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# Mapping of a new stripe rust resistance locus *Yr57* on chromosome 3BS of wheat

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**Abstract** A common wheat landrace, AUS27858, from the Watkins collection showed low seedling stripe rust response against Australian *Puccinia striiformis* f. sp. *tritici* pathotypes. Genetic analysis of stripe rust resistance indicated the involvement of two independent resistance loci *YrAW1* and *YrAW2*. *YrAW1* was mapped in chromosome 4AL and formally named *Yr51*. Ninety seeds of a heterozygous $F_3$ line (HSB#5474; *YrAW2yrAW2*) were grown individually to produce a segregating population referred to as single gene segregating population #5474 (SGSP#5474) to map *YrAW2*. Monogenic segregation at the *YrAW2* locus was confirmed among the SGSP#5474. *YrAW2* was located in chromosome 3BS through DArT-based bulked segregant analysis. SGSP#5474 was advanced to $F_6$ generation and was phenotyped for detailed mapping. Test of allelism with *Yr4*, previously located on chromosome 3BS, showed 5.2 ± 1.3 % recombination between *YrAW2* and *Yr4*. Since there is no other stripe rust resistance gene located in the distal part of chromosome 3BS, *YrAW2* was formally named *Yr57*. Markers *gwm389* and *BS00062676* flanked *Yr57* at genetic distances of 2.0 and 2.3 cM, proximally and distally, respectively. These markers were genotyped on a set of Australian and Indian wheat cultivars and the absence of resistance-linked alleles of *gwm389* and *BS00062676* markers was shown in cultivars known to lack *Yr57*. These markers would be useful in marker-assisted pyramiding of *Yr57* with other marker-tagged major and minor genes. The genetic stock carrying *Yr57* singly has been deposited with the Australian Winter Cereal Collection, Tamworth, Australia, and it is accessioned as AUS91463.

**Keywords** Wheat · Stripe rust · Molecular mapping · Validation of markers

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## Introduction

Wheat (*Triticum aestivum* L.) is affected by three rust diseases; stripe rust, leaf rust and stem rust. Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is currently the most damaging wheat disease worldwide. During the mid-1980s, stripe rust caused significant losses in the eastern wheat belt of Australia (Wellings 2007). An epidemic of stripe rust occurred in eastern Australia during 2003 due to a new pathotype that was first detected in Western Australia in 2002 (Wellings et al. 2003). Due to this incursion, annual crop protection costs were estimated to be A$40–90 million (Wellings 2007). Annual losses of

A$127 m due to stripe rust infection of wheat in Australia were estimated (Murray and Brennan 2009).

Stripe rust resistance is conditioned by two types of genes, often referred to as major and minor genes (Bariana 2003). Major (all stage resistance; ASR) genes confer high levels of resistance against avirulent pathotypes and are rendered ineffective by new pathotypes in a short span of time, if deployed singly. On the other hand, minor (adult plant resistance; APR) genes provide low level of resistance at the post-seedling stages and combinations of three to four APR genes are needed to condition commercially acceptable levels of resistance. These combinations are expected to be durable; however, in some crop seasons, congenial conditions and high inoculum load may result in more than the expected disease levels on genotypes carrying these combinations.

A successful breeding for resistance program dwells on the availability of genetically diverse sources of resistance and their deployment in combinations. The knowledge of pathotypic variation in the target pathogen is equally important for the positive outcome. Rust resistance genes *YrA*, *Yr6* and *Yr7* were rendered ineffective within the first decade of initial introduction of stripe rust in Australia (Wellings and McIntosh 1990). Virulence for *Yr17* was detected in Pst pathotype 104 E137A−Yr17+ during the 1999 crop season. Combinations of *Yr17* with *YrA*, *Yr6* and *Yr7* remained effective until the detection of pathotype 134 E16A+Yr17+ in 2006 (Wellings 2007). Although not present in many Australian wheat cultivars, *Yr27* remained effective until the detection of pathotype 134 E16A+J+Yr27+ in 2008 and a pathotype 134 E16A+Yr17+Yr27+ with combined virulence for *Yr17* and *Yr27* was detected in 2010. This pathotypic evolution in Pst population stressed the need for continuous discovery and deployment of effective sources of stripe rust resistance.

Digenic segregation for stripe rust response was observed in an AUS27858/Westonia $F_3$ population. Two distinct low infection types (1-NN to 2C and 0;) were observed among lines that showed monogenic segregation (Bariana and Bansal unpublished results). The locus expressing seedling response ranging from 1-NN to 2C was temporarily named *YrAW1*. Randhawa et al. (2014) located *YrAW1* in chromosome 4AL and named it *Yr51*. The second locus was temporarily named *YrAW2*. This study describes genomic location of *YrAW2* and identification of markers closely linked with it for its marker-assisted selection in breeding programs.

## Materials and methods

### Host materials

Ninety seeds from an AUS27858/Westonia-derived heterozygous (*YrAW2yrAW2*) $F_3$ family HSB#5474 were sown and 82 plants were individually harvested to produce single gene segregating population (SGSP#5474). SGSP#5474 was advanced to produce a recombinant inbred line (RIL) $F_6$ population which is referred to as RIL#5474 in the text.

A line carrying *YrAW2* was crossed with Rubric (*Yr4*) to determine genetic relationship of *YrAW2* with *Yr4*. $F_1$ plants were grown in the greenhouse to produce $F_2$ population. $F_2$ seeds were sown in the field during the 2012 crop season and 136 $F_2$ plants were successfully harvested. *YrAW2*/Rubric $F_3$ population was used for allelism studies. A set of Australian and Indian cultivars (Table 3) was used for validation of *YrAW2*-linked markers.

### Pathogen materials

The Australian Pst pathotype, 134 E16A+Yr17+ Yr27+, Plant Breeding Institute (PBI) culture number 617, was used to screen SGSP#5474 and RIL#5474 and *YrAW2*/Rubric $F_3$ populations. *YrAW2*/Rubric $F_3$ population was also tested against the pre-2002 Pst pathotype, 110 E143A+ (444). Seven Australian Pst pathotypes 134 E16A+ (572), 134 E16A+Yr17+ (599), 134 E16A+Yr17+Yr27+ (617), 110 E143A+ (444), 108 E141A+ (420), 104 E137+ (414) and 150 E16A+ (598) were used to test two resistant RILs to ascertain wider effectiveness of *YrAW2*. Virulence/avirulence formulae of Pst pathotypes were presented in Randhawa et al. (2014), except pathotype 150 E16A+. This pathotype differs from 134 E16A+ for virulence on *Yr10* and *Yr24*.

### Stripe rust tests

Twenty seeds of each $F_3$ line were sown in 9-cm pots filled with a potting mixture (pinebark and river sand in the ratio of 2:1). In the case of RILs, eight seeds of each line and four lines per pot were sown. Parents

AUS27858, Westonia and Rubric were included as controls in respective experiments. Cultivar Morocco was used as the susceptible control. Greenhouse screening procedure is described in Randhawa et al. (2014).

## DNA isolation and quantification

Total genomic DNA was isolated from seedlings of all test material following the procedure described in Bansal et al. (2014). Quantification of DNA was performed using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies). DNA dilutions of final 30 ng/µl concentration were prepared.

## Bulked segregant analysis (BSA)

Based on the phenotypic data, resistant and susceptible bulks were made by pooling equal amounts of DNA from 20 homozygous resistant (*YrAW2YrAW2*) and 20 homozygous susceptible lines (*yrAW2yrAW2*), respectively. BSA was performed using high-density DArT array Wheat *Pst*I (*Taq*I) 3 (http://www.diversityarrays.com).

## Saturation of chromosome 3BS map

Simple sequence repeat (SSR), insertion site-based polymorphism (ISBP) and expressed sequence tag (EST)-based STS markers that were previously mapped on chromosome 3BS were tested on contrasting bulks along with parents (Table 2). Primer sequences of markers were obtained from the GrainGenes 2.0 database (http://wheat.pw.usda.gov). Markers that showed repeatable polymorphisms between contrasting bulks and parents were mapped on RIL#5474 population to saturate the chromosomal region containing *YrAW2*.

In addition, 33 single-nucleotide polymorphism (SNP) markers (Wilkinson et al. 2012) surrounding the SSR marker *gwm389* in chromosome 3BS were tested on contrasting bulks and parents using competitive allele-specific polymorphism (KASP) assay. The resistance-linked SNPs were tested on whole RIL population.

## KASP genotyping

For each SNP, two allele-specific forward primers and one common reverse primer were obtained from the Cereals DB website (Wilkinson et al. 2012). The 8 µl reaction volume contained 4 µl of 2× KASP mix (KBioscience), 0.11 µl assay mix (containing 12 µM each allele-specific forward primer and 30 µM of reverse primer), 3 µl of genomic DNA (30 ng/µl) and 0.89 µl of autoclaved dd$H_2O$. KASP mix contains universal FRET (fluorescence resonance energy transfer) cassettes (FAM and HEX), ROX$^{TM}$ passive reference dye, Taq polymerase, free nucleotide and $MgCl_2$ in optimised buffer. Reactions were performed using 96-well PCR plate in Mx3005P qPCR system (Agilent technologies). The following cycling conditions were used: 15 min at 94 °C; 10 touchdown cycles of 20 s at 94 °C, 60 s at 65–57 °C (dropping 0.8 °C per cycle); and 26–35 cycles of 20 s at 94 °C, 60 s at 57 °C. Reading was taken by fluorescence detection of the reactions at 40 °C for 30 s, and the data were analysed using MxPro qPCR software. Genotyping of a test assay displays three observable genotyping groups, FAM (homozygote), HEX (homozygote) and mixed (heterozygote).

## PCR amplification and gel electrophoresis

For amplification of SSR, ISBP and STS (EST and DArT based) markers, PCR reactions were performed in T100$^{TM}$ thermal cycler (BioRad) in a volume of 10 µl reaction mixture containing 50 ng of genomic DNA according to PCR conditions described earlier (Randhawa et al. 2014).

The amplified PCR products were resolved in 2.5 % agarose (Amresco) gel stained with GelRed$^{TM}$ (Biotium) and scanned under UV gel documentation system (UVP-GelDoc-It). GeneRuler$^{TM}$ 1 Kb ladder (Fermentas) was used to determine allele sizes.

For better resolution, amplified products were run in a LICOR 4300 DNA analyser. For this, forward primers were 5′-tailed with M13 sequence (CACGACGTTGTAAAACGAC), such that the entire forward primer would look like 5′-CACGACGTTGTAAAACGAC XXXXXXXXXXXXXXX-3′, where the Xs denote the microsatellite-specific primer sequence. PCR was conducted in 10 µl reaction containing 30 ng/µl of genomic DNA, 0.02 µM dNTP, 1× Immolase PCR buffer (Bioline) containing $MgCl_2$, 0.05 µM forward primer (M13-tailed), 0.2 µM reverse primer, 0.005 µM 5′-fluorescently labelled M13 dye (M13-700 or M13-800) and 0.02 U of Immolase DNA polymerase (Bioline). Following an initial denaturing

step of 95 °C for 10 min, PCR amplification was performed for 33 cycles with the touchdown profile as explained previously.

### Polyacrylamide gel electrophoresis

Electrophoresis was carried out in the 4300 DNA Analyser (LICOR) using 6.5 % KB$^{Plus}$ gel matrix (denaturing gel containing urea and TBE). Two 28-cm-long plates were cleaned with Kim wipes using 70 % ethanol solution. Twenty-five microlitres of bind silane (50 µl of bind silane to 10 ml of 100 % ethanol) was mixed with 25 µl of 10 % acetic acid and resultant solution was applied using cotton swab to area below the edge of notched plate where wells will form. Plates were kept horizontally for 5 min to air dry. KB$^{Plus}$ (6.5 %) gel matrix (20 ml) was mixed with 150 µl of APS (10 % w/v) and 25 µl of TEMED. The resultant gel solution was poured into the sandwich of plates using 60-cc syringe. Comb was fitted onto the notched area and plates were tight clamped. Gel was kept for 30 min to polymerise. Comb was pulled out slowly to form nice wells. Gel was pre-run for 30 min using Saga Lite Electrophoresis software. Standard KB$^{Plus}$ buffer (1×) containing TBE was used as running buffer. Ten microlitres of gel loading dye (98 % formamide, 10 mM EDTA pH 8.0 and 0.5 % basic fuchsin as tracking dye) was added to the PCR product. PCR product was denatured at 95 °C for 5 min in thermocycler, immediately put on ice and covered to avoid exposure to light. Samples were loaded into wells using 8-channel 0.2-mm Hamilton syringe. Gel was run for 1–2 h depending upon size of the amplified product.

### Statistical analyses and genetic mapping

To establish the goodness of fit of observed segregation with the expected genetic ratios, 1:2:1 and 1:1 in $F_3$ and RIL populations, respectively, and to detect marker–trait linkages, Chi-squared tests were performed. The genotypic status of each RIL with respect to the resistance gene under study was deduced from the seedling stripe rust response data. MAP MANAGER version QTXb20 (Manly et al. 2001) was used to calculate recombination fractions. Kosambi (1943) mapping function was used to convert recombination fractions into centimorgans (cM). Logarithm of odds (LOD) score of 3.0 was used to determine significance of genetic linkages between molecular markers and the resistance locus. For a visual inspection of map order, maps were constructed and aligned using MapChart software (Voorrips 2002).

## Results

### Inheritance studies

The SGSP#5474 was tested at the seedling stage against the Pst pathotype 134 E16A+Yr17+Yr27+ and was classified into three categories, namely homozygous resistant (*YrAW2YrAW2*—IT0;), segregating (*YrAW2yrAW2*—IT0; and IT3+) and homozygous susceptible (*yrAW2yrAW2*—IT3+). Single gene segregation at the *YrAW2* locus was observed among the SGSP#5474 (Table 1). The RIL#5474 population was tested at the seedling stage and RILs were classified as homozygous resistant (IT0;) and homozygous susceptible (IT3+). Monogenic segregation for stripe rust response variation at the *YrAW2* locus was confirmed among the RIL#5474 population (Table 1).

Two homozygous resistant (*YrAW2YrAW2*) RILs were tested against seven Pst pathotypes to assess effectiveness of *YrAW2* against a range of virulence combinations. Both resistant RILs produced IT 0 to 0; against all seven Pst pathotypes (Fig. 1) indicating the broad effectiveness of *YrAW2*.

**Table 1** Frequency distribution of AUS27858/Westonia-derived SGSP#5474 and RIL#5474 populations when tested against Pst pathotype 134 E16A+Yr17+Yr27+ at the seedling stage

| Genotype | Number of families | | $\chi^2_{(1:2:1)}$ |
|---|---|---|---|
| | Observed | Expected | |
| SGSP#5474 | | | |
| *YrAW2YrAW2* | 22 | 20.5 | 0.11 |
| *YrAW2yrAW2* | 42 | 41 | 0.02 |
| *yrAW2yrAW2* | 18 | 20.5 | 0.30 |
| Total | 82 | 82 | 0.43 |
| RIL#5474 population | | | $\chi^2_{(1:1)}$ |
| *YrAW2YrAW2* | 38 | 39.5 | 0.57 |
| *yrAW2yrAW2* | 41 | 39.5 | 0.57 |
| Total | 79 | 79 | 0.114 |

Table value of $\chi^2$ at $P = 0.05$ and 1 *d.f.* = 3.84 and at $P = 0.05$ and 2 *d.f.* = 5.99

**Table 2** List of different markers used for saturation of chromosome 3BS map

| Marker type | Marker name | Reference |
|---|---|---|
| *SSR* | *gwm389*, *gwm493*, *gwm533* | Röder et al. (1998) |
| | *gpw8100*, *cfb3530* | Sourdille et al. (2004) |
| | *wmc430* | Somers et al. (2004) |
| | *barc12*, *barc17*, *barc75*, *barc87*, *barc180*, *barc238* | Song et al. (2005) |
| | *cfb5006*, *cfb5007*, *cfb5010*, *cfb5025*, *swm13* | Diéguez et al. (2014) |
| ISBP | *cfp37*, *cfp54*, *cfp55*, *cfp132*, *cfp140*, *cfp3131*, *cfp3132*, *cfp3296* | Paux et al. (2006, 2008) |
| EST-STS | *mag2095* | Xue et al. (2008) |
| | *sts3B15*, *sts3B1.1* | Liu and Anderson (2003) |
| DArT-STS | *sun159* (*wPt-342*), *sun163* (*wPt-733477*), *sun164* (*wPt-734202*), *sun160* (*wPt-6043*), *sun165* (*wPt-742222*), *sun166* (*wPt-744251*) | Diversity Arrays Technology |
| SNP | *BS00004074*, *BS00011438*, *BS00003814*, *BS00010844*, *BS00009440*, *BS00009992*, *BS00010945*, *BS00012316*, *BS00021849*, *BS00062676*, *BS00012531*, *BS00011532*, *BS00011373*, *BS00011806*, *BS00009476*, *BS00010849*, *BS00012127*, *BS00022190*, *BS00032912*, *BS00067242*, *BS00007446*, *BS00079522*, *BS00009393*, *BS00022154*, *BS00022971*, *BS00033209*, *BS00100706*, *BS00003596*, *BS00001335*, *BS00064778*, *BS00056327*, *BS00056684*, *BS00071225* | Wilkinson et al. (2012) |

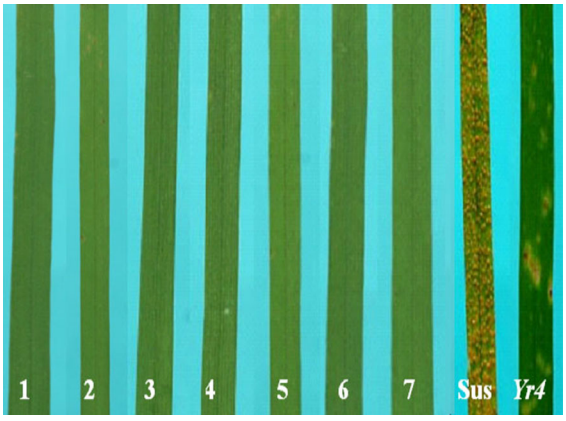


**Fig. 1** Infection types produced by a homozygous resistant line carrying *Yr57* against Pst pathotypes. *1* 104 E137A+, *2* 108 E141A+, *3* 110 E143A+, *4* 134 E16A+, *5* 134 E16A+Yr17+, *6* 134 E16A+Yr17+Yr27+, *7* 150 E16A+, *Sus* Susceptible control (Morocco) and *Yr4* Rubric with 134 E16A+Yr17+ Yr27+

## Molecular mapping

### *Chromosomal location of YrAW2*

Six chromosome 3BS located DArT markers showed association with *YrAW2* in BSA (Table 3). The sequences of linked DArT clones were obtained from the Diversity Array Technology Pty Ltd, Australia, and were used to design sequence tagged site (STS) markers *sun159* (*wPt-342*), *sun160* (*wPt-6043*), *sun163* (*wPt-733477*), *sun164* (*wPt-734202*), *sun165* (*wPt-742222*) and *sun166* (*wPt-744251*). These markers were tested on contrasting bulks and parents. Marker *sun163* showed polymorphism between parents and contrasting bulks and was subsequently tested on the entire RIL#5474. Marker *sun163* amplified product only in one of the parents indicating its dominant nature and was mapped 3.9 cM away from *YrAW2*. These results placed *YrAW2* in chromosome 3BS. Primer sequences of marker *sun163* are: forward (5′GGTCGTGGCGTTTAAAGAAA3′) and reverse (3′CAACCAGTGCTATCCCACCT5′).

### *Saturation of 3BS map*

Thirty-four markers [SSR, ISBP and STS (EST and DArT based)] were used to saturate the *YrAW2* carrying region of the chromosome 3BS and 11 markers were polymorphic among resistant and susceptible bulks. Out of 33 SNPs tested, six showed polymorphism between parents and contrasting bulks. The linked and polymorphic markers were mapped on the entire RIL#5474 population, and a linkage map of 17 markers (one DArT-STS, nine SSR, one EST-STS, six SNP markers) and *YrAW2* was constructed (Fig. 2). The linkage map spanned over a total genetic

**Table 3** Validation of *Yr57*-linked markers *gwm389* and *BS00062676* on modern wheat cultivars

| Cultivars/RIL | *gwm389* (bp) | *BS00062676* (SNP allele) |
|---|---|---|
| AUS27858 | 150 | A |
| Westonia | 145 | G |
| Australian cultivars | | |
| Camm, Calingiri, Derrimut, Diamondbird, EGA Bonnie Rock, EGA Gregory, Giles, H45, Hartog, Livingston, Mace | 145 | G |
| Janz | 147 | G |
| Carnamah, Sunlin, Sunzell | 155 | G |
| Sunsoft 98, Gladius | 160 | G |
| Kelalac | Null | G |
| Indian cultivars | | |
| PBW343, PBW550, HD2733 | 145 | G |
| DBW17 | 147 | G |

distance of 31.8 cM. Markers *gwm389* and *BS00062676* flanked *YrAW2* at genetic distances of 2.0 cM and 2.3 cM, proximally and distally, respectively (Fig. 2).

*Test of allelism*

Stripe rust resistance gene *YrRub* (*Yr4*) was previously mapped on chromosome 3BS of Rubric by Bansal et al. (2010). In order to ascertain the uniqueness of *YrAW2*, a RIL#5474-3 was crossed with Rubric and $F_3$ population was developed. RIL#5474-3/Rubric $F_3$ population was tested against a doubly avirulent Pst pathotype 134 E16A+Yr17+Yr27+ and *Yr4*-virulent pathotype 110 E143A+. RIL#5474-3 and Rubric produced IT0; and IT;C, respectively, (Fig. 1) against pathotype 134E16A+Yr17+Yr27+. Rubric was susceptible against 110 E143A+. No $F_3$ line produced homozygous susceptible response (IT3+) indicating close repulsion linkage between *YrAW2* and *Yr4*. Fifteen recombinant genotypes were observed among 146 $F_3$ lines, and a recombination value of $5.2 \pm 1.3$ % was calculated using the maximum-likelihood method of Allard (1956). Tests with *Yr4*-virulent pathotype 110 E143A+ enabled confirmation of genotypic status *YrAW2* carrying lines. The differential responses of *YrAW2* and *Yr4* against Pst pathotypes 134 E16A+Yr17+Yr27+ and 110 E143A+ and recombination between these loci

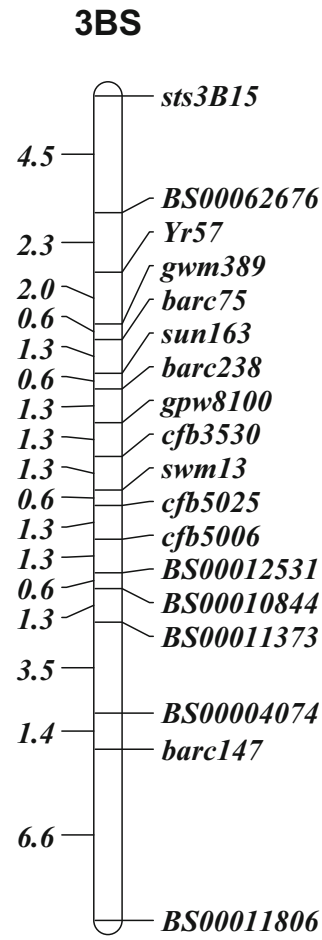


**Fig. 2** Genetic linkage map of chromosome 3BS showing location of stripe rust resistance gene *Yr57*

confirmed the unique identity of *YrAW2* and hence it was permanently named *Yr57*.

*Validation of Yr57-linked markers*

Closely linked markers *gwm389* and *BS00062676* were genotyped on a set of 19 Australian and four Indian wheat cultivars that do not carry *Yr57* (Table 3). Marker *gwm389* amplified 150 bp product in the resistant parent AUS27858 and 145 bp in the susceptible parent Westonia. All test cultivars carried alleles of different sizes ranging from 145 to 160 bp; the *Yr57*-linked 150 bp allele was, however, not observed in any of these cultivars. The distally mapped SNP marker *BS00062676* amplified *Yr57*-linked 'A' allele in AUS27858 and *Yr57* carrying resistant RIL, whereas all test cultivars carried the 'G'

allele for this marker. Thus, markers *gwm389* and *BS00062676* can be used for marker-assisted selection of this gene in different backgrounds.

## Discussion

This study located a previously uncharacterised stripe rust resistance gene *Yr57* on chromosome 3BS. Tests on RIL#5474-3 with pre- and post-2002 Pst pathotypes demonstrated broad effectiveness of *Yr57*. *Yr57* showed close repulsion linkage with *Yr4*, previously located in this chromosome. Genetic linkages among rust resistance genes located in the same chromosome are not uncommon in wheat. Ren et al. (2012) reported linkage distance of 36.5 ± 6.8 cM between *Yr52* and *Yr39* on chromosome 7BL based on allelic tests. Zhou et al. (2014) estimated genetic distance of 12.3 cM between *YrC591* (Li et al. 2009) and *Yr59*. Singh et al. (2001) reported close repulsion linkage (2.4 ± 0.5 cM) between *Lr17b* and *Lr37*. Knowledge of genetic relationships among rust resistance genes located in the same chromosome is important for their pyramiding with other resistance genes and genes conditioning other agronomic/economic traits.

Different marker technologies [SSR, ISBP, SNP and STS (EST and DArT based)] were used to enrich the chromosome 3BS map and markers linked with *Yr57* were identified. These included the SSR marker *gwm389* and the SNP marker *BS00062676* (Fig. 2). Robustness of proximal marker *gwm389* and distal marker *BS00062676* for marker-assisted selection of *Yr57* was demonstrated through the absence of resistance-linked alleles of these loci in 23 common wheat cultivars known to lack this gene. Markers for stripe rust resistance genes *Yr15* (Zakari et al. 2003) and *Yr51* (Randhawa et al. 2014), that are currently effective against all Australian Pst pathotypes, have been reported and validated across diverse genetic backgrounds. Similarly, markers for APR gene *Yr18* (Lagudah et al. 2006) and *Yr36* (Uauy et al. 2005) are also available. Markers *gwm389* and *BS00062676* can be used for marker-assisted pyramiding of *Yr57* with the above-mentioned seedling and APR genes in new wheat cultivars to achieve durable stripe rust control. Use of parents that carry marker-tagged resistance/other economic traits would make the marker-assisted selection more economical.

*Yr47* (Bansal et al. 2011) and *Yr51* (Randhawa et al. 2014) were previously identified from the Watkins collection, and *Yr57* adds to the contribution of this collection in providing new genetic variation for stripe rust resistance. Six more genotypes are currently being investigated for potentially new sources of seedling resistance to stripe rust (Bariana and co-workers unpublished results). These observations demonstrate the value of this historic collection in the identification of yet uncharacterised sources of resistance. Chromosomal location of new sources of resistance through monosomic analysis in the last century was very slow in comparison to the twenty-first century molecular genotyping technologies. The 90 K SNP map is now available for wheat (Wang et al. 2014), and SNPs from this region will be used to fine map the *Yr57*-carrying chromosome 3BS region with the eventual aim of molecular cloning of this locus.

Chromosome 3BS also carries durable stem rust resistance gene *Sr2* (McIntosh et al. 1995). Based on comparative mapping distances (data not shown), *Yr57* maps more than 5 cM distal to the marker *gwm533* that is very closely linked with *Sr2* (Spielmeyer et al. 2003). These estimates suggest that *Yr57* and *Sr2* can be combined. This project is part of the Australia–India collaboration and therefore *Yr57* is currently being backcrossed into Australian and Indian wheat cultivars through marker-assisted selection. Recurrent parents carry marker-tagged stem rust and leaf rust resistance genes. Care will be taken to select triple rust resistant backcross derivatives for use as donors in wheat breeding programs in Australia, India and elsewhere. Seed of genetic stock carrying *Yr57* singly, accessioned as AUS91463, is available from the Australian Winter Cereal Collection, Tamworth.

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