An Adult Plant Stripe Rust Resistance Gene Maps on Chromosome 7A of Australian Wheat Cultivar Axe

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Abstract

Australian wheat cultivar Axe produced resistant to moderately resistant stripe rust responses under field conditions and was exhibiting seedling response varying from 33C to 3+ under greenhouse conditions. Experiments covering tests at different growth stages (2^{nd}, 3^{rd} and 4^{th} leaf stages) demonstrated the clear expression of resistance at the 4^{th} leaf stage under controlled-environment greenhouse conditions. A recombinant inbred line (RIL) population was developed from the Axe/Nyabing-3 (Nyb) cross. Genetic analysis of Axe/Nyb RIL population in the greenhouse at the 4^{th} leaf stage showed monogenic inheritance of stripe rust resistance. Selective genotyping using the iSelect 90K Infinium SNP genotyping array was performed and the resistance locus was mapped to long arm of chromosome 7A and named *Yr75*. The Axe/Nyb RIL population was genotyped using a targeted genotype-by-sequencing (tGBS) assay and the resistance-linked SNPs were converted into kompetitive allele specific PCR (KASP) markers. These markers were tested on the entire Axe/Nyb RIL population and markers *sunKASP_430* and *sunKASP_427* showed close association with *Yr75* in Axe/Nyabing-3 RIL population. A high-resolution mapping family of 1032 F_2 plants from the Axe/Nyb cross was developed and genotyped with *sunKASP_430* and *sunKASP_427* and these markers flanked *Yr75* at 0.3 cM and 0.4 cM, respectively. These markers covers 1.24Mb of the physical map of Chinese Spring and can be used for future map-based cloning of this gene.

Introduction

Stripe rust or yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), ranks high among fungal diseases of wheat worldwide. This disease has the potential to cause almost 1 billion AUD of losses in Australia (Murray and Brennan 2009). Previously, this disease was endemic to cooler wheat-growing regions, but in the last two decades it has adapted to relatively warmer regions causing worldwide expansion and leading to many destructive pandemics (Ali et al. 2014).

The global progression and rapid evolution of stripe rust has led to increased application of fungicides, effectively decreasing the economic losses in several epidemics (Carmona et al. 2020), but the use of fungicides is neither environment-friendly nor cost-effective control measure. Experiments have also been conducted on the biological control of stripe rust, but no significant reduction in severity of disease has been noted. This approach is also not practically viable (Reiss and Jørgensen 2017; Feodorova-Fedotova et al. 2019). Both these control measures are resource-demanding and unprofitable for farmers with small land holdings in developing nations. In contrast, breeding for rust resistance is considered a preferred method to effectively control this disease due to its eco-friendly and economic nature (Bariana et al. 2007a).

Resistance to stripe rust has been classified into two broad classes (Bariana 2003; Chen 2005) often referred to as overall resistance (also known as all stage resistance; ASR) and adult plant resistance (APR). Genes that condition ASR follow gene-for-gene model (Flor 1942) and exhibit high levels of resistance. Deployment of ASR genes individually in wheat cultivars makes them vulnerable to breakdown through acquisition of virulence in pathogen populations (Bariana et al. 2016). The APR genes express resistance at post-seedling stages and show non-hypersensitive reactions and slow disease development on genotypes carrying these genes. This type of resistance has also been referred to as partial or slow rusting resistance and is considered more durable (Caldwell 1968). Some APR genes conferred pleiotropic resistance towards multiple diseases including stripe rust, leaf rust, stem rust and powdery mildew. These include *Yr18/Lr34/Sr57/Pm38*; *Yr29/Lr46/Sr58/Pm39* and *Yr46/Lr67/Sr55/Pm46* (https://wheat.pw.usda.gov/GG3/wgc). A third category of resistance that expresses clearly at the 3rd to 4th leaf stages, but behaves like ASR in expression and can be referred to as mid-stage resistance (MSR) (Chhetri et al. 2016a), for example; *Yr58* (Chhetri et al. 2016b), *Lr48* (Nsabiyera et al. 2016) and *Lr49* (Nsabiyera et al. 2020).

To date, 83 stripe rust resistance genes have been formally designated (https://wheat.pw.usda.gov/GG3/wgc) and most of these genes belong to the ASR category. The availability of various high-throughput genotyping platforms (e.g. DArT array, DArTseq, 40K and 90K SNP arrays, genotyping-by-sequencing) have accelerated the discovery of new disease resistance loci and the development of trait-linked molecular markers. Moreover, complete genome assembly of common wheat genotype Chinese Spring IWGSC RefSeq v1.0 (IWGSC, 2018) and IWGSC RefSeq v2.0 (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v2.0/) have offered opportunities for fine mapping and map-based cloning of loci that control economic tratis.

Stripe rust resistance genes *Yr26* (Wu et al. 2018), *Yr29* (Cobo et al. 2019) and *Yr47* (Qureshi et al. 2017) have recently been fine mapped. Resistance genes *Yr5*, *Yr7*, *YrSp* (Marchal et al. 2018), *Yr15* (Klymiuk et al. 2018), *Yr18/Lr34* (Krattinger et al. 2009), *Yr36* (Fu et al. 2009) and *Yr46* (Moore et al. 2015) have been cloned using these new tools. Axe, an Australian cultivar released in 2015 by Australian Grain Technologies, was susceptible at the seedling stage against Australian Pst pathotypes and showed high level of resistance at the adult plant stage. A recombinant inbred line (RIL) population was developed for genetic analysis of stripe rust resistance in cultivar Axe and molecular mapping of gene (s) involved in expression of resistance.

Materials And Methods

Population development

Cultivar Axe was crossed with a susceptible genotype Nybing-3, a selection from cultivar Nyabing. F_1 seeds were grown and harvested separately for producing the F_2 population. Individual F_2 seeds were planted 10 cm apart in the field and each plant was harvested separately. A single spike was harvested from each greenhouse grown F_3 family to generate an F_4 population. Similarly, single head harvest from F_4 generation led to the production of an F_5 population. Single seed from each F_5 line was grown and the whole plant was harvested to raise an F_6 generation. The final set of 151 lines is referred to as the Axe/Nyb recombinant inbred line (RIL) population.

Greenhouse screening

The Axe/Nyb RIL population was screened in the greenhouse against the most prevalent Pst pathotype 134E16A+Yr17+Yr27+ (Plant Breeding Institute culture no. 617). Eight to 10 seeds from each RIL (four RILs per pot) were sown in 9-cm diameter pots filled with a potting mixture comprising of composted pine bark and sand in a 2:1 ratio, followed by fertilizer treatment (25g Aquasol®; Hortico Pty. Ltd., Revesby, NSW, Australia /10L of water for 100 pots). Seedlings were fertilised weekly with Urea at the same rate as Aquasol. Inoculations were carried out at the 4^{th} leaf stage in a specialized inoculation chamber by atomising urediniospores of Pst pathotype 134E16 A+Yr17+Yr27+ suspended in light mineral oil (Isopar L, 5 mg spores 10 ml^{-1} of oil as solvent) using a hydrocarbon propellant pressure pack. After inoculation, plants were moved to luke-warm water-filled steel trays covered with polythene hoods in the incubation room set at 9-12 °C. Following 24 hours of incubation, plants were transferred to microclimate rooms (automated temperature and irrigation control) set at 17 °C. Rust response assessments were performed 16 -18 days after inoculation using a 0 to 4 scale described in McIntosh et al. (1995.) The Axe/Nyb RILs were classified as homozygous resistant (HR), homozygous susceptible (HS) and segregating (this class can have very low frequency and sometime could represent a mixture).

Molecular mapping

DNA extraction

DNA from 10-12 days old seedings of each Axe/Nyb RIL and parents was extracted and quantified following a modified CTAB method outlined in Bansal et al. (2014).

Chromosomal location of stripe rust resistance

Selective genotyping

For identification of chromosomal location of the resistance locus, selective genotyping was carried out on eight resistant and eight susceptible RILs using an Illumina iSelect 90K Infinium SNP genotyping array (Wang et al. 2014). The stripe rust response-linked SNPs were converted into kompetitive allele-specific polymerase chain reaction (KASP) assays and were tested on parental lines (Axe and Nyb) using the protocol described by LGC (Laboratory of the Government Chemist) genomics, UK (www.biosearchtech.com/ngs). The KASP markers which gave clear clusters were genotyped on the entire RIL population for construction of a linkage map.

KASP genotyping

All KASP assays in this study were genotyped following the procedure described in Pakeerathan et al. (2019).

Detailed mapping using a targeted genotyping-by-sequencing (tGBS) assay

Once the genomic region that controls stripe rust resistance was identified through selective genotyping, we used targeted genotyping-by-sequencing (tGBS) assay for saturation of the map. This service was provided by Agriculture Victoria, Agribio, Bundoora, Victoria. The purpose of the tGBS assay was to capture additional polymorphisms between parents by sequencing the genomic region of interest. The genetic map was constructed using the R package ASMap (Taylor and Butler 2017) and imputations for missing SNP data were performed using Impute v2.2 (Sargolzaei et al. 2014).

Marker design

An automated bioinformatic pipeline PolyMarker (Ramirez-Gonzalez et al. 2015) was used for designing KASP markers from the SNPs associated with stripe rust resistance identified through tGBS mapping. Allele specific primers A1 and A2 were tagged with diagnostic sequences for fluorescent dyes FAM (gaaggtgaccaagttcatgct) and HEX (gaaggtcggagtcaacggatt) at their 5□, respectively.

High-resolution mapping

A set of seven KASP markers from the tGBS assay were genotyped on the entire Axe/Nyb RIL population (154 lines) and these markers were incorporated into the initial map. Following this, a large mapping population of 1032 F_2 plants (2064 gametes) from an Axe/Nyb cross was developed. DNA from this population was extracted using a modified SDS (sodium dodecyl sulfate) DNA extraction protocol (M. Pourkheirandish personal communication). Leaf tissue from individual F_2 plants was collected in 96 wells plate and two ball bearings and 450µl of extraction buffer (100mM Tris-HCl pH 8.0, 50mM EDTA pH 8.0, 500mM NaCl and 10mM 2-mercaptoethanol) was added in each well and samples were crushed in a tissue lyser (1600 MiniG^{TM}) for 3 minutes. After crushing, 60µl of 10% SDS buffer was added and samples were inverted vigorously for homogenous mixing. Samples were then incubated at 65 °C for 60 min and the plate was centrifuged for 1 min at 3600 rpm. After centrifugation, 200µl of 7.5 M ammonium acetate was added and the plate was shaken vigorously, followed by incubation for 60 min at 4 °C. After centrifugation at 1000 rpm for 1 min, 300µl of chloroform was added and the plate was again shaken vigorously, followed by centrifugation for 10 min at 4800 rpm. One hundred µl of supernatant was added to100µl of chilled isopropanol in new plate and

mixed gently. After centrifugation for 10 min at 4800 rpm, the isopropanol was removed and the pellet was resuspended in 100µl of 1M Tris HCl (pH 8) containing 10mM of RNase, with incubation at 37 °C for 1-2 hours.

Yr75 Flanking markers were tested on a large F_2 population. Recombinants were phenotyped in the F_3 generation against Pst pathotype 134E16A+17+27 to refine the location of the stripe rust resistance locus.

Statistical analysis and genetic mapping

Chi-squared (χ^2) analysis was used to identify segregation distortion among markers located near the resistance locus. The KASP and SSR marker data were converted to 'A' for Axe allele and 'B' for Nyb allele and 'H' for heterozygotes for mapping. Genetic linkage maps were constructed using MapManager QTXb20 (Manly et al. 2001) with the Kosambi map function (Kosambi 1943) and then presented graphically using MapChart version 2.3 (Voorrips 2002). The physical position of markers that flanked the resistance locus and structural variation in the genomic region was investigated using the tool Pretzel (Keeble-Gagnère et al. 2019) which compares genetic and physical maps.

Results

Greenhouse screening

The Axe/Nyb RIL population was inoculated with the Pst pathotype 134 E16A+Yr17+Yr27+ under greenhouse conditions at the 2nd, 3rd, and 4th leaf stages. Expression of resistance was not clear at the 2nd to 3rd leaf stages; however, a clear expression of resistance was observed at the 4th leaf stage. The resistant parent Axe produced infection type (IT) ;1-C and the susceptible parent produced IT3+ (Figure 5.1). Infection types among resistant RILs ranged from IT1C to IT23C. These results suggested that resistance carried by Axe does not typically belong to the either of the currently defined ASR or APR categories.

The Axe/Nyb RILs were classified as homozygous resistant (HR=1C to 23C) and homozygous susceptible (HS=3+). Eighty-four lines were placed in the HR class, while 67 were categorised as HS. Chi-squared analysis of stripe rust response variation among RILs conformed to segregation at a single locus (Table 5.1). The underlying stripe rust resistance locus was named *YrAxe*.

Table 1
Frequency distribution of Axe/Nyb RILs when tested against Pst pathotype 134E16A+Yr17+Yr27+

Phenotype	Number of RILs		χ^2 (1:1)
	Observed	Expected	
Homozygous resistant	84	75.5	0.96
Homozygous susceptible	67	75.5	0.96
Total	151	151	1.92
Table value of χ^2 at P = 0.05 and 1df = 3.84			

Molecular mapping of *YrAxe*

Selective genotyping with the 90K SNP array was used to identify the chromosomal location of *YrAxe*. Of 306 linked SNPs, 140 SNPs were mapped in chromosome 7AL, 55 in 7BL and seven on 7DL of the wheat consensus map (Wang et al. 2014). Among the 7AL SNPs, 128 SNPs spanned a region of 708,363,089 bp to 726,684,629 bp of the Chinese Spring physical map (IWGSC RefSeq v1.0) and showed strong linkage with *YrAxe*. One SNP from each representative LD block (28 SNPs in total) was converted into a KASP assay. Two KASP markers *KASP_34640*, and *KASP_38710*, clearly differentiated the parents (Table 5.2) and *KASP_39562* produced heterozygous allele for Nyb.

Table 2
List of *YrAxe*-linked KASP markers developed from the iSelect 90K SNP genotyping

Marker	SNP	Position in 90K map (cM)*	Primer sequence		
			Allele 1[a]	Allele 2[b]	Common primer
KASP_34640	[A/G]	216.36	Aagataaaatcgtccatcaagtct	aagataaaatcgtccatcaagtcc	atgaaggacgacgcagacac
KASP_38710	[T/C]	212.97	Ggagatgacaggtcaaaactatcat	ggagatgacaggtcaaaactatcac	tatgcgtaggccttcccgta
KASP_39562	[T/C]	Not known	Cacggataggctcttgcttt	cacggataggctcttgcttc	tgggaagcttatgcctggt

* marker position in 90K SNP consensus map (Wang et al. 2014), [a] A1 primer labeled with FAM: GAAGGTGACCAAGTTCATGCT

[b] A2 primer labeled with HEX: GAAGGTCGGAGTCAACGGATT

YrAxe was mapped on the long arm of chromosome 7A. The SSR marker, *cfa2040*, previously mapped on chromosome 7A was used to confirm the map location of linked SNP markers and was genotyped on the entire Axe/Nyb RIL population. *YrAxe* was flanked by *cfa2040* and *KASP_34640*. Since no other stripe rust resistance gene was previously located in the region flanked by *cfa2040* and *KASP_34640*, *YrAxe* was permanently named *Yr75*.

Detailed mapping of *Yr75*

Low-resolution mapping

Seven SNPs from the tGBS assay that showed close association with *Yr75* were converted into KASP markers (*sunKASP_425, sunKASP_426, sunKASP_427, sunKASP_428, sunKASP_429, sunKASP_430, sunKASP_431*). Primer sequences of these markers are given in Table 3. These markers differentiated parents clearly and were genotyped on the entire Axe/Nyb RIL population. A genetic map carrying *Yr75* was developed including two 90K SNPs and *cfa2040*. Marker *sunKASP_427* mapped at 0.7 cM proximally to *Yr75* while su*nKASP_430* co-segregated with the gene (Figure 2a). The physical positions of flanking markers in Chinese Spring (IWGSC RefSeq v1.0) were at 719,076,651 (*sunKASP_427*) and 717,832,538 bp (su*nKASP_430*) (Figure 3).

Table 3
List of *Yr75*-linked KASP markers developed from the tGBS assay

Marker	tGBS marker name	SNP	Super scaffold	Position in IWGSC v1.0 wheat genome (bp)	Primer sequence		
					Allele 1	Allele 2	Common
sunKASP_425	scaffold134444|TaGBSv2-5557_274769	[T/C]	31	714,587,271	tcatggtcaagttcaacggt	tcatggtcaagttcaacggc	tcgctaggggagtgc
sunKASP_426	scaffold43271-1|TaGBSv2-5558_88393	[C/G]	32	715,122,740	ctattgccctcctcatcgatg	ctattgccctcctcatcgatc	gagggaggaagcg
sunKASP_427	scaffold96044|TaGBSv2-11657_1099665	[A/G]	32	719,076,651	cgacgacgtccaaagactaat	cgacgacgtccaaagactaac	gcaacaacacacta
sunKASP_428	scaffold43271-1|TaGBSv2-5562_2434232	[T/A]	32	717,468,579	cacagccgcggaaagact	cacagccgcggaaagaca	tgcggttgccttgttat
sunKASP_429	scaffold43271-1|TaGBSv2-11654_2223053	[T/C]	32	717,257,400	agccgctcgaactacattttt	agccgctcgaactacattttc	gctgtgcacagtatg
sunKASP_430	scaffold43271-1|TaGBSv2-11655_2798191	[A/G]	32	717,832,538	agcgcagagagatgcagt	agcgcagagagatgcagc	agacctgcggtagct
sunKASP_431	scaffold96044|TaGBSv2-5564_877551	[C/A]	34	718,854,537	tggtccgccatgaagtcc	tggtccgccatgaagtca	acgaccttctgcacg

High-resolution mapping

Markers *sunKASP_425, sunKASP_426, sunKASP_427, sunKASP_428, sunKASP_429, sunKASP_430* and *sunKASP_431* from the Axe/Nyb low resolution map were genotyped on 1032 individual F_2 plants (2064 gametes) to refine recombination in the region. *KASP_34640* and *KASP_38710* did not produce clear clusters for heterozygotes therefore were not included in mapping. Marker *cfa2040* is a multiallelic locus (amplify alleles on chromosome 7A, 7B and 7D), size difference was only 2bp (7A) which can be assessed clearly in the RIL population, but it is very difficult to score in F_2 population, therefore this marker was also not used for genotyping. Thirty recombinants were identified and grown for progeny testing. These progenies were scored at the 4^{th} leaf stage and a high-resolution map was constructed. Markers *sunKASP_427* and *sunKAS_430* flanked *Yr75* at 0.4 cM, and 0.3 cM proximally and distally, respectively (Figure 2b).

Discussion

This study demonstrated the expression of stripe rust resistance at the 4th leaf stage in wheat cultivar Axe and the underlying locus was mapped on the long arm of chromosome 7A. As none of the previously known stripe rust resistance genes mapped in this region, the resistance locus was permanently named *Yr75*. The expression of stripe rust resistance at post seedling stages was previously described by Chhetri et al. 2016 in a landrace W195 from India and in an Australian cultivar Sentinel by Chemayek (2016). This class of genes should be treated as a different category and ideally fit into the MSR category and unlike typical APR genes *Yr18* and *Yr29* (Lagudah et al. 2006; McIntosh 1992; Singh 1992).

This investigation used the state-of-the-art technologies and sequence information for detailed mapping of the chromosome 7A region that carried *Yr75*. Several studies have used an integrated approach for development of molecular markers for rust resistance which include *Lr49* (Nsabiyera et al. 2020), *Yr47* (Qureshi et al. 2017) and *Yr26* (Wu et al. 2018).

The Axe/Nyb map was saturated through a targeted genotyping-by-sequencing (tGBS) assay that generates sequence data for genomic regions surrounding known exome-derived SNPs. SNPs identified from the tGBS analysis, 90K Infinium assay and SSR marker *cfa2040* led to the development of a low-resolution map with 0.7 cM interval with *sunKASP_427* and *Yr75-sunKASP_430*. Markers *cfa2040* and *KASP_34640* were located in the tGBS scaffolds scaffold43271-1 and scaffold96044, respectively, from which *Yr75*-flanking markers were devloped. Due to the lack of good clustering and similar positioning in the scafoolds these markers were not genotyped in the high resolution population. Markers devloped from tGBS SNPs were genotyped on a high-resolution Axe/Nyb F_2 population and genetic distances of 0.4 cM for *sunKASP_427* (11 recombinants) and 0.3 cM for *sunKASP_430* (10 recombinants) from *Yr75* were obsereved. These markers were devloped from two scaffolds (scaffold96044 and scaffold43271-1) and were represented in the superscaffold 32 of the IWGSC v1.0 genome assembly (IWGSC. 2018). The tGBS approach has been used for mapping and development of molecular markers for only a few genes including *Yr82* (Pakeerathan et al. 2019) *Sr26* (Qureshi et al. 2018) and *Yr26* (Wu et al. 2018) in wheat.

Despite the low genetic distance (1.24 Mb physical disctance; IWGSC RefSeq v1.0 genome assembly) between closely linked markers *sunKASP_427* and *sunKASP_430* and *Yr75*, we were unable to validate these markers on a diverse set of wheat genotypes, which suggested either chromosomal rearrangement or the supressed recombination in this region. *Yr75* is located near the telomere of the 7A chromosome, where frequent recombination should be expected. The existence of suppressed recombination is also supported by previous studies on pleiotropic locus *Sr15*/ *Lr20/Pm1* (Sears and Briggle 1969; Watson and Luig 1966) on chromosome 7AL, which showed a total genetic distance of 9.6 cM flanked by markers *psr148* and *psr687* (Gale 1995). The fine mapping of chromosome 7AL later revealed that the markers *cdo347, psr121*, and *ksuh9* covered a region of 30 cM (Peña et al. 1997). Genetic mapping of the *Lr20-Pm1* in three different F_2 populations also revealed that markers at the distal end were completely linked with *Lr20-Pm1* locus, but at the proximal end there was a discrepancy compared to previous map for this locus (Neu et al. 2002). These results also suggested reduced recombination in this region and/or chromosomal rearrangements. Suppressed recombination was also reported in another study for root lesion nematode gene *Rlnn1* which is tightly linked with *Sr15*/ *Lr20* locus (Jayatilake et al. 2013). These results supported the hypothesis that terminal region of chromosome 7A is one of the complex region resulted due to several chromosomal abberations (Badaeva et al. 2007). To move forward, different appraoches such as flow sorting of chromosome of the line carrying the target locus could be helpful in saturating the region and finding closer markers. Further study on this region by using advanced genomic techniques including sequence capture and CRIPSR mediated genetic manipulation will facilitate the unexplained reason for complexity of this region.

In conclusion, this study provided a new adult plant stripe rust resistance gene *Yr75* and tightly linked markers for marker-assisted selection for this gene in breeding material following parental polymorphism check. These results will be useful in map based cloning of *Yr75*.

Declarations

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Grain Research Development Corporation, Australia.

Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

Availability of data and material:

All data are given in the manuscript

Code availability:

Publicly available software's are used in this study

Authors' contributions:

UB & HB planned the study, MK drafted the manuscript; MK & MG developed segregating population and performed phenotyping, KF and NQ performed genotyping and tGBS analysis, UB and MK designed the KASP primers and MK and PB performed KASP genotyping, UB, HB, KF, and NQ edited the manuscript.

Key message:

An adult plant stripe rust resistance gene *Yr75* was fine mapped on chromosome 7AL which is a step closure for future map-based cloning for this gene.

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Figures

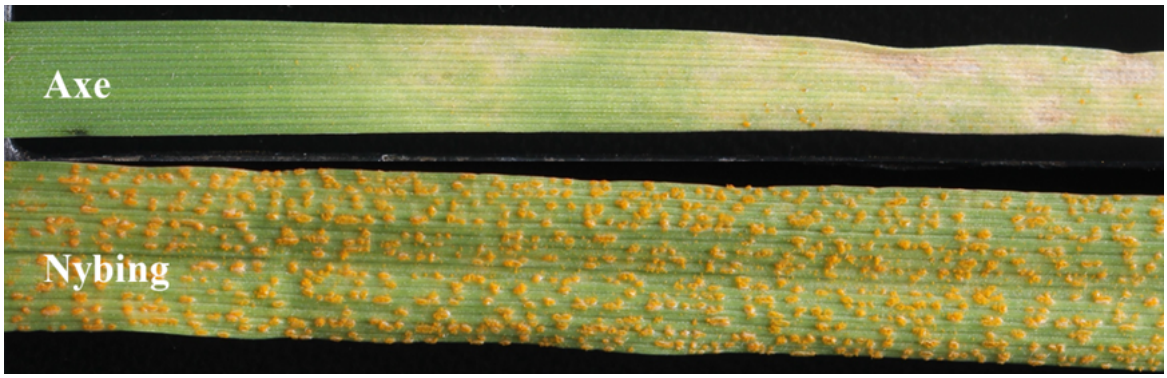

Figure 1

Infection types produced by parents Axe and Nyb against Pst pathotype134E16 A+Yr17+Yr27+ at 4th leaf stage

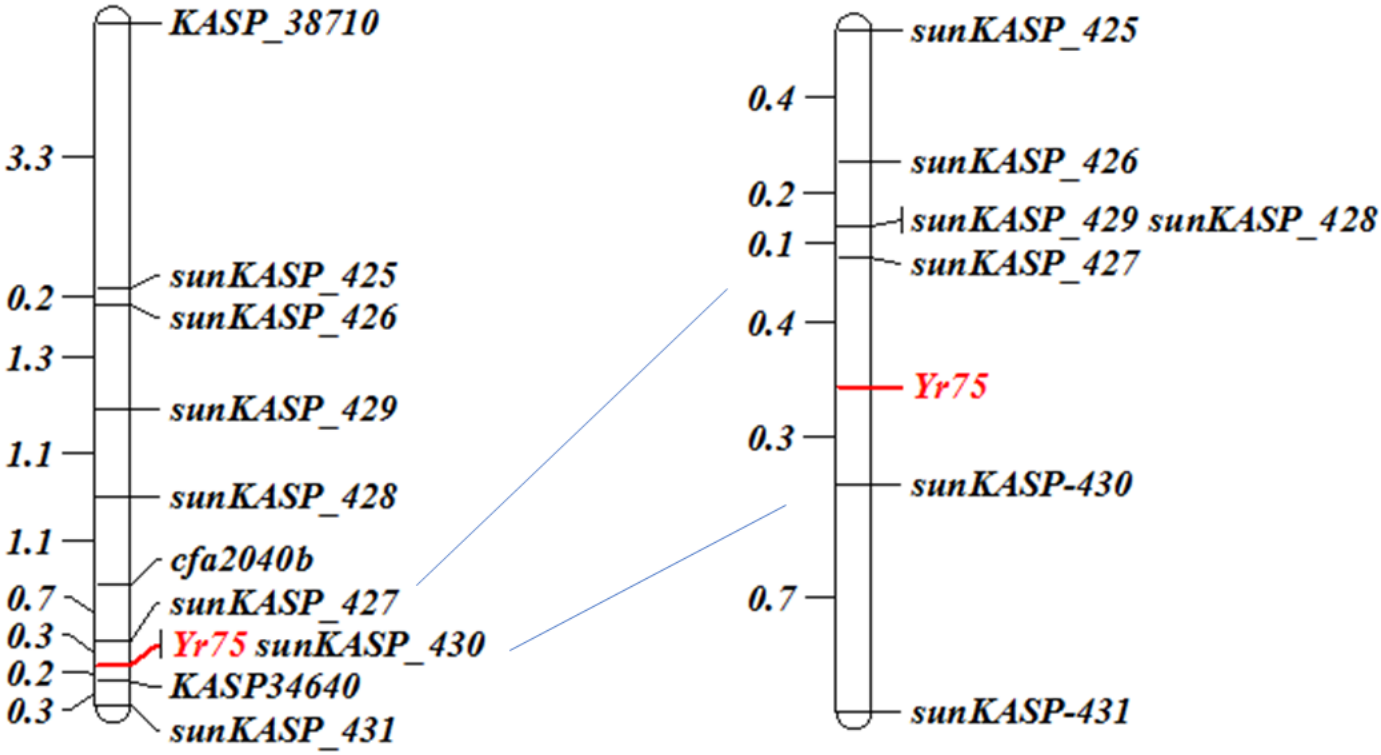

Figure 2

Low-resolution map of chromosome 7AL of Axe/Nyb RIL population (a) and high-resolution map of Axe/Nyb F2 population (b)

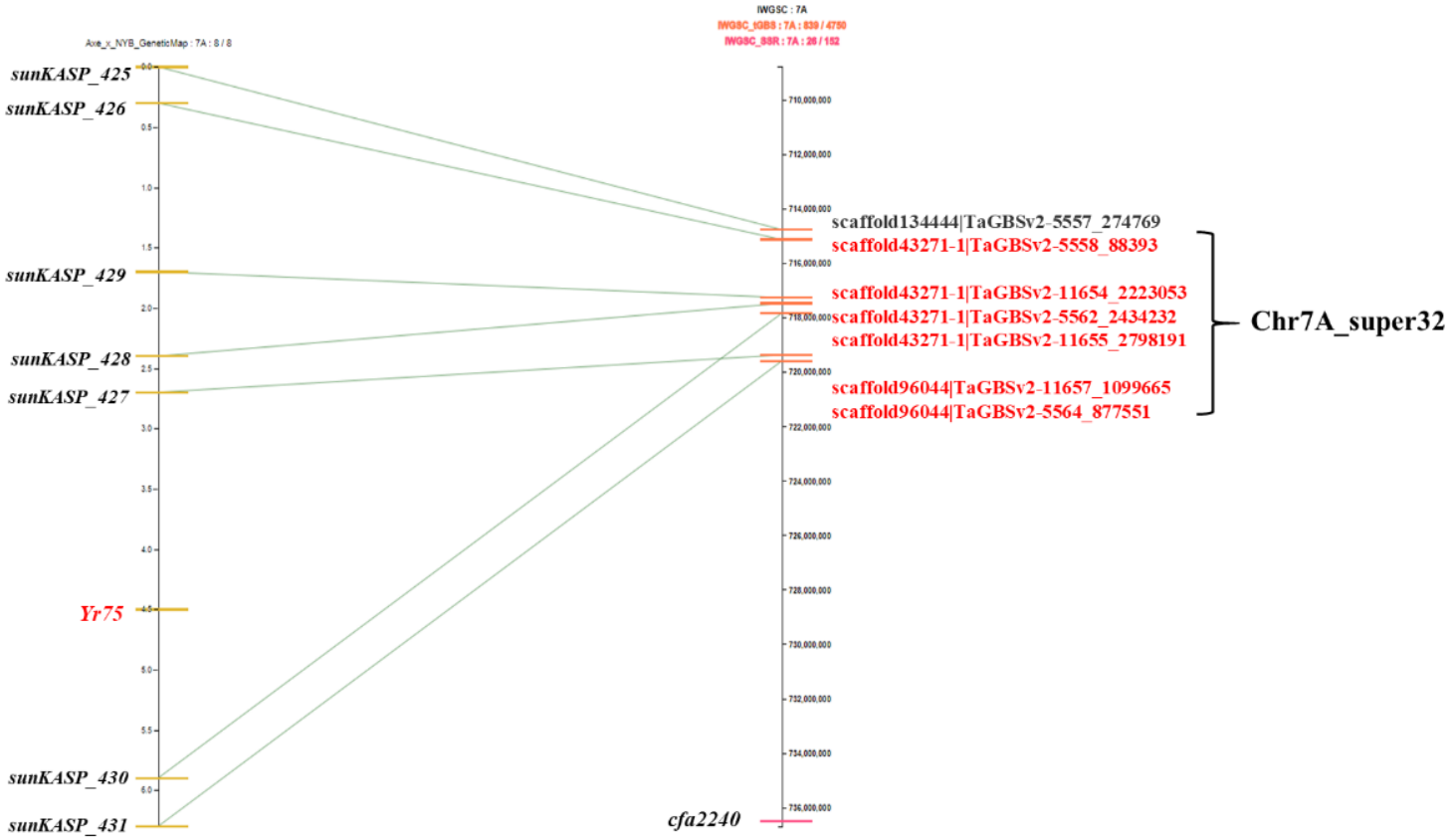

Figure 3

Position of Yr75 linked markers on chromosome 7AL in the IWGSC v1.0 Chinese Spring genome assembly. Figure constructed using Pretzel (Keeble-Gagnère et al. 2019)