

# Adult Plant Stem Rust Resistance in Durum Wheat Glossy Huguenot – Mapping, Marker Development and Validation

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## Research Article

**Keywords:** Rust Resistance, Wheat Glossy, kompetetive allele

**Posted Date:** December 10th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1133072/v1>

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# Abstract

An F<sub>3</sub> population from a Glossy Huguenot (GH)/Bansi cross used in a previous Australian study was advanced to F<sub>6</sub> for molecular mapping of adult plant stem rust resistance. Maturity differences among F<sub>6</sub> lines confounded assessments of stem rust response. GH was crossed with a stem rust susceptible F<sub>6</sub> recombinant inbred line (RIL), GHB14 (M14), with similar maturity and an F<sub>6:7</sub> population was developed through single seed descent method. F<sub>7</sub> and F<sub>8</sub> RILs were tested along with the parents at different locations. The F<sub>6</sub> individual plants and both parents were genotyped using the 90K single nucleotide polymorphism (SNP) wheat array. Stem rust resistance QTL on the long arms of chromosomes 1B (*QSrGH.cs-1BL*) and 2A (*QSrGH.cs-2AL*) were detected. *QSrGH.cs-1BL* and *QSrGH.cs-2AL* were both contributed by GH and explained 22% and 18% adult plant stem rust response variation, respectively, among GH/M14 RIL population. RILs carrying combinations of these QTL reduced more than 14% stem rust severity compared to those that possessed *QSrGH.cs-1BL* and *QSrGH.cs-2AL* individually. *QSrGH.cs1BL* was demonstrated to be the same as *Sr58/Lr46/Yr29/Pm39* through marker genotyping. Lines lacking *QSrGH.cs-1BL* were used to Mendelise *QSrGH.cs-2AL*. Based on genomic locations of previously catalogued stem rust resistance genes and the *QSrGH.cs-2AL* map, it appeared to represent a new APR locus and was permanently named *Sr63*. SNP markers associated with *Sr63* were converted to kompetitive allele specific PCR (KASP) assays and were validated on a set of durum cultivars.

## Key Message

Adult plant stem rust resistance locus, *QSrGH.cs-2AL*, was identified in durum wheat Glossy Huguenot and Mendelised as *Sr63*. Markers closely linked with *Sr63* were developed.

## Introduction

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is one of the most devastating fungal diseases affecting wheat production worldwide. The detection of *Pgt* isolate Ug99 (Pretorius et al. 2000) and its variants with additional virulence led to a global research program (<https://www.globalrust.org/>). Subsequent appearance of highly virulent non-Ug99 races in Ethiopia (Olivera et al. 2012, 2015) and Germany (Olivera et al. 2017), and an outbreak of *Pgt* race TTTTF in Italy (<http://rusttracker.cimmyt.org/?p=7083>; Babiker et al. 2017; Bhattacharya 2017) on both common wheat and durum highlighted the need for continuous discovery of new resistance genes and monitoring of variation among *Pgt* populations.

Of the 62 stem rust resistance genes that have been described; most belong to the race-specific all-stage resistance (ASR) category. These genes generally confer high to moderate levels of resistance to avirulent races but are often short lived due to the emergence of new races with virulence for commercially deployed resistance genes. A few adult plant (APR) stem rust resistance genes include *Sr2* (McFadden 1930), *Sr55* (Herrera-Foessel et al. 2014), *Sr56* (Bansal et al. 2014), *Sr57* and *Sr58* (Singh et al. 2015). APR genes typically reduce the growth rate of the pathogen (Herrera-Foessel et al. 2012) and may be race non-

specific. APR genes are also known to interact with ASR genes to enhance their expression (Ayliffe et al. 2013; Bariana et al. 2001; Lagudah 2011).

Many stem rust resistance genes in hexaploid wheat were introgressed from wild relatives or progenitors including *Sr2*, *Sr22* and *Sr35* (Fedak 2015). Among the tetraploid species of wheat, *T. turgidum* ssp. *durum*, is the main species grown commercially. Durum, the 10th most important crop in the world with annual production of approximately 36 million metric tons, accounts for 5% of global wheat production (Bassi and Sanchez-Garcia 2017). Although the Mediterranean basin accounts for ~75% of global durum production, durum is grown to a lesser extent worldwide. For example, in Sub-Saharan Africa, Ethiopia is the largest wheat producer, with nearly 40% of its wheat growing area under durum production (Bergh et al. 2012) and accounting for 90% of durum production in that region (Sall et al. 2019). Despite its economic importance and value as a source of stem rust resistance genes in common wheat far less information is available on stem rust resistance in tetraploid wheat than in common wheat.

Many commercial durum cultivars carry stem rust resistance genes *Sr8b*, *Sr9e* and *Sr13a-d* allelic variants either singly or in different combinations (Toor et al. 2013; Letta et al. 2013, Gill et al 2021), with only *Sr13a-c* being broadly effective against the Ug99 lineage. The adult plant stem rust resistance gene *Sr2*, which was introgressed into hexaploid wheat from tetraploid Yaroslav emmer, has remained effective for over a century (Ellis et al. 2014; <https://globalrust.org/gene/sr2>), but it is not known to be present in any durum cultivar. Relatively few studies have described new stem rust resistance genes including APR in durum genotypes. Herrera-Foessel et al (2011) reported the presence of *Sr58/Lr46/Yr29* in durum cultivar Quetru and it was later detected in a large number of durum cultivars (Li et al. 2020). Haile et al. (2012) identified minor QTL against Ug99. Toor et al. (2013) screened 73 tetraploid landraces from the Watkins Collection and identified lines with varying levels of APR. Using association mapping on 183 elite durum accessions, Letta et al. (2013) identified 13 QTL located on chromosomal region where no stem rust resistance gene was reported previously; however, further characterization is required to determine the effectiveness and distribution of those QTL.

According to Hare (1997) Glossy Huguenot is a waxless selection believed to be derived from the Algerian durum variety Huguenot, an awnless selection from Medeah. Both Huguenot and Medeah were cultivated in Western Australia between 1890 and 1922 (Valder 1900). Hare (1997) crossed Glossy Huguenot (GH, Aus2499) with the stem rust susceptible durum genotype Bansi and based on analysis of F<sub>3</sub> lines concluded that stem rust resistance in Glossy Huguenot was conferred by a single dominant APR gene. The possibility of a second gene was not ruled out. With the objective to map the adult plant stem rust resistance in Glossy Huguenot we developed a recombinant inbred line (RIL) F<sub>6:7</sub> population and conducted QTL analysis using the 90k single nucleotide polymorphism (SNP) wheat array and identified resistance-linked molecular markers.

## Materials And Methods

### Plant materials

Initially,  $F_3$  population from a cross of adult plant resistant parent GH and susceptible parent Bansi were enhanced to  $F_{5:6}$  RIL generation and the population was named GHB. This population was screened at the University of Sydney Plant Breeding Institute, Cobbitty in 2003-2010. The segregation for maturity and stripe rust among this population affected stem rust assessments. Hence, a set of 210  $F_{6:7}$  RILs from a cross between GH and a stem rust susceptible but stripe rust resistant RIL GHB14 (M14) was developed through single seed descent method. M14 was similar in maturity to GH.  $F_7$  and  $F_8$  generations were tested for adult plant stem rust response variation.

A set of 20 Australian durum cultivars and 155 lines from 39th International Durum Screening Nursery (IDSN) from CIMMYT were used for marker validation. Parents Glossy Huguenot and M14 were included as controls.

## Evaluation of stem rust response

Adult plant stem rust phenotyping was done on the parents and 192  $F_7$  and  $F_8$  RILs during 2014 and 2015 using race RTR (Singh 1991) at Ciudad Obregon, Mexico and  $F_8$  RILs in 2019 at Njoro, Kenya. Stem rust response variation was scored based on disease severity (0 -100%). Pearson's correlation coefficients across years and locations were computed using R (R Core Team 2020, <https://www.r-project.org/>).

## Genetic map construction and statistical analysis

DNA from  $F_6$  generation was isolated according to Lagudah et al. (1991). A set of 192 RILs and parental lines were genotyped using the Infinium 90K wheat SNP array described in Wang et al. (2014), following the manufacturer's instructions (Illumina Ltd, USA). A high-density linkage map was constructed from 7,811 markers using ASMap (Taylor and Butler 2017), an interface to MSTmap (Wu et al. 2008) for the R environment (R Core Team 2020, <https://www.r-project.org/>). Genetic distances were estimated using the Kosambi function (Kosambi 1943). Linkage groups were formed using the criterion of  $p > 1e-6$  for each pairwise marker association. Linkage groups were assigned to the 14 chromosomes using marker location in a durum consensus map that contained most of the markers (Maccaferri et al. 2015). Rust severity data from trials were analysed with a mixed linear model using ASReml-R (Butler et al. 2009), treating years as a fixed effect and genotypes and replicates as random effects. The QTL analysis was performed using multivariate whole genome average interval mapping (Broman et al. 2003; Verbyla and Cullis 2012).

In addition, we also used MapManager version QTXb20 (Manly et al. 2001) for linkage map construction. The Kosambi mapping function (Kosambi 1943) was used to convert recombination fractions into centiMorgans (cM). The redundant markers were excluded using the command "hide redundant loci", option and the phenotypic data imported into MapManager. QTL cartographer (Wang et al. 2012) was used for composite interval mapping (CIM) with 1000 permutations. Graphical representation of the linkage map was generated using MapChart (Voorrips 2002).

## Molecular marker analysis and marker validation

Simple sequence repeat (SSR) markers previously assigned to different wheat chromosomes were obtained from the GrainGenes website (<https://wheat.pw.usda.gov/GG3/>). Kompetitive allele specific PCR (KASP) assays for trait-linked SNPs were obtained from PolyMarker ([http://www.polymarker.info/designed\\_primers](http://www.polymarker.info/designed_primers)). All KASP markers were tested on the entire RIL population. PCR reactions were performed in 96-well plates in a total volume of 8 µl, comprising of 25-30 ng of genomic DNA, 4 µl of KASP master mix (LGC Biosearch Technologies, Hoddesdon, UK) and 0.11 µl of assay mix containing 12 mM of each allele-specific forward primer, and 36 mM of reverse primer. The cycling conditions were: 15 min at 95°C, 10 touchdown cycles of 20 sec at 94°C, 60 sec at 65°C (dropping 0.8°C per cycle), 25 cycles of 20 sec at 94°C, and finally 60 sec at 57°C. Fluorescence detection of PCR products was done using Bio-Rad CFX96 manager v 3.1 software. If genotyping clusters were not formed after initial amplification, 5 to 8 additional amplification cycles were performed, and the samples read again. SNPLr46G22, linked to the *Lr46/Sr58/Yr29* APR locus (Lagudah unpublished) was used as a KASP marker to genotype the mapping population based on Kandiah et al. (2020). KASP\_32429 was amplified using allele specific primers 1. Agtgaatgcattgctcataaaaatt, 2. Agtgaatgcattgctcataaaaatg and a common primer cattaaccagtaaccaccaaagg. Allele primers 1 and 2 were tagged with FAM and HEX, respectively.

## Assessment of QTL interaction

Mean stem rust severities across sites/years for RIL carrying GH and M14 alleles for peak markers were compared to assess QTL interaction and to determine their individual mean contributions towards reductions in stem rust severity. Least significant differences (LSD) were calculated to compare mean disease severities of RILs carrying different combinations of QTL using R (<https://www.r-project.org/>).

## Results

### Adult plant rust responses

GH exhibited 10 to 20% stem rust severities across all field trials whereas the responses of the susceptible parent M14 ranged from 50 to 100%. The stem rust responses of RILs ranged from 10 to 80% in 2014 and 2019 trials, whereas 20 to 100% disease severities were noted in 2015. Figure 1 shows the distribution of disease severity during three seasons. The high inoculum pressure in 2015 compared to the other seasons resulted in higher disease severities across whole population.

### QTL mapping

The map of the GH/M14 RIL population (192 lines) comprised 23 linkage groups, each of which mapped with high confidence to one of the 14 durum chromosomes. A high-quality map was generated based on 7,812 SNPs distributed across all 23 linkage groups. There were 1,293 recombination bins, resulting in an average of six markers per bin covering a total map length of 1,937.2 cM.

Significantly positive correlations among the rust severity data among the three trials were observed (Table 1). One QTL each was detected on the long arms of chromosomes 1B and 2A in each growing season and these were named *QSrGH.cs-1BL* and *QSrGH.cs-2AL*, respectively. Both QTL were contributed by GH. The positions of SNP markers associated with both QTL are shown in Figure 2, and the QTL intervals, likelihood of odds (LOD) scores and phenotypic variation explained (PVA) are presented in Table 2.

Table 1  
Pearson correlation coefficient of stem rust severity of GH × M14 RILs

	<b>Obregon 2014</b>	<b>Obregon 2015</b>	<b>Kenya 2019</b>	<b>P value</b>
Obregon 2014	-	0.65	-	$1.69 \times 10^{-18}$
		-	0.21	0.015
Obregon 2015	-	-	0.24	0.004

Table 2  
Quantitative trait loci detected for stem rust resistance in the Glossy  
Huguenot/M14 RIL population using QTL Cartographer and 'R'

Chromosome	Trial	Marker	Position (cM)	LOD	R <sup>2</sup>
<b>QTL Cartographer</b>					
1B	Mexico-2014	<i>IWB73674</i>	66.94	8.28	14
	Mexico-2015			22.00	41
	Kenya-2019			1.96	5
2A	Mexico-2014	<i>IWB6412</i>	437.08	18.60	31
	Mexico-2015			10.44	14
	Kenya-2019			3.83	10
<b>R</b>					
1B	Mexico-2014	<i>IWB29684</i>	51.05	2.90	8
		<i>IWB5732</i>	61.53	4.00	11
		<i>IWA3388</i>	107.93	3.10	9
	Mexico-2015	<i>IWB5732</i>	61.53	5.40	15
		<i>IWB61688</i>	85.60	4.20	12
2A	Mexico-2014	<i>IWB6412</i>	437.08	6.90	19
		<i>IWB9506</i>	440.47	8.30	22
		<i>IWB58452</i>	456.30	7.30	20
	Mexico-2015	<i>IWB34603</i>	439.19	10.39	27
		<i>IWB9506</i>	440.47	11.30	29
		<i>IWB58452</i>	456.30	10.40	27
	Kenya-2019	<i>IWB34895</i>	423.45	3.00	8
<i>IWB9506</i>		440.47	3.95	11	

*QsRGH.cs-1BL* explained 14 and 41% of the phenotypic variation in stem rust severity during the 2014 and 2015 seasons, but its contribution (5%) in 2019 field trial was quite low. *QsRGH.cs-2AL* explained high phenotypic variation (31%) than *QsRGH.cs-1BL* (14%) among the RILs in 2014, but its contribution was much lower in 2015 (14%) and 2019 (10%) (Table 2). Results obtained by both QTL analysis (R package and QTL Cartographer) methods were comparable.



Several SSR markers previously mapped to chromosomes 1B and 2A were used to define the target regions. Only *gpw1077-1B* among nine SSR markers was polymorphic between the parents and was used for mapping (Figure 3). Markers *gwm265*, *gwm382*, *gwm312* and *cfa2164* from chromosome 2A were polymorphic between the parents and all were used in mapping, only *gwm312* mapped close to the QTL and is included in the map (Figure 3). Based on sequence comparisons of the SNP markers linked to the QTL with the Chinese Spring (CS) Ref Seqv2.1, wild emmer wheat Zavitan Ref V2 and the durum wheat Svevo RefV1, *QSrGH.cs-1BL* was mapped in a 691-693 Mb interval in CS, 679- 682 Mb in Zavitan and 661-664 interval Mb in Svevo. The *QSrGH.cs-2AL* was located between 683 and 696 Mb in the CS and Zavitan and at about 674 Mb in the Svevo genomes (Table 3).

Table 3  
Location of *QSrGH.cs-1BL* and *QSrGH.cs-2AL* associated SNPs in the wild emmer wheat Zavitan (Zv) Seq v2.0, and durum wheat Svevo (Sv) Seq v1.0 chromosome sequence and CS RefSeq v2.1

SNP/Gene	Zv	Sv	CS
Chromosome 1BL			
<i>IWB5732</i>	691,692,800	661,593,905	679,342,244
<i>IWB73674</i>	693,746,374	664,540,889	682,555,776
Chromosome 2AL			
<i>IWB6412</i>	683,293,540	674,726,092	683,586,715
<i>IWB9506</i>	686,885,098	678,288,685	687,152,150
<i>IWB58452</i>	696,228,559	-	696,305,895
<i>Sr21</i>	713,411,071	704,410,507	713,856,641

## Mendelisation of *QSrGH.cs-2AL*

*QSrGH.cs-1BL* was mapped to the chromosome 1B region reported to carry the pleiotropic gene *Lr46/Yr29/Sr58/Pm39*. This QTL was therefore considered to be either *Sr58* or a closely linked gene. Genotyping with *Sr58/Lr46/Yr29/Pm39* (hereafter referred to as *Sr58*)-closely linked marker *csLV46G22* predicted its presence in GH. The GH/M14 RILs that carried the *Sr58*-linked *csLV46G22* allele were removed and the remaining RIL population was used to Mendelise *QSrGH.cs-2AL*. Thirty-seven RILs were scored resistant (mean stem rust severity 52.1%) and 36 were susceptible (stem rust severity 64.5%). The phenotypic data were converted into genotypes; 'A' for GH and 'B' for M14 and incorporated into the GH/M14 map. *QSrGH.cs-2AL* was flanked by markers *IWB32429* (2.7 cM) and *IWB4881* (3.0 cM) (Figure 3). This locus was named *Sr63*.

## Development of KASP assays and validation

The SNP markers linked to *Q<sub>SrGH</sub>.cs-2AL* (Figure 3,4) were converted to KASP assays. Only *IWB32429* was reliably scored as a diagnostic KASP (*KASP\_32429*). As expected, the KASP marker co-segregated with the respective SNP from which it was developed. *KASP\_32429* was also used for genotyping a panel of durum cultivars. No cultivar carried the *Sr63*-linked *KASP\_32429* allele indicating the absence of *Q<sub>SrGH</sub>.cs-2AL* (Table 4). Of the 155 durums from CIMMYT, 2 showed the *Sr63* diagnostic SNP (**Table S1**). This marker will be useful for marker assisted introgression of this APR allele. Marker *csLV46G22* diagnostic for *Sr58/Lr46/Yr29* has been described previously (Cobo et al. 2018; Kandiah et al. 2020)

Table 4  
Marker validation on a panel of durum wheats

Durum cultivars	<i>Sr58 (CsLV46G22)</i>	<i>KASP_32429</i>
Glossy Huguenot	<i>A</i>	<i>T</i>
M14	<i>B</i>	<i>G</i>
Altar84	<i>A</i>	<i>G</i>
Arrivato	<i>A</i>	<i>G</i>
Bansi	<i>B</i>	<i>G</i>
Bellaroi	<i>A</i>	<i>G</i>
Caparoi	<i>A</i>	<i>G</i>
DBA-Aurora	<i>A</i>	<i>G</i>
Gundaroi	<i>A</i>	<i>G</i>
Hyperno	<i>A</i>	<i>G</i>
Jandaroi	<i>A</i>	<i>G</i>
Kalka	<i>A</i>	<i>G</i>
Lillaroi	<i>A</i>	<i>G</i>
Penne	<i>A</i>	<i>G</i>
Rostine	<i>A</i>	<i>G</i>
Saintly	<i>A</i>	<i>G</i>
Tamaroi	<i>A</i>	<i>G</i>
Tjilkuri	<i>A</i>	<i>G</i>
WID802	<i>A</i>	<i>G</i>
Wollaroi	<i>A</i>	<i>G</i>
Yallaroi	<i>A</i>	<i>G</i>
Yawa	<i>A</i>	<i>G</i>

## Assessment of QTL interaction

GH contributed both QTL *QSrGH.cs-1BL* and *QSrGH.cs-2AL* and the expression of 15 to 20% stem rust severity proves the interaction of these QTL. However, the RIL population was divided into four classes; *QSrGH.cs-1BL+QSrGH.cs-2AL*, *QSrGH.cs-1BL*, *QSrGH.cs-2AL* and no QTL (Table 5, Fig. 5). The average disease severity over the three seasons for RILs with both *QSrGH.cs-1BL* and *QSrGH.cs-2AL* was 33.6%, whereas RILs carrying *QSrGH.cs-1BL* and *QSrGH.cs-2AL* individually displayed disease severities of

49.2% or 52.1%, respectively. The average disease severity of RILs lacking both QTL was 64.0%. The phenotypic effects of *QSrGH.cs-1BL* and *QSrGH.cs-2AL* were statistically similar in years 2014 and 2019, but in 2015 the disease severity in RILs carrying *QSrGH.cs-1BL* was significantly lower than RILs carrying *QSrGH.cs-2AL* (Table 5, Fig. 5).

Table 5

Comparison of stem rust severities of Glossy Huguenot/M14 RILs carrying combination of *QSrGH.cs-1BL* and *QSrGH.cs-2AL* with those possessing these QTL individually or lacking both loci

QTL	No of RILs	Disease Severity			Average
		2014	2015	2019	
<i>QSrGH.cs-1BL+ QSrGH.cs-2AL</i>	43	25.6 <sup>a</sup>	46.0 <sup>a</sup>	29.2 <sup>a</sup>	33.6
<i>QSrGH.cs-1BL</i>	41	48.2 <sup>b</sup>	63.2 <sup>b</sup>	33.7 <sup>ab</sup>	48.3
<i>QSrGH.cs-2AL</i>	37	43.7 <sup>b</sup>	79.0 <sup>c</sup>	33.7 <sup>bc</sup>	52.1
NIL	36	59.9 <sup>c</sup>	91.4 <sup>d</sup>	42.3 <sup>c</sup>	64.5
Means of disease severity followed by different letters (a, b, c and d) are significantly different based on LSD test at					
P = 0.05. Same letter shows non-significance (ns). LSD: least significant difference					

## Discussion

Although *Pgt* race TTKSK (aka Ug99) detected in Uganda in 1998 (Pretorius et al. 2000) posed a major threat to wheat production globally, durum cultivars were not significantly affected by that race or its derivatives, presumably due to the widespread presence of resistance gene *Sr13* and its alleles (H.S. Bariana unpublished results). However, other *Pgt* races virulent for these genes were reported (Singh et al. 2011). Race analysis following a severe stem rust outbreak in Ethiopia in 2013-14 indicated the presence of TTKSK (Ug99), JRCQC and TKTF (Olivera et al. 2015). Only 5% of durum cultivars had effective resistance against the non-Ug99 races JRCQC and TKTF (Letta et al. 2013; Olivera et al. 2015). The *Pgt* race involved in a stem rust outbreak in Sicily in 2016 that affected both common wheat and durum wheat was typed as TTTTF, which is virulent on *Sr13* (<http://www.globalrust.org/races/ttttf>; Bhattacharya 2017). Although there is now evidence for allelic variation following the cloning of *Sr13* and different sequence variants (haplotypes) respond differently to the *Pgt* races (Zhang et al 2017), breeding material is highly unlikely to contain all haplotypes.

We identified two consistent stem rust resistance QTL, *QSrGH.cs-1BL* and *QSrGH.cs-2AL*, explaining high levels of variation in stem rust response in Glossy Huguenot. Hare (1997) concluded that a single APR gene was present in Glossy Huguenot, but the wide variation in maturity in his materials would have been a confounding factor in determining reliable differences among his materials. *QSrGH.cs-1BL* was

mapped in the same region as the pleiotropic APR gene *Sr58*. Molecular isolation of this locus will shed further light on this. Marker analysis with the linked marker, *csLV46G22*, also suggested the presence of *Sr58* in GH. However, a higher level of resistance conferred by *Sr58* remains a query to be resolved, but it is well known that disease resistance genes confer higher levels of protection at lower ploidy level. A high level of expression by this chromosome 1BL locus has been overserved for stripe rust resistance (Pakeerathan et al. 2019). Although, the ASR gene *Sr21* is also located on the long arm of chromosome 2A and the current knowledge indicates that distinctive ASR and APR genes are unlikely to be allelic. Moreover, we located *Q<sub>SrGH.cs-2AL</sub>* (Table 3) in the interval 683-696 Mb in CS-Refv2.1 (Zhu et al. 2021) whereas *Sr21* was located at 713 Mb (Chen et al. 2018). Since there is no previously reported APR gene in this region *SrGH* is named *Sr63*.

Although the most widely used APR gene *Sr2* in common wheat originates from the tetraploid Yaroslav emmer, no known durum cultivar is known to carry this gene. Li et al. (2020) suggested the presence of *Sr58* in several durum cultivars. Tests of tetraploid landraces in the 'Watkins Collection' showed varying degrees of APR to stem rust (Toor et al. 2013). Genome-wide association mapping of seedling and adult plant resistance in a panel of 280 durum wheats (Megerssa et al. 2021) identified regions on chromosomes 5B, 6A and 7A for field resistance. No QTL was identified on the long arms of chromosomes 1B or 2A. Test in Ethiopia on durum mapping populations indicated the involvement of chromosome 1BL (Bariana and Bansal unpublished results).

High levels of resistance to Ug99, has been achieved by combining APR genes in hexaploid wheat (Pretorius et al. 2017). Comparison of disease severities of RILs carrying *Sr58* and *SrGH* (*Sr63*) in combination with those carrying these genes individually demonstrated positive interaction causing reduced stem rust severity and it is even evident from responses of GH in different environments. All lines of a panel of durum accessions in this study were positive when tested with a marker that is linked with *Sr58*. *Sr63* linked allele was detected only in two out of 175 lines indicating that it is likely to be rare in durum cultivars and this gene can be introgressed into breeding populations using the KASP marker developed in this study.

## Declarations

### Acknowledgements

We thank Dr W. Spielmeyer for helpful suggestions and Dr R. Hare for providing GH/B F3 population. This project was supported by funds provided through Grains Research and Development Corporation, Australia (CSP00161)

### Author contribution statement

HB and ES conceived and designed the study and initiated the crosses and phenotyping. RM generated the GHxM14 RILs. MR, J H-E, BB and RPS did phenotyping. KF and MH did 90k SNP genotyping. AW did initial QTL analysis. XX and SC prepared DNA and XX and GP helped with KASP marker analysis. CC and

UB performed QTL and statistical analysis and generated the maps. RM wrote the manuscript. All authors read and approved the manuscript

### Conflict of interest

The authors declare that they have no conflict of interest.

### Data availability

90k SNP data is available on request

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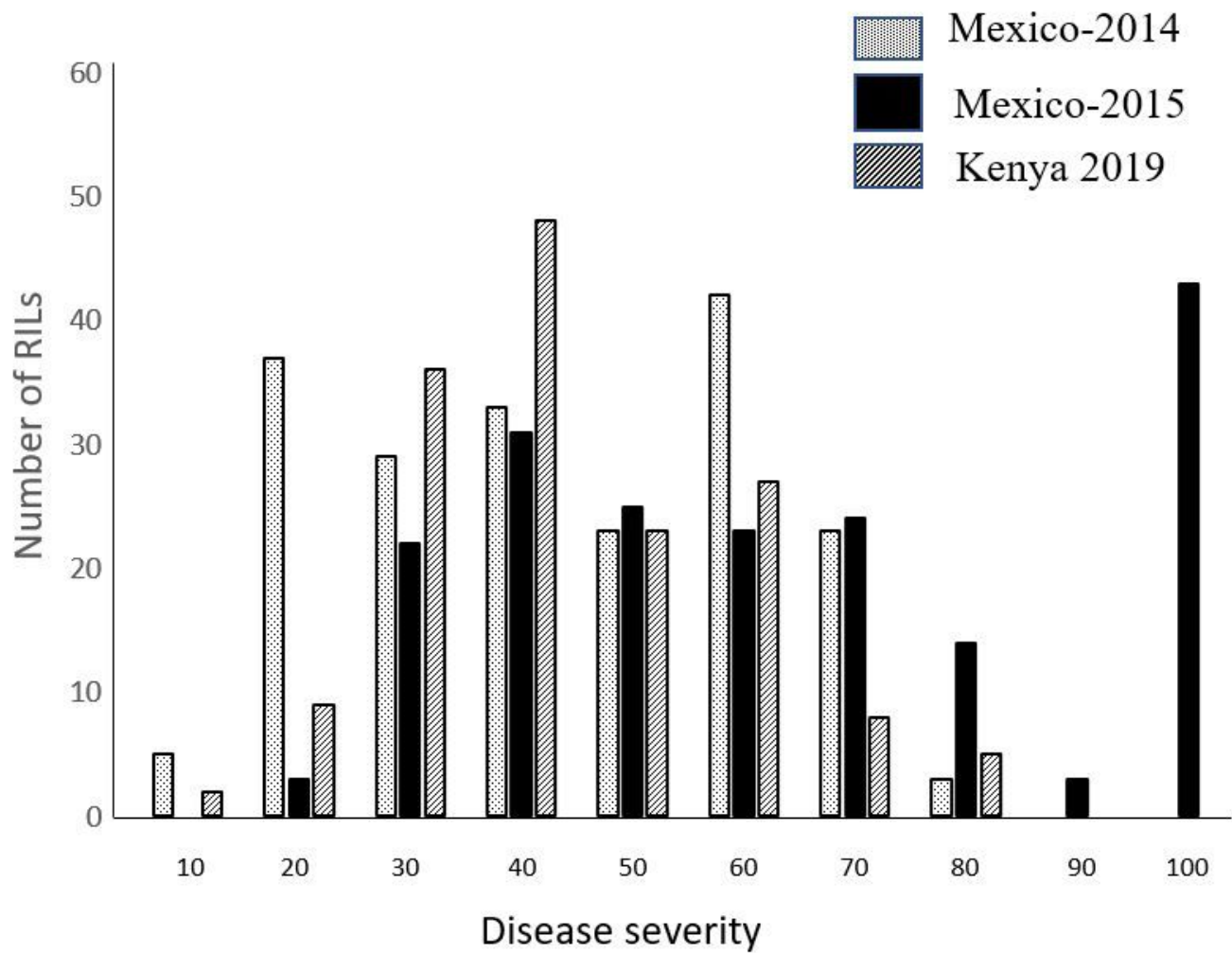
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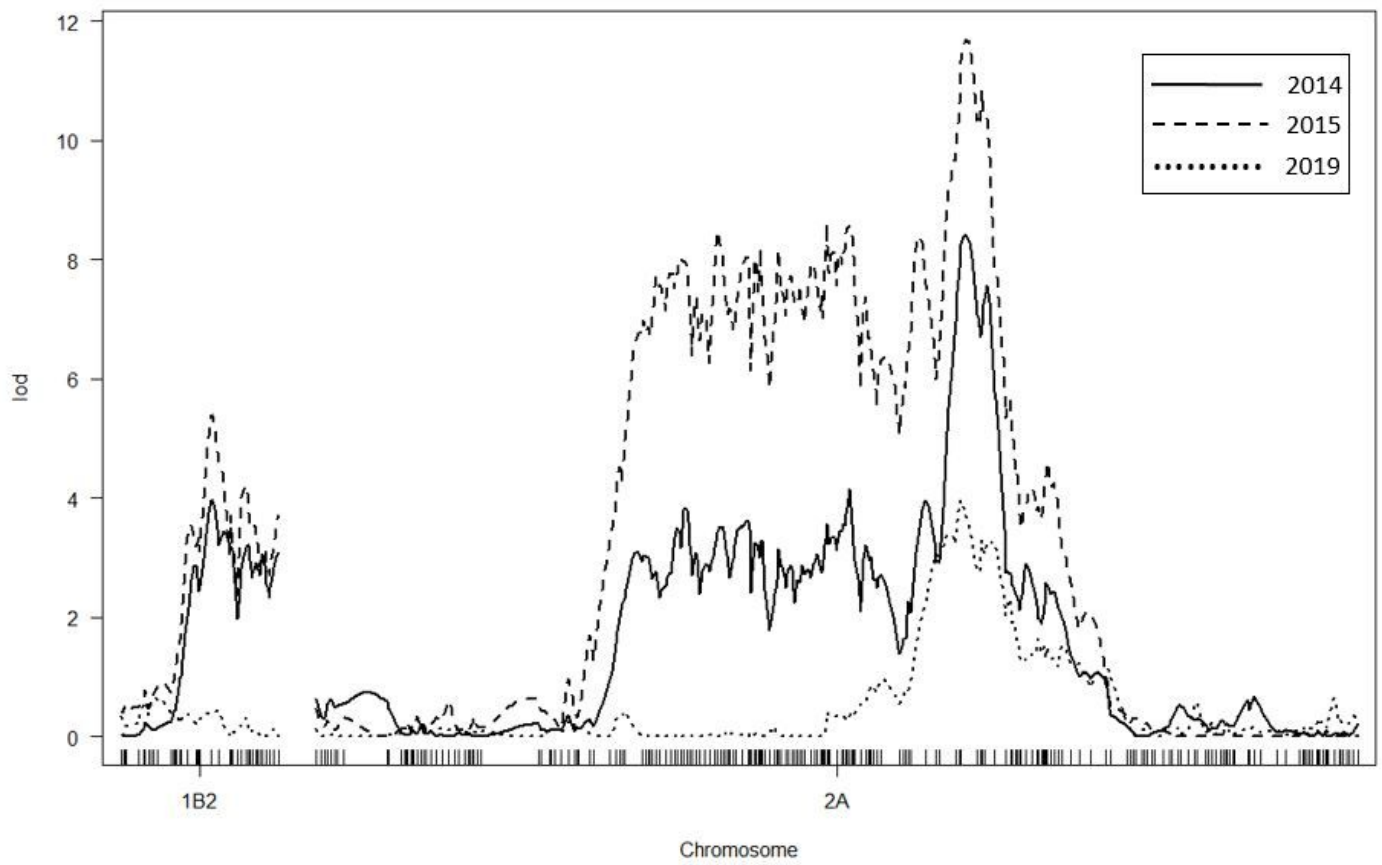
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## Figures



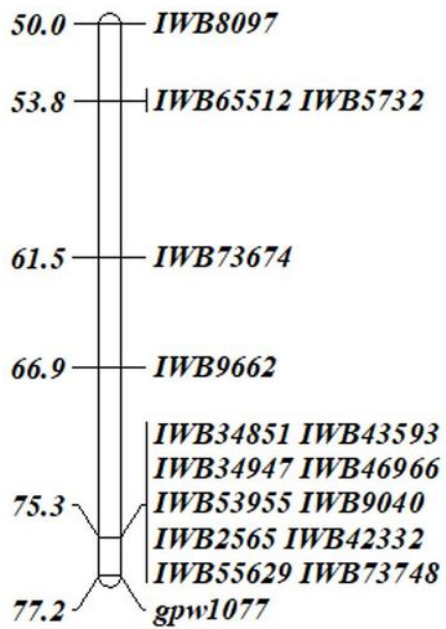
**Figure 1**

Distribution of rust severities in RILs from cross Glossy Huguenot/M14 over three years

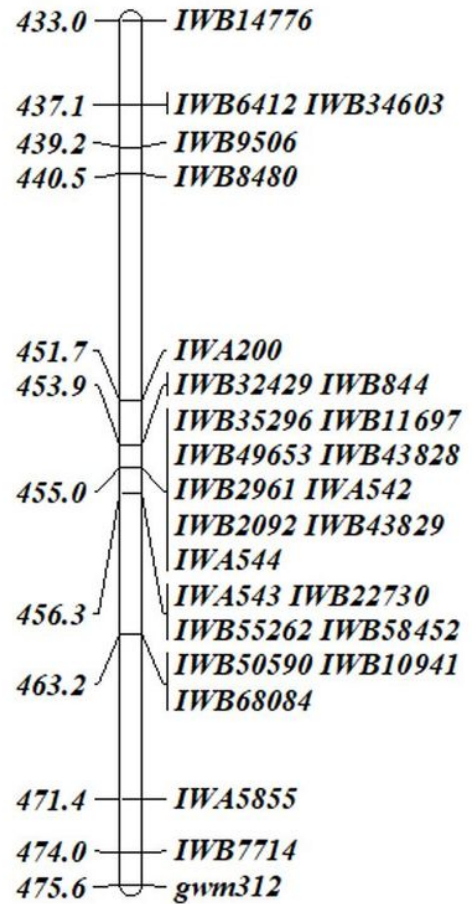


**Figure 2**

Main scan plot showing the position of major of the QTLs identified during 3 crop seasons.



**QsrGH.cs-1BL**



**QsrGH.cs-2AL**

**Figure 3**

Map position of SNP markers linked to QTLs on chromosomes 1BL and 2AL

PI 272557/DV92

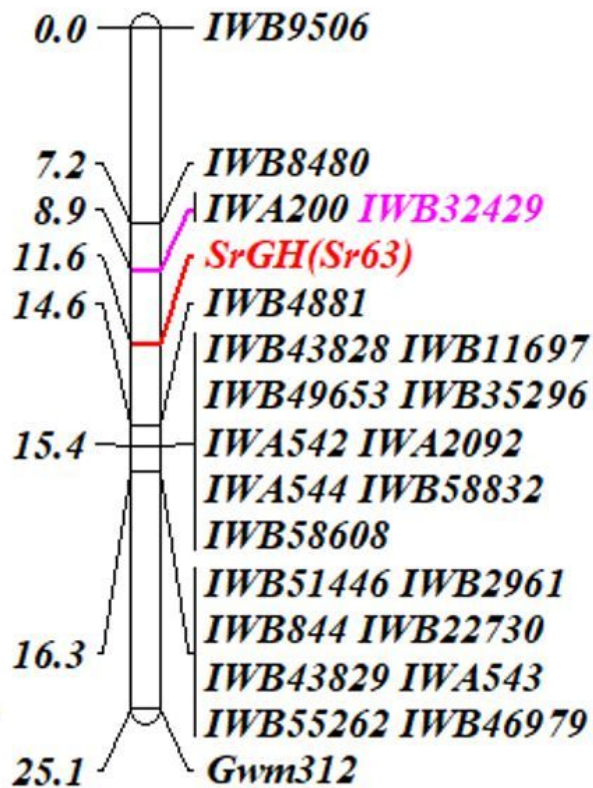
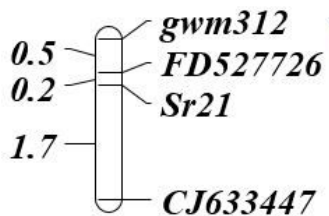
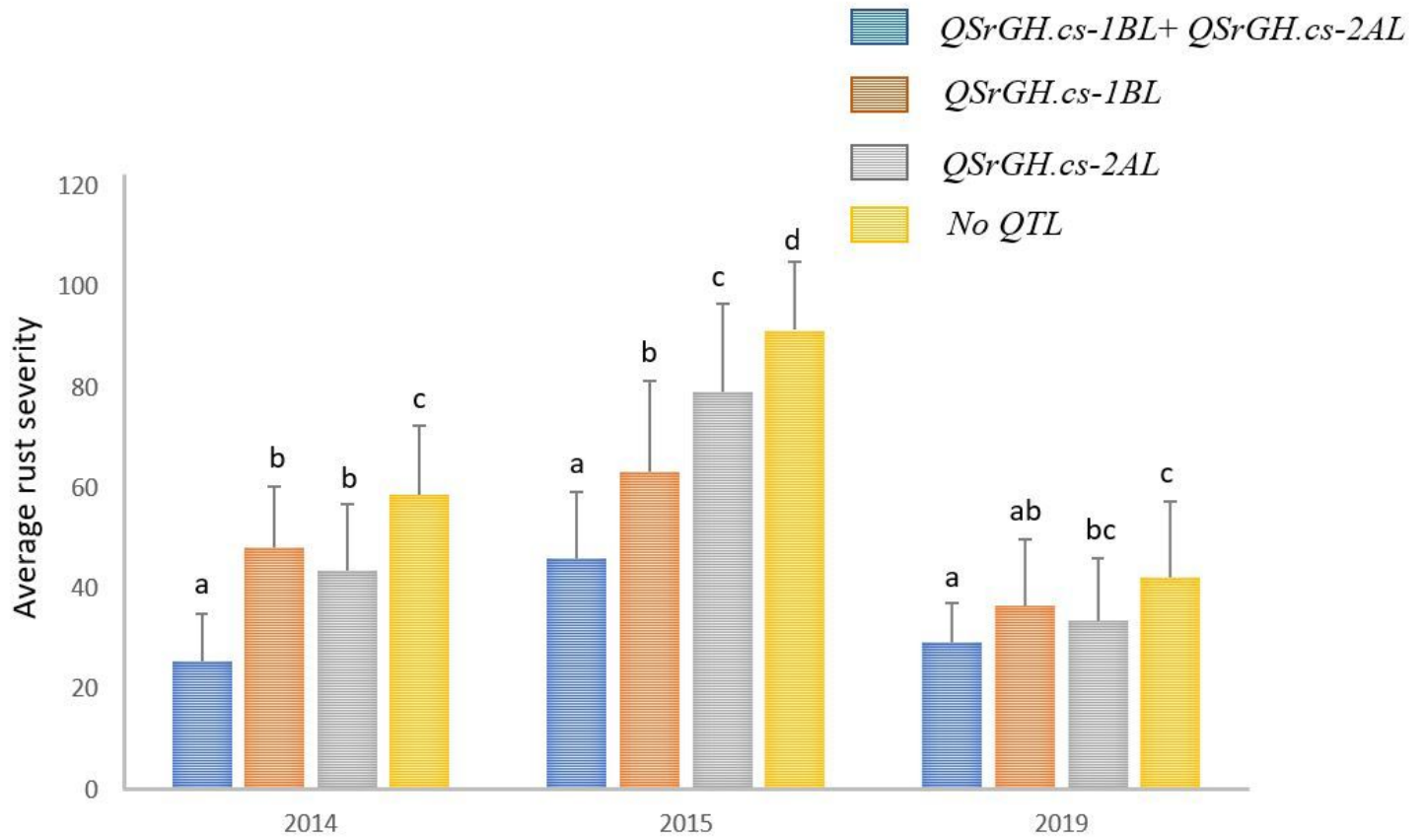


Figure 4

GH/M14 map showing location of Sr63 and its comparison with Sr21 in chromosome 2A



**Figure 5**

Mean stem rust severities of Glossy Huguenot /M14 RILs carrying individual QTL and QTL combinations during the 3 seasons. Different letters (a, b, c and d) are significantly different based on LSD test at P = 0.05. Same letter shows non-significance (ns). LSD: Least Significant Difference

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1Sr63Final.docx](#)