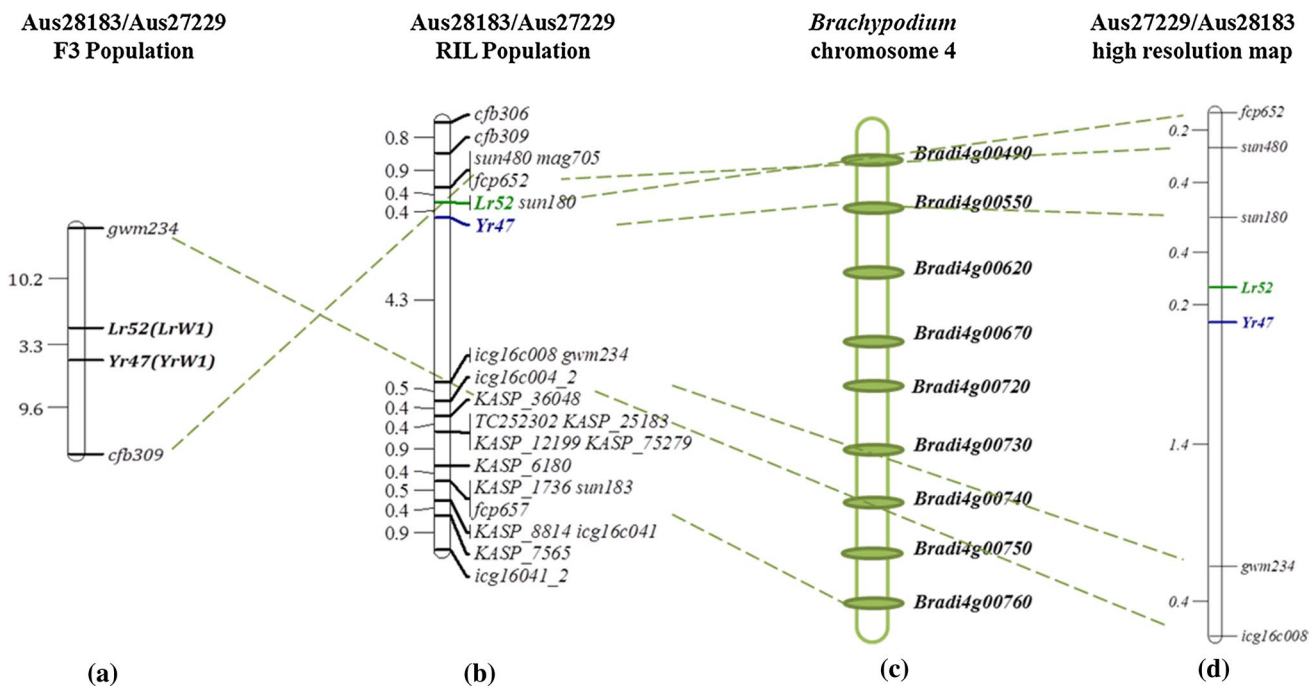

Fig. 2 Genetic linkage map of chromosome 5BS: **a** Aus28183/Aus27229 F_3 (Bansal et al. 2011) **b** Aus28183/Aus27229 F_6 RIL **c** *B. distachyon* chromosome 4 marked with significant hits with wheat survey sequences within the *Yr47*/*Lr52* interval and **d** High resolution map of Aus27229/Aus28183

Table 4 Validation of closely linked marker *sun180* on Australian and Nordic wheat genotypes

Cultivars	*sun180*
Control AUS28183 (*Lr52* and *Yr47*)	200 bp
Control AUS27229 (NIL)	195 bp
Australian wheats	
AGT Katana, Axe, Banks, Baxter, Beaufort, Batavia, Bolac, Calingiri, Carnamah, Cobra, Catalina, Chara, Corack, Correll, Crusader, Dart, Derrimut, Diamondbird, EGA Bonnie Rock, EGA Bounty, EGA Wedgetail, EGA Gregory, EGA Wylie, EGA Burke, Elmore CL PLus, Emu Rock, Envoy, Espada, Estoc, Forrest, Fortune, Gauntlet, Gazelle, GBA Sapphire, Giles, Gladius, Grenade CL Plus, Harper, Impala, Impose CL Plus, Janz, Justica CL Plus, King Rock, Kord CL Plus, Kunjin, Lancer, Lang, Lincoln, Livingston, Mace, Mackellar, Magenta, Mansfield, Merinda, Merlin, Naparoo, Orion, Phantom, Preston, Scout, SF Adagio, SF Scenario, Spitfire, SQP Revenue, Sunco, Sunguard, Sentinel, Shield, Strzelecki, Suntop, Sunvale, Sunzell, Sunvex, Trojan, Ventura, Waagan, Westonia, Wallup, Wedin, Wyalkatchem, Wylah, Yitpi, Young, Yandanooka	195 bp
Nordic Wheats	
Apu, Aros, Atson, Avle, Bastian, Batalj, Bjarne, Bjorke, Blanka, Børsum, Boru, Brons, Canon, Dacke, Dala, Dalarna, Diamant ll, Drabant, Dragon, ELS 6404 - 102 - 3, Ergo, Eroica, Extra Kolben, Fagott, Fram l, Fram ll, Fylgia l, Fylgia ll, Haarajärvi ME0102 Apu, Halland, Hildur, Holger, Horsmanaho ME201 Timantti, J-03, Järvenkylä ME0302 Timantti, JO 3524, JO 8023, Jokikylä ME0505 Apu, Kadett, Kalle, Kanzler, Kärn, Kärn ll, Kimmo, Kiuru, Kenya Farmer, Kota, Kraka, Laitiala AP0103, Landvårkveite, Lantvete från Dalarna, Lantvete från Halland, Lavett, Manu, Monola ME1301, Møystad, MS 273 - 150, Nana, Naxos, Nemares, Nora, Norrøna, østby, Pansar ll, Pansar lll, Polkka, Pompe, Pondus, Prins, Progress, Pudel, Rang, Reno, Renodlat Sammetsvete, Rida, Ring, Rival, Rollo, Rubin, Rudolf Rubin, Runar, Ruso, Saffran, Safir, Sappo, Sibirian, Skirne, Sleipner, Snøgg II, Snøgg l, Sober, Sol ll, Sol lV, Sopu, Sport, Stava, Steiner, Storvik sjundeå, Svenno, Tarso, Thule ll, Thule lll, Timantti, Timantti Paavo, Tjalve, Touko, Troll, Trym, Ulla, Vakka, Viking, Vinjett, Vitus, Walter, William, WW 20299, Zebra, Diamant, Kosack, Sigyn ll	195 bp

Yr47 and *Lr52*. Six backcross derived genotypes carrying *Yr47* and *Lr52* in the wheat cultivar Ventura background produced a 200 bp amplicon with *sun180*, demonstrating its usefulness for marker-assisted selection.

Discussion

Breeding for durable rust resistance is challenging due to continuous evolution of virulence in pathogen populations.

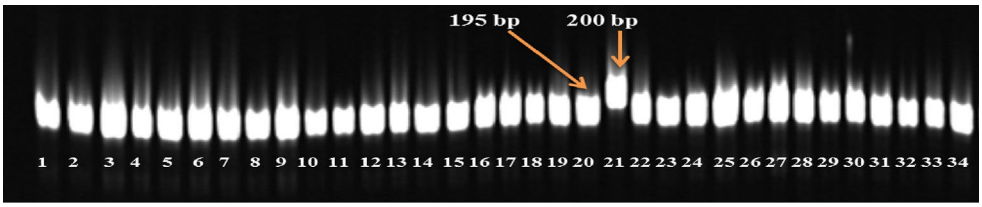

Fig. 3 Amplicons produced by Australian and Nordic cultivars along with parental lines. Lanes 1-19 Australian cultivars, 22–34 Nordic cultivars; *lane 20* susceptible parent Aus27229 producing 195 bp amplicon; *lane 21* resistant parent Aus28183 producing 200 bp amplicon with marker *sun180*

Pyramiding of two or more genes in new cultivars is essential to reduce the rate of breakdown of host resistance due to evolution of virulence in pathogen populations. This can be best achieved through the incorporation of modern molecular technologies, such as marker-assisted selection, in wheat improvement programs. Bansal et al. (2011) identified a genetic association between rust resistance genes *Yr47* and *Lr52*, with a recombination distance of 3.3 cM based on F_3 analysis. The advancement of the F_3 population to F_6 in this study reduced this distance to 0.4 cM. Likewise the genetic distance between the flanking markers *gwm234* and *cfb309* was also significantly reduced (Fig. 1b). Mapping of markers developed from various genomic resources changed the orientation of the genetic map originally reported in Bansal et al. (2011). Marker *cfb309* mapped 1.3 cM distal to *Lr52*, whereas *gwm234* mapped 4.3 cM proximal to *Yr47*. This marker order corresponds with that reported by Alfares et al. (2009), where *cfb306* was completely linked with the *Skr* gene and *gwm234* mapped 0.3 cM proximal to it. Further, high resolution mapping reduced the total genetic distance between markers *fcp652* and *icg16c008* to 3 cM, compared with 5.1 cM in the lower resolution map developed using the RIL population. The identification of recombinants in the high resolution mapping increased the genetic distance distal to *Lr52* (*sun180* which co-segregated in the low resolution map was mapped 0.4 cM distal to *Lr52*), but decreased the distance proximal to *Yr47*. Recombination breakpoints and the ratio of physical to genetic distance determines the position of target locus in the region (Dawson et al. 2016) and, therefore, the recombinants identified in this study can be used to provide a step forward for map-based cloning of *Yr47* and *Lr52*. Two high resolution mapping studies involving *Tsn1* (Lu et al. 2006) and *Snn3-B1* (Shi et al. 2016) loci on the short arm of chromosome 5B of wheat also resulted in the identification of closely linked markers.

The availability of extensive genomic resources provided critical information for saturating the *Yr47* and *Lr52* region. The Chinese Spring genomic sequences proved to be the best resource in this study for saturating the region. Markers developed from the Chinese Spring flow-sorted chromosome survey sequence contigs (IWGSC 2014) showed a higher level of polymorphism compared to markers developed from other genomic resources. The chromosome survey sequence contigs successfully identified the orthologous region in chromosome 4 of *Brachypodium distachyon* and allowed the development of 60 new SSR markers within the chromosome arm 5BS region containing *Yr47* and *Lr52*. High colinearity between the wheat and *Brachypodium* genomes in this region led to the development of closely linked marker (*sun180*) to *Yr47* and *Lr52* (supplementary Table S1 and Fig. 2c). The syntenic interval in *Brachypodium,* delineated by genes *Bradi4g00490* and *Bradi4g00550,* carries four genes with putative kinase domains. There appears to be a gap between *Bradi4g00550* and *Bradi4g00620*. While markers developed from the chromosome arm 5BS physical map and iSelect 90 K wheat SNP genotyping were useful as well, but they did not yield markers closely linked to *Yr47* and *Lr52*. The high resolution genetic linkage map constructed in this study will be useful for map-based cloning of *Yr47* and *Lr52*. The recent development of a high-quality wheat reference genome sequence combined with the availability of the physical map and BAC clones will facilitate faster determination of the physical region carrying *Yr47* and *Lr52*. For example, the nine genes in the syntenic interval in *Brachypodium* were contained in nine scaffolds of the most recent publicly released assembly of the bread wheat genome (TGACv1, http://plants.ensembl.org/) developed by The Centre for Genome Analysis (TGAC) at the Earlham Institute, the United Kingdom. The nine TGAC scaffolds had a total length of 1.273 Mbp, compared to 115 Kbp for the nine CSS contigs in which the same *Brachypodium* genes were located (supplementary Table S2). This 11-fold increase in availability of assembled genome sequence illustrates the potential for faster map-based gene cloning in the near future. Validation of markers developed from bi-parental mapping populations on diverse genotypes carrying and/or lacking the target locus is essential to demonstrate their diagnostic value in marker-assisted selection (Sharp et al. 2001; Bariana et al. 2016). The absence of the *Yr47*- and *Lr52*-linked *sun180* allele in 84 Australian

and 120 Nordic wheat cultivars demonstrated its robustness for use in marker-assisted selection of *Yr47* and *Lr52* in these genetic backgrounds. Markers linked with several widely effective rust resistance genes have been reported in the last decade and protocols are available on MASWheat website (http://maswheat.ucdavis.edu/) for use in breeding programs. The marker *sun180* is currently being used in the marker-assisted pyramiding of *Yr47* and *Lr52* with *Lr34*/*Yr18*/*Sr57*, *Lr67*/*Yr46*/*Sr55* and *Sr22* in the Australian Cereal Rust Control Program to produce triple rust resistant material based on ASR and APR genes to achieve durable rust control.

Author contribution statement NQ drafted the manuscript; HB and UB developed segregating population; NQ, HB and UB did rust phenotyping; BK, TW, JF, ES provided primer information; NQ did marker work; UB, KF and MH designed primers; UB, HB, BK, TW, JF, ES, KF and MH edited the manuscript.

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Compliance with ethical standards

Conflict of interest All authors have read the manuscript and declare that they have no conflict of interest.

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