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Identification and mapping of resistance to stem rust in the European winter wheat cultivars Spark and Rialto

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Abstract A mapping population of 126 doubled haploid (DH) lines derived from a cross between the English winter wheat cultivars Spark and Rialto was evaluated for response to *Puccinia graminis* f. sp. *tritici* in the greenhouse and in artificially inoculated field plots at two locations over 3 years (2011, 2012 and 2013). Genetic analysis indicated the involvement of two seedling genes (*Sr5* and *Sr31*, contributed by Rialto) and three adult plant resistance genes. QTL analyses of field data showed the involvement of three consistent effects QTL on chromosome arms 1BS (contributed by Rialto), and 3BS and chromosome 5A (contributed by Spark) in the observed resistance to stem rust. These QTLs explained average phenotypic variation of 78.5, 9.0 and 5.9 %, respectively. With the presence of virulence for *Sr5* and absence of *Sr31* virulence in the field, the QTL detected on 1BS (*QSr.sun-1BS*) was attributed to the major seedling resistance gene *Sr31*. The QTL located on chromosome arm 3BS (*QSr.sun-3BS*) was closely associated with SSR marker *gwm1034*, and the QTL detected on 5A (*QSr.sun-5A*) was closely linked with SSR marker *gwm443*. DH lines carrying the combination of *QSr.sun-3BS* and *QSr.sun-5A* exhibited lower stem rust responses indicating the additive effects of the two APR genes in reducing disease severity. The markers identified in this study can be useful in pyramiding these QTLs with other major or minor genes and marker assisted selection for stem rust resistance in wheat.

Keywords Stem rust · *Puccinia graminis* · APR · Genetic mapping · QTL

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Introduction

Wheat, one of the world's most important staple food crops, faces several production challenges due to abiotic factors like drought and heat stress, and biotic factors such as pests and diseases. Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is one of the most important diseases of wheat on a global basis. It causes severe devastation, capable of reducing an apparently healthy looking wheat crop to broken stems and shrivelled grains in <1 month (Singh et al. 2008b). In susceptible cultivars, yield loss due to stem rust can be as high as 100 % (Singh and Rajaram 2002).

Historically, stem rust has been a significant problem in the major wheat-growing regions of Africa, the Middle East, Asia, Australia and New Zealand (Saari and Prescott 1985). Stem rust has been

controlled in many parts of the world through the deployment of resistance genes (Bariana et al. 2004; Singh et al. 2004; Park 2008) and the eradication of common barberry plants in North America (Roelfs et al. 1992). Australian wheat production has also been well protected from stem rust by the deployment of cultivars carrying multiple genes for resistance (Park 2007; Bansal et al. 2008). However, the detection of a stem rust race TTKSK ("Ug99") in Uganda in 1998 (Pretorius et al. 2000), combined with rapid evolution of new virulences and migration to different countries within Africa [Kenya, Ethiopia, Sudan, Tanzania, South Africa and Yemen (Singh et al. 2006; Pretorius et al. 2010; Singh et al. 2011a; Hale et al. 2014) and Iran (Nazari et al. 2009)], has again raised the global threat posed by stem rust. The movement of members of the "Ug99" lineage has been predicted to follow the same migratory route as *Yr9*-virulence, which in the late 1980s is believed to have arisen from the Eastern Africa highlands and then spread to Asia (Singh et al. 2006).

The East African highlands are known as a hot spot for the evolution and survival of new wheat rust pathotypes. The narrow genetic basis of resistance to stem rust in countries like Ethiopia (Beteselassie et al. 2007) has also contributed to the vulnerability of available genetic resources to the pathogen population. Recent stem rust outbreaks have occurred in Ethiopia, causing yield losses up to 100 % in severely affected areas. These epidemics first occurred in the 2013/2014 growing season and continued in 2014/2015. The pathotype responsible, TKTTF, affected the widely grown cultivar Digalu, which was known to carry the stem rust resistance gene *SrTmp* (Hodson 2015).

Genetic resistance is considered the most effective and sustainable way of controlling stem rust, particularly for farmers in developing countries where the use of fungicides is often unaffordable. To date, 58 genes conferring resistance to *Pgt* have been designated in wheat (McIntosh et al. 2014). Most of these genes confer seedling resistance, which are easily manipulated in a breeding programme but frequently overcome by new pathogen pathotypes (Park 2008; Singh et al. 2009; Herrera-Foessel et al. 2011). On the other hand, adult plant resistance (APR) based on minor, slow-rusting genes are believed to offer more durable resistance to the rust diseases of wheat. Although such minor resistance genes do not confer adequate levels of resistance individually, good levels of protection can be achieved by combining 4–5 of them (Singh et al. 2000). While polygenic APR to rust in wheat is considered durable and race non-specific, examples exist of single APR genes such as *Sr2/Yr30*, *Lr34/Yr18/Sr57*, *Lr46/Yr29/Sr58* and *Lr67/Yr46/Sr55* that have also proved to be durable (William et al. 2003; Herrera-Foessel et al. 2011; Singh et al. 2012).

Molecular markers play a significant role in the evaluation and identification of both major and minor genes (Suenaga et al. 2003; Collard and Mackill 2008). The development of molecular markers that are closely linked to target traits is important in expediting reliable selection and pyramiding of different genes in wheat breeding. This makes the breeding programme and selection process faster and more cost efficient (Kaur et al. 2008). Genetic mapping has been used effectively in identifying regions in the wheat genome that confer resistance to *Pgt* (Haile and Röder 2013). Molecular markers are available for several stem rust resistance genes including *Sr2* (Spielmeyer et al. 2003; Mago et al. 2011), *Sr13* (Klindworth et al. 2007; Periyannan et al. 2014), *Sr22* (Khan et al. 2005; Periyannan et al. 2011), *Sr24* and *Sr26* (Mago et al. 2005), *Sr31* (Das et al. 2006), *Sr32* (Mago et al. 2013), *Sr33* (Periyannan et al. 2008), *Sr35* (Zhang et al. 2010), *Sr39* (Mago et al. 2009), *Sr40* (Shuangye et al. 2009), *Sr45* (Periyannan et al. 2014), *Sr52* (Qi et al. 2011), *Sr53* (Liu et al. 2011), *Sr55* (Herrera-Foessel et al. 2014), *Sr56* (Bansal et al. 2014) and *Sr57* (Spielmeyer et al. 2005).

In field tests in Australia, the European winter wheat cultivars Spark and Rialto were moderately resistant and highly resistant, respectively, against predominant Australian pathotypes of *Pgt*. In the current study, we characterised the genetic basis of the stem rust resistance observed in both cultivars through integrated greenhouse and field studies.

Materials and methods

Plant material

A DH winter wheat mapping population was generated from a cross between Spark (Pedigree: Moulin/Tonic) and Rialto (Pedigree: Haven/Fresco). The population was developed at the John Innes Centre (Simmonds et al. 2014) and introduced to Australia by

the Plant Breeding Institute Cobbitty (PBIC) in 2005. A total of 126 DH lines were used for greenhouse and field stem rust phenotyping and QTL mapping.

Greenhouse phenotyping

Seedling tests were conducted under controlled greenhouse conditions at PBIC. The mapping population and parents, along with 35 Australian stem rust differential lines (McIntosh et al. 1995), were evaluated for seedling response against three pathotypes of *Pgt* (98-1,2,3,5,6 [PBIC Accession Number 781219]; 343-1,2,3,5,6 [840837] (virulent to *Sr5* and avirulent to *Sr31*); and 21-0 [540129] (avirulent to both *Sr5* and *Sr31*)) maintained in liquid nitrogen in the PBIC rust collection. Eight to 10 seedlings per line were evaluated by growing in 9-cm plastic pots. The pots were fertilised with complete fertiliser (Aquasol®, Hortico Pty. Ltd., Revesby, NSW, Australia) at the rate of 25 g/10L water for 200 pots. After fertilising, seeds were sown and covered with potting mix. Pots were moved to microclimate rooms maintained at 15–20 °C until they were ready for inoculation. Methods of sowing, disease assessment and pathotype nomenclature are explained in McIntosh et al. (1995). Seedlings were inoculated with each pathotype at the 1–1.5 leaf stage by atomising urediniospores suspended in light mineral oil (Isopar™ L, Australasian Solvents and Chemicals Pty. Ltd., Springwood, Queensland, Australia @ 10 mg urediniospores per 10 ml oil per 200 pots) using a hydrocarbon propellant pressure pack. To avoid contamination, the spray equipment was washed in alcohol and rinsed in running tap water between successive inoculations. Inoculated plants were incubated and placed in water-filled steel trays covered with moistened polythene sheet that maintained 100 % relative humidity within a greenhouse room. Inoculated seedlings were incubated for 48 h at a temperature maintained between 20 and 25 °C. The incubated seedlings were then transferred to a temperature and irrigation-controlled greenhouse growth rooms at 24–26 °C until ready for disease assessment. Individual DH lines were classified as resistant or susceptible at seedling growth stages based on infection type scale described by McIntosh et al. (1995).

Field phenotyping

The parents and the DH lines were assessed in the field for stem rust severity at PBIC in 2011, 2012 and 2013. In 2013, all lines were sown at two sites, Horse Unit (HRU) and Lansdowne (LDN). PBI is located at 34°1′2.20″S, 150°40′3.79″E. HRU and LDN are located at 34°1′15.79″S, 150°39′32.37″E and 34°1′18.43″S, 150°39′44.73″E, respectively. Approximately 20–30 seeds were planted evenly in a 0.75-m row with a spreader strip comprising stem rust susceptible genotypes adjacent to each row. The two parental lines were included at the beginning, middle and end of each nursery as controls. Urediniospores of selected mixtures of relevant Australian *Pgt* pathotypes [98-1,2,3,5,6 (781219), 343-1,2,3,5,6 (840837), 34-2,12,13 (840552), 34-1,2,7 +*Sr38* (010130) in 2011 and 98-1,2,3,5,6, 343-1,2,3,5,6, 34-2,12,13, 34-1,2,7 +*Sr38* and 98-1,2,3,5,6,7 (030312) in 2012 and 2013] suspended in mineral oil (Isopar™ L) were misted over the spreader using an ultra-low-volume applicator (Microfit", Micron sprayer Ltd. Bromyard, Herefordshire, UK) on clear afternoons when there was likelihood of overnight dew formation. The pathotypes used were virulent to *Sr5* and avirulent to *Sr31*.

Adult plant disease responses were assessed visually and scored based on disease severity and host response following the modified Cobb scale (Peterson et al. 1948). Disease scoring was performed at anthesis and repeated after 10 days. Coefficient of infection (CI) value was calculated by combining the disease severity and host response data, by multiplying severity by a constant for host response where R and TR, MR, MR-MS, MS, MSS, and $S = 0.10$, 0.25, 0.50, 0.75, 0.90 and 1.00, respectively. For example, a disease score of "60MS" was represented by $CI = 60 \times 0.75 = 45$.

Statistical analysis and QTL mapping

Chi-squared (χ^2) analysis was used to evaluate the goodness of fit of observed to expected segregation ratios to estimate the number of genes conferring resistance. The program Map Manager QTX20 (Manly et al. 2001) was used to construct linkage maps. Inclusive composite interval mapping (ICIM) (Wang et al. 2007) was used to detect the QTLs. QTLs were considered significant at $P < 0.05$, with logarithm of odds (LOD) score determined by 1000 iterations for each trait (Doerge and Churchill 1996). Linkage groups were assigned to chromosomes on the basis of previously mapped and known genomic

positions of the markers. Centimorgan (cM) values were calculated using the Kosambi (1943) mapping function. A total of 281 markers were used to localise the QTLs in the mapping population (Simmonds et al. 2014). QTL figures were drawn using MapChart V2.1 (Voorrips 2002). A one-way ANOVA was conducted, and the least significant difference (LSD) was used to verify the differences between the varieties or genotypes at $P = 0.05$.

Results

Parental response in the greenhouse and field

Under greenhouse conditions, seedlings of Rialto produced infection types (IT) "1+" to "2−" when tested with *Pgt* pathotypes 98-1,2,3,5,6 and 343-1,2,3,5,6 (*Sr5*-virulent and *Sr31*-avirulent). The ITs were similar to those produced by the *Sr31* control genotype Mildress (Table 1). However, with pathotype 21-0 (avirulent to both *Sr5* and *Sr31*), Rialto produced IT "0;" typical to that produced by the differential genotype Reliance carrying *Sr5*. On this basis, the seedling genes *Sr5* and *Sr31* were postulated in Rialto. In contrast, Spark showed IT "3+" against the three *Pgt* pathotypes and was therefore considered to lack effective seedling resistance to the pathotypes used (Table 1). When evaluated for adult plant stem rust response under field conditions, Rialto consistently exhibited high levels of resistance, while Spark was moderately susceptible across experiments/years to the *Pgt* pathotypes used (Table 1).

Progeny response at seedling stage

The DH population was also assessed in the greenhouse for response to stem rust. With a classification of IT "3" or less considered resistant, and greater than IT "3" as susceptible, the population segregated for a single dominant gene ($\chi^2 = 1.16$, $P > 0.281$ and $\chi^2 = 0.97$, $P > 0.325$), apparently *Sr31*, with the *Sr5*-virulent pathotypes 98-1,2,3,5,6 and 343-1,2,3,5,6 (Table 2). Three conspicuous ITs ("0;", "2−" to "2-C" or "3+") were observed in the population when tested against pathotype 21-0 (avirulent to *Sr5*): 86 lines (69.9 %) displayed a resistant reaction ("0;" or "2−" to "2-C") and 37 (30.1 %) were susceptible ("3" to "3+"), while three lines did not germinate. The chi-squared analysis supported the involvement of two genes ($\chi^2 = 1.35$, $P > 0.246$) from Rialto, consistent with the presence of seedling genes *Sr5* and *Sr31* (Table 2). The genotypes that showed IT "0;" were postulated to carry either *Sr5* or *Sr5* +*Sr31*, whereas those showing ITs "2−" to "2-C" were postulated to carry only *Sr31*.

Progeny response at adult plant stage

The parents and the DH lines were assessed for stem rust severity against *Pgt* pathotypes in the field in 2011, 2012 and 2013 (two locations). Consistent

Table 1 Seedling and adult plant responses of parental lines and differential controls Reliance (*Sr5*) and Mildress (*Sr31*) to *Puccinia graminis* f. sp. *tritici* pathotypes used in greenhouse and field

Parents	Greenhouse ITs				Adult plant response (CI)			
	98-1,2,3,5,6	343-1,2,3,5,6	21-0	Postulated gene (s)	2011[a]	2012[b]	2013HRU[c]	2013LND[d]
Spark	3+	3+	3+	None	63	54	63	67.5
Rialto	1+	2−	0	*Sr5* + *Sr31*	1.25	1.25	5	2.5
Reliance	33+	33+	0	*Sr5*				
Mildress	1+	2=	1	*Sr31*				

CI = coefficient of infection, cultivars in bold are differentials used for greenhouse test

[a] 98-1,2,3,5,6; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6

[b] 98-1,2,3,5,6; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6; 98-1,2,3,5,6,7

[c] 98-1,2,3,5,6,7; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6

[d] 98-1,2,3,5,6,7; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6

Table 2 Frequency distribution and chi-squared values of 126 doubled haploid lines tested with the three *Puccinia graminis* f. sp. *tritici* pathotypes in the greenhouse (seedlings) and 3 years in field (adult plants)

Growth stage	Pathotype	Observed segregation		Ratio	χ^2	*P* value	Gene
		Resistant	Susceptible				
Seedling	21-0	86	37	3:1	1.35	0.246	*Sr31* +*Sr5*
Seedling	98-1,2,3,5,6	57	69	1:1	1.16	0.281	*Sr31*
Seedling	343-1,2,3,5,6	57	68	1:1	0.97	0.325	*Sr31*
Adult plant	2011[a]	107	19	7:1	0.77	0.384	*Sr31*, *SrAPR1*, *SrAPR2*
Adult plant	2012[b]	105	21	7:1	2.00	0.167	*Sr31, SrAPR1, SrAPR2*
Adult plant	2013HRU[c]	104	22	7:1	2.83	0.092	*Sr31, SrAPR1, SrAPR2*
Adult plant	2013LDN[d]	104	21	7:1	2.11	0.146	*Sr31, SrAPR1, SrAPR2*

χ^2 at P = 0.05 (1 d.f.) = 3.84

[a] 98-1,2,3,5,6; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6

[b] 98-1,2,3,5,6; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6; 98-1,2,3,5,6,7

[c] 98-1,2,3,5,6,7; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6

[d] 98-1,2,3,5,6,7; 34-2,12,13; 34-1,2,7 +*Sr38* and 343-1,2,3,4,5,6

variation in stem rust infection was observed across the DH lines in all four experiments. Transgressive segregation was observed in some lines, which were either more resistant or more susceptible than the parents across all experiments. A total of 11 lines (8.73 %) that displayed high ITs ("3+") to all three pathotypes at the seedling stage also showed high disease responses in the field (CI values of "70" to "90"), indicating a lack of seedling resistance and APR in these lines. Ten lines (7.94 %) that were susceptible to pathotypes 98-1,2,3,5,6 and 343-1,2,3,4,5,6, but resistant to pathotype 21-0 (postulated to carry *Sr5*), were also susceptible in the field because of the ineffectiveness of *Sr5* against the field pathotypes. As expected, in the absence of pathotypes virulent to *Sr31* in field, all DH lines that carried *Sr31* (singly or in combination with *Sr5*) were highly resistant in the field (CI value of "0.1–7.5"). In this case, 57 (45.24 %) lines that showed resistance to pathotypes 98-1,2,3,5,6 and 343-1,2,3,4,5,6 at the seedling stage were also resistant in the field. However, 47 of the lines (37.30 %) that were susceptible to pathotypes 98-1,2,3,5,6 and 343-1,2,3,4,5,6 at the seedling stage were resistant to moderately susceptible at adult plant growth stages, indicating the presence of gene(s) conferring APR.

The population showed the same pattern of segregation across all experiments, and chi-squared analysis supported the involvement of three genes (segregation ratio 7:1, Table 2). When chi-squared analysis was performed using 75 lines lacking *Sr31,* segregation within the population fitted a genetic ratio of 3:1, indicating the presence of two APR genes in the DH population.

QTL analysis

Inclusive composite interval mapping (ICIM) of field data with 281 markers revealed the involvement of resistance loci on chromosome arms 1BS and 3BS and chromosome 5A across all data sets. The QTL detected on chromosome arm 1BS, temporarily designated *QSr.sun-1BS*, was associated with the marker *gwm18*, where the major seedling resistance gene *Sr31* was also located and contributed by Rialto. The QTL located on chromosome arm 3BS, designated *QSr.sun-3BS*, was closely associated with SSR marker *gwm1034*, and the QTL detected on chromosome 5A (*QSr.sun-5A*) was associated with SSR marker *gwm443*. The resistance effects of QTLs *QSr.sun-3BS* and *QSr.sun-5A* were both contributed by Spark.

QTL *QSr.sun-1BS* (*Sr31*) explained 73–84 % of the phenotypic variation. The highest phenotypic variation (84 %) was explained in 2011 and in 2013HRU (LOD = 39.8), while the lowest phenotypic variation (73 %) was explained in 2012 (LOD = 29.78) (Table 3). The phenotypic variation explained by the QTL *QSr.sun-3BS* varied from 6 % to 12 %. The

largest APR variance was explained in 2013LDN (12 %) with an LOD value 3.5, whereas the lowest was in 2012 explaining phenotypic variance of 6 % with the LOD value 1.59 at the *gwm1034* locus (Table 3; Fig. 1a). The chromosome 3BS comprised a total of 20 loci covering a total distance of 122.9 cM with an average distance between markers of 6.15 cM. The QTL *QSr.sun-5A* (LOD value of 1.09–1.80) explained 4.43–7.30 % of the phenotypic variation with *gwm443* being the closest marker (Table 3; Fig. 1b). This QTL had the largest effect on stem rust resistance (7.30 %) in 2013HRU and lowest (4.43 %) in 2012.

The effect of different QTL combinations on stem rust response of non-*Sr31* carrying DH lines

The resistance conferred by each QTL alone and in combination was assessed by predicting the genotype of each DH line for the different QTL combinations based on the presence/absence of the most closely linked flanking markers. Lines that carried *QSr.sun-3BS* only produced comparatively lower disease responses (35.75 %) than those with QTL *QSr.sun-5A*, while DH lines carrying the two genes in combination showed lower stem rust responses (20.6 %) than lines with no gene or a single gene (Table 4). The mean stem rust severity of 20 lines (54.1 %) with no QTL was higher than lines with any QTL, whether present singly or in combination. Further analysis was performed using one-way ANOVA to see whether there were statistically significant differences in the effects of the QTLs and their combination. The results indicated that there was statistical difference between groups of all means except those of QTL *QSr.sun-3BS* and *QSr.sun-5A*.

Discussion

The study supported the presence of *Sr5* and *Sr31*, two major seedling stem rust resistance genes, in Rialto. In contrast, Spark did not carry seedling resistance genes effective against the pathotypes used in this study. Previously, Singh et al. (2008a) also reported the presence of *Sr5* and *Sr31* in Rialto. Gene *Sr5* is often cited as the “immunity” gene; while the infection type conferred by this gene is usually very low (“0” to “;”), it gives relatively higher infection types when transferred to susceptible genetic backgrounds (Luig and Rajaram 1972). Virulence to *Sr5* occurs in many geographical areas and hence the gene is usually not effective in the field (Luig 1983; Vanegas et al. 2008). Linkage analysis based on the phenotypic data generated against *Pgt* pathotype 21-0 mapped *Sr5* on chromosome arm 6DS associated with DArT marker *wPt-3879* (data not shown). Previously, this gene was also mapped to the same region with the flanking markers *barc183* and *wPt-3879* (Prins et al. 2011). The resistance gene *Sr31* is located in the 1BL/1RS translocation and has contributed significantly to protecting wheat crops from stem rust. It is very common in European, Chinese, USA and Indian wheat varieties (McIntosh et al. 1995) and has been deployed extensively worldwide, and remained effective and

Table 3 QTLs detected for adult plant resistance to stem rust in Spark/Rialto doubled haploid population

Year/location	Chromosome	Marker	R^2	LOD	Source of resistance
2011	1B	*gwm18*	84.00	39.76	Rialto
	3B	*gwm1034*	9.00	2.43	Spark
	5A	*gwm443*	6.69	1.65	Spark
2012	1B	*gwm18*	73.00	29.78	Rialto
	3B	*gwm1034*	6.00	1.59	Spark
	5A	*gwm443*	4.43	1.09	Spark
2013HRU[a]	1B	*gwm18*	84.00	39.89	Rialto
	3B	*gwm1034*	9.00	2.57	Spark
	5A	*gwm443*	7.30	1.80	Spark
2013LDN[b]	1B	*gwm18*	82.00	35.76	Rialto
	3B	*gwm1034*	12.10	3.5	Spark
	5A	*gwm443*	6.20	1.52	Spark

R^2 = % phenotypic variation

[a] 2013 experiment at Horse Unit

[b] 2013 experiment at Lansdowne

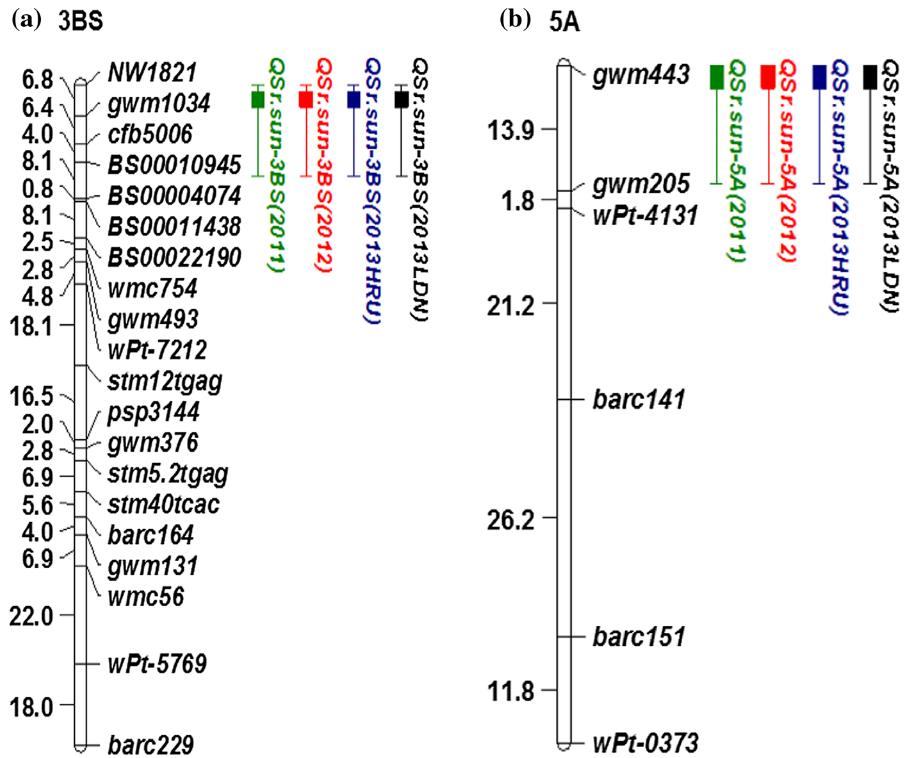

Fig. 1 QTLs for adult plant resistance to stem rust detected on chromosome 3BS (*QSr.sun-3BS*), 5A (*QSr.sun-5A*) in the Spark/Rialto doubled haploid population. *Vertical bars* to the *right* of the markers indicate QTL regions. Intervals between markers are given in cM to the *left* of the map

Table 4 Mean stem rust severities of non-*Sr31* doubled haploid lines carrying different QTLs or their combination

QTL	Average stem rust severity in different years (%)					No. of lines
	2011	2012	2013HRU	2013LDN	Average	
No QTL	54.76	47.31	54.50	59.68	54.06	20
QSr.sun-3BS	36.26	35.41	35.68	35.64	35.75	33
QSr.sun-5A	40.34	40.26	39.80	44.01	41.11	19
QSr.sun-3BS + *QSr.sun-5A*	20.88	20.85	19.84	20.81	20.60	37

was considered durable until the detection of *Pgt* pathotype TTKSK ("Ug99") in Uganda, which carries virulence for this gene (Pretorius et al. 2000; Das et al. 2006; Singh et al. 2008c). However, this gene is still used effectively in some wheat-growing countries where *Sr31* virulence does not occur. Although effective in Australia, *Sr31* has not been used widely in Australia due to the association of the rye introgression with a problem of dough stickiness (McIntosh 1988).

This study detected three consistent QTLs on chromosome arms 1BS, 3BS and chromosome 5A, all of which contributed field resistance and reduced stem rust severity significantly. Because *Sr31* is effective against Australian *Pgt* pathotypes, the QTL detected on chromosome arm 1BS is most likely due to the major seedling resistance gene *Sr31* or, less likely, a gene conferring APR. Previously, Letta et al. (2013) reported a highly significant QTL in tetraploid wheat associated with stem rust resistance near the centromere of chromosome 1B. The authors explained that in the case of hexaploid wheat, the markers identified in their study could tag either *Sr14* in the centromeric region of 1B (McIntosh 1980) or *Sr31* in the short arm of 1B (Zeller 1973). Through monosomic analysis, *Sr14* was mapped on the long arm of chromosome 1B (McIntosh 1980). Letta et al. (2013) concluded that the QTL detected on chromosome 1B is likely *Sr14* because this gene originated from tetraploid wheat, while *Sr31* is present in hexaploid

wheat germplasm only. In another study, a QTL associated with stem rust resistance was reported in a hexaploid spring wheat near the centromere of chromosome 1BS (Yu et al. 2011). Yu et al. (2012) also reported that some of the stem rust resistance genes identified in spring wheat were not found in winter wheat because they represent different gene pools. Thus, further testing and mapping of the common markers could help to clearly understand and determine whether the QTL *QSr.sun-1BS* and *Sr31* are equivalent.

The second consistent QTL, *QSr.sun-3BS*, was located on chromosome arm 3BS where it mapped close to the SSR marker *gwm1034*. Previously, several stem rust QTLs have been reported on chromosome arm 3BS in different populations (Kaur et al. 2009; Haile et al. 2012; Njau et al. 2013; Singh et al. 2013a). However, in all of these studies, the authors stated that the QTL identified was likely *Sr2*. In a separate study (Getie 2015), the parents and a resistant bulk of ten lines carrying *QSr.sun-3BS* were screened with two *Sr2* linked markers *gwm533* (Spielmeyer et al. 2003) and *csSr2* (Mago et al. 2011). Marker analysis based on these two markers indicated the absence of *Sr2* in both parents and DH lines tested. *Sr2* is also associated with the dark pigmentation trait pseudo-black chaff (PBC) (Hare and McIntosh 1979). In this study, PBC was not detected in either of the parents or any of the DH lines. Combined, these results indicate that the APR observed in Spark and in the population is highly unlikely to be controlled by *Sr2*. Studies of APR to leaf rust in the Spark/Rialto population (Getie 2015) found a QTL on the short arm of chromosome 3B at a genetic distance of 6.2 cM from the SSR marker *gwm1034*. However, the phenotypic data for leaf rust segregated independently of that for stem rust and therefore it is unlikely that the QTL located on chromosome arm 3BS for stem rust is associated with the gene identified in the same region as conferring APR to leaf rust. The third consistent QTL detected on chromosome 5A for APR to stem rust resistance was very minor and contributed by the moderately susceptible parent Spark. Although consistently detected, the LOD values of *QSr.sun-5A* were lower than the threshold. This could be either that the effect of the QTL is very minor or the presence of lower number of markers in this region. Use of more tightly linked markers could greatly improve the QTL mapping resolution and the LOD values. Stem rust QTLs in chromosome 5A were also reported in previous studies in different genetic backgrounds (Bhavani et al. 2011; Njau et al. 2013; Singh et al. 2013b; Letta et al. 2014). However, because of differences in the markers linked to these QTLs, it is difficult to determine whether QTL *QSr.sun-5A* detected in this study is same as these previously reported QTLs. Further work is therefore required to resolve the relationships between these loci.

Stem rust responses of lines carrying *QSr.sun-3BS* and *QSr.sun-5A* in combination were lower than in lines that carried either of the genes, clearly indicating the additive nature of the QTLs. This was further determined by one-way ANOVA that the combinations of the two QTLs were statistically significant from either of the QTLs based on LSD value obtained. Singh et al. (2011b) reported that cultivars carrying combinations of 4–5 APR genes, each with minor to intermediate effects, could provide a high level of resistance in the field. In view of this, there would be significant merit in combining both of these QTLs with other major or APR genes to stem rust resistance including *Sr2/Yr30*, *Lr34/Yr18/Sr57/Pm38*, *Lr46/Yr29/Sr58/Pm39* and *Lr67/Yr46/Sr55/Pm46*. Minor genes with additive effects to the rust diseases are common in wheat germplasm (Johnson 1988; Singh et al. 2011b) and sufficient levels of APR are usually provided by several minor genes, each with a small to intermediate effect in reducing disease severity (Singh et al. 2008b). In order to provide adequate level of resistance in wheat varieties, the QTLs identified in this study can be useful in combination with other major or minor genes for developing germplasm with durable resistance to stem rust.

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