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ORIGINAL ARTICLE

Mapping a gene on wheat chromosome 4BL involved in a complementary interaction with adult plant leaf rust resistance gene *LrSV2*

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Abstract

Key message **A complementary gene to *LrSV2* for specific adult plant leaf rust resistance in wheat was mapped on chromosome 4BL, tightly linked to *Lr12/31*.**

Abstract *LrSV2* is a race-specific adult plant leaf rust (*Puccinia triticina*) resistance gene on subdistal chromosome 3BS detected in the cross of the traditional Argentinean wheat (*Triticum aestivum*) variety Sinvalocho MA and the experimental line Gama6. The analysis of the cross of R46 [recombinant inbred line (RIL) derived from Sinvalocho MA carrying *LrSV2* gene and the complementary gene *Lrc-SV2* identified in the current paper] and the commercial variety Relmo Siriri (not carrying neither of these two genes) allowed the detection of the unlinked complementary gene *Lrc-SV2* because the presence of one dominant allele of both is necessary to express the *LrSV2*-specific adult plant resistance. *Lrc-SV2* was mapped within a 1-cM interval on chromosome 4BL using 100 RILs from the cross Sinvalocho MA × Purple Straw. This genetic system resembles the *Lr27+31* seedling resistance reported in the Australian varieties Gatcher and Timgalen where interacting genes map at similar chromosomal positions. However, in high-resolution maps, *Lr27* and *LrSV2* were already mapped to adjacent intervals on 3BS and *Lrc-SV2* map position on 4BL is distal to the reported *Lr12/31*-flanking microsatellites.

Introduction

Considering the economic importance of wheat leaf rust disease and the benefits of its genetic control, the identification of new sources of resistance and linked molecular markers for marker-/genomic-assisted introgression become an objective of wheat molecular breeding (Huerta-Espino et al. 2011). The genetic bases of host–pathogen interactions in diseases like rusts were originally characterized by Flor (1956) by using the flax rust pathosystem. This author proposed the gene for gene theory that states that resistance reaction, known as "hypersensitive response" (HR), occurs only when there is at least one resistance gene (R) in the host and the corresponding avirulence allele (*avr*) in the pathogen. He did not describe complementary genes in flax rust pathosystem. Gene complementation, known in standard genetics as double or duplicate epistasis, is the interaction between two genes in a genotype to produce a particular phenotypic trait, while they do not have individual effects in separate genotypes. This interaction can only be observed by studying the inheritance of the trait in the progeny of crosses between individuals with contrasting alleles for both *loci*. Several reports involving complementary genes for plant pathogen resistance were published, including wheat rusts (Baker 1966; Singh and McIntosh 1984; Buell and Somerville 1997; Fukuta et al. 1998; Herrera-Foessel et al. 2005; Wu et al. 2007; Geffroy et al. 2008; Dracatos et al. 2016). In wheat, the best described complementary action was observed for genes *Lr27* and *Lr31* in the Australian hexaploid wheat varieties Gatcher and Timgalen

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(Singh and McIntosh 1984). This genetic system, denominated *Lr27+31*, confers leaf rust resistance since the seedling stage, known as all stage resistance or ASR (McIntosh et al. 1995). Park and McIntosh (1994) noted that all pathotypes virulent for the adult plant resistance or APR gene *Lr12* also showed virulence for *Lr27+31*. Singh et al. (1999) observed that when cultivars carrying *Lr27* were crossed with a line carrying *Lr12*, F1 seedlings gave intermediate but clearly resistant infection types and segregation among F2 seedlings fitted a 9:7 resistant/susceptible segregation ratio, as expected for dominant complementary genes. These authors suggested that *Lr27* could be a genetic modifier of *Lr12* that allows its expression at the seedling stage. In addition, they confirmed the presence of *Lr12* in Timgalen, supporting the prediction of Park and McIntosh (1994) that *Lr12* is either completely linked to *Lr31* or that it is in fact the same gene. Also in hexaploid wheat, Dracatos et al. (2016) reported two complementary genes for ASR to wheat stripe rust in the variety Avocet that were designated as *Yr73* and *Yr74* and mapped to the long arm of chromosomes 3D (3DL) and 5B (5BL), respectively. In durum wheat (*Triticum turgidum* var. *durum*), Herrera-Foessel et al. (2005) suggested the presence of complementary genes for leaf rust APR in varieties Jupare C2001, Hualita and Pohowera when observing F3 segregations that fitted a 1:8:7 ratio for homozygous resistant/segregating/homozygous susceptible. The resistance in Jupare C2001 showed the same specificity as *Lr27+Lr31* in bread wheat, and in a follow-up study, the homozygous seedling-susceptible F3 lines were all susceptible to an *Lr12* avirulent race at adult stage, suggesting that in durum wheat *Lr31* is not same as *Lr12* because some of the susceptible lines should carry *Lr31* (Singh R., *personal communication*).

LrSV2 gene was identified and mapped to subdistal chromosome 3BS as a single dominant leaf rust APR gene in the traditional Argentinean durable resistant bread wheat variety Sinvalocho MA (Ingala et al. 2012). In a subsequent work, *LrSV2* was fine mapped in a genetic interval of 0.45 cM, delimited by SSRs markers swm13 and gwm533 (Diéguez et al. 2014). A recombinant inbred line (R46) derived from Sinvalocho MA which carried gene *LrSV2* was used to introgress this gene into different susceptible commercial varieties, including Relmo Siriri (Si). During this introgression, an F2 population of Si × R46 was obtained and used for further improving the genetic mapping of *LrSV2*. With the same purpose, an F8 RILs population and an F2 population from the cross Sinvalocho MA × Purple Straw (SV × P) were developed.

The objective of the present study was to identify additional recombinants within the genetic interval containing *LrSV2* among the F2 population of Si × R46 and the RIL and F2 populations of SV × P by using flanking molecular markers. This led to the identification and mapping of a complementary gene interacting with *LrSV2*.

Materials and methods

Plant material

Line R46 was selected among a wheat (*Triticum aestivum*) recombinant inbred lines (RILs) population from the cross of the durable leaf rust-resistant Argentinean cultivar Sinvalocho MA (SV) with the susceptible experimental line Gama6 (G6). This RIL carries the seedling resistance gene *LrG6* and the adult plant resistance genes *LrSV1* and *LrSV2* (Ingala et al. 2012). The *LrSV2* gene provides resistance at adult stage to the Castelar02-Gama1R (Ca02-G1R) race of *Puccinia triticina* which is virulent on *LrSV1* (Ingala et al. 2012). The commercial variety Relmo Siriri (Si) and the cultivar Purple Straw (P) were susceptible to this race.

Three segregating populations were used: 521 F2 plants from the cross R46 × Si, an F8 population of 100 RILs obtained by single-seed descendent (SSD) from the cross between SV and P and 388 F2s from the cross SV × P.

Plants were grown either at the experimental field in Castelar, province of Buenos Aires, Argentina (34°S–58°W) during the standard wheat-growing season or in the greenhouse at temperatures between 15 and 25 °C in one-liter pots with 5 plants per pot.

Rust inoculation

Plants were artificially inoculated either at the second leaf stage (seedling) or at flag leaf stage (adult plant) using *Puccinia triticina* race Ca02-G1R (nomenclature MBGJ, according to the North American System of Long and Kolmer 1989), which at adult plant stage shows an incompatible reaction (Mains and Jackson 1926) in the presence of the *LrSV2* gene (Ingala et al. 2012).

Infections were carried out spraying a suspension of 20 mg of urediospores in 50 ml of water with one drop of Tween 20 per 50 one-liter pots. Incubation was performed in moist chambers (100% humidity) for 16 h at 18–20 °C. Afterward, plants were kept in the greenhouse at temperatures that ranged between 15 and 25 °C. Reactions were scored after 14–21 days, according to Mains and Jackson's scale (Mains and Jackson 1926).

DNA isolation

DNA was isolated from leaf tissue. Briefly, 300 mg of dehydrated leaf tissue was mechanically ground by the Tissue Lyser (Quiagen) and 0.8 ml of extraction buffer (100 mM Tris–HCl pH 7.5, 700 mM NaCl, 50 mM EDTA

pH 8) at 65 °C was added. After the incorporation of 100 µl of 10% CTAB and 100 µl of 20% SDS, samples were incubated under gentle shaking at 65 °C for 60 min. Once at room temperature, 900 µl of chloroform/isoamyl alcohol (24:1) was added and, after 5 min of gentle mixing by inversion, centrifuged for 10 min at 14,000 rpm. The upper aqueous phase was incubated with 5 µl of RNase (10 µg/µl) for 60 min at 37 °C. After a second chloroform/isoamyl alcohol extraction, 510 µl of cold isopropanol was added to the aqueous phase and centrifuged for 10 min at 14,000 rpm. The pellet was washed with 1 ml 70% ethanol and dissolved in 50 µl H_2O. The amount of purified DNA was estimated by using a spectrophotometer (ND-1000, NanoDrop®).

Marker development

The sequences of the primers used to amplify microsatellites gwm149 and gwm251, reported to flank *Lr12* by Singh and Bowden (2011), were aligned to the wheat 4B sequence contigs of the TGAC (The Genome Analysis Center http://www.tgac.ac.uk/) local database. Selected contigs were used to BLAST (Basic Local Alignment Search Tool, Altschul et al. 1990) against *Brachypodium distachyon* sequences at Gramene (gramene.org) and Phytozome (phytozome.org) in order to define a *B. distachyon* interval putatively syntenic to the wheat *Lr12*-containing interval. The *B. distachyon* transcripts annotated in this interval were used to identify wheat 4B contigs spanning the target region. Primers suitable to amplify microsatellites on these contigs were designed using the program WEBSAT (Martins et al. 2009) and named FSs (listed in Table S1).

Amplification of DNA markers by PCR

PCR amplifications were performed with 50 ng of genomic DNA as template in 20 µl reactions with 1.5 mM de $MgCl_2$, 0.16 µM dNTPs, 0.08 µM of each primer and 0.032 units/µl of Taq DNA polymerase (Invitrogen) in its corresponding buffer (10×, without $MgCl_2$). Thermal cycling consisted of seven cycles of 30 s at 94 °C, 30 s at 62 (reducing 1 °C per cycle) and 30 s at 72 °C; 31 of cycles 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C; 11 cycles of 30 s at 94 °C, 30 s at 56 °C and 30 s at 72 °C. Primer sequences were as described: *barc* (Song et al. 2005), *cfa* (Sourdille et al. 2001), *cfd* (Guyomarc'h et al. 2002), *cnl* (M Sorrels, http://wheat.pw.usda.gov/GG2/index.shtml), *gwm* (Röder et al. 1998), *gpw* (Sourdille et al. 2004), *psp* (Bryan et al. 1997), *stm* (Hayden et al. 2006), *swm* (Diéguez et al. 2014), *wmc* (Somers et al. 2004), and *wms* (M Röder, personal communication).

Visualization of PCR products

After cycling, one volume of loading buffer (10 mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol and 95% deionized formamide) was added and after 5-min incubation at 95 °C, 7 µl was loaded in 5% denaturing polyacrylamide gels and electrophoresed in 0.5× TBE buffer for 90 min at 60 W. The separated fragments were silver-stained as follows: 5-min incubation in 0.05% acetic acid–10% ethanol, 10-min incubation in 1.5 g/l $AgNO_3$ plus 1.5 ml/l formaldehyde, 15 s wash in double distilled water and incubation in 15 g/l NaOH plus 2 ml/l formaldehyde until PCR products were visible. Staining was stopped with 0.05% acetic acid–10% ethanol.

Data analysis

Segregations were tested by binomial exact test.

Marker association was evaluated by BSA (bulk segregant analysis, Michelmore et al. 1991) on 12 resistant and 11 susceptible RILs from the cross SV×P.

Data matrixes were prepared as follows: Resistant parent alleles were scored as 1, alleles from the susceptible parent as 0 and heterozygotes as H. Linkage groups and the genetic distances were calculated using Carthagene (de Givry et al. 2005) at min LOD 3.0 and maps were drawn by using MapChart software (Voorrips 2002).

Results

Inheritance of *LrSV2*-specific resistance in the cross of RIL46 (*LrSV2 Lrc-SV2*) × Relmo Siriri (*lrSV2 lrc-SV2*)

RIL46 (R46) is a recombinant inbred line derived from SV that carries *LrSV2* gene on chromosome 3BS, and Relmo Siriri (Si) is a commercial variety susceptible to *P. triticina*

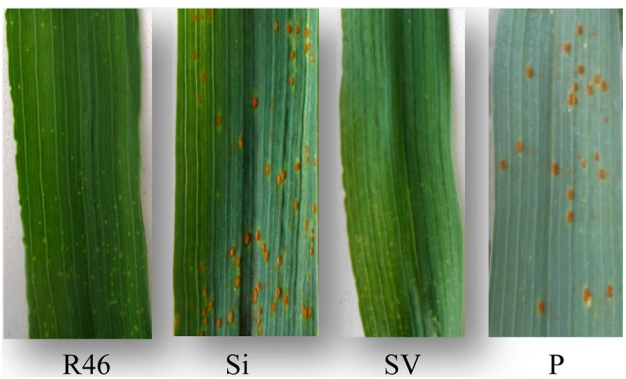

Fig. 1 Flag leaves infected by *Puccina triticina* race Ca02-G1R

race Ca02-G1R that identifies *LrSV2* (Fig. 1). During marker-assisted introgression of *LrSV2* in wheat cultivar Si, 521 F2 plants from the cross R46 × Si were grown at the experimental field. For fine mapping purposes, they were genotyped with the *LrSV2*-flanking markers cfb3417 and gwm533 (Diéguez et al. 2014) in order to identify recombinants within this genetic interval. Both markers were codominant and allowed a clear distinction between homozygous and heterozygous plants. The closest marker, microsatellite swm13 distal 0.15 cM from *LrSV2* gene (Diéguez et al. 2014) was not used in this study because it was dominant in this cross and would therefore require progeny tests to distinguish between heterozygous and dominant homozygous plants.

Twenty-four recombinants were found between markers cfb3417 and gwm533 out of 521 tested F2s, which represents a genetic distance of 2.3 cM. Progeny tests were performed by inoculating thirteen-to-twenty F3 progenies from each recombinant F2 at the flag leaf stage using rust race Ca02-G1R (Table 1). For a single dominant gene like *LrSV2*, in these recombinants it is expected that rust reaction cosegregated with one of the tightly linked *LrSV2*-flanking markers, as the possibility of a double crossover is highly improbable. In other words, according to the position of recombination breakpoint with respect to the *LrSV2* gene, the genotype for rust reaction (deduced from F3 inoculations) and one of the two *LrSV2*-flanking markers should be the same. For example, F2 recombinants s3, s35, s65, s88, s283, s299, s408 and s483 were assumed as not carrying *LrSV2* because their F3 progenies were homozygous susceptible and one of the *LrSV2*-flanking markers was homozygous for the allele of the susceptible parent (Si). However, progenies of recombinants s11, s83, s263 and s411 were homozygous susceptible for rust reaction, while the F2s were homozygous for the resistant parent allele for one of the *LrSV2*-flanking markers and heterozygous for the other (Table 1). Therefore, their F3 progenies were expected to be either all resistant or to segregate following a 3:1 proportion

Table 1 Genotype for the *LrSV2*-flanking markers in the 24 recombinant F2s from the cross R46 × Si and rust phenotype upon *P. triticina* race Ca02-G1R artificial infection at adult stage of their F3 progeny

RIL 46 × Relmo Siriri						
F2			F3			
Recombinant	*LrSV2*-flanking markers (3BS)		Ca02G1R (flag leaf)			
	cfb3417	gwm533	R	S	*p* 3:1	*p* 9:7
s3	H	0	0	18	< 0.001	< 0.001
s11	1	H	0	15	< 0.001	< 0.001
s35	0	H	0	19	< 0.001	< 0.001
s65	H	0	0	17	< 0.001	< 0.001
s83	H	1	0	19	< 0.001	< 0.001
s88	0	H	0	18	< 0.001	< 0.001
s104	0	H	7	9	< 0.01	0.23
s155	H	1	11	7	0.14	0.43
s167	H	1	13	3	0.41	< 0.05
s191	H	1	15	4	0.47	< 0.05
s240	H	1	13	7	0.21	0.28
s263	H	1	0	17	< 0.001	< 0.001
s283	0	H	0	19	< 0.001	< 0.001
s298	H	1	17	0	< 0.01	< 0.001
s299	H	0	0	18	< 0.001	< 0.001
s313	H	1	13	7	0.21	0.28
s369	H	1	20	0	< 0.01	< 0.001
s408	0	H	0	20	< 0.001	< 0.001
s411	H	1	0	18	< 0.001	< 0.001
s437	H	1	19	0	< 0.01	< 0.001
s441	1	H	12	5	0.43	0.17
s462	0	H	8	5	0.21	0.46
s483	H	0	0	19	< 0.001	< 0.001
s509	H	1	14	5	0.53	0.09

1: R46 allele, 0: Si allele, H: heterozygous, R: resistant, S: susceptible. *p*: probability value for a binomial exact test

of resistant and susceptible plants. This fact, added to the segregations observed in several F3 progenies that fitted a 9-resistant/7-susceptibility ratio (Table 1), allowed to hypothesize that resistance conferred by *LrSV2* gene was modified by an independent complementary gene. In this cross, R46 carries *LrSV2* and a complementary gene temporally termed *Lrc-SV2*, and both genes were absent in Si.

In a map developed with 1308 F2 plants from the cross SV × G6, Diéguez et al. (2014) positioned the gene *LrSV2* in the same chromosome region as *Lr27*, reported as complementary to *Lr31* by Singh and McIntosh (1984). In turn, *Lr31* was mapped on chromosome 4BL, completely linked or identical to *Lr12* (Singh et al. 1999). The availability of flanking markers for *Lr12* (Singh and Bowden 2011) allowed to test the hypothesis of the influence of this and/or other tightly linked genes in this region on the expression of *LrSV2*-specific resistance.

When the *Lr12*-flanking microsatellites gwm149 and gwm251 were tested in the parental lines, SV, G6 and the derived line R46 showed the marker alleles associated with the presence of *Lr12*, but Si showed a higher molecular weight allele for both, as was observed in cultivar Thatcher that does not carry *Lr12* (Singh and Bowden 2011).

The F3 progenies of s11, s83, s263 and s411, homozygous R46 parental type for one of the *LrSV2*-flanking markers and heterozygous for the other, were homozygous susceptible for rust reaction and showed the Si allele in homozygous condition for the *Lr12*-flanking markers (Table 2). Therefore, it was assumed that these F2s lack the *Lr12*-containing interval which either contains or is tightly linked to the complementary gene *Lrc-SV2*.

F2 plants s104, s313 and s462 were heterozygous for both gwm149 and gwm251. In their F3 progenies, the resistance was observed only when there was at least one copy of both intervals on 3BS and 4BL (individuals s104-27, s104-28, s104-30, s104-31, s104-32, s104-33, s104-34, s313-1, s313-5, s313-16, s462-1, s462-3 and s462-9, bold in Table 2). In recombinants s104 and s462, the position of the crossover within the *LrSV2* interval should be distal to this gene because the F3ss104-27, s104-28, s104-30, s104-31, s104-32, s104-33, s104-34, s462-1, s462-3 and s462-9 were resistant and therefore assumed to carry both genes. On the contrary, in recombinant s313 the crossover should be proximal to *LrSV2* because the F3 s313-2, even when homozygous for the complementary gene linked to the *Lr12*-containing interval, was susceptible due to the lack of *LrSV2*. This F3 plant, together with plants s104-29 and s104-36, showed that the presence of *Lrc-SV2* was not enough to observe *LrSV2*-specific resistance (Table 2). Thus, it was concluded that recombinants s104, s313 and s462 were heterozygous for genes *LrSV2* and *Lrc-SV2* in agreement with the hypothesis of two complementary genes that fit a 9-resistant/7-susceptible ratio in F3 progenies.

Inheritance of the *LrSV2*-specific resistance in the cross Sinvalocho (*LrSV2 Lrc-SV2*) × Purple Straw (*LrSV2 lrc-SV2*)

The variety Purple Straw (P) was susceptible to rust race Ca02-G1R at the flag leaf stage (Fig. 1), and the flanking markers for *LrSV2* and *Lr12* genes were polymorphic with respect to those of R46 and SV.

A population of 100 recombinant inbred lines (RILs) from the cross of SV × P was used to analyze both the reaction to rust race Ca02-G1R and flanking markers for *LrSV2* and *Lr12*. The segregation of the intervals containing *LrSV2* and *Lr12* followed a 1:1 ratio ($p = 0.5$–0.7 and $p = 0.3$–0.5, respectively). Both parental lines and the RIL population were susceptible to race Ca02-G1R at seedling stage. When these RILs were inoculated at the flag leaf stage, the segregation of resistance and susceptibility also followed a 1:1 proportion as would be expected for a monogenic trait ($p = 0.3$–0.5). Therefore, it was assumed that P lacks one of the two complementary genes. The resistance was associated with SV markers flanking *Lr12* region on 4BL which complemented either SV or P alleles of the *LrSV2* gene. Thus, it was concluded that SV carries both *LrSV2* and the complementary gene *Lrc-SV2* and P lacks only the later.

In order to support this hypothesis, the variety P (susceptible but with a functional allele of *LrSV2*, see above) was crossed to G6 which is also susceptible but shows the *Lr12*-flanking marker alleles associated with the presence of this gene. Five F1 plants from this cross were inoculated both at seedling and at flag leaf stage with race Ca02-G1R. At seedling all showed a susceptible reaction, while a moderate resistance (infection type 1 + 2 ++) was observed at adult stage, as opposed to the susceptible phenotype of both parents (Fig. 2). This showed that one dose of each interval is sufficient to confer resistance to race Ca02-G1R at the flag leaf stage. In agreement with the observed segregations, it was assumed that SV and R46 carry both genes, G6 only *Lrc-SV2*, P a resistance allele of *LrSV2* alone and Si lacks both complementary genes.

Molecular mapping of the *LrSV2*-complementary gene

With the aim of developing a map of the 4BL region containing the *LrSV2*-complementary gene, provisionally termed *Lrc-SV2*, 53 additional molecular markers reported to map on the long arm of chromosome 4B and seven SSR markers developed from the available Chinese Spring survey sequence (see "Materials and methods") were tested for polymorphism between the parental lines SV and P. Among these 60 markers, eight did not amplify a discrete band and sixteen did not show polymorphism between parental lines. The remaining 36 were polymorphic and tested on

Table 2 Rust phenotype upon *P. triticina* race Ca02-G1R artificial inoculation at adult stage and *LrSV2*- and *Lr12*-flanking markers genotype in the F3 progeny of the indicated recombinant F2s from the cross R46 × Si

RIL 46 × Relmo Siriri						
F2	F3					
	Progeny number	Ca02G1R (flag leaf)	*LrSV2*-flanking markers (3BS)		*Lr12*-flanking markers (4BL)	
			Distal cfb3417	Proximal gwm533	Distal gwm149	Proximal gwm251
s11	1	S	1	H	0	0
	2	S	1	H	0	0
	3	S	1	1	0	0
	4	S	1	0	0	0
s83	1	S	1	1	0	0
	3	S	1	1	0	0
	6	S	1	1	0	0
	17	S	H	1	0	0
s104	25	S	0	0	0	0
	26	S	0	0	0	0
	27	**R**	**0**	**1**	**1**	**1**
	28	**R**	**0**	**H**	**1**	**1**
	29	S	0	0	H	H
	30	**R**	**0**	**H**	**H**	**H**
	31	**R**	**0**	**H**	**H**	**H**
	32	**R**	**0**	**H**	**H**	**H**
	33	**R**	**0**	**H**	**1**	**1**
	34	**R**	**0**	**H**	**1**	**1**
	35	S	0	H	0	0
	36	S	0	0	1	1
	37	S	0	H	0	0
s263	5	S	H	1	0	0
	9	S	H	1	0	0
	11	S	1	1	0	0
	17	S	1	1	0	0
	16	S	1	1	0	0
s313	**1**	**R**	**1**	**1**	**1**	**1**
	2	S	0	1	1	1
	5	**R**	**1**	**1**	**H**	**H**
	12	S	H	1	0	0
	16	**R**	**H**	**1**	**1**	**1**
s411	2	S	H	1	0	0
	6	S	H	1	0	0
	10	S	0	1	0	0
	16	S	1	1	0	0
s462	**1**	**R**	**0**	**1**	**H**	**H**
	3	**R**	**0**	**H**	**H**	**H**
	4	S	0	H	0	0
	9	**R**	**0**	**H**	**1**	**H**

1: R46 allele, 0: Si allele, H: heterozygous, R: resistant, S: susceptible

12 resistant and 11 susceptible RILs from the cross SV × P. Thirteen of these polymorphic markers (barc109, barc174, barc292, cfa2149, cfd54, gpw2328, gpw3229, gwm314, gwm538, $stm513_{tgtc}$, wmc47, wms935 and wms940) were not found to be associated with resistance. The remaining 23, together with *Lr12*-flanking markers gwm149 and gwm251, were evaluated in the whole RIL population ($n = 100$). All fitted a 1:1 proportion ($p > 0.1$) and formed a

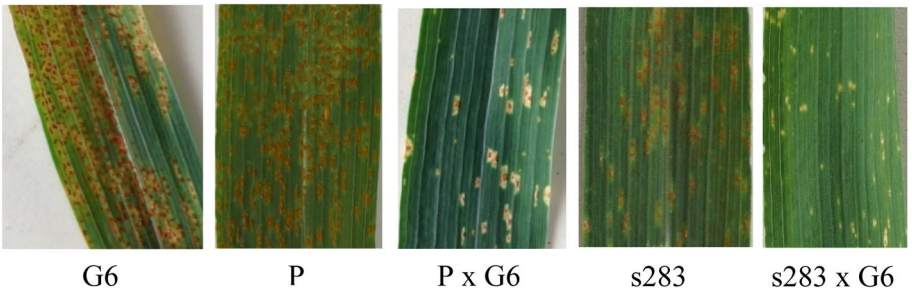

Fig. 2 Flag leaves infected by *Puccina triticina* race Ca02-G1R

linkage group including *Lrc-SV2*, the only gene determinant of the resistance to race Ca02-G1R that was segregating in this population. In this map, *Lrc-SV2* cosegregated with wmc692 within a 1-cM interval between markers FSs34 and gpw4388 (Fig. 3). Microsatellites gwm149 and gwm251, reported as *Lr12*-flanking markers by Singh and Bowden (2011), were positioned in a cluster together with gwm375 at 2 cM proximal to *Lrc-SV2*.

In order to refine the genetic map of this region, 388 F2s from the same cross were genotyped with the reported *Lr12*-flanking markers and barc163 to identify recombination events around *Lrc-SV2*. Recombinant F2 plants were selfed, and F3 lines with the recombinant gametes in homozygous condition were identified. Five-to-ten F4 progenies of this selected F3 plant were rust inoculated at adult stage with *P. triticina* race Ca02-G1R. Among them, 6 recombinants were found between the reported *Lr12*-flanking markers and *Lrc-SV2* (0.8 cM).

In both populations derived from the cross SV × P (100 RILs + 388 F2s), *Lrc-SV2* mapped distal to the position reported for *Lr12* in the cross Thatcher × Tc*Lr12* (Singh and Bowden 2011).

In the cross R46 × Si, where both complementary genes *LrSV2* and *Lrc-SV2* were segregating, F2 recombinants s3, s35, s65, s88, s283, s299, s408 and s483 had homozygous-susceptible F3 progenies and one of the *LrSV2*-flanking markers was also homozygous for the allele of the susceptible parent (Table 1). The genotyping of additional markers within the *LrSV2*-interval on 3BS that became available after the fine mapping of this gene (Diéguez et al. 2011) indicated that recombinant s283 was heterozygous for *LrSV2* resistance gene on 3BS and the fact that its F3 progeny was homozygous susceptible suggested the absence of the complementary gene *Lrc-SV2* on 4BL. Moreover, F4 progeny from this recombinant in which the gamete carrying a crossover within the *LrSV2* interval on 3BS was made homozygous was crossed to G6 (susceptible but assumed to carry *Lrc-SV2*, see above). Fifteen F1 plants from this cross were inoculated both at seedling and at flag leaf stage with race Ca02-G1R. All showed a susceptible reaction at seedling while at adult stage were resistant (0;1—infection type), as opposed to the susceptible phenotype of both parents (Fig. 2). Therefore, it was concluded that s283 carried *LrSV2* but lacked *Lrc-SV2*. Twenty markers from the map in Fig. 3 were genotyped in these s283 F4 plants. A recombination breakpoint was found between the cluster containing the reported *Lr12*-flanking markers gwm149 and gwm251 (R46 genotype) and the *Lrc-SV2* gene (susceptible Si phenotype) as shown in Fig. 3.

Taken together, results from the RIL population and F2s from 2 crosses place *Lrc-SV2* distal to the *Lr12* position reported by Singh and Bowden (2011).

Discussion

The APR gene *LrSV2* for leaf rust resistance was identified in the wheat durable resistant cultivar Sinvalocho MA, together with other leaf rust resistance genes. This gene was originally detected by analyzing the cross Sinvalocho MA by the experimental line Gama6 and mapped to subdistal chromosome 3BS (Ingala et al. 2012). In the present report, the analysis of the F2 population from the cross R46 (recombinant inbred line carrying the *LrSV2* gene derived from the cross SV × G6) and the commercial variety Relmo Siriri (Si) allowed the detection of an unlinked complementary gene on chromosome 4BL, provisionally termed *Lrc-SV2*. The two genes act in a complementarily way since the presence of both dominant alleles were necessary to determine the *LrSV2*-specific adult plant leaf rust resistance. This interaction was not observed in SV × G6 cross where a monogenic segregation for *LrSV2* was reported (Ingala et al. 2012). The fact that 13 out of the 15 molecular markers linked to *Lrc-SV2* on 4BL tested in this cross were monomorphic (Table S2) supports the hypothesis that SV and G6 most probably carry the same allele for this gene. Only markers gwm6 and gwm107, which mapped 8.9 cM distal and 16.6 cM proximal, respectively, to *Lrc-SV2* in the cross SV × P (Fig. 3) were polymorphic between SV and G6 (Table S2). On the contrary, 19 out of 24 tested molecular markers linked to *Lrc-SV2* showed polymorphism between SV and Si, supporting the absence of *Lrc-SV2* in this last variety. In agreement with these observations, the region containing *Lrc-SV2* and linked markers did not segregate

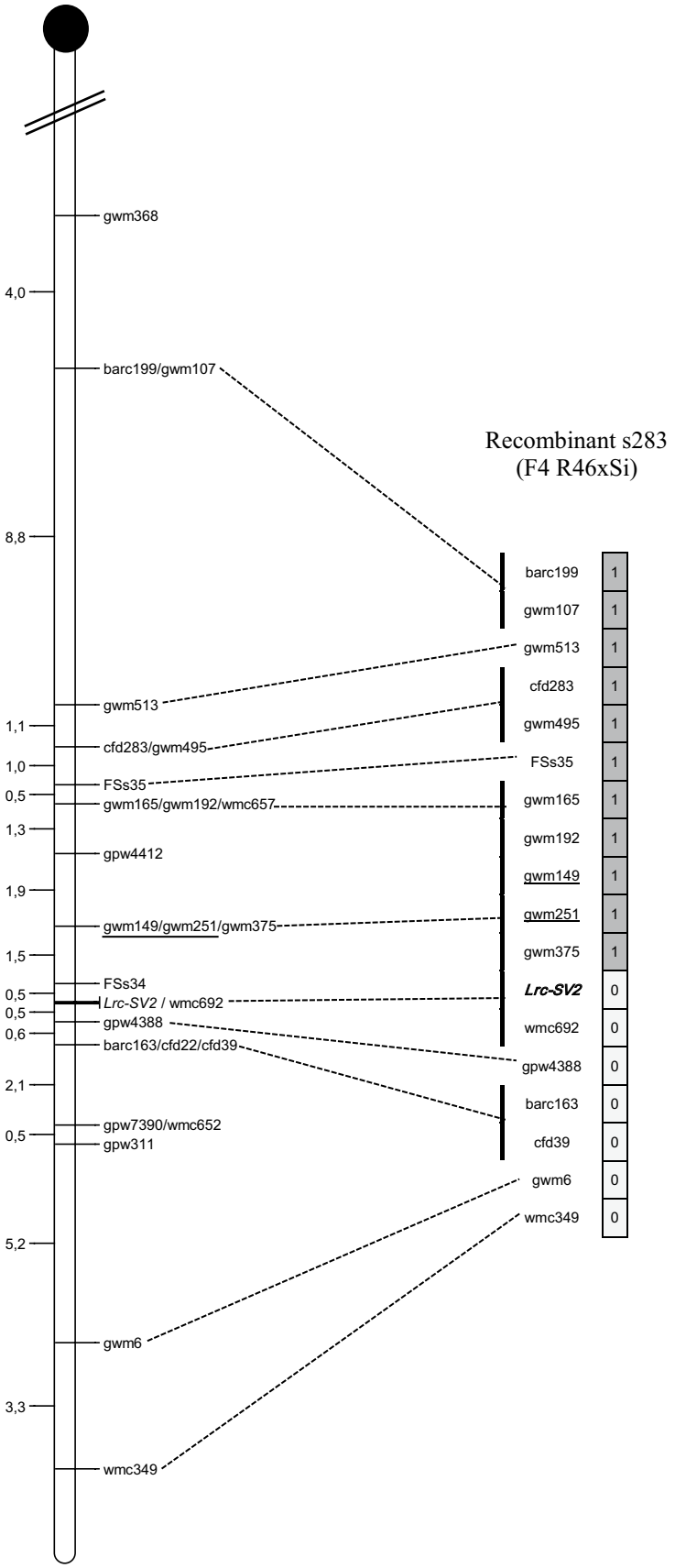

Fig. 3 Left: genetic map of chromosome 4BL, developed using a population of 100 RILs from the cross SV × P. The centromere is indicated by a circle, and the genetic distance, in cM, is shown on the left. Right: genotype of recombinant s283 (F4 R46 × Si). 1 = homozygous R46, 0 = homozygous Si. Microsatellites reported as *Lr12*-flanking markers are underlined

in the cross SV × G6, but it was segregating in the crosses R46 × Si and SV × P and the F1 from the cross G6 × P showed resistance at adult stage while both parents were susceptible. In Chinese Spring (CS), a variety reported to carry *Lr31* (Singh and McIntosh 1984) and/or *Lr12* (McIntosh et al. 1995), the 14 molecular markers linked to *Lrc-SV2* tested were monomorphic with respect to SV (Table S2). Sears (1988) traced the history of Chinese Spring, initially named Chinese White, obtained by the Plant Breeding Institute (Cambridge, England). Shortly after, it was introduced in Argentina and may have been used in wheat breeding through the Argentinian cultivar 38MA because this cultivar was developed from a cross between Barleta and Chino, a Chinese introduction that may have been Chinese Spring (Dyck 1991). The cultivar SV was developed from the cross Klein Sin Rival by 38MA, and therefore, SV may have inherited this chromosomal region from 38MA, explaining the genetic similarity to Chinese Spring. The analysis of the rust resistance at seedling and adult stages of crosses to nullitetrasomic CS lines lacking 7D (and therefore *Lr34* to avoid its pleiotropic effect) will be done to test whether *Lr31* from CS can complement *LrSV2*. However, it should be noted that CS might carry not only *Lr31* and *Lr12* but other genes, including *Lrc-SV2* that could be identified only if races with the corresponding avirulent genes are available.

The segregation 9 resistant/7 susceptible in a F2 (or in segregating F3 progenies like here) not only fit the expected ratios for two dominant complementary genes, but also for a dominant resistance gene together with an unlinked recessive suppressor gene (Herrera-Foessel et al. 2005). While reports on complementary gene interaction for rust resistance in wheat are scarce, being the only well-studied example the *Lr27+Lr31* system, there are several reports on suppressor genes (Bai and Knott 1992; Nelson et al. 1997; Knott 2000; Assefa and Fehrmann 2000). However, in the gene interaction described here, not only a population where both genes were segregating (R46 × Si) was analyzed but also two populations where either of them was segregating (*LrSV2* on 3BS in the cross SV × G6 and *Lrc-SV2* on 4BL in SV × P). In these last two cases, it should be postulated that both genes act as mutually recessive suppressors, supporting the hypothesis that at least one copy of both genes is necessary to express the resistant phenotype. An alternative hypothesis may be dosage dependence of resistance (Sacco et al. 2001). Resistance expression may be triggered as a consequence of the interaction of both a threshold of host reaction gene products and pathogenicity gene products. In the *Triticum aestivum–P triticina* gene for gene system, Kolmer and Dyck (1994) showed that host resistance and avirulence in the pathogen may be completely dominant, incompletely dominant or recessive. Disease expression among different host–pathogen genotype combinations is usually assessed by visual rating of the infection types. However, the level of expression between homozygous and heterozygous combinations, both in the host and pathogen, could, in some instances, be detected by counting number of pustules (Saione et al. 1993). The fact that a moderate resistance was observed at adult stage in the F1 progeny of the cross G6 × P could be because in the F1 both genes were in heterozygous condition. Alternatively, examples in which differences may not be visually detected may be attributed to the clustering of genes or the presence of other genes not detected, but participating in the interaction in which the number of doses in the heterozygous is high enough that the threshold level is overcome and dominance fully expressed. This variation in copy number may imply gene dosage effects and quantitatively influence the expression of resistance genes.

Other rust resistance genes and QTLs have been mapped on chromosome 4BL. QTLs for seedling leaf rust resistance were mapped close to wmc349 (Naz et al. 2008) and between cfd22 and gwm165 in a GWAS (genome-wide association study) using a panel of 1596 wheat accessions (Li et al. 2016). *Yr50* for seedling stripe rust resistance was also mapped on 4BL, although there are no common markers mapped between this and our study, the authors postulate that the gene was presumably transferred from *Thinopyrum intermedium* into Chinese wheat-susceptible cultivars with no pedigree relationship to Sinvalocho MA (Liu et al. 2013). Also, QTLs for adult plant stripe and leaf rust resistance were located between microsatellites gwm495 and gwm368 (William et al. 2006) and the APR gene *Lr49* was mapped between barc163 and wmc349 (Bansal et al. 2008). Interestingly, a strong effect QTL for adult plant leaf rust resistance in Chinese environment was found by Rosewarne et al. (2015). This QTL significantly reduced the AUDPC (area under the disease progress curve) when present alone but in combination with a QTL on 3BS reaches disease severity levels similar to those observed in lines with a QTL on 7DS, presumably *Lr34*. The authors named the QTL on chromosome 4 as QLR.cim-4BS, but the diagnostic marker wPt-6209 was placed between gwm251 and gwm149, the reported *Lr12*-flanking markers on 4BL (Tan et al. 2006). However, they postulate that the 3B locus is not *Lr27* because, according to the seedling tests, none of the parents carry the complementary pair *Lr27+31* and both QTL were shown to be able to act independently in addition to additively. This last is also different to the *LrSV2+c-SV2* system described here where one copy of both genes is needed in order to get the resistant phenotype.

Provided that the appropriate races of the pathogen are available, the detection of resistance genes is possible even in varieties commonly used as rust-susceptible parents in the developing of mapping populations. Here we describe the presence of an active allele of *LrSV2* in the variety Purple Straw, reported as susceptible to rust infection for more than 50 years in Argentina (Sacco et al. 1998). This variety was

originated in Australia, where it was selected at the beginning of the twentieth century among the first wheat introductions into that country. There are no records of resistance genes so far identified in it. Probably, the Purple Straw allele of *LrSV2* is different from the one present in Sinvalocho, given that the flanking markers swm13 and gwm533 and 17 markers mapped within the interval were polymorphic (Diéguez, unpublished). The availability of several functional alleles of a resistance gene is interesting because they might have different specificities and could be useful not only for breeding purposes but also for basic studies on the resistance mechanism.

Interacting genes *LrSV2* and *Lrc-SV2* described here mapped at similar chromosomal positions on 3BS and 4BL, respectively, reported for complementary genes *Lr27* and *Lr31*. The *P. triticina* race Ca02G1R which was used to identify *LrSV2+c-SV2* at adult stage was also avirulent on seedlings carrying *Lr27+31* (Ingala et al. 2012). However, while *Lr27+31* were active at the seedling stage, Sinvalocho behaved as susceptible at seedling and *LrSV2+c-SV2* were not detected until later developmental stages like the flag leaf, supporting the hypothesis that they are not the same genes. In addition, the availability of a physical map of 3B (Paux et al. 2008) and the high number of markers that could be developed after its sequencing (Choulet et al. 2010, 2014) allowed high-resolution mapping of *Lr27* and *LrSV2* using 1340 and 3404 F2s, respectively. In these fine maps, *Lr27* mapped proximal to the marker DOX_1, while *LrSV2* was distal from it (Mago et al. 2011; Diéguez et al. 2011). Considering that *Lr31* is allelic or the same as *Lr12*, as suggested by Singh et al. (1999), the position of *Lr31* and *Lrc-SV2* is not coincident, since in the genetic map *Lr12* was flanked by microsatellites gwm149 and gwm251 (Singh and Bowden 2011) and in the map developed here, the *LrSV2*-complementary gene was positioned distal to a cluster composed by these two markers and gwm375. The size of the mapping population analyzed by Singh and Bowden was 230 F2s plants and in the present study was 100 RILs + 388 F2s. Both populations allowed the construction of low- to medium-resolution maps, and therefore, they do not allow to resolve unambiguously if both genes are alleles or closely linked, and the possibility that an increase in the number of segregating individuals could lead to coincident positions cannot be ruled out. Alternatively, given the clustering tendency observed in resistance gene *loci* (Michelmore and Meyers 1998; Leister 2004), the possibility that they are different genes cannot be discarded. The marker order of the eight common markers between our map and that reported by Röder et al. (1998) is conserved, and fourteen of the SSRs mapped here were also physically allocated using the CS deletion stocks (Sourdille et al. 2004). Most of them map to the distal bin 4BL1-0.86-1 but the most proximal markers barc199, gwm107, gwm368 and gwm513 that were positioned in C-4BL5-0.71 and C-4BS4-0.37, in agreement with the order observed here. In conclusion, either *Lr12* or a tightly linked gene on 4BL is a complementary gene required for the expression of the *LrSV2*-specific adult plant leaf rust resistance. Whether there is something unique about these chromosome regions in wheat that two linked complementary gene systems are found there is not known but the origin of each of the two members of the complementary system could be explained by duplication and divergence. Indeed, several rust resistance loci were reported as allelic series or tightly linked genes in wheat, including the leaf rust resistance genes *Lr2*, *Lr3*, *Lr14* and *Lr22* (McIntosh et al. 1995). Complex loci for disease resistance were also described in maize, barley, flax and lettuce (Michelmore and Meyers 1998; Leister 2004).

The closely linked codominant flanking markers for the *Lrc-SV2* gene will be used in the analysis of larger populations (> 1000) with the aim of developing a fine map where the genetic distances are representative of the physical ones. This, together with the availability of a non-gridded BAC library of the cultivar Sinvalocho (Marande et al. 2012), constitutes an ideal scenario to attempt its positional cloning.

Both *Lr27+31* and *LrSV2+c-SV2* genetic systems differ from the "integrated decoy" hypothesis described by Cesari et al. (2014) where resistance *loci* comprise two tightly linked genes, most of which are transcribed in opposite directions with a relatively short intergenic region. The description of the molecular mechanism underlying *LrSV2+c-SV2* complementary action must wait until the products of both genes were identified.

Resistance genes are frequently studied in a single contrasting cross; however, this work highlights the importance of analyzing different crosses in order to uncover genetic interactions between unlinked genes.

Author contribution statement Conceived and designed the experiments: MJD FS. Performed the experiments: MJD CP LF GF MS MAD GIRY ML MFP LI ARC FS. Analyzed the data: MJD CP LF GF MS FS. Wrote the paper: MJD FS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Assefa S, Fehrmann H (2000) Resistance to wheat leaf rust in *Aegilops tauschii* Coss. and inheritance of resistance in hexaploid wheat. Genet Resour Crop Evol 47(2):135–140

Bai D, Knott DR (1992) Suppression of rust resistance in bread wheat (*Triticum aestivum* L.) by D-genome chromosomes. Genome 35:276–282

Baker EP (1966) Isolation of complementary genes conditioning crown rust resistance in the oat variety Bond. Euphytica 15:313. https://doi.org/10.1007/BF00022174

Bansal UK, Hayden MJ, Venkata BP, Khanna R, Saini RG, Bariana HS (2008) Genetic mapping of adult plant leaf rust resistance genes *Lr48* and *Lr49* in common wheat. Theor Appl Genet 117(3):307–312. https://doi.org/10.1007/s00122-008-0775-6

Bryan GJ et al (1997) Isolation and characterization of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Buell CR, Somerville SC (1997) Use of Arabidopsis recombinant inbred lines reveals a monogenic and a novel digenic resistance mechanism to *Xanthomonas campestris pv campestris*. Plant J. 12(1):21–29. https://doi.org/10.1046/j.1365-313X.1997.12010021.x

Cesari S, Bernoux M, Moncuquet P, Kroj T, Dodds PN (2014) A novel conserved mechanism for plant NLR protein pairs: the "integrated decoy" hypothesis. Front Plant Sci 5:606. https://doi.org/10.3389/fpls.2014.00606

Choulet F, Wicker T, Rustenholz C, Paux E, Salse J, Leroy P, Schlub S, Le Paslier M-C, Magdelenat G, Gonthier C, Couloux A, Budak H, Breen J, Pumphrey M, Liu S, Kong X, Jia J, Gut M, Brunel D, Anderson JA, Gill BS, Appels R, Keller B, Feuillet C (2010) Megabase level sequencing reveals contrasted organization and evolution patterns of the wheat gene and transposable element spaces. Plant Cell 22:1686–1701

Choulet et al (2014) Structural and functional partitioning of bread wheat chromosome 3B. Science 345:1249721. https://doi.org/10.1126/science.1249721

de Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CARTHAGENE: multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21(8):1703–1704

Diéguez MJ, Pergolesi MF, Petignat C, Velásquez M, Ingala L, López M, Darino M, Choulet F, Paux E, Feuillet C, Sacco F (2011) Towards positional cloning of *LrSV2*, a specific adult plant wheat leaf rust resistance gene identified in the durable resistant variety Sinvalocho MA. In: 21st international Triticeae Mapping Initiative workshop, Mexico DF, Mexico

Diéguez MJ, Pergolesi MF, Velasquez SM, Ingala L, López M, Darino M, Paux E, Feuillet C, Sacco F (2014) Fine mapping of LrSV2, a race-specific adult plant leaf rust resistance gene on wheat chromosome 3BS. Theor Appl Genet 127:1133–1141. https://doi.org/10.1007/s00122-014-2285-z

Dracatos PM, Zhang P, Park RF et al (2016) Complementary resistance genes in wheat selection 'Avocet R' confer resistance to stripe rust. Theor Appl Genet 129:65. https://doi.org/10.1007/s00122-015-2609-7

Dyck PL (1991) Genetics of adult-plant leaf rust resistance in "Chinese Spring" and "Sturdy" wheat. Crop Sci 31:309–311

Flor HH (1956) Mutations in flax rust induced by ultraviolet radiation. Science 124:888–889

Fukuta Y, Tamura K, Hirae M, Oya S (1998) Genetic analysis of resistance to green rice leafhopper (*Nephotettix cincticeps* UHLER) in rice parental line. Norin-PL6, using RFLP marker. Breed Sci 48:243–249

Geffroy V, Sévignac M, Billant P, Dron M, Langin T (2008) Resistance to *Colletotrichum lindemuthianum* in *Phaseolus vulgaris*: a case study for mapping two independent genes. Theor Appl Genet 116:407–415. https://doi.org/10.1007/s00122-007-0678-y

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Hayden MJ, Stephenson P, Logojan AM, Khatkar D, Rogers C, Elsden J, Koebner MD, Snape JW, Sharpe PJ (2006) Development and genetic mapping of sequence-tagged microsatellite (STMs) in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 113:1271–1281

Herrera-Foessel SA, Singh RP, Huerta-Espino J, Yuen J, Djurle A (2005) New genes for leaf rust resistance in CIMMYT durum wheats. Plant Dis 89:809–814

Huerta-Espino J, Singh RP, Germán S et al (2011) Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179:143. https://doi.org/10.1007/s10681-011-0361-x

Ingala L, López M, Darino M, Pergolesi MF, Diéguez MJ, Sacco F (2012) Genetic analysis of leaf rust resistance genes and associated markers in the durable resistant wheat cultivar Sinvalocho MA. Theor Appl Genet 124(7):1305–1314. https://doi.org/10.1007/s00122-012-1788-8

Knott DR (2000) Inheritance of resistance to stem rust in Medea durum wheat and the role of suppressors. Crop Sci 40:98–102. https://doi.org/10.2135/cropsci2000.40198x

Kolmer JA, Dyck PL (1994) Gene expression in the *Triticum aestivum–Puccinia recondita* f. sp. *tritici* gene-for-gene system. Phytopathology 84(4):437–440

Leister D (2004) Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance genes. Trends Genet 20(3):116–122

Li G, Xu X, Bai G, Carver BF, Hunger R, Bonman JM, Kolmer J, Dong H (2016) Genome-wide association mapping reveals novel QTL for seedling leaf rust resistance in a worldwide collection of winter wheat. Plant Genome. https://doi.org/10.3835/plantgenome2016.06.0051

Liu J, Chang Z, Zhang X, Yang Z, Li X, Jia J, Zhan H, Guo H, Wang J (2013) Putative *Thinopyrum intermedium* -derived stripe rust resistance gene *Yr50* maps on wheat chromosome arm 4BL. Theor Appl Genet 126(1):265–274

Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525–529

Mago R, Tabe L, McIntosh RA, Pretorius Z, Kota R, Paux E, Wicker T, Breen J, Lagudah ES, Ellis JG, Spielmeyer W (2011) A multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), left rust (*Lr27*) and powdery mildew. Theor Appl Genet 123(4):615–623

Mains EB, Jackson HS (1926) Physiologic specialization in the leaf rust of wheat; *Puccinia triticina* Erikss. Phytopathology 16:89–120

Marande W, Vautrin S, Bellec A, Prat E, Beydon G, Pergolesi MF, Diéguez MJ, Sacco F, Berges H (2012) Non-gridded BAC libraries to isolate genomic regions involved in different cellular processes of interest in wheat varieties. In: 22nd international Triticeae Mapping Initiative workshop, Fargo, USA

Martins WS, Lucas DCS, De Souza Neves KF, Bertioli DJ (2009) WebSat—a web software for microsatellite marker development. Bioinformation 3(6):282–283

McIntosh RA, Welllings CR, Park RF (eds) (1995) Wheat rusts: an atlas of resistance genes. Kluwer Academic publishers, Dordrecht

Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8(11):1113–1130

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Naz AA, Kunert A, Lind V, Pillen K, Léon J (2008) AB-QTL analysis in winter wheat: II. Genetic analysis of seedling and field resistance against leaf rust in a wheat advanced backcross population. Theor Appl Genet 116(8):1095–1104

Nelson JC, Singh RP, Autrique JE, Sorrells ME (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37:1928–1935. https://doi.org/10.2135/cropsci1997.0011183X003700060043x

Park F, McIntosh RA (1994) Adult plant resistances to *Puccinia recondita f.* sp. *tritici* in wheat. N Z J Crop Hortic Sci 22(2):151–158. https://doi.org/10.1080/01140671.1994.9513819R

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Berges H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C (2008) A physical map of the 1 gigabase bread wheat chromosome 3B. Science 322:101–104

Röder M, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rosewarne GM, Li ZF, Singh RP, Yang EN, Herrera-Foessel SA, Huerta-Espino J (2015) Different QTLs are associated with leaf rust resistance in wheat between China and Mexico. Mol Breed 35:127

Sacco F, Suárez EY, Naranjo T (1998) Mapping of the leaf rust resistance gene *Lr3* on chromosome 6B of Sinvalocho MA wheat. Genome 41:686–690

Sacco F, Suárez EY, Saione HA, Tanos BE (2001) Dosage effect of *Lr3* gene in the *Triticum aestivum–Puccinia recondita* specific interaction. J Phytopathol 149:583–588

Saione HA, Favret EA, Franzone PM, Sacco F (1993) Host genetic analysis by using *Puccinia recondita tritici* induced mutants for increased virulence. J Phytopathol 138:225–232

Sears ER (1988) History of the Chinese Spring aneuploids. In: Miller TE, Koebner RMD (eds) Proceedings of 7th international wheat genetics symposium, Cambridge, England, pp 3–6

Singh S, Bowden RL (2011) Molecular mapping of adult-plant race-specific leaf rust resistance gene *Lr12* in bread wheat. Mol Breed 28:137–142. https://doi.org/10.1007/s11032-010-9467-4

Singh RP, McIntosh RA (1984) Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum* II. Cytogenetic studies. Can J Genet Cytol 26:736–742

Singh D, Park RF, McIntosh RA (1999) Genetic relationship between the adult plant resistance gene *Lr12* and the complementary gene *Lr31* for seedling resistance to leaf rust in common wheat. Plant Pathol 48:567–573

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus W, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Guyomarc'h H, Baron C, Gandon B, Chiquet V, Artiguenave F, Edwards K, Foisset N, Dufour P (2001) Improvement of the genetic maps of wheat using new microsatellite markers. Plant Anim Genome IX, Abstracts 167

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi LL, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Tan M-K, Sharp PJ, Howes Q (2006) Genetics of grain dormancy in a white wheat. Aust J Agric Res 57(11):1157–1165. https://doi.org/10.1071/AR06101

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered 93(1):77–78

William HM, Singh RP, Huerta-Espino J, Palacios G, Suenaga K (2006) Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. Genome 49(8):977–990

Wu J, Ding J, Du Y, Xu Y, Zhang X (2007) Genetic analysis and molecular mapping of two dominant complementary genes determining resistance to sugarcane mosaic virus in maize. Euphytica 156:355–364. https://doi.org/10.1007/s10681-007-9384-8