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ORIGINAL PAPER

Molecular mapping of stripe rust resistance gene *Yr51* in chromosome 4AL of wheat

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Abstract
Key message **This manuscript describes the chromosomal location of a new source of stripe rust resistance in wheat. DNA markers closely linked with the resistance locus were identified and validated.**
Abstract A wheat landrace, AUS27858, from the Watkins collection showed high levels of resistance against Australian pathotypes of *Puccinia striiformis* f. sp. *tritici*. It was reported to carry two genes for stripe rust resistance, tentatively named *YrAW1* and *YrAW2*. One hundred seeds of an F3 line (HSB#5515; *YrAW1yrAW1*) that showed monogenic segregation for stripe rust response were sown and harvested individually to generate monogenically segregating population (MSP) #5515. Stripe rust response variation in MSP#5515 conformed to segregation at a single locus. Bulked segregant analysis using high-throughput DArT markers placed *YrAW1* in chromosome 4AL. MSP#5515 was advanced to F6 and phenotyped for detailed mapping. Novel wheat genomic resources including chromosome-specific sequence and genome zipper were employed to develop markers specific for the long arm of chromosome 4A. These markers were used for further saturation of the *YrAW1* carrying region. *YrAW1* was delimited by 3.7 cM between markers *owm45F3R3* and *sun104*. Since there was no other stripe rust resistance gene located in chromosome 4AL, *YrAW1* was formally named *Yr51*. Reference stock for *Yr51* was lodged at the Australian Winter Cereal Collection, Tamworth, Australia and it was accessioned as AUS91456. Marker *sun104* was genotyped on a set of Australian and Indian wheat cultivars and was shown to lack the resistance-linked *sun104*-225 bp allele. Marker *sun104* is currently being used for marker-assisted backcrossing of *Yr51* in Australian and Indian wheat backgrounds.

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Introduction

Global wheat production is affected significantly by stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst). The historic breakdown of stripe rust resistance gene combination *Yr9* and *Yr27* in the 'Veery' derivatives alarmed wheat-growing nations. Even though more than 50 stripe rust resistance genes have been identified in wheat (McIntosh et al. 2011), virulent races of the pathogen continue to emerge rapidly to overcome resistance genes. Stripe rust resistance can be classified as all stage resistance (ASR) or adult plant resistance (APR) on the basis of their expression at different growth stages. Various terms have been used to describe these two types of resistance (Bariana 2003).

Deployment of ASR genes singly does not often provide durable resistance due to the emergence of virulence in pathogen populations. Pyramiding of two or more genes in a single genotype can be difficult using conventional selection system based on bioassays, especially in the event of resistance genes expressing similar infection types and absence of epistatic interactions. Recent developments in molecular biology have provided

phenotype neutral selection technology based on marker-trait associations.

Identification of markers closely linked with disease resistance genes has progressed in the last decade through the development of high-throughput and cost-effective genotyping facilities. One of the first high-throughput platforms in wheat, diversity arrays technology (DArT), exploits independent chip hybridization of genome representation for diversity assessment of tested genomes and can test hundreds to thousands of genomic loci in parallel (Jaccoud et al. 2001; Akbari et al. 2006). This approach can be more efficient using high-throughput next-generation sequencing (NGS) platforms for genome representations sequencing referred to as genotyping-by-sequencing (GBS) and can identify several hundred thousand genome tags (Poland et al. 2012). Another approach includes the use of advances in wheat genome sequencing and NGS technologies to develop SNP chips for wheat with 9,000 sequences of wheat transcriptome and with 92,000 markers from the wheat genome sequence (E. Akhunov personal communication, http://wheat.pw.usda.gov/ggpages/9K_assay_available.html). All these technologies individually or in combination can be used to fine map the gene of interest.

Wheat landraces are valuable sources of genetic diversity for resistance to biotic and abiotic stresses. A common wheat landrace, AUS27858, was observed to be resistant against a range of Australian Pst pathotypes both under the greenhouse and field conditions. It was demonstrated to carry two genes for seedling resistance based on analysis of AUS27858/Westonia F3 population (Bariana and Bansal unpublished results). F3 lines segregating at a single locus were identified, and monogenically segregating populations (MSPs) were developed. Stripe rust resistance genes were temporarily named *YrAW1* and *YrAW2*. This investigation was planned to determine chromosomal location of *YrAW1*.

Materials and methods

Host materials

One hundred seeds from the F3 family HSB#5515 (*YrAW-1yrAW1*) were grown and harvested individually to develop monogenically segregating population MSP#5515. A recombinant inbred line (RIL) F6 population (89 lines) was subsequently developed from MSP#5515.

Pathogen material

Pst pathotype, 134 E16A+Yr17+Yr27+ (culture number 617), was used for testing MSP#5515 and F6 RIL population. Two resistant and two susceptible RILs were also tested against six Australian Pst pathotypes 134 E16A+ (572), 134 E16A+Yr17+ (599), 134 E16A+Yr17+Yr27+ (617), 110 E143A+ (444), 108 E141A+ (420), and 104 E137+ (414). Avirulence/virulence formulae of Pst pathotypes used are presented in Table 1.

Greenhouse screening

Twenty seeds of each F3 line were sown in 9-cm pots filled with a mixture of pine bark and river sand in the ratio of 2:1. In the case of RILs, six seeds of each line and four lines per pot were sown. Parents AUS27858 and Westonia were included as controls. Ten grams of water-soluble fertilizer Aquasol® was dissolved in 10 l of tap water and applied to 100 pots. A single application of nitrogenous fertilizer urea was applied at the same rate as Aquasol® to 7-day-old seedlings.

Twelve-day-old seedlings (two leaf stage) were inoculated by atomising Pst pathotype 134 E16A+Yr17+Yr27+ urediniospores suspended in light mineral oil (Isopar L) using a hydrocarbon propellant pressure pack. Inoculated

Table 1 Virulence/avirulence formulae of Pst pathotypes used

Pst pathotype	Culture no.	Virulnce/avirulence formulae
104 E137A+	414	*Yr2*, *Yr3*, *Yr4*, *Yr34/Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr33*, *Yr35*, *Yr36*, *Yr37*, *Yr47*, *YrA*, *YrSp*
108 E141A+	420	*Yr2*, *Yr3*, *Yr4*, *Yr6*, *YrSD*, *YrSu*, *YrND*, *YrA*, *Yr34/Yr1*, *Yr5*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr33*, *Yr35*, *Yr36*, *Yr37*, *Yr47*, *YrA*, *YrSp*
110 E143A+	444	*Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *YrSD*, *YrSu*, *YrND*, *YrA*, *Yr34/Yr1*, *Yr5*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr33*, *Yr35*, *Yr36*, *Yr37*, *Yr47*, *YrA*, *YrSp*
134 E16A+	572	*Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *YrA/Yr1*, *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr33*, *Yr34*, *Yr35*, *Yr36*, *Yr37*, *Yr47*, *YrSp*
134 E16A+Yr17+	599	*Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *YrA/Yr1*, *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr27*, *Yr32*, *Yr33*, *Yr34*, *Yr35*, *Yr36*, *Yr37*, *Yr47*, *YrSp*
134 E16A+Yr17+Yr27+	617	*Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *YrA/Yr1*, *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr27*, *Yr32*, *Yr33*, *Yr34*, *Yr47*, *YrSp*

seedlings were incubated at 9–12 °C for 24 h on trolleys covered with polythene hoods to provide 100 % humidity in a temperature controlled cool room. Inoculated seedlings were then moved to a microclimate growth room maintained at 17 ± 2 °C. Seedling responses were scored on a 0–4 scale as described in Bariana and McIntosh (1993).

Molecular mapping

DNA isolation and quantification

Genomic DNA was isolated from seedlings of MSP#5515, MSP#5515-derived F6 RIL population and parents AUS27858 and Westonia following the procedure described on the Diversity Arrays Technology (DArT) Pty. Ltd. website (http://www.diversityarrays.com). DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies). DNA dilutions with final concentration of 50 ng/μl were prepared.

Bulked segregant analysis

Bulked segregant analysis (BSA) was performed to establish the genomic location of *YrAW1* in MSP#5515. Equal amounts of DNA from 20 homozygous resistant and 20 homozygous susceptible lines were bulked together to constitute resistant and susceptible bulks, respectively. High-density DArT array Wheat *Pst*I (*Taq*I) 3 (http://www.diversityarrays.com) was used for BSA.

Saturation of chromosome 4AL map

Fourteen simple sequence repeat (SSR) markers (*gpw356*, *gpw1142*, *gpw2139*, *gpw3030*, *gpw4153*, *gpw5095*, *gpw7051*, *barc52*, *barc78*, *barc153*, *barc1172*, *gwm160*, *gwm350* and *cfd31*) mapped previously in chromosome 4AL (Somers et al. 2004; Sourdille et al. 2004) were used to saturate the *YrAW1* carrying genomic region. Primer sequences of SSR markers were obtained from the GrainGenes 2.0 database (http://wheat.pw.usda.gov).

A set of 24 expressed sequenced tags (ESTs) were selected from the 4AL4-0.80-1.00 deletion bin (http://wheat.pw.usda.gov/cgi-bin/weSTSql/map_locus.cgi). ESTs amplifying 4AL-specific alleles were selected by comparing amplification profile images of each EST. Forty-four EST-based sequence tagged site (eSTS) markers were designed from selected ESTs using Primer3 software (http://frodo.wi.mit.edu/) and tested on resistant and susceptible bulks together with parents. Resistance-linked markers were subsequently genotyped on the entire RIL population to generate linkage map. In addition, 136 gene-based markers (Xue et al. 2008; Jakobson et al. 2012) mapped in chromosome 4AL were also tested on bulks and parents. Polymorphic markers were genotyped on RIL population.

To further saturate the region using more targeted approach, we used 454 survey sequence of chromosome 4A and virtual ordering of identified coding sequences using synteny with barley EST map and genomic sequences of rice, *Brachypodium* and *Sorghum*—the 4A genome zipper (Hernandez et al. 2012). Additionally, during the construction of physical map of the *QPm.tut-4A* gene region, the *gwm160* locus was anchored to the zipper (Jakobson et al. 2012 and unpublished data) using sequences of *psr160*, *psr119*, and *cdo454* markers (GrainGenes 2.0, http://wheat.pw.usda.gov) which are flanking the region (Paillard et al. 2003). For example, on collinear rice chromosome 6 (R6), the region encompasses 0.74 Mb and on the 4AL zipper it contains 89 genes. Twenty-eight of these genes were selected for marker development to cover the *Yr51* region. Homologous wheat 4AL sequence scaffolds from the 4A survey sequence were selected using genes from the zipper syntenic region. The selected scaffolds were annotated and used to develop a set of primers using exon–exon and exon-intergenic sequence approach to enhance chromosome specificity of resultant markers (unpublished). Primers were designed using Primer3 software (http://frodo.wi.mit.edu/) and the markers were designated as "sun" (Sydney University) and "owm" (Olomouc Wheat Marker).

PCR amplification

For PCR amplification of SSR and sequence tagged site STS markers, assays were performed in 10-μl reaction mixture containing 0.2-mM dNTPs, 1× Immolase PCR buffer (Bioline), 0.2 mM each of forward and reverse primer, 50 ng of genomic DNA, and 0.2 U of Immolase DNA polymerase (Bioline). Following an initial denaturing step of 95 °C for 10 min, PCR amplifications were performed for 40 cycles with the touchdown profile: 30 s at 92 °C, 30 s at 65 °C, and 30 s at 72 °C. Following the first cycle, the annealing temperature was reduced by 1 °C per cycle for the next five cycles. A final extension step at 72 °C for 7 min was performed.

The amplified PCR products were resolved in 2.5 % agarose (Amresco) gel stained with GelRed™ (Biotium) and scanned under UV gel documentation system (UVP-GelDoc-It). GeneRuler™ 1-Kb ladder (Fermentas) was used to determine allele sizes. Markers that did not show polymorphism on agarose gels were resolved in 8 % denaturing gel [19:1 (acrylamide: bis-acrylamide solution), 1× TBE, 8 M Urea], stained with 1× solution of SYBR®Gold (Invitrogen) in ddH_2O, and visualized in UV gel documentation system. The Quick-Load® 50-bp DNA ladder (New England Biolabs) was used to determine allele sizes.

Data analyses and genetic mapping

Chi-squared analyses were performed to determine the goodness-of-fit of observed segregation with the expected genetic ratios (1:2:1 and 1:1 in F_3 and RIL population, respectively) and to detect marker-trait linkages. The genotypic status of each RIL with respect to the resistance gene under study was deduced from seedling stripe rust response data. Recombination fractions were calculated with the MAP MANAGER version QTXb20 (Manly et al. 2001) and converted to centimorgans (cM) using the Kosambi mapping function (Kosambi 1944). Logarithm of odds (LOD) score of 3.0 was used to determine significance of genetic linkages. MapChart software (Voorrips 2002) was used to construct and align three genetic maps for a visual inspection of map order.

Results

Inheritance studies

YrAW1 produced infection type (IT) ;1-nn and a relatively higher IT2C was observed in some experiments. The MSP#5515 was tested at the seedling stage against the Pst pathotype 134E16A+Yr17+Yr27+. It was classified into three categories, namely: homozygous resistant (;1-nn), segregating (;1-n, 3+), and homozygous susceptible (3+). Monogenic segregation of *YrAW1* in MSP#5515 was confirmed (Table 2). The segregating families (*Yr51yr51*) included a low proportion of resistant individuals indicating the recessive mode of inheritance of resistance. The susceptibility of F1 plants from crosses of *YrAW1* stock (AUS91456) with susceptible cultivars confirmed the recessive nature of this gene. MSP#5515-derived RIL population was tested at the seedling stage, and RILs were classified as homozygous resistant (;1-n) and homozygous susceptible (3+). Chi-squared analysis of stripe rust response variation conformed to single gene ratio (Table 2).

Table 2 Frequency distribution of AUS27858/Westonia-derived MSP#5515 and MSP#5515-derived RIL population when tested against Pst pathotype 134 E16A+Yr17+Yr27+ at the seedling stage

Genotype	Number of families		$\chi^2_{(1:2:1)}$
	Observed	Expected	
MSP#5515			
YrAW1YrAW1	24	22	0.18
YrAW1yrAW1	43	44	0.02
yrAW1yrAW1	21	22	0.05
Total	88	88	0.25
MSP#5515-derived RIL population			$\chi^2_{(1:1)}$
YrAW1YrAW1	42	42.5	0.006
yrAW1yrAW1	43	42.5	0.006
Total	85	85	0.012

Table value of $\chi^2_{(1:2:1)}$ at $P = 0.05$ and 2df = 5.99 and $\chi^2_{(1:1)}$ at $P = 0.05$ and 1df = 3.84

Multi-pathotype tests

Resistant RILs (*YrAW1YrAW1*) produced IT ;n–;1-nn and susceptible RILs (*yrAW1yrAW1*) produced IT 3+ against six Pst pathotypes 134 E16A+, 134 E16A+Yr17+, 134 E16A+Yr17+Yr27+, 110 E143A+, 108 E141A+, and 104 E137+ (Fig. 1). These results supported the effectiveness of *YrAW1* against a range of Australian Pst pathotypes carrying virulence for stripe rust resistance genes present in the global wheat germplasm.

Molecular mapping

Chromosome location of ***YrAW1***

DArT markers based BSA identified association of 14 DArT markers with *YrAW1* in the long arm of chromosome 4A. List of linked DArT markers and their map locations on the consensus DArT map (Diversity Array Technology Pty Ltd, Australia, personal communication) are given in Table 3. Linked DArT markers were converted into STS

Fig. 1 Infection types produced by a homozygous resistant line carrying *Yr51* with Pst pathotypes *1*) 134 E16A+, *2*) 134 E16A+Yr17+, *3*) 134 E16A+Yr17+Yr27+, *4*) 110 E143A+, *5*) 108 E141A+, and *6*) 104 E137A+ and the susceptible control Morocco

Table 3 List of STS markers derived from DArT clone sequences and their locations on DArT consensus map (Diversity Array Technology Pty Ltd, Australia, personal communication)

STS markers	DArT clones	DArT consensus map (cM)
sun108	*wPt-5003*	87.86
sun105	*wPt-3795*	87.86
sun103	*wPt-0150*	99.79
sun111	*wPt-6966*	100.32
sun106	*wPt-4487*	102.74
sun102	*rPt-7987*	102.74
sun112	*wPt-731166*	103.08
sun114	*wPt-742051*	103.09
sun109	*wPt-5172*	104.60
sun107	*wPt-4620*	104.60
sun104	*wPt-763*	104.60
sun101	*rPt-0238*	104.60
sun110	*wPt-6176*	105.83
sun113	*wPt-731374*	106.90

markers (*sun101*, *sun102*, *sun103*, *sun104*, *sun105*, *sun106*, *sun107*, *sun108*, *sun109*, *sun110*, *sun111*, *sun112*, *sun111* and *sun114*) and tested on contrasting bulks and parents. Two STS markers (*sun104* and *sun106*) that generated repeatable polymorphisms between parents and contrasting bulks were tested on the entire MSP#5515 RIL population. Both STS markers behaved as dominant markers and amplified products in one of the parents only. Marker *sun106* did not amplify any product in AUS27858, and on the other hand parent Westonia was null for marker *sun104*. Markers *sun104* and *sun106* were mapped 2.5 cM and 1.8 cM distal and proximal to *YrAW1*, respectively. These results confirmed the location of *YrAW1* in chromosome 4AL. Since there was no other stripe rust resistance located in chromosome 4AL, *YrAW1* was formally named *Yr51*.

Saturation of 4AL map

Simple sequence repeat, eSTS, and gene-based markers were used to saturate the *Yr51* carrying region of chromosome 4AL. Of 14 SSR markers tested, five markers *gpw7051*, *gwm855*, *gwm160*, *mag3273* and *barc78* were mapped distal to *Yr51* (Fig. 2). Four eSTS markers (*sun139*, *sun140*, *sun154*, and *sun155*) flanked *Yr51*. The markers *sun139* and *sun140* were mapped 8.2-cM distal to *Yr51*, and *sun154* and *sun155* were mapped at 1.8-cM proximal to *Yr51* (Fig. 2).

Of 136 gene-based markers, six showed polymorphism between parents and contrasting bulks. These markers were genotyped on the entire MSP#5515 RIL population. Marker *owm45F3R3* mapped 1.2-cM proximal to *Yr51*.

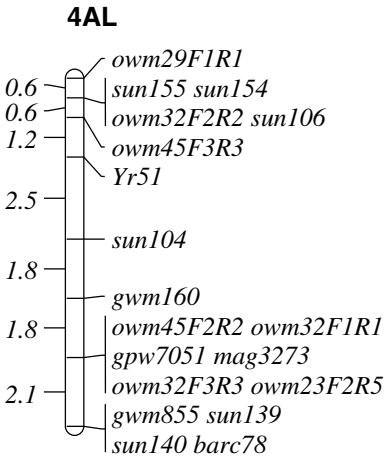

Fig. 2 Genetic linkage map of chromosome 4AL showing location of stripe rust resistance gene *Yr51* based on DArT-derived STS, SSR, eSTS and the 4AL zipper-derived *owm* markers in the MSP#5515 RIL population

A linkage map consisting of 18 markers (2 STS, 4 eSTS, 5 SSR, and 7 gene-based markers) was constructed using phenotypic and genotypic data of MSP#5515 RIL population (Fig. 2). The sequences of markers (except SSR) used in the linkage map are given in Table 4. The linkage map spanned over a total genetic distance of 10.6 cM.

Validation of *Yr51*-linked markers

Since *Yr51* is not present in modern wheat genotypes, positive validation was not feasible. Markers *owm45F3R3* and *sun104* were genotyped on a set of 27 Australian and 13 Indian wheat lines to check the absence of *Yr51*-linked alleles of these markers, often referred to as negative validation (Table 5). Marker *sun104* amplified 225 bp in resistant parent AUS27858 and null in susceptible parent Westonia. All test cultivars did not amplify the *Yr51*-linked 225 bp allele indicating the usefulness of this marker in marker-assisted selection of this gene in these backgrounds. We did not get meaningful results with marker *owm45F3R3*, presumably due to differences in chromosomal rearrangements in this region. Therefore, *sun104* can be used for marker-assisted selection of *Yr51* in wheat genotypes lacking the resistance-linked 225-bp allele.

Discussion

Intensive cereal improvement and global spread of elite wheat germplasm led to a decrease in genetic diversity (Feuillet et al. 2008). To replenish the gene pool of modern varieties, landraces and uncultivated wheat relatives can serve as a valuable source of genetic variation. The

Table 4 Primers polymorphic in the *Yr51* region and designed in this study using different genomic resources

Marker	Forward sequence	Reverse sequence
Gene-based markers		
owm23F2R5 (*Os06g0107600*)[a]	CATGGTGTCCCTCGTCAAG	AGGTAGAGCGTCTCGTGCAG
owm29F1R1 (*Os06g0106100*)	CATCACAGGCTCTTTCAGCA	GCTCGTGGAGAGACCAAGAC
owm32F1R1 (*Os06g0105800*)	ACGGTCTTCCTTCGTGGGTA	ACGCTCACGACATCGCTAAT
owm32F2R2 (*Os06g0105800*)	GGATCTCCTACGCTCTCGTG	TTGATCCAGATACAACAGGACAT
owm32F3R3 (*Os06g0105800*)	CGCCCCCAAGAAAGTTGTAT	TGCAAACGAGGACACATTTC
Owm45F2R2 (*Os06g0107700*)	GGCTCGTCTACACCAACGAC	TTGGGGTCTTTAGGCATGAG
Owm45F3R3 (*Os06g0107700*)	CGCAACAGGGACCGGTAT	GAGCTGCTGGTCGGAACTC
DArT-STS markers		
sun104 (*wPt-763*)	TGCTATGTGCGTGATGATGA	TTACATGCTCCAGCGACTTG
sun106 (*wPt-4487*)	TGCACACAAGGAGAGGAGTG	AGAGGACAGTGCCCGTGTAG
eSTS markers		
sun139 (*BF483646.1*)	TTTGGTCGGTTGGTTTGTTT	CCCCGACATCATCCTTTTTA
sun140 (*BF483646.2*)	CCGCACATATACATATAACCTCAA	CCTCCCTGTGCACAAACATA
sun154 (*BE444404.1*)	ATATTAGGGGCAAGCAAGCA	TCTCCCCAAGAACACCAAAC
sun155 (*BE444404.2*)	GTTTGGTGTTCTTGGGGAGA	ATTCCAACCTGCCCTGTATG

[a] Owm markers developed from syntenic region of rice genome

Table 5 Validation of *Yr51*-linked marker *sun104* (*wPt-763*) on diverse wheat genotypes

Cultivars/RIL	Allele size (bp)
AUS27858 and *Yr51* carrying resistant RIL (AUS91456)	225 bp
Westonia and susceptible RIL	Null
Australian genotypes	
Braewood, Calingiri, Camm, Carinya, Carnamah, Derrimut, Diamondbird, EGA Bonnie Rock, EGA Gregory, Ellison, Frame, Giles, Gladius, Goldmark, H45, Halberd, Kellalac, Kukri, QAL2000, Rubric, Sunsoft 98, Sunlin, Sunvale, Sunzell, Tatiara, Ventura, Wyalkatchem	Null
Indian genotypes	
HD2402, PBW502, PBW343, PBW533, PBW550, FLW2, FLW6, K9107, HD2733, WH542, DBW17, PBW343 + *Lr24* + *Lr28*, PBW343*2/Kukuna	Null

transfer of favorable genes from wild relatives of wheat often accompany with unwanted genes, whereas use of landraces in wheat improvement has not shown such disadvantages.

Isolation of *YrAW1* in MSP#5515 singly enabled the confirmation of its monogenic inheritance and was located in chromosome 4AL through BSA using DArT markers. It was named *Yr51* and shown to be effective against key Australian Pst pathotypes tested. Using SSR markers from the wheat composite map (wheat.pw.usda.gov/GG2/index.shtml), the precise location of *Yr51* (Fig. 2a) in the most distal deletion bin 4AL4-0.80-1.00 of chromosome 4A was determined.

During the evolution of common wheat, chromosome 4AL has undergone translocations and inversions. Two reciprocal translocations events, pericentric and paracentric inversions in 4AL, have been previously reported (Devos et al. 1995). First translocation occurred at the diploid level between chromosome 4AL and 5AL. Then, a pericentric inversion took place before another translocation between 4AL and 7BS at the tetraploid stage. Paracentric inversion resulted in modern 4AL chromosome containing segments of 7BS, 5AL, ancestral 4AL and proximal segment of the ancestral 4AS (Naranjo et al. 1987; Devos et al. 1995; Miftahudin et al. 2004; Hernandez et al. 2012). Berkman et al. (2012) reported that 13 % genes has been translocated from 7BS to 4AL, and 13 genes in chromosome 7BS appear to have originated from 4AL. Due to complex composition of the chromosome 4A, mapping is a challenging task. To saturate the *Yr51* region, several marker resources were explored. The public domain markers included SSR, EST (GrainGenes 2.0, http://wheat.pw.usda.gov) and "*mag*" markers (Xue et al. 2008). In addition, to identify markers closely linked with *Yr51* we utilized a large synteny study of 4A chromosome specific survey sequence with barley, rice, *Brachypodium*

and *Sorghum* genomes—the 4A genome zipper (Hernandez et al. 2012).

The targeted marker development approach using the 4A genome zipper and 4AL survey sequence resulted in saturation of the *Yr51* region with eight additional gene-based markers. Similarly, synteny-based approach using rice genome was used in high-density mapping and positional cloning projects in wheat (e.g., Distelfeld et al. 2004; Yan et al. 2004; Valárik et al. 2006). However, in many cases micro-collinearity in the region of interest was interrupted (Distelfeld et al. 2004; Valárik et al. 2006). In case of the 4A genome zipper utilizing four syntenic genomes, the breaks in collinearity of one genome could be bypassed by synteny in the other (Hernandez et al. 2012). In addition, the use of wheat sequence scaffolds for primer design increases effectiveness of PCR and specificity of products (Staňková et al. unpublished results). On the other hand, designing multiple primers pairs for single gene revealed multiple locations of genes from which markers *owm32* and *owm45* were developed (Fig. 2b). This observation could account for frequent gene duplication events and pseudogene evolution in wheat as described by Wicker et al. (2011). Markers *owm45F3R3* and *sun104* flanked *Yr51* at a genetic distance of 1.2 and 2.5 cM on the proximal and distal sides, respectively.

The closely linked marker *sun104* was negatively validated in a set of 40 genetically diverse wheat genotypes. Although the marker *owm45F3R3* mapped more closer to *Yr51*, it was not successfully validated in the absence of its resistance-linked allele among these 40 genotypes. Comparative sequence data from 4A Zipper (data not presented) indicated chromosomal rearrangements in this region. Marker *sun104* can be used in marker-assisted pyramiding of *Yr51* with other genes for which markers are available.

This project is part of the Australia–India collaboration, and therefore *Yr51* 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 Australian, India, and elsewhere. Seed of genetic stock carrying *Yr51* singly has been deposited with the Australian Winter Cereal Collection Tamworth and it has been accessioned as AUS 91456.

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