Mapping of Stripe Rust Resistance Gene Yr72 in Two Common Wheat Landraces

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Article

Mapping of Stripe Rust Resistance Gene *Yr72* in Two Common Wheat Landraces

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Abstract: Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is a significant challenge to global wheat production. Common wheat landraces AUS27507 and AUS27894 displayed stripe rust resistance against several commercially prevailing Pst pathotypes. These genotypes were crossed with a stripe rust susceptible landrace AUS27229 to understand inheritance of resistance and to determine genomic location(s) of underlying gene(s). F_3 generations of crosses AUS27507/AUS27229 and AUS27894/AUS27229 showed monogenic segregation for stripe rust resistance under greenhouse conditions. The absence of segregation for stripe rust response among the AUS27507/AUS27894-derived F_3 population suggested that both genotypes carry the same gene. The stripe rust resistance gene carried by AUS27507 and AUS27894 was tentatively named *YrAW4*. Bulked segregant analysis placed *YrAW4* in the long arm of chromosome 2B. The AUS27507/AUS27229 F3 population was enhanced to develop an F_6 recombinant inbred line (RIL) population for detailed mapping of chromosome 2BL. DArT-based SSR, STS and KASP markers were employed to enrich the 2BL map. DArT-based STS markers *sun481* and SNP marker *IWB12294* flanked *YrAW4* proximally (1.8 cM) and distally (1.2 cM), respectively. Deletion mapping placed *sun481* in the deletion bin 2BL-5. All stripe rust resistance genes, previously located on chromosome 2BL, neither express infection type like *YrAW4*, nor are mapped in the deletion bin 2BL-5. Hence *YrAW4* represented a new locus and was formally named *Yr72*. The robustness of *IWB12294* marker in marker-assisted selection was demonstrated by amplification of and alternative allele to that linked Yr72 in 21 common wheat background.

Keywords: wheat; stripe rust; KASP marker; STS marker; marker validation

1. Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is one of the most destructive diseases of wheat worldwide [1,2]. The first stripe rust incursion in Eastern Australia occurred in 1979 [3,4] and in Western Australia in 2002 [5]. Subsequently it caused several epidemics, including the most recent in 2022, with devastating impacts on the agricultural industry with approximate yield reduction of 84% [6]. Now stripe rust pathogen has a history of 44 years in Australia and it still remains a major constraint for wheat production with an estimated average annual loss of A$ 127 m [7].

Although more than 80 loci for stripe rust resistance have been characterized and formally named [https://wheat.pw.usda.gov/GG3/wgc; visited on July 21, 2023], most of these belong to the

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race-specific category. Resistance genes that condition ASR often succumb to the acquisition of virulence in pathogen populations. Wellings and McIntosh[8] summarised the evolution of virulence in Pst population and concluded the stepwise attainment in virulence for stripe rust resistance genes *YrA*, *Yr6* and *Yr7* individually and in different combinations. The other commercially important event included the detection of virulence for *Yr17* in 1999. All derivatives of the 1979 introduction (104 E137A-) were virulent on genotypes carrying *Yr3* and *Yr4*. In contrast, the 2002 introduction (134 E16A+) and all its current derivatives are avirulent on *Yr3* and *Yr4*. In addition, this group carried virulence for resistance genes *Yr8* and *Yr9* that have not been deployed widely in Australian wheat cultivars. Stripe rust resistance genes *Yr6*, *Yr7*, *Yr9*, *Yr17* and *Yr27* have been postulated in spring wheat cultivars grown worldwide. Pathotypes carrying virulence for these have been reported in many countries including Australia.

All stage resistance (ASR) and adult plant resistance (APR) are two main categories of resistance to rust diseases in wheat. ASR genes protect plants throughout all growth stages, while APR genes provide post-seedling resistance. To achieve long-lasting control, it's essential to combine ASR and APR genes in a single cultivar which is now possible through advancement in molecular technologies. It has enabled efficient mapping of resistance genes and identification of closely linked markers using whole genome scanning [9] using different high-throughput platforms such as Diversity Arrays Technology (DArT) [10], genotyping-by-sequencing (GBS) [11], selective genotyping (SG) [12], and SNP-genotyping arrays [13]. The development of ordered draft sequence of the 17-gigabase with more than 75,000 genes positioned along 21 chromosomes of wheat [14] adds further impetus to mapping and marker development research. Bariana et al. [15] identified genotypes carrying four to six QTL for stripe rust resistance and a genotype combining closely linked genes *Yr15* and *Yr24* were identified by Zakeri et al. [16].

To achieve durable resistance to stripe rust, discovery and characterisation of diverse sources of resistance is essential. Bariana and co-workers screened the Watkins Collection of common wheat landraces [17] and identified genotypes for detailed genetic analysis. Daetwyler et al. [18] predicted the involvement of several chromosomes in conditioning stripe rust resistance among this collection of landraces. Two landraces AUS27507 and AUS27894 exhibited seedling resistance against a range of Pst pathotypes in Australia. This study covers genomic location of resistance carried by these genotypes and identification of closely linked markers with the underlying gene(s).

2. Materials and Methods

2.1. Plant Material

AUS27507 and AUS27894 from France and Spain, respectively, were crossed with a susceptible landrace AUS27229. F_3 populations comprising 106 and 100 families were developed from AUS27507/AUS27229 and AUS27894/AUS27229 crosses. Only AUS27507/AUS27229 F3 was advanced to generate an F_6 recombinant inbred line (RIL) population for further analysis. AUS27894 and AUS27507 were crossed to determine the allelic relationship of the gene(s) carried by these genotypes. Twenty-one Australian wheat cultivars were used to validate markers closely linked with the resistance gene identified in this study.

2.2. Greenhouse Tests

Twenty seeds of each AUS27507/AUS27229 F_3 line were sown in 9 cm plastic pots filled with mixture of pine bark and river sand (2:1 ratio). Parental genotypes and the susceptible control Morocco were sown in each experiment. AUS27894/AUS27507 F_3 families and AUS27507/AUS27229 F_6 RIL population were evaluated against Pst pathotype 134 E16A+17+27+ (Plant Breeding Institute, Cobbitty culture no. 617).

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2.3. Isolation of Genomic DNA

Leaf tissue was collected from 12 day-old seedlings in 2 ml Eppendorf tubes and was dried on silica gel for 72 hours. Genomic DNA was extracted using the modified CTAB method [19] and quantified using Nano Drop spectrophotometer (NanoDrop® ND1000).

2.4. Bulked Segregant Analysis

Bulked segregant analysis (BSA) was performed on resistant and susceptible bulks prepared by pooling equal amount of DNA from 20 homozygous resistant (HR) and 20 homozygous susceptible (HS) F_3 lines, respectively. The F_1 artificial bulk was prepared combining an equal amount of DNA from rest of the population. DNA of bulks and F_1 were sent to Diversity Arrays Technology Canberra for BSA using high density DArT array Wheat *Pst*I (*Taq*I) 3 (http://www.diversityarrays.com).

2.5. STS and SSR Genotyping

Sequence of resistance linked DArT clones (kindly provided Dr A. Kilian) was used to develop STS markers using the Primer3 program [22] (http://primer3.sourceforge.net/). In addition, 52 SSR markers previously mapped on chromosome 2BL (http://wheat.pw.usda.gov) were also genotyped to enrich the genetic map [19–21]. PCRs were carried out in 10 µl reaction volume containing 2 µl of 30 ng/µl DNA, 1 µl of 1X PCR buffer containing $MgCl_2$, 0.75 µl of dNTPs, 0.4 µl of forward (1.25 mM) and reverse (5.0 mM) M13 tagged primers, 0.1 µl (0.5mM) of M13 fluorescent tag and 0.04 µl of Taq DNA polymerase. PCR amplification conditions described in Randhawa et al. [22] were used. Markers polymorphic on parents and showing strong linkage among HR and HS lines were evaluated on the entire AUS27507/AUS27229 RIL population. Electrophoresis of PCR products was carried out on 2.5-3% agarose (Amresco) gel stained with GelRed™ (Biotium). PCR products were visualized using UV gel documentation system. Markers which could not be differentiated on agarose gel were separated on polyacrylamide gel using Analyzer Gene ReadIR 4300, Li-COR sequencing system (Li-COR Bio-sciences, USA) after denaturing PCR product at 95 °C for five minutes.

2.6. SNP Genotyping

Twenty-seven SNPs showed strong linkage with the stripe rust resistance locus in the AUS27507 AUS27894. Closely linked SNPs (*IWA5694*, *IWA5839*, *IWA6130*, *IWA6334*, *IWA6417*, *IWA7265*, *IWB10417*, *IWB10455*, *IWB12294*, *IWB20875*, *IWB21638*, *IWB23209*, *IWB24984*, *IWB25015*, *IWB28191*, *IWB31823*, *IWB33668*, *IWB43166*, *IWB45530*, *IWB45652*, *IWB49793*, *IWB49793*, *IWB62498*, *IWB62757*, *IWB62762*, *IWB69000* and *IWB79078*) were used to design kompetitive allele specific PCR assays (KASP) with two allele-specific and one common primer. These markers were genotyped on the parents and the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) was used for comparing KASP amplifications among different genotypes following the protocol given at LGC website (http://www.ksre.ksu.edu/igenomics/doc1363.ashx).

2.7. Deletion Bin Mapping of Linked Markers

The DNA samples of chromosome 2BL deletion stocks [del2BL-9, del2BL-3, del2BL-7, del2BL-5 and del2BL-6 [23]; kindly provided by Dr Evans Lagudah were used to confirm the location of resistance gene. Chinese Spring (CS) was used as control.

2.8. Data Analysis

Goodness of fit of observed segregation data to the expected genetic ratios was tested through Chi-squared analysis. Linkage map was constructed using Mapmaker version 3.0 [24] and recombination fractions were transformed to centi Morgans (cM) using Kosambi mapping function [25]. Final genetic linkage map was constructed using MapChart [26].

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3. Results

3.1. Inheritance Studies

Seedling stripe rust responses of AUS27507 and AUS27894 varied from 2C to 3C (Figure 1), when evaluated against Pst pathotype 134 E16A+17+27+. Stripe rust screening results of F_3 populations derived from crosses AUS27507/AUS27229 and AUS27894/AUS27229 are presented in Table 1. Both populations showed monogenic inheritance of stripe rust resistance. Both parents and homozygous resistant F_3 lines produced similar stripe rust responses suggesting the presence same gene. AUS27507 and AUS27894 were crossed and F_3 families were generated. All 80 F_3 families produced responses similar to parents confirming presence of the same gene in both genotypes. The resistance locus segregating in both populations was temporarily named *YrAW4*. The AUS27507/AUS27229 derived population was advanced to generate F_6 RIL population. Segregation for *YrAW4* among the RIL population was confirmed (Table 1).

Table 1. Distribution of F_3 families and RIL lines when tested with Pst pathotype 134E16A+Yr17+Yr27+.

Stripe Rust Response	Infection Type	No of lines Observed	χ^2 Expected	(1:2:1) or (1:1)
F3 AUS27507/AUS27229				
Homozygous Resistant	23C to 3C	24	24.8	0.00
Segregating	23C-3C, 3+	58	49.5	1.50
Homozygous Susceptible	3+	17	24.8	2.40
Total		99	99.0	3.90
F3 AUS27894/AUS27229				
Homozygous Resistant	23C to 3C	19	19.3	0.00
Segregating	23C-3C, 3+	44	38.5	0.80
Homozygous Susceptible	3+	14	19.3	1.40
Total		77	77.1	2.20
AUS27507/AUS27229 RIL populations				
Homozygous Resistant		53	51.0	0.08
Homozygous Susceptible		49	51.0	0.08
Total		102	102.0	0.16

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Figure 1. Range of infection types produced by AUS27507 (a) IT 2C, (b) 3C and (c) AUS27229 IT 3+.

3.2. Molecular Mapping

Twenty homozygous resistant and homozygous susceptible lines each from AUS27507/AUS27229 F_3 population were used to construct contrasting bulks. These bulks were subjected to BSA using DArT markers. DArT markers *wPt-2397, wPt-665550, wPt-7161, wPt-8916, wPt-1722, wPt-4197, wPt-6542, wPt-9104.2, wPt1650.2, wPt-2185, wPt-0510, wPt-0948, wPt-3632, wPt-7350, wPt-9104.1, wPt-9190, and wPt-9336* showed linkage with *YrAW4*. All these markers are located in chromosome 2BL. Linked DArT clones were converted to STS markers and tested on the AUS27507/AUS27229 RIL population. The STS primers were named as "sun" (Sydney University). Four STS markers *sun483* (*wPt-2397*), *sun481* (*wPt-665550*), *sun482* (*wPt-7161*) and *sun484* (*wPt-8916*)

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showed polymorphism between parents and were genotyped on the entire RIL population. Markers *sun481* and *sun482* flanked *YrAW4* at genetic distances of 1.8 cM (proximally) and 2.7 cM (distally), respectively (Figure 2).

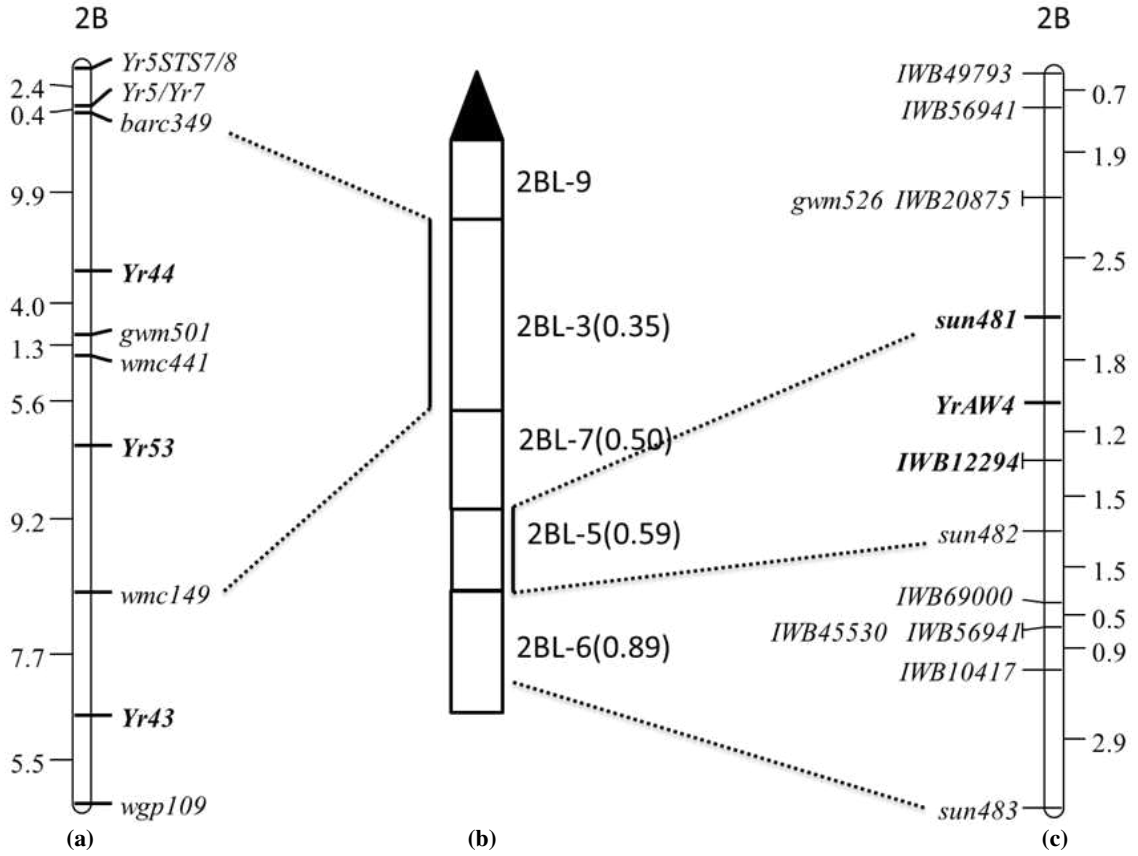

Figure 2. Diagrammatic representation of (a) stripe rust resistance genes *Yr5/Yr7*, *Yr44*, *Yr53* and *Yr43* as given in Xu et al. 2013 (b) deletion bins (c) genetic linkage map of AUS27507/AUS27229 RIL population showing the location of *YrAW4*.

3.3. Deletion Mapping of Flanking Markers

YrAW4-linked STS markers *sun481* and *sun482* were genotyped on DNA of the 2BL deletion stocks: del2BL-9, del2BL-3, del2BL-7, del2BL-5 and del2BL-6 to determine their precise genomic locations. Chinese Spring, AUS27507, AUS27894 (resistant parents) and AUS27229 (susceptible parent) were used as controls. The amplicons produced by deletion stocks were compared with Chinese Spring. Marker *sun482* amplified the expected product in del 2BL-5, del2BL-6 and CS, whereas *sun481* amplified the target band only in del 2BL-5 and CS. These results placed the closest marker *sun481* in the deletion bin 2BL-5 (Figure 2b,c).

3.4. Saturation of Chromosome 2BL using SNP Markers

AUS27507/AUS27229 RIL population was genotyped using 90K Infinium SNP wheat array. Twenty-one SNPs markers on chromosome 2BL mapped close to *YrAW4* and spanned 759 to 784 Mbp region in physical map of Chinese Spring (IWGSC RefSeq_V2.0). These were converted into KASP assays, and those showing parental polymorphisms were genotyped on the entire population (Figure 2). Eight KASP markers were incorporated into the AUS27507/AUS27229 chromossome 2BL map (Table 3, Figure 2). The final map included 1 SSR, 3 STS and 8 KASP markers (Figure 2c). The SNP marker *IWB12294* mapped 1.2 cM distal to *YrAW4* and 1.5 proximal to *sun482* (Figure 2). Sequences of the primers for markers included in the final map are given in Tables 2 and 3.

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Table 2. Primer sequences of closely linked STS markers derived from DArT- markers spanning the *YrAW4* region.

STS Markers	DArT- Markers	Forward a	Reverse
sun481	*wPt-665550*	GGCCAAGGTATGTTGATCGT	TCCAAATGAAACCAGGAAGG
sun482	*wPt-7161*	GCTCCTCTCGTTGATTGAG	GATGCAAAGGGAGAAAGCTG
sun483	*wPt-2397*	CAGTAGCATCCAACCCACT	GGGACGGATGATGAGACAGA

[a] CACGACGTTGTAAAACGAC represents M13 sequence.

Table 3. Kompetitive Allele-Specific polymerase chain reaction (KASP) markers, designed from SNPs spanning the *YrAW4* region.

SNPs	Allele 1 [a]	Allele 2 [b]	Common Primer
IWB49793	CCATGTTCAAGGGTCTCAATTA	CCATGTTCAAGGGTCTCAATTC	TCGCCTGATATATTCACAACCTTT
IWB56941	AGGAGGAATGGAAGTTTGATACT	AGGAGGAATGGAAGTTTGATACG	TGCAGAAAATGACAGCCTGA
IWB20875	AGAGTAGCATGCGAGTCTT	AGAGTAGCATGCGAGTCTC	CAAGCACTTTACAGGTTTCCG
IWB12294	TCCTCCGGCATTCCTCT	TCCTCCGGCATTCCTCG	AGCGTGCTGTACTTCGCC
IWB69000	TGTTCTAGCTAACCAGGCAA	CTGTTCTAGCTAACCAGGCAG	CGAGCCAGAGTCTGAGACCA
IWB45530	GAAACCAGCCGCAGAGTAT	TGAAACCAGCCGCAGAGTAC	GCAGTCAAGTTTTGGAACTGG
IWB56941	AAGGAGGAATGGAAGTTTGATACT	AAGGAGGAATGGAAGTTTGATACG	TGCAGAAAATGACAGCCTGA
IWB10417	TTTTCTTGTAGGAGGCAAAGATTTCAA	TTCTTGTAGGAGGCAAAGATTTCAC	TGATAATGTAAGCAGGAGTGTGCAATGTA

[a] Allele 1 labeled with FAM: GAAGGTGACCAAGTTCATGCT; [b] Allele 2 labeled with HEX: GAAGGTCGGAGTCAACGGATT.

3.5. Validation of YrAW4 Linked Markers

A set of Australian common wheat and durum wheat cultivars was tested with flanking markers *IWB12294* and *sun481*. *IWB12294* produced SNP allele 'G' in the resistant parent AUS27507 and allele 'T' in susceptible parent AUS27229 (Table 4 and Figure 3). SSR marker amplified *YrAW4* linked $sun481_{240bp}$ and $sun481_{100bp}$ allele in resistant and susceptible parents, respectively (Table 4). Amplification of the 'T' allele and the $sun481_{100bp}$ allele in all test cultivars confirmed the usefulness of these markers in marker assisted selection of *YrAW4* in breeding programs.

Table 4. Validation of closely linked STS marker *sun481* and SNP marker *IWB12294* on Australian cultivars.

Cultivar and RIL	*sun481* (bp)	*IWB12294* (G:T)
AUS27507 and HR RIL	240	G
AUS27229 and HS RIL	100	T
Calingiri	100	T
Carnamah	250+120+100	T
Derrimut	120+100	T
DiamondBird	250+100	T
EGA Bellaroi (durum)	120+100	T

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EGA BonnieRock	130+120+100	T
EGA Gregory	250+130+100	T
EmuRock	250+120+100	T
Espada	250+120	T
Forest	250+120+100	T
Giles	255+100	T
Gladius	250+120+100	T
Hyperno (durum)	250+100	T
Magenta	250+120+100	T
Merlin	250+120+100	T
Orion	250+120+100	T
Scout	120+100	T
Spitfire	250+120+100	T
Sunvale	255+250+100	T
Ventura	100	T
Wyalkatchem	250+100	T

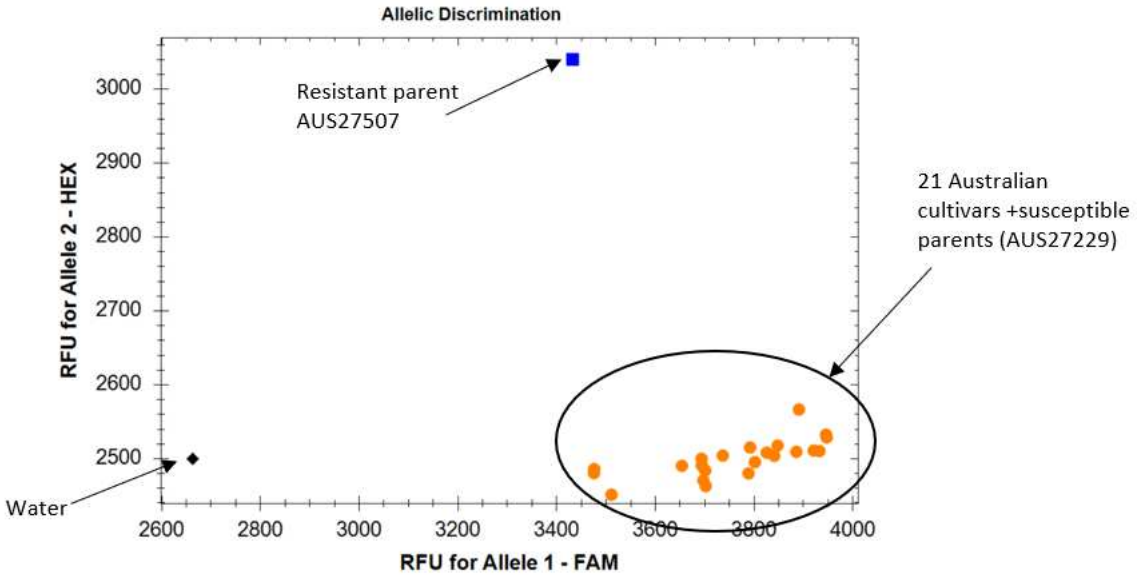

Figure 3. Allelic discrimination plot of SNP marker *IWB12294* using KASP assay. Allele 2 (blue) represents AUS27507 carrying *YrAW4* and Allele 1 (orange dots) represents Australian cultivars and susceptible parent AUS27229.

4. Discussion

This study identified a new stripe rust resistance locus in landraces AUS27507 and AUS27894. Different genomic resources were used to determine the genomic location and to identify closely linked markers for marker assisted selection of this gene. Absence of segregation among AUS27894/AUS27507 F3 population demonstrated presence of the same gene in both genotypes. The resistance locus was temporarily named *YrAW4*. DArT based BSA indicated the location of *YrAW4* in chromosome 2BL and deletion mapping of flanking DArT markers refined the location of *YrAW4* in the deletion bin 2BL-5. Markers *sun481* and *sun482* mapped 1.8 cM proximal and 2.7 cM distal to *YrAW4*, respectively. The target genomic region was enriched by converting *YrAW4*-linked SNP markers to KASP assays. Marker *IWB12294* mapped 1.2 cM distal to *YrAW4* and 1.5 cM proximal to *sun482* (Figure 2c).

Seedling stripe rust resistance genes *Yr5*, *Yr7*, *YrSp*, *Yr43*, *Yr44* and *Yr53* were previously reported in the long arm of chromosome 2B (2BL) [https://wheat.pw.usda.gov/GG3/wgc]. *Yr5* and *Yr7* were mapped 21cM away from centromere. *Yr7* was proved as an allele of *Yr5* [27]. *Yr5* produce

IT from 0; to; against Australian Pst pathotype 134 E16A+ and its derivatives and *Yr7* is not effective against this group of pathotypes and hence *YrAW4* cannot be either of these genes. Moreover, *Yr5*-linked marker, *Yr5STS7/8* [28], Digenic segregation among *YrAW4*/*YrSp* F2 population (H.S. Bariana unpublished results) also excluded the possibility of *YrAW4* to be *YrSp*.

Yr43 [29], *Yr44* [30] and *Yr53* [30] produce IT 2 on a 0 to 9 scale, which is much lower than IT 2C-3C (on a 0 to 4 scale) produced by *YrAW4*. Xu et al. [30] placed flanking markers for *Yr43*, *Yr44* and *Yr53* in the chromosomal deletion bin 2BL-3, which is next to the centromeric deletion bin 2BL-9 (Figure 2a, 2b). The *YrAW4*-linked marker *sun481* was mapped in the deletion bin 2BL-5. Based on infection type and deletion bin location, we believe that *YrAW4* is a unique locus and therefore, *YrAW4* was formally named *Yr72*.

The usefulness of markers closely linked with the resistance gene requires validation across potential backgrounds in which the target gene has to be transferred. Presence of the resistance-linked allele in genotypes carrying the target gene is referred to as 'positive validation' and the absence/amplification of an alternate allele in cultivars lacking the target locus is called 'negative validation'. Since *YrAW4* has not yet been deployed in modern cultivars, 'negative validation' was performed. Markers *sun481* and *IWB12294* were negatively validated among a set of Australian wheat cultivars including two durum wheat genotypes (Table 4 and Figure 3). These robust markers can be used (Tables 2 and 3) for marker assisted selection of *Yr72* in these backgrounds.

Markers linked with genes *Yr15* [31], *Yr51* [22] and *Yr57* [32] that are effective against currently predominant Pst pathotypes and race non-specific APR genes *Yr18* [33], *Yr36* [34] and *Yr46* [35] are available to deploy different combinations of these genes in future wheat cultivars. SNP markers linked with economic traits are preferred by breeding companies for their amenability for high-throughput testing. *YrAW4* linked SNP marker *IWB12294* offers this opportunity (Table 3) for marker assisted selection and marker-assisted pyramiding of this gene with other ASR and APR genes. *YrAW4* is currently being transferred to leading Australian cultivars through marker assisted selection.

Author Contributions: This study was planned by H.B. and U.B. and H.B., U.B. and H.M. created the mapping population. H.B and M.C. performed phenotyping in the greenhouse. M.H. and D.W. carried out bulk segregant analysis. U.B. and M.C. developed STS and KASP markers and mapped those on the RIL population. The manuscript was ddrafted by M.C., U.B and H.B and was read and approved by all authors.

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Informed Consent Statement: Not applicable as this study did not involve humans.

Data Availability Statement: All data included in this publication.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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