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ORIGINAL PAPER

A multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew

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Abstract *Sr2* is the only known durable, race non-specific adult plant stem rust resistance gene in wheat. The *Sr2* gene was shown to be tightly linked to the leaf rust resistance gene *Lr27* and to powdery mildew resistance. An analysis of recombinants and mutants suggests that a single gene on chromosome arm 3BS may be responsible for resistance to these three fungal pathogens. The resistance functions of the *Sr2* locus are compared and contrasted with those of the adult plant resistance gene *Lr34*.

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Introduction

The resistance gene *Sr2* was transferred from tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccon*) into common wheat (*Triticum aestivum* L.) in the 1920s (McFadden 1930) and has since been effective against all known pathotypes of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*). The *Sr2* response is characterized by partial resistance, which restricts the number and size of uredinia and is expressed only in adult plants. The gene was mapped to chromosome arm 3BS by Hare and McIntosh (1979) and associated with a trait called pseudo-black chaff (PBC), which causes a variable and genotype-dependent pigmentation of stems and/or glumes (McFadden 1939; Hare and McIntosh 1979). In a fine mapping study, PBC could not be separated from *Sr2* by genetic recombination (Kota et al. 2006).

The leaf rust (caused by *P. triticina*) resistance gene *Lr27* was also mapped to chromosome arm 3BS and co-segregated with *Sr2* in families consisting of 289 lines (Singh and McIntosh 1984a, b). In contrast to *Sr2*, *Lr27* resistance is race specific and requires a complementary gene (*Lr31*) on chromosome 4B. Although seedling resistance conferred by *Lr31* is dependent on the presence of *Lr27*, there is evidence that *Lr31* may be identical to *Lr12*, which alone confers race-specific adult plant resistance (Singh et al. 1999). *Lr27* may also be allelic to another adult plant leaf rust resistance gene (*SV2*) (M. Dieguez, pers. comm.).

In this study, we investigated the genetic relationship of *Lr27* and *Sr2* in more detail by mapping *Sr2* and *Lr27* in a high-resolution mapping family. We also isolated susceptible mutants for both genes to investigate the possibility that stem rust and leaf rust resistance may be conferred by the same gene. Furthermore, given the previous findings that the multiple rust resistance genes *Lr34/Yr18* and

Lr46/Yr29 provide partial resistance to powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) (Spielmeyer et al. 2005; Lillemo et al. 2008), we further investigated the likelihood that *Sr2* confers resistance to powdery mildew.

Materials and methods

Plant material and mapping family

Sr2 was transferred to hexaploid wheat by crossing "Yaroslav" emmer with the susceptible "Marquis" (McFadden 1930). Stem rust-resistant variety "Hope", which was one source of *Sr2* in early breeding programs, was selected from progeny of the interspecific cross (McFadden 1930). Two accessions of "Yaroslav" used in this study were obtained from the Australian Winter cereal collection. They were differentiated from other emmer accessions and shown to be identical to Hope, but not to Marquis, with respect to *Sr2*-specific markers (Mago et al. 2011). An F_2 mapping family was derived from a cross between cv. Chinese spring (CS) and the chromosome substitution line CS (Hope 3B), which carries *Sr2*. The high-resolution mapping family was generated by screening 1,340 F_2 seeds (equivalent to 2,680 gametes) with flanking simple sequence repeat (SSR) markers *gwm389* and *gwm533* as described in Kota et al. (2006). The mapping family was reduced to 17 recombinants by placing *Sr2* between wEST marker *CA640157* and *gwm533* (Kota et al. 2006).

Seedling leaf rust screen

To screen for leaf rust resistance gene *Lr27*, 1-week-old seedlings were inoculated in the glasshouse at the Plant Breeding Institute, Cobbitty, Australia with the leaf rust race 10-1,2,3 (culture accession no. 347) using the protocol described by McIntosh et al. (1995). Rust responses were scored 10–14 days post-infection according to the scale proposed by Stakman et al. (1962).

Adult plant stem rust test in the field

Stem rust resistance screening was done on adult plants in the field in 2009 with the rust races 98-1,2,3,5,6 (culture no. 279) and 34-1,2,7 + Sr38 (culture no. 565) using the protocol described by Kota et al. (2006). Lines were scored as resistant or susceptible based on the level of infection on parental lines.

Adult plant stem rust testing in glasshouse

For testing the stem rust response of adult plants in the glasshouse, entries CS(Hope3B) (resistant), 72-2 deletion mutant (susceptible), four M_5 sister lines of mutant M39 (39-1, 39-2, 39-3, 39-4), four M_5 sister lines of mutant M40 (40-1, 40-2, 40-3, 40-4) and four M_5 sister lines of mutant M48 (48-1-1, 48-1-2, 48-2-1, 48-2-2) were grown in plastic cones in a glasshouse as described by Pretorius et al. (2007). Hartog (resistant) and two susceptible lines, entry 37 from the 2nd ISRTN 07 and Kariega, were included as controls. Two replications, arranged over two trays, were planted. Depending on the number of seeds available, the total number of plants tested ranged from two to eight per entry. When most entries were at anthesis (46 days after planting), plants were inoculated by spraying with a suspension of urediniospores of isolate UVPgt60 (race PTKST) in sterile, distilled water (1 mg/ml) containing Tween 20®. As much as 100 ml of suspension was applied as uniformly as possible per replication using a pressurized atomizer (Pretorius et al. 2007). Immediately after inoculation, plants were placed in a plastic tent (dew chamber) on a glasshouse bench flooded with water. The plants were removed from the dew chamber after 24 h and maintained in a greenhouse at 18–25°C. Stem rust severities and reaction types on the last internodes were scored 15 days after inoculation. The most common response for an entry over the two replications was recorded. A similar adult plant glasshouse assay was conducted in Canberra to test mutants M161 and M174 with the stem rust race 98-1, 2, 3, 5, 6.

Powdery mildew testing

A glasshouse isolate of powdery mildew, caused by *B. graminis* f. sp. *tritici*, was maintained on susceptible plants. Screening of test genotypes (generally four biological replicates per genotype) was conducted by growing test plants in a growth cabinet (25°C, 16 h day, 18°C, 8 h night). At approximately 5 weeks after sowing (late tillering), plants were transferred to the glasshouse and inoculated with mildew as follows. Test plants were randomized and laid on their sides on the glasshouse bench, and sporulating mildew spreader plants were shaken gently overhead. A fine mist of spores settled onto the test plants, which were then placed in shallow tubs and watered from below, without disturbance of the leaves. Phenotypes were recorded at approximately 11 days after inoculation.

Scoring pseudo-black chaff

PBC was scored as a qualitative trait based on the presence of dark pigmentation, which accumulates on stem internodes both in glasshouse and field-grown plants. As the plants matured, pigmentation was also scored on the glumes. From each mutant, approximately 20 individuals per M_6 family were screened for PBC in the glasshouse.

Mutagenesis and mutant screens

Mutagenesis of the resistant line CS(Hope3B) was done using both γ-irradiation and ethylmethane sulfonate (EMS). A dosage of 20 krads was used for irradiation, which was chosen based on LD50 as described in Mago et al. (2004). For EMS treatment, the seeds were soaked for 24 h in water at room temperature after which EMS (0.4 or 1%) was added and treated for 12 h at 28°C with shaking. The solution was decanted and seeds washed under tap water for several hours to remove residual EMS and grown in the field to produce M_1 heads. Field screening for stem rust mutants was done by sowing approximately 20 M_2 seeds from single heads in 60 cm field rows. Over 10,000 M_2 field rows derived from single heads (includes γ-irradiated and 0.4% EMS treated) were screened for loss of stem rust resistance in four field seasons at the University of Sydney Plant Breeding Institute, Cobbitty, Australia. In each season, field rows were scored at least twice at the adult plant stage before and after anthesis. Irrigation was used to maintain moist conditions during the infection process. The test plot area included susceptible infection rows, single spikes of which were inoculated by injecting 2–5 ml of a water-based *P. graminis* f. sp. *tritici* urediniospore suspension directly into a stem internode at 1–2 m intervals. For leaf rust mutant screening, M_2 seeds (1% EMS treated) from single spikes were sown in large metal trays in the glasshouse and screened for *Lr27* mutants at the seedling stage, as described above. Mutants were scored for leaf rust, stem rust and powdery mildew in the M5 and M7 generations.

PCR amplification of markers

PCR reactions were performed in 20 µl volumes with 2–4 µl (100 ng) DNA template, 0.2 mM dNTP, 10 pmol of each primer, 1.5 mM MgCl2, 1× GoTaq Flexi buffer and 0.5 U GoTaq Flexi Taq polymerase (Promega). The primer sequences and PCR conditions are shown in Table 1.

Results

High-resolution map of *Sr2*

The stem rust resistance gene *Sr2* was previously positioned between wEST marker *CA640157* and SSR marker *gwm533* on chromosome arm 3BS using a high-resolution mapping family generated from Chinese Spring (CS) × CS(Hope3B) (Fig. 1) (Kota et al. 2006). We used the tightly linked marker *CA640157* to start a chromosome walk by screening a chromosome 3B-specific BAC library of CS (Šafář et al. 2004). The physical map of the *Sr2* region was then completed by utilizing BAC contig information from the early stages of physical map construction of chromosome 3B (Paux et al. 2008). The *Sr2* region was contained within contig 11 (1.2 Mb) of the 3B physical map, which was sequenced and annotated as part of a larger study of gene density and transposable elements (Choulet et al. 2010; Breen et al. 2010). The CS sequence was used to develop markers and define the *Sr2* region in more detail (Fig. 1). EST-derived marker *DOX_1* recombined with *Sr2* on the distal side in RIL#7, and marker *RKO_1* was separated by one recombination event from *Sr2* on the proximal side through RIL#10 (Fig. 1). These two recombinants were among F_2 progeny derived from 2,680 gametes; therefore, *DOX_1* and *RKO_1* delineate a genetic interval of approximately 0.07 cM, which represents approximately 570 kb in CS (Fig. 1). Five additional markers, *BEX_2*, *CD882879*, *CA746621*, *RKO_2* and *MSF_2*, were developed from the intervening sequence and co-segregated with *Sr2*. All these markers were within features annotated on the Chinese Spring 3B physical map of Choulet et al. (2010), with the exception of CD882879 and CA746621. Wheat EST CD882879 has 92% identity with sequences at 469 kb in the 1.2 Mb CS sequence (Genbank accession FN645450), and CA746621 has 87% identity with sequences at 393 kb in the FN645450 sequence. No recombination event was detected in the approximate 450 kb sequence between markers *BEX_2* and *MSF_2*. The marker *csSr2* was previously published as the most useful marker to select for *Sr2* in a wide range of genetic backgrounds (Mago et al. 2011).

Leaf rust resistance and powdery mildew resistance co-locate with *Sr2*

The leaf rust response was evaluated in the parental lines and seven critical recombinants to estimate the genetic linkage between *Lr27* and *Sr2*. The parental lines showed clear differences in response to leaf rust at the seedling stage with CS (Hope3B), which contains both *Lr27* + *Lr31*, showing moderate resistance (infection type (IT); 12X), whereas CS (containing *Lr31*, but not *Lr27*) was susceptible (IT 33+). Hope (presumably containing only *Lr27*) and Marquis, presumably lacking both *Lr27* and *Lr31*, were susceptible while Yaroslav emmer (donor of *Sr2* and *Lr27*) was highly resistant probably due to the presence of additional gene(s) (Fig. 2). The infection types on recombinant lines were similar to the parental types (Table 2). Leaf rust resistance and *Sr2* co-segregated in seven critical recombinant lines (Fig. 1) confirming that race-specific leaf rust resistance expressed in seedlings is tightly linked to *Sr2* (Singh and McIntosh 1984a).

In the glasshouse when powdery mildew was allowed to proliferate, the level of mildew infection was noted to be lower on CS(Hope3B) than on CS. The initial observation was confirmed in repeated and replicated

Table 1 Primer sequences and PCR conditions for the amplification of the markers co-segregating with *Sr2*

Marker	Primer sequence	PCR conditions
MSF_2	F-GAAAAGATATGTCCTTTGGTGG	95°C—2 min (1 cycle)
	R-GAAGATTCGCAACTTCTTCAGC	30 cycles of
		95°C—30 s
		62°C—40 s
		72°C—1 min
		1 cycle
		72°C—5 min
		15°C—1 min
RKO_2	F-AAATCTAACCAAGGATAATCAATC	95°C—2 min (1cycle)
	R-ACATCAAGCATCCTTTCCGTTTGC	30 cycles of
		95°C—30 s
		58°C—40 s
		72°C—2 min
		1 cycle
		72°C—5 min
		15°C—1 min
CA746621	F-GGGTGCACATCCATTGACTTT	95°C—2 min (1cycle)
	R-TTCTCTCAAGAGCGGGTGCT	30 cycles of
		95°C—30 s
		58°C—40 s
		72°C—50 s
		1 cycle
		72°C—5 min
		15°C—1 min
CD882879	F-CCGTCCACCGGGAACAAGACC	95°C—2 min (1cycle)
	R-GCTCCTCGCGATGACCCCAC	30 cycles of
		95°C—30 s
		58°C—40 s
		72°C—50 s
		1 cycle
		72°C—5 min
		15°C—1 min
BEX_2	F-TGCGGGTTCAAGAACGTCAAC	95°C—2 min (1cycle)
	R-GTTCATGTCCGTGATGATCA	30 cycles of
		95°C—30 s
		58°C—40 s
		72°C—50 s
		1 cycle
		72°C—5 min
		15°C—1 min

glasshouse experiments with plants at the late tillering stage (approximately 5 weeks after sowing), with consistent results (Fig. 3). The resistance response of CS(Hope3B) was accompanied by necrotic lesions, which may have contributed to limiting fungal growth. Although infected leaves of Hope showed some necrosis, there was more fungal growth on Hope than on CS(Hope3B). Marquis was susceptible and Yaroslav emmer highly resistant probably due to other effective resistance genes. When the seven recombinant lines were inoculated with powdery mildew, lines resistant to stem rust and leaf rust were also resistant to powdery mildew. Recombinants susceptible to both rusts were also susceptible to powdery mildew, indicating that the resistance to three different fungal pathogens was not separated by recombination (Table 2).

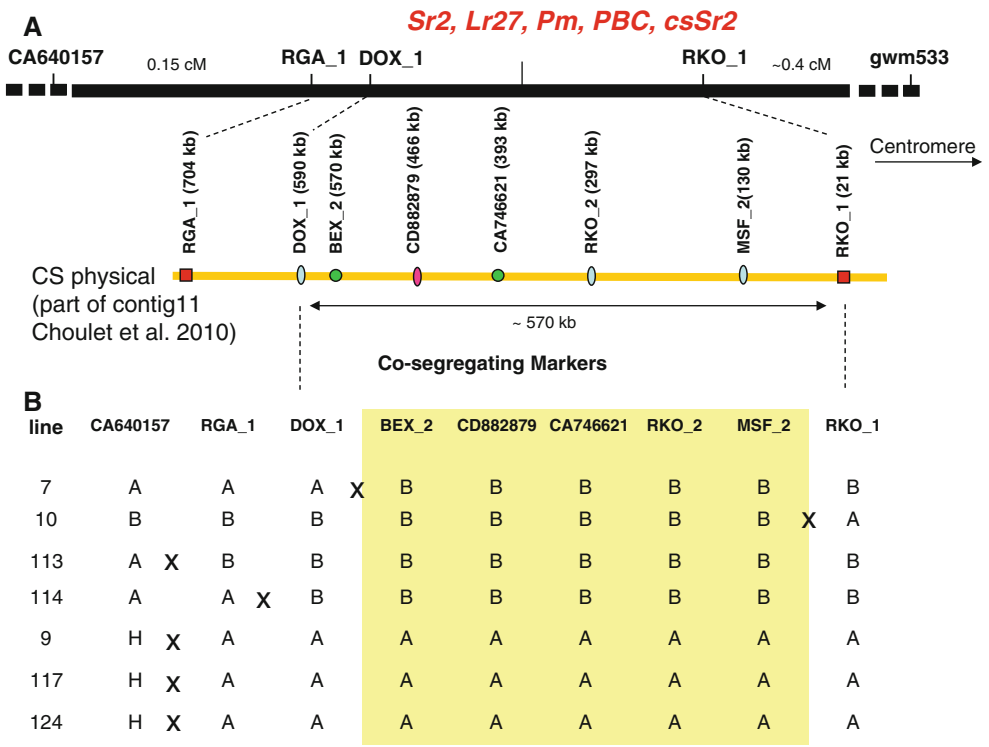

Fig. 1 **a** High-resolution genetic map of the *Sr2* region on chromosome arm 3BS and the corresponding physical map of the susceptible haplotype of Chinese Spring. The physical map and annotation are part of contig 11 (1.2 Mb) published by Choulet et al. 2010. Wheat ESTs CD882879 and CA746621 mapped to 469 kb and 393 kb, respectively, in the published contig 11 sequence. The contig 11 DNA sequence was previously submitted to GenBank under accession number FN645450. **b** A subset of seven critical recombinants from the mapping family CS × CS(Hope3B) was used for fine mapping of markers and genes (**a** genotype = Chinese Spring, **b** genotype = CS(Hope3B). These seven recombinants define the genetic interval in CS that corresponds to *Sr2* region (highlighted by *shading*) and were selected from a set of 17 recombinants originally reported between *CA640157* and *gwm533* (Kota et al. 2006)

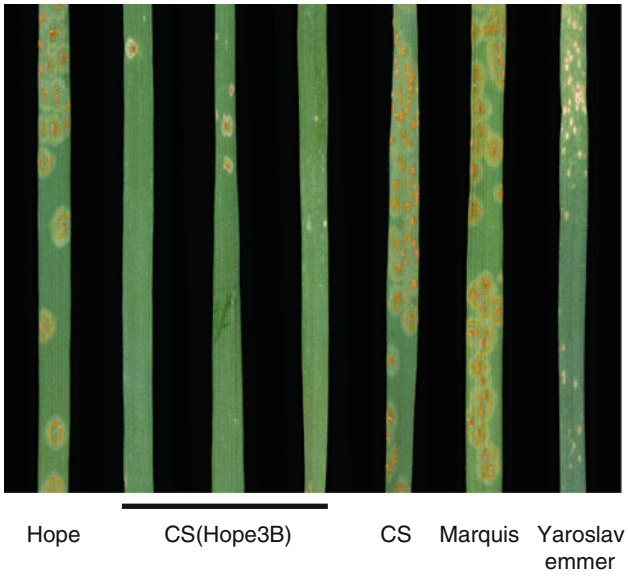

Fig. 2 Leaf rust infection types on first leaves of parental lines 14 days post-inoculation. The following genotypes are shown: Hope, Chinese Spring (Hope 3B) chromosome substitution line, Chinese Spring, Marquis and Yaroslav emmer

Table 2 Summary of known rust resistance genotypes of parental lines and stem rust, leaf rust and powdery mildew scores of parental and recombinant lines

Genotype	*Sr2*	*Lr27*	*Lr31*	Stem rust	Leaf rust	Powdery mildew
Parental						
Yaroslav emmer	+	+	?	R	R	R
Marquis	−	−	−	S	S	S
Hope	+	+	−	R	S	MR
CS	−	−	+	S	S	S
CS (Hope3B)	+	+	+	R	R	R
Recombinants						
RL#7				R	R	R
RL#10				R	R	R
RL#113				R	R	R
RL#114				R	R	R
RL#9				S	S	S
RL#117				S	S	S
RL#124				S	S	S

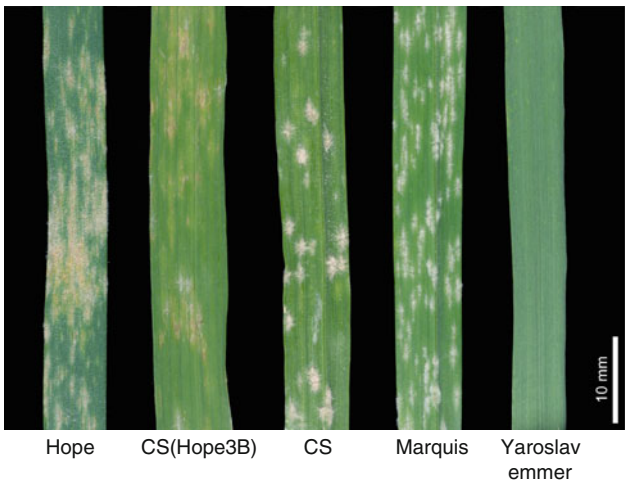

Fig. 3 Powdery mildew infection on leaves of 5-week-old plants (+11 days) grown in the glasshouse. The following genotypes are shown: Hope, Chinese Spring (Hope 3B) chromosome substitution line, Chinese Spring, Marquis and Yaroslav emmer

Five independent mutants lack resistance to stem rust, leaf rust and powdery mildew

Putative mutants lacking stem rust resistance and PBC were recovered from gamma-irradiated seed along with at least one of the resistant sibs from the same M_2 family. Loss of the *Sr2* phenotype in individual plants in the CS background was difficult to ascertain. Progeny tests of putative mutants were conducted in the following field season. A total of four homozygous mutants that lacked stem rust resistance and PBC were recovered. Markers flanking the *Sr2* locus failed to amplify from these mutants indicating that loss of resistance in each case was the result of a large deletion. These mutants also lost resistance to leaf rust and powdery mildew.

Given the possibility that *Sr2* and *Lr27* resistance may be encoded by the same gene, we screened other M_2 families from 1% EMS-treated seed for loss of seedling leaf rust resistance. Nineteen leaf rust-susceptible mutants were recovered among 4,000 M_2 families with loss of flanking markers, suggesting that these lines also contained large deletions. Subsequent progeny testing of these mutants in the field showed they had also lost adult plant stem rust resistance and PBC. Testing of a subset of these mutants demonstrated that they had lost powdery mildew resistance under glasshouse conditions. Although EMS treatment is expected to induce point mutations or small deletions, it is possible that the high concentration of EMS (1%) used in the current study may have led to large deletions of the chromosome.

Five putative leaf rust-susceptible mutants, M39, M40, M48, M161 and M174, retained flanking markers, *DOX_1* and *RKO_1*, and co-segregating markers, *BEX_2*, *CD882879*, *CA746621*, *RKO_2* and *MSF_2*, suggesting that they may be point mutations or small deletions. Progeny testing of M_5 and M_7 families confirmed the loss of leaf rust resistance in these mutants (Table 3). M39, M40 and M48 were also grown in the field in 2009 to evaluate their response to stem rust. M39 and M40 were scored as homozygous susceptible to stem rust and lacked PBC, although 1 out of 20 plants in the family M39 may have retained small amounts of PBC in the field. Several M48 progeny displayed PBC and diminished stem rust infections, indicating either a partial knockout phenotype or segregation. Plants were individually harvested from field rows and progeny of four plants from each mutant were grown in the glasshouse. Progeny derived from M40 lacked PBC, indicating that this mutant was homozygous. In M39, two of the four families contained one or two plants with small amounts of PBC suggesting either a partial knockout phenotype, or that the line may still be segregating, although previously it appeared homozygous for loss of stem rust and leaf rust resistance. PBC was clearly visible on several plants in three of four M48 families, confirming the previous observations of partial loss of PBC and stem rust resistance in the field.

Three mutants (M39, M40 and M48) were also tested as adult plants for stem rust resistance under glasshouse conditions in the Republic of South Africa using a Ug99-related *Pgt* isolate that was virulent on the lines at the seedling stage. Although differences in rust responses were less pronounced than expected between resistant wild-type CS(Hope3B) and the susceptible deletion mutant 72-2 (Fig. 4), the levels of stem rusting on M_5 progeny of these mutants were clearly higher than on the wild type with the exception of one family derived from M48, which was resistant (Table 3). These glasshouse results confirmed the more pronounced phenotypes of mutants M39 and M40 in comparison to M48. Mutants M161 and M174 also showed higher level of stem rust as compared to CS(Hope3B) in glasshouse tests conducted in Canberra.

Mutants M39, M40, M48, M161 and M174 were tested for response to powdery mildew in the glasshouse and the infection phenotypes of all mutants were distinguishable from the resistant parent (Table 3). CS(Hope 3B) showed little or no proliferation of mildew and the development of necrotic patches on infected leaves by 11 days after inoculation. All plants (between 4 and 7 per genotype) of M39, M48 and M161 showed less necrosis and clearly more proliferation of mildew than CS(Hope 3B). M40 and M174 appeared to be susceptible with no large patches of necrosis.

Table 3 Disease scores of CS(Hope3B), mutants and controls grown under field and glasshouse conditions

	Powdery mildew	Leaf rust	Stem rust			
			Field	Glasshouse		
				Sister lines	Stem rust score	Classification
CS/Hope3B	R	R	R		10MS	R
72-2 del mutant	S	S	S		40S	S
Mutant 39	MR[a]	S	S	39-1	40MSS	S
				39-2	40S	S
				39-3	20MSS	MS
				39-4	50MSS	S
Mutant 40	MS[a]	S	S	40-1	30S	S
				40-2	40MSS	S
				40-3	40S	S
				40-4	20S	MS
Mutant 48	MR[a]	S	S	48-1-1	40S	S
				48-1-2	40S	S
				48-2-1	5MS	R
				48-2-2	30MSS	S
Mutant 161	MR[a]	S	nt		MS[b]	S
Mutant 174	MS[a]	S	nt		MS[b]	S
Hartog (res control)	nt	nt	nt		5MS	R
Line 37 ISRTN 07 (sus control)	nt	nt	nt		50S	S
Kariega (sus control)	nt	nt	nt		30S	S

nt not tested

[a] Compared to CS(Hope3B)

[b] Tested in Canberra

Discussion

In this study, stem rust resistance gene *Sr2*, leaf rust resistance gene *Lr27* and powdery mildew resistance could not be separated by recombination. Three independent mutants were isolated that lacked resistance to stem rust, leaf rust and powdery mildew. Loss of leaf rust resistance in these mutants could have resulted from mutations in the complementary gene *Lr31* on chromosome 4B; however, there is no evidence that this gene is involved in stem rust and powdery mildew resistances determined at the *Sr2* locus. The resistance response to powdery mildew was associated with necrosis of leaf cells. Because mutants retained markers that were flanking and co-segregating with the *Sr2* locus, it is possible that mutants carry small deletions or point mutations that resulted in the loss or disruption of a single gene and loss of resistance to three fungal pathogens. However, there are reports that a small number of wheat genotypes express *Lr27* specificity without conferring stem rust resistance (Singh and McIntosh 1984a, b and Singh pers. comm.) suggesting that *Sr2* and *Lr27* may recombine and are therefore encoded by separate genes. To our knowledge, no wheat line has been reported where the reciprocal is true, i.e., carrying *Sr2* and lacking *Lr27*. A possible explanation of these observations is that *Lr27* and *Sr2* are encoded by the same gene, but the stem rust resistance conferred by *Sr2* is not expressed or is difficult to detect in certain backgrounds. For example, the Australian cultivar "H45" was predicted to encode a functional *Lr34* gene based on DNA data, although it is susceptible to stripe rust and leaf rust, indicating that the intact *Lr34* gene is not sufficient for resistance in this background (Lagudah et al., unpublished). Because the physical map used in this study was derived from the susceptible haplotype Chinese Spring, it is also possible that these mutants harbor larger deletion events if the resistant haplotype in Hope has diverged significantly from CS. A final conclusion about the molecular basis of *Sr2*, *Lr27* and *Pm* resistance must await the cloning of these genes.

There are notable similarities between the *Sr2* locus and the recently cloned resistance gene *Lr34* on chromosome 7DS. A single gene confers adult plant resistance to leaf rust (*Lr34*) and stripe rust (*Yr18*) and causes necrosis of the tips of the flag leaves, a trait commonly referred to as leaf tip necrosis (Krattinger et al. 2009). *Lr34* was also linked to the powdery mildew resistance gene (*Pm38*) (Spielmeyer et al. 2005) and loss of function mutants were susceptible to powdery mildew, indicating that a single gene was effective against three fungal pathogens. In this study, we demonstrate that a multiple resistance locus on 3BS also confers resistance to two rusts and powdery mildew, which was associated with necrosis of leaf cells. In a previous

Fig. 4 Stem rust infection on stems after anthesis on *1* CS(Hope3B), *2* 72-2 deletion, *3* Hartog (resistant control) and *4* line 37 ISRTN 07 (susceptible control)

study, *Sr2* was also associated with leaf chlorosis under high-temperature conditions (Brown 1997).

There are also notable differences between *Sr2* and *Lr34*. For example, the race non-specific, adult plant resistance gene *Sr2* is associated with race-specific leaf rust resistance gene *Lr27* that can be scored in the seedling stage. However, the expression of the resistance is dependent on the presence of *Lr31*. Both *Lr34* and *Yr18* are considered race non-specific genes that are effective only at the adult plant stage, although *Lr34* resistance can be seen on seedlings under specific low-temperature conditions (Dyck 1977). From the analysis of mutants, it is possible that a single gene is responsible for both the *Sr2* and *Lr27* resistances. Similar observations were made with *Lr34*, which was associated with the expression of additional leaf rust and stem rust resistance that was not directly encoded by *Lr34* (Dyck and Samborski 1982; German and Kolmer 1992; Spielmeyer et al. 2008; Kolmer et al. 2011; Hiebert et al. 2010). These interactions are particularly pronounced in Thatcher (Tc)-derived germplasm. Loss of function mutants for *Lr34/Yr18/Pm38/Ltn1* also eliminated adult plant stem rust resistance in the Tc background confirming that *Lr34* rather than tightly linked genes is responsible for the enhanced resistance effect (Krattinger et al. 2009). Components of stem rust resistance expressed in the presence of *Lr34* were recently mapped to chromosomes 2BL and 6DS providing important insights into the genetic basis of stem resistance in Tc and Tc derivatives (Kolmer et al. 2011; Hiebert et al. 2010). The adult plant resistance gene *Lr67/Yr46*, which was recently mapped to chromosome 4DL, was also associated with adult plant stem rust resistance in the Tc background, suggesting positive interactions between race non-specific adult plant resistance and that unlinked rust resistance genes may occur rather frequently and such interactions may contribute to the durability of this class of resistance genes in wheat (Dyck 1987; Hiebert et al. 2011; Herrera-Foessel et al. 2011).

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