Plant Disease • 2019 • 103:1166-1171 • https://doi.org/10.1094/PDIS-06-18-1055-RE

Molecular Mapping of Stripe Rust Resistance Gene *Yr81* in a Common Wheat Landrace Aus27430

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Abstract

The deployment of diverse sources of resistance in new cultivars underpins durable control of rust diseases. Aus27430 exhibited a moderate level of stripe rust resistance against *Puccinia striiformis* f. sp. *tritici* (Pst) pathotypes currently prevalent in Australia. Aus27430 was crossed with the susceptible parent Avocet S (AvS) and subsequent filial generations were raised. Monogenic segregation observed among Aus27430/AvS F_3 families was confirmed through stripe rust screening of an F_6 recombinant inbred line (RIL) population, and the resistance locus was temporarily named *YrAW5*. Selective genotyping using an Illumina iSelect 90K wheat SNP bead chip array located *YrAW5* in chromosome 6A. Genetic mapping of the RIL population with linked 90K SNPs that were converted into PCR-based marker assays, as well as SSR markers previously mapped to chromosome 6A, confirmed the chromosomal assignment for *YrAW5*. Comparative analysis of other stripe rust resistance genes located in chromosome 6A led to the formal designation of *YrAW5* as *Yr81*. Tests with a marker linked with *Yr18* also demonstrated the presence of this gene in Aus27430. *Yr18* interacted with *Yr81* to produce stripe rust responses lower than those produced by RILs carrying these genes individually. Although *gwm459* showed higher recombination with *Yr81* compared with the other flanking marker *KASP_3077*, it amplified the AvS allele in 80 cultivars, whereas *KASP_3077* amplified AvS allele in 67 cultivars. Both markers can be used in marker-assisted selection after confirming parental polymorphism.

The concept of genome mapping dates back to the landmark publication covering the genetic map of six sex-linked genes on a fruit fly chromosome through classical linkage analysis (Morgan and Cattell 1912). The principles of genetic mapping and linkage analysis remain the same, but recent technological advances now facilitate the mapping of genes responsible for both simple and complex traits in a faster and more precise manner (Semagn et al. 2006). The genetic analysis of rust resistance genes in wheat involves crossing of the resistant genotype with a susceptible parent to produce segregating populations followed by screening against relevant pathogen isolates (Bariana and Bansal 2017). In the case of involvement of more than one gene in controlling resistance, the isolation of individual components and development of single locus segregating populations enables detailed characterization (Bariana and Bansal 2017).

Sears (1954) identified a series of monosomic plants lacking one of the homolog of 21 chromosomes individually and 41 telocentric chromosomes from the wheat genotype Chinese Spring. Endo and Gill (1996) later identified 436 chromosome deletions by C-banding and developed the respective deletion stocks of common wheat. These pioneering studies facilitated the genetic and physical mapping of genes in the wheat genome. The chromosomal locations of several stripe rust resistance genes were determined through monosomic analysis using Sear's cytogenetic stocks (Bariana and McIntosh 1993; Bariana et al. 2002; Chen et al. 1996; Eriksen et al. 2004; McDonald et al. 2004; McIntosh et al. 1995). Molecular marker technologies have now largely replaced cytogenetic techniques. Methods such as bulked segregant analysis (BSA; Michelmore et al. 1991), selective genotyping (SG; Xu et al. 2008), and whole genome scanning (Edae et al. 2016) using DNA marker technologies such as Infinium SNP bead chip arrays (Wang et al. 2014) and DArTseq (https://www.diversityarrays.com/technology-and-resources/dartseq/dart-application/) can be used to identify the chromosomal locations of resistance genes. Following the identification of markers linked with a target locus in BSA and SG, genotyping of the entire mapping population using the linked markers is performed to estimate genetic linkage and to detect close trait-marker associations (Collard et al. 2005). Molecular technologies facilitate faster and higher resolution mapping of rust resistance genes, compared with monosomic analysis (Bansal et al. 2011; Bariana et al. 2006, 2016; Basnet et al. 2013; Cheng et al. 2014; Dracatos et al. 2016; Feng et al. 2013; Herrera-Foessel et al. 2015; Lu et al. 2014; McIntosh et al. 2015 - 2016; Randhawa et al. 2014, 2015; Xu et al. 2013; Zhang et al. 2013; Zhou et al. 2014a, b).

The Australian Grains Gene Bank holds a collection of phenotypically diverse hexaploid wheat landraces originating from 32 different countries, known as the 'Watkins Collection'. This collection was compiled by A. E. Watkins in the 1920s to 1930s through the Board of Trade in London (Miller et al. 2000). These landraces harbor valuable genetic variation for resistance to biotic and abiotic stresses (Pasam et al. 2017). The common wheat landrace from China, Aus27430, showed resistance to predominant Australian *Puccinia striiformis* f. sp. *tritici* (Pst) pathotypes at the seedling stage and produced moderately resistant to moderately susceptible (MRMS) response at the adult plant stage. This study describes the inheritance and chromosomal location of the all stage stripe rust resistance carried by Aus27430 and its interaction with adult plant resistance (APR).

Materials and Methods

Plant materials. A single plant selection from the common wheat landrace Aus27430, obtained from the Australian Winter Cereal Collection (AWCC), Tamworth, Australia (now Australian Grains Gene Bank, Horsham), was crossed with the stripe rust susceptible genotype Avocet S (AvS), and an $F_{5:6}$ recombinant inbred line (RIL) population comprising 130 lines was developed. A set of 81 Australian

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Funding: This research was supported by the Australian Grains Research and Development Corporation (GRDC) through the Australian Cereal Rust Control Program, project no. US0063.

The authors declare no conflict of interest.

Accepted for publication 10 January 2019.

wheat cultivars was used to test polymorphism of markers linked with the all stage resistance gene carried by Aus27430.

Greenhouse screening. The Aus27430/AvS F_3 population was initially tested under greenhouse conditions against Pst pathotype 134 E16A$^+$Yr17$^+$Yr27$^+$ to determine the mode of inheritance of resistance following the procedure outlined in McIntosh et al. (1995), and later the Aus27430/AvS RIL population was tested. Infection type (IT) variation was scored after 14 days of inoculation on a 0–4 scale described in McIntosh et al. (1995). More than usual necrosis or chlorosis was denoted by letters 'N' and 'C', respectively. Similarly, '-' and '+', respectively, explained slight variations in the expression of an IT. Twenty seeds of each F_3 family and eight to 10 seeds of each RIL were sown in 9-cm-diameter plastic pots filled with potting mix (80% composted pine bark and 20% coarse sand) supplemented with the balanced fertilizer Aquasol (20 g/10 liters of water for 200 pots). The parents and susceptible genotype Morocco were included as controls. Seedlings were grown in microclimate rooms maintained at 20 ± 2°C until inoculated and were fertilized with urea 1 week after sowing at the same rate as Aquasol. At the two-to-three leaf stage, seedlings were inoculated using Pst pathotype 134 E16A$^+$Yr17$^+$Yr27$^+$. The F_3 families were classified homozygous resistant (HR; showing ITs 1 to 3c), segregating (SEG; ITs 1 to 23c and 3+), and homozygous susceptible (HS; IT 3+) based on seedling stripe rust responses. The RILs were categorized as HR and HS.

Tests were also conducted on the parents and four each HR and HS RILs using five Pst pathotypes; 134 E16A$^+$Yr17$^+$, 104 E137A$^-$Yr17$^+$, 110 E143A$^+$, and 108 E141A$^+$ at the two-leaf stage.

Field evaluation. Adult plant stripe rust response assessments were conducted at University of Sydney Plant Breeding Institute experimental sites in three replications in 2016 and two replications in 2018. The RIL population and parents were sown as eight to 10 seeds in hill plots. To facilitate rust infection in the field, a row of susceptible spreader was sown after every five hill plots horizontally and after every four rows vertically. Spreader rows were inoculated with a mixture of Pst pathotypes 110 E143A$^+$ and 134 E16A$^+$Yr17$^+$Yr27$^+$ in the field.

Adult plant stripe rust response variation under field conditions was recorded using the 1–9 scale described by Bariana et al. (2007b), where 1 = very resistant, 2 = resistant, 3 = resistant to moderately resistant, 4 = moderately resistant, 5 = moderately resistant to moderately susceptible, 6 = moderately susceptible, 7 = moderately susceptible to susceptible, 8 = susceptible, and 9 = very susceptible. Adult plant stripe rust responses were recorded when the susceptible parent AvS showed a score of 8. Preliminary observations were also made from booting stage to identify HS RILs.

Molecular analysis. *DNA extraction.* The modified CTAB method described in Bansal et al. (2014b) was used to extract DNA from 10-day-old leaves of each F_3 family, RIL, and parent. The DNA samples were quantified using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies).

Molecular mapping. SG was performed on eight HR and eight HS Aus27430/AvS F_3 families using the Illumina iSelect 90K Infinium SNP genotyping array (Wang et al. 2014) to determine chromosomal location of the resistance locus.

Saturation of chromosome region on 6AS. Linked SNP markers from the Infinium genotyping array were converted to Kompetitive Allele-Specific PCR (KASP; LGC genomics, UK) assays and used to genotype Aus27430 and AvS. Polymorphic KASP markers were then assayed on the entire RIL population. In addition, six SSR markers: *gwm459*, *gwm334*, *barc206*, *barc3*, *barc23*, and *gwm494*, previously mapped on chromosome 6A (Somers et al. 2004), were used to confirm the location of *YrAW5* on chromosome 6AS. Primer sequences of these SSR markers were taken from the GrainGenes 2.0 database (https://wheat.pw.usda.gov/).

PCR amplification of KASP markers. KASP assays were performed following protocols given at the LGC genomics website (https://www.lgcgroup.com/products/kasp-genotyping-chemistry/#.WvKUz6SFOUk) using a CFX96 TouchTM real-time PCR detection system (BioRad, U.S.A.). The data were analyzed using Bio-Rad CFX Manager Software (BioRad) described in Bariana et al. (2016).

Genotyping for known APR gene markers. The *Lr34/Yr18*-linked marker *csLV34* (Lagudah et al. 2006), *Lr67/Yr46*-linked SNP marker *TM4* (Moore et al. 2015), *Lr46/Yr29*-linked marker *Lr46_SNP1G22* (E. S. Lagudah, *personal communication*), and *Sr2/Yr30*-linked marker *csSr2* (Mago et al. 2011) were tested for the presence of these genes in Aus27430 following protocols given in the respective publication.

Data analyses. χ^2 analyses were conducted to test the goodness of fit of observed phenotypic segregation to the theoretically expected genetic ratios. Genetic linkage analysis was performed with Map Manager QTXb20 (Manly et al. 2001) using the Kosambi mapping function (Kosambi 1943). Linkage values with logarithm of odds (LOD) scores of 3.0 and above were considered statistically significant. Genetic maps were constructed using MapChart software (Voorrips 2002).

Fig. 1. Infection types produced by Aus27430 (1C) and Avocet S (3+) when infected with Pst pathotype 134 E16A+Yr17+Yr27+ at the seedling stage.

Results

Seedling tests. Aus27430 expressed ITs 1-c to 23c, whereas the susceptible parent AvS showed IT 3^+ (Fig. 1), when infected with Pst pathotype 134 E16A+Yr17+Yr27+. Tests on the F_3 population showed monogenic segregation (22 HR: 68 SEG: 33HS; $\chi^2_{1:2:1}$ = 3.34, nonsignificant at P = 0.05 and 2 df), and the underlying resistance locus was temporarily named *YrAW5*. Stripe rust response variation among segregating F_3 families indicated incomplete dominance of inheritance. The monogenic segregation for stripe rust resistance observed among the F_3 families was confirmed on Aus27430/AvS RIL population (Table 1). The parents, four HR, four HS, and AvS were tested with other Pst pathotypes (Table 2). Aus27430 and HR lines produced low IT (1-c to 23c), and AvS and HS lines produced high IT (3+), indicating that *YrAW5* is effective against all these Pst pathotypes.

Mapping of *YrAW5*. The iSelect 90K SNP arrays-based SG revealed association of 45 SNP markers from the short arm of chromosome 6A with *YrAW5*. KASP assays designed for the linked SNPs were tested on the parental genotypes Aus27430 and AvS. Seven KASP markers allowing unambiguous genotype calling were screened on the entire Aus27430/AvS RIL population. Six SSR markers previously mapped on chromosome 6A also showed polymorphism between parents and were tested on the entire RIL population to confirm the chromosomal location of *YrAW5*. Markers *KASP_3070* and *gwm459* flanked *YrAW5* at genetic distances of 2.7 cM and 6.4 cM distally and proximally, respectively (Fig. 2). The sequences of KASP markers used in the linkage map are listed in Table 3.

Detection of known APR. Parents Aus27430 and AvS were tested with markers linked to APR genes *Lr34/Yr18*, *Lr67/Yr46*, *Lr46/Yr29*, and *Sr2/Yr30*. The presence of the *Lr34/Yr18*-linked 150-bp allele in Aus27430 suggested the presence of this gene, whereas amplification of products alternate to those linked with other APR genes indicated their absence.

Field tests. Under field conditions, Aus27430 showed a moderately resistant to moderately susceptible (MRMS) response (score 5), and the susceptible parent AvS was scored 8 to 9 (S-VS). Aus27430 was observed to carry *Yr18* based on the linked marker *csLV34* and leaf tip necrosis associated with this gene. The entire Aus27430/AvS RIL population was tested with *csLV34* and was partitioned into four genotypic classes; *YrAW5*, *Yr18*, *Yr18*+*YrAW5*, and no known gene (Table 4). The rust response data recorded on a 1–9 scale were converted into disease severity according to Bansal et al. (2014a). The RILs carrying *YrAW5* (ITs 1c to 3c) and *Yr18* singly did not differ significantly for mean rust severity under field conditions. RILs possessing *YrAW5* and *Yr18* in combination resulted in significant reduction in average disease severity during the crop seasons 2016 (28.3%) and 2018 (30%) when compared with RILs that carried these genes singly or lacked both genes. The RILs carrying neither *Yr18* nor *YrAW5* produced about 70 to 80% disease severities under field conditions. These results indicated that *YrAW5* interacts additively with the APR gene *Yr18* to lower disease severity.

Polymorphism of *YrAW5* linked molecular markers. Eighty-one Australian wheat cultivars were tested with *YrAW5* flanking markers *KASP_3077* and *gwm459*. The marker *KASP_3077* produced AvS haplotype (A:A) in 67 cultivars, whereas *gwm459* produced the 126-bp specific to AvS in 80 cultivars. Gazelle was the only cultivar that carried the Aus27430 allele at both markers, whereas, 13 cultivars carried the Aus27430 allele for the *KASP_3077* (Table 5). These results indicate that both markers can be used for marker-assisted breeding following polymorphism checks on potential parents.

Table 1. Phenotypic distribution of Aus27430/AvS-derived RIL population when tested with Pst pathotype 134 E16A+Yr17+Yr27+ at the seedling stage under greenhouse conditions[a]

Response class	Response	Number of RILs		$\chi^2_{1:1}$
		Observed	Expected	
Seedling stage	Infection type			
Homozygous resistant	1C to 3C	57	65	0.98
Homozygous susceptible	33+ to 3+	73	65	0.98
Total		130	130	1.96

[a] Table value of χ^2 at P = 0.05 and 1 df is 3.841.

Table 2. Infection types produced by parents, homozygous resistant, and homozygous susceptible lines against different Pst pathotypes

Pathotype	Aus27430	HR lines	HS lines	Avocet S
134 E16A+Yr17+	2c	1c to 23c	3+	3+
104 E137A-Yr17+	2c	1-c to 23c	3+	3+
110 E143A+	23c	2c to 23c	3+	3+
108 E141A+	23-c	2c to 23c	3+	3+

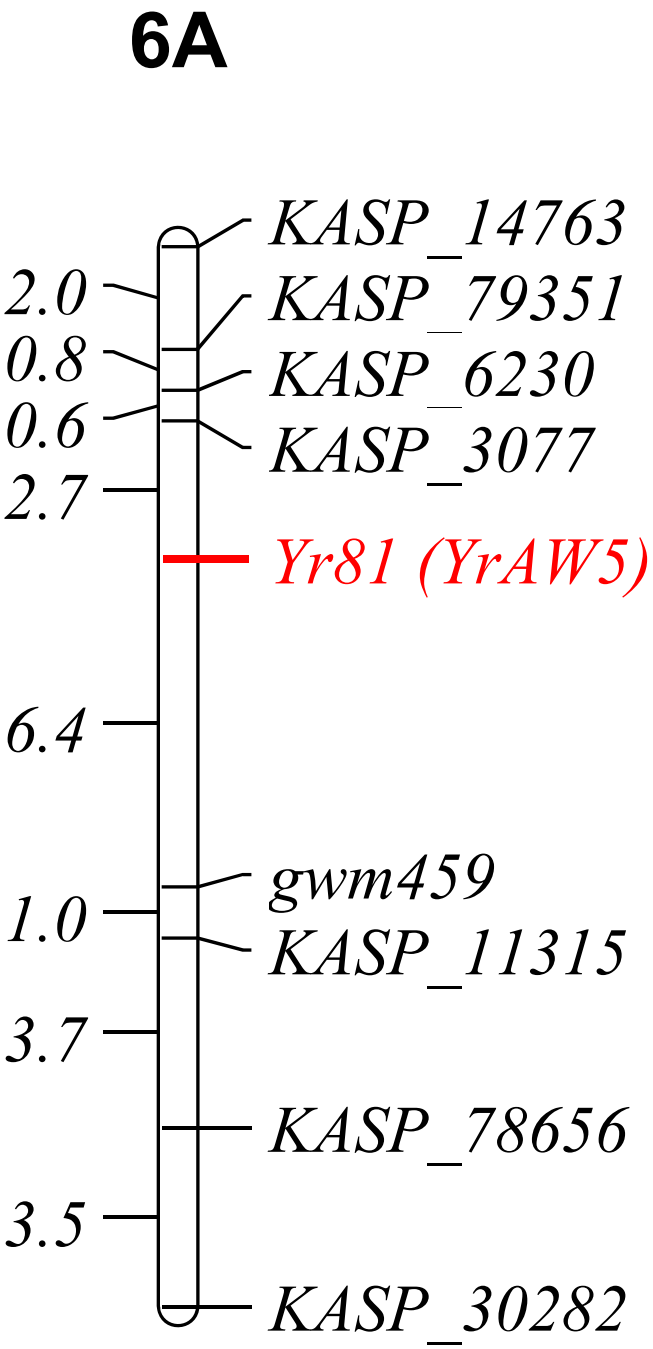

Fig. 2. Genetic linkage map of chromosome 6AS showing location of *YrAW5* using KASP and SSR markers in a Aus27430/AvS RIL population.

Discussion

Breeding for resistance to rust diseases in wheat is a continuous process, as the pathogens causing these diseases evolve to acquire virulence for resistance genes present in commercial cultivars (Bariana 2003; Bariana et al. 2007a; McIntosh and Brown 1997). The release of rust resistant wheat cultivars is important to reduce input costs and to increase profitability for growers. The uninterrupted release of rust resistance cultivars requires the continuous discovery and characterization of diverse sources of resistance. Genetic analysis of stripe rust resistance in the landrace genotype Aus27430 detected a putatively new stripe rust resistance gene, and it was temporarily named *YrAW5*. Several new rust resistance loci have been identified in pre-Green Revolution wheat genotypes in the last decade. Bansal et al. (2011) identified *Yr47* in landraces Aus28183 and Aus28187 using a procedure similar to that followed in this study. Other studies have resulted in the formal designations of *Yr51* (Randhawa et al. 2014) and *Yr57* (Randhawa et al. 2015) from the landrace Aus27858. Chhetri (2015) also identified a stripe rust resistance locus in landraces Aus27507 and Aus27894 from the Watkins Collection and named it *Yr72*. Recently, Nsabiyera et al. (2018) identified an adult plant stripe rust resistance gene *Yr80* in the landrace Aus27284. Similarly, Bansal et al. (2015) reported a new stem rust resistance locus *Sr49* in Aus28011.

Stripe rust resistance from Aus27430 was located in the short arm of chromosome 6A using SG. Stripe rust resistance genes *Yr38* (Marais et al. 2006) and *Yr42* (Marais et al. 2009) are also located on chromosome 6A. These genes are present in translocated segments from *Aegilops sharonensis* (*Yr38*) and *Ae. neglecta* (*Yr42*) and produce lower infection types than that produced by *YrAW5*. Hence, it is highly unlikely for *YrAW5* to be *Yr38* or *Yr42*. Seven QTL for stripe rust resistance have been identified on chromosome 6A, including *QYrex.wgp-6AS* (linked markers *gwp56*, *gwm334*) in cultivar Express (Lin and Chen 2009), *QYr.uga-6AS* (*wPt-671561*, *wPt-7840*) in Pioneer 26R61 (Hao et al. 2011), *QRYr6A*0.2 (*barc3*, *wPt-7063*) in AvS (Lillemo et al. 2008), *QRYr6A*0.2 (*gwm427*, *wmc256*) in AvS (William et al. 2006), *QRYr6A*0.2 (*wPt-0959*, *378849*) in AvS (Rosewarne et al. 2012), *QRYr6A*0.3 (*wPt-1642*, *gwm617*) in Platte (Vazquez et al. 2012), and *YrQ3* (*gwm334*, *gwm169*) in Xichang 76-9 (Cao et al. 2012). We compared the position of linked markers from above studies in Somers et al. (2004) and DArTseq map (https://www.diversityarrays.com/technology-and-resources/genetic-maps/). *YrAw5* was mapped 6.5 cM distal to *gwm459*, whereas all other QTL linked markers were located proximal to *gwm459* (Table 6), indicating that *YrAW5* represents a new locus for all stage stripe rust resistance and was therefore formally designated *Yr81*.

This investigation characterized an all stage resistance gene *Yr81* in Aus27430, and an attempt was made to uncover additional APR in this genotype. The presence of the APR gene *Yr18* was observed in Aus27430. This gene interacted with *Yr81* to lower disease severity at the adult plant stage, suggesting that *Yr18* is a good candidate for pyramiding to achieve durable control of stripe rust. It was surprising to note that responses of the ASR gene *Yr81* and the APR gene *Yr18* did not differ significantly. It could presumably be due to additional resistance present in the background as indicated by stripe rust severity variation of 40 to 100% among RILs lacking both *Yr81* and *Yr18*. In another study, Chhetri et al. (2016) showed the additive interaction of adult plant resistance gene *Yr46*/*Lr67*/*Sr55* with the all stage resistance gene *Yr58* (detected at the fourth leaf stage) in wheat genotype W195.

Various countries across Africa, Central Asia, and Europe are battling with new strains of stripe rust (http://www.fao.org/news/story/en/item/469467/icode/). This is one of the most damaging diseases worldwide and can result in up to 60% yield loss (https://rusttracker.cimmyt.org/). Deployment of diverse sources of rust resistance in new wheat cultivars will help to avoid stripe rust epidemics in the future. Assessment of polymorphisms of trait-linked markers across target recurrent parents underpins their implementation in breeding program for marker assisted pyramiding (Bariana and Bansal 2017). The amplification of *KASP_3077* and *gwm459* alleles alternate to those linked with *Yr81* in 82.7 and 98.8% of cultivars tested demonstrated their usefulness in marker-assisted selection

Table 4. Interaction of stripe rust resistance genes *YrAW5* and *Yr18* among Aus27430/AvS F_6 RIL population under field conditions[a]

Gene	No of RILs	Average (3 replications) 2016	Average (2 replications) 2018
YrAW5	24	43.07	50.79
Yr18	37	38.61	42.19
YrAW5+Yr18	33	28.55*	30.00*
NIL	36	71.11	81.81
LSD (5%)		7.10	10.71

[a] * = Statistically significant.

Table 5. Australian cultivars tested for polymorphism with *Yr81*-linked markers *KASP_3077* and *gwm459*

Cultivars	*KASP_3077*	*gwm459* (bp)
Aus27430	G:G	132
Avocet S	A:A	126
Gazelle	G:G	132
Beaufort, Emu Rock, Envoy, GBA Sapphire, Mackellar, Mansfield, Naparoo, Orion, Phantom, Scout, Sentinel, SF Adagio, Yandanooka	G:G	126
AGT Katana, Axe, Baxter, Bolac, Calingiri, Carnamah, Catalina, Chara, Cobra, Corack, Correll, Crusader, Dart, Derrimut, Diamondbird, EGA Bonnie Rock, EGA Bounty, EGA Burke, EGA Gregory, EGA Wedgetail, EGA Wylie, Elmore CL PLus, Estoc, Forrest, Fortune, Gauntlet, 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, Magenta, Merinda, Merlin, Preston, SF Scenario, Shield, Spitfire, SQP Revenue, Strzelecki, Sunco, Sunguard, Suntop, Sunvale, Sunvex, Sunzell, Trojan, Ventura, Waagan, Wallup, Wedin, Westonia, Wyalkatchem, Wylah, Yitpi, Young	A:A	126

Table 3. Primer sequences for KASP markers used to map *YrAW5* on chromosome 6AS

Marker	cM[a]	Allele 1[b]	Allele 2[c]	Common/reverse
KASP_3077	4.7	attccaaagtaattggcaacaggttca	ccaaagtaattggcaacaggttcg	tgtggagcgtgacaatgaggaagtt
KASP_79351	5.6	ggctgaatcactggtggataacatt	gctgaatcactggtggataacatc	ggacttttagcagtaaacccatgatcaaa
KASP_6230	5.7	aggagtatacatatttgtcgtaaggattta	ggagtatacatatttgtcgtaaggatttg	gatacataggacagggtatcgccaa
KASP_14763	7.0	ggtcctgtttagagtggagcgt	gtcctgtttagagtggagcgc	gcaaggagcgcaacctggactt
KASP_11315	13.5	ccttcaacgacctgactgccaat	cttcaacgacctgactgccaag	cgaaggcgacgcggccgtt
KASP_78656	22.9	gaagaattcgcatattcaggcgcaa	aagaattcgcatattcaggcgcag	tcgactctacccagcaaacttcctt
KASP_30282	33.3	ggcggacgtggcaaggatgat	gcggacgtggcaaggatgac	cggagcgtggcaggcgcaa

[a] Genetic map position of the SNP markers in the 90K snp assay (Wang et al. 2014).
[b] A1 primer labeled with FAM: GAAGGTGACCAAGTTCATGCT.
[c] A2 primer labeled with HEX: GAAGGTCGGAGTCAACGGAT.

Table 6. Markers linked with QTL located in the short arm of chromosome 6A

QTL	Left marker	cM	Right marker	cM
QYrex.wgp-6AS	*gwp56*	Not known	*gwm334*	1
QYr.uga-6AS	*wPt-671561*[a]	6	*wPt-7840*	23
QRYr6A.2	*barc3*[b]	27	*wPt-7063*	-
QRYr6A.2	*gwm427*	67	*wmc256*	80
QRYr6A.2	*wPt-0959*	14	*378849*	-
QRYr6A.3	*wPt-1642*	99	*gwm617*	75
YrQ3	*gwm334*	1	*gwm169*	81

[a] Distance in cM is according to https://www.diversityarrays.com/technology-and-resources/genetic-maps/.
[b] Distance in cM is according to Somers et al. (2004).

of this resistance source. Gazelle was the only cultivar that carried resistance-linked alleles for both markers. It is hard to prove the presence of *Yr81* in Gazelle due to the presence of additional resistance inherited from parents K1056 (advanced breeding line), VPM1, and Vasco. Several stripe rust resistance gene-marker associations have been reported recently (Chhetri et al. 2016; McIntosh et al. 2015 - 2016; Nsabiyera et al. 2018; Qureshi et al. 2018; Randhawa et al. 2014, 2015) and are being used to deploy marker-tagged stripe rust resistance genes in Australian wheat backgrounds. *Yr81* represents an additional intermediate-effect all stage resistance source to achieve genetic diversity for stripe rust resistance in future wheat cultivars.

Acknowledgments

The first author thanks Australia Awards for the scholarship.

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