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ORIGINAL PAPER

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Fine genetic mapping fails to dissociate durable stem rust resistance gene *Sr2* from pseudo-black chaff in common wheat (*Triticum aestivum* L.)

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Abstract The broad-spectrum stem rust resistance gene *Sr2* has provided protection in wheat against *Puccinia graminis* Pers. f. sp. *tritici* for over 80 years. The *Sr2* gene and an associated dark pigmentation trait, pseudo-black chaff (PBC), have previously been localized to the short arm of chromosome 3B. In a first step towards the positional-based cloning of *Sr2*, we constructed a high-resolution map of this region. The wheat EST (wEST) deletion bin mapping project provided tightly linked cDNA markers. The rice genome sequence was used to infer the putative gene order for orthologous wheat genes and provide additional markers once the syntenic interval in rice was identified. We used this approach to map six wESTs that were collinear with the physical order of the corresponding genes on rice chromosome 1 suggesting there are no major re-arrangements between wheat and rice in this region. We were unable to separate by recombination the tightly linked morphological trait, PBC from the stem rust resistance gene suggesting that either a single gene or two tightly linked genes control both traits.

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Introduction

The *Sr2* gene in wheat has conferred durable, adult-plant resistance to stem rust caused by *Puccinia graminis* f. sp. *tritici*. *Sr2* plays an important role in wheat production throughout the world as reflected by its presence in many wheat cultivars (McIntosh 1988; McIntosh et al. 1995; Rajaram et al. 1988; Roelfs 1988). Resistance conferred by *Sr2* is characterized by a non-hypersensitive, partial resistance response with varying levels of disease under field conditions. The effect of *Sr2* can be masked by the presence of other effective rust resistance genes, which in addition to its recessive inheritance can complicate the selection process in breeding programs. In addition, pseudo-black chaff (PBC), a phenotypic trait that causes varying degrees of dark pigmentation on the stem internodes and glumes (Fig. 1a, b), has been associated with the presence of *Sr2* (Hare and McIntosh 1979). The PBC phenotype facilitates the selection of the breeding lines carrying *Sr2* (Hare and McIntosh 1979), but high levels of PBC expression (especially on glumes) are thought to reduce yield and farmer acceptance in some circumstances (Sheen et al. 1968).

In recent years, comparative mapping studies in plants revealed conservation of gene order across species and within large genetic linkage blocks (Devos 2005; Moore et al. 1995). This is particularly true for species of the grass family which include all the important cereal crops. The rice genome sequence together with a large set of publicly available wheat expressed sequence tag (wESTs) provides a rich resource for comparative mapping studies (Sorrells et al. 2003). There are currently over 620,000 wESTs in databases, of which over 8,200 unique wESTs have been mapped to defined chromosome deletion bins using deletion stocks of wheat (Qi et al. 2004; http://wheat.pw.usda.gov/NSF/progress_mapping.html).

The *Sr2* gene was first mapped by monosomic analysis to the short arm of chromosome 3B (Hare and

Fig. 1 Expression and absence of PBC in *Sr2* and non-*Sr2* wheat varieties, respectively. **a** *L* Three stem sections displaying PBC in lines containing *Sr2*, *R* three stem sections displaying lack of PBC and susceptibility to stem rust: **b** Absence (*L*) and presence (*R*) of PBC on the glumes of wheat plants

McIntosh 1979) followed by the identification of tightly linked microsatellite markers (Spielmeyer et al. 2003). One of the microsatellite markers (*Xgwm533*) was informative across a wide range of genotypes and is being applied in molecular breeding (Spielmeyer et al. 2003). Previously, extensive collinearity was reported between rice chromosome 1 and wheat chromosome 3 (Munkvold et al. 2004; Liu and Anderson 2003). In the present study, we used the rice genome sequence and data from the wEST deletion bin mapping project to identify additional wheat markers in a small target region flanking *Sr2*. This paper reports the fine mapping of *Sr2* and its association with PBC, to lay the groundwork for the positional cloning of *Sr2* and to elucidate the molecular basis of a broad-spectrum rust resistance gene.

Materials and methods

Plant material

To map the *Sr2* gene, we initially used a mapping population consisting of 53 F_3 lines (Mapping family 1) derived from a cross between cv. Chinese Spring (CS) and the chromosome substitution line CS (Hope 3B), a CS backcross line containing a 3B chromosome pair from cv. Hope, which carries *Sr2*. Subsequently, a high-resolution mapping family was generated by screening 1,344 F_2 seeds (equivalent to 2,688 gametes) from the same cross with two SSR markers (*Xgwm389* and *Xgwm533*) which amplify co-dominant markers from the parental lines and flank the *Sr2*. One hundred and seven F_2 genotypes that incorporated recombination events within the *Xgwm389–Xgwm533* interval constituted the high-resolution mapping family (Mapping family 2).

High throughput DNA extraction

DNA isolation of the F_2 individuals was carried out by using a half seed extraction method. The seed was cut into halves, i.e. an endosperm section and an embryo section. Individual endosperm sections were placed in wells of 96-well plates. The half seeds were crushed using a stainless steel ball bearing using a Retsch MM300 mixer mill (Retsch, Germany). After a short spin at 1,000 rpm, 300 µl of pre-warmed (65°C) extraction buffer (0.1 M Tris–HCl pH8.0, 0.05 M EDTA pH8.0 and 25% SDS) was added to each well. Samples were incubated at 65°C for 1 h and cooled at 4°C for 30 min. One hundred and fifty microliter of 6 M ammonium acetate was added and shaken vigorously before leaving it at 4°C for 30 min. The plate was centrifuged at 3,000 rpm for 30 min and the supernatant was transferred to a fresh deep well plate containing 180 µl of iso-propanol/well, mixed thoroughly and left at room temperature for 5 min to precipitate before centrifugation at 3,000 rpm for 30 min. The pellet was washed with 250 µl of 70% ethanol and air dried before being re-suspended overnight in 150 µl of water at 4°C. The plate was centrifuged at 3,000 rpm for 30 min and 50 µl of the supernatant was transferred to a fresh microtitre plate for storage at −20°C. Four microliter of this DNA was used to perform PCR. The embryo sections of the seeds of interest were subsequently germinated for the desired F_2 lines.

Sr2/PBC screening

Approximately 24 F_3 seeds from a subset of 30 F_2 plants (recombinants between markers *XBE401794* and *Xgwm533*) were tested for the presence or absence of PBC under glasshouse conditions. PBC was scored as a qualitative trait, which was based on the presence of blackening around the stem internodes. As the plant

Table 1 BlastN search results of wESTs in deletion bin 3BS-8 (0.78–1.00) with rice chromosome 1

Locus	EST	BlastN	Rice chromosome	BAC/PAC clones	Accession	Distance from telomere (Mb)	BlastX_description
WHE0445_G11_M21ZS	BE404656	3e-43	1	P0482C06	AP002845	0.5	Putative co-repressor protein (*Oryza sativa*)
WHE0331_F10_L19ZS	BE426676	7e-48	1	P0439B06	AP002882	0.6	Putative acetoacyl-CoA-thiolase (*O. sativa*)
WHE1651-1654_ C21ZS	BE591959	2e-29	1	OSJNBb0032H19	AP003219	0.7	Putative receptor serine/threonine kinase (*O. sativa*)
WHE2164_A01_A02ZS	BQ169082	3e-40	1	P0698G03	AP002747	1.0	Putative nodulin (*O. sativa*)
WHE1109_B12_C23ZS	BE443202	4e-61	1	P0698A04	AP002868	1.1	P0698A04.11 (*O. sativa*)
WHE2321_C04_E07ZS	BF484268	1e-27	1	P0684C01	AP002487	1.2	Similar to nucleoside triphosphatase, putative; protein id: At2g01275.1 [*Arabidopsis thaliana*]
WHE2102_F09_K18ZS	BQ487452	1e-08	1	P0460E08	AP003233	1.4	P0445D12.9 [*O. sativa* (japonica cultivar-group)]
WHE2158_A05_B10ZS	BF293537	7e-26	1	P0504H10	AP002526	1.7	Probable microsomal signal peptidase 22 kDa subunit (SPase 22 kDa
WHE2158_A05_B10ZS	CD453848	7e-26	1	P0037C04	AP002526	1.7	Probable microsomal signal peptidase 22 kDa subunit (SPase 22 kDa subunit) (SPC22)
WHE0007.B12R000701	BE438292	2e-22	1	P0408F06	AP002538	1.8	Expressed protein; protein id: At3g18370.1, supported by cDNA: gi_15983786 [*A. thaliana*]
WHE0427_A05_B09ZS	BQ162511	5e-30	1	OSJNBa0083M16	AP003214	1.9	OSJNBa0083M16.18 [*O. sativa* (japonica cultivar-group)]
WHE0804_C07_F14ZS	BQ166024	2e-12	1	OSJNBa0083M16	AP003214	1.9	Unnamed protein product [*Oryza sativa* (japonica cultivar-group)]
WHE1801_H02_O03ZS	BQ160312	6e-13	1	P0443D08	AP003250	2.0	P0443D08.11 [*O. sativa* (japonica cultivar-group)]
WHE1755-1758_ L17ZS	BE637769	5e-39	1	P0009G03	AP002522	2.8	Unknown [*A. thaliana*]
WHE1755-1758_K23ZS	BE637850	1e-110	1	P0452F10	AP003434	7.1	Putative receptor protein kinase PERK1 [*O. sativa* (japonica cultivar-group)]
WHE1071-1074_ C14ZS	BE489782	2e-77	1	P0509B06	AP002093	6.0	Unnamed protein product [*O. sativa* (japonica cultivar-group)]
WHE0492_E02_I04ZS	BM138503	6e-24	1	P0672C09	AP003546	22.2	Putative protein (fragment); protein id: At4g33000.1 [*A. thaliana*]
WHE2205_D02_G03ZS	BF291730	1e-25	1	P0431H09	AP003248	28.5	P0431H09.11 [*O. sativa* (japonica cultivar-group)]

The first 13 wESTs identified closely related rice genes within a contiguous sequence of approximately 2 Mb at the end of rice chromosome 1S

matures, a distinctive pigmentation of the glumes occurs that has also been associated with the presence of the stem rust resistance gene *Sr2*. Two replicates containing the homozygous F_4 seeds of the 30 F_2 individuals were rust tested with *Puccinia graminis* Pers. f. sp. *tritici* at three field sites. The homozygous F_4 lines of the 30 F_2 individuals were developed as follows: each of the 24 F_3 lines representing a given F_2 individual was screened with markers *Xgwm533* and a new PCR-based marker derived from a wheat BAC (Bac9R, unpublished data) that flanked *Sr2* on the distal side. Only those F_3 lines from each F_2 individual that were homozygous recombinant for the two flanking markers were selected. Seeds from these F_3 lines constituted the homozygous F_4 seed for a given F_2 individual and were planted in the field for assessment of stem rust response. Approximately 20 F_4 seeds from a given F_3 plant were sown in 60 cm field rows. 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* uredospore suspension directly into a stem internode at 1–2 m intervals. F_3 lines were scored as homozygous resistant or homozygous susceptible based on the level of infection in the parental lines. In addition to the *Sr2* screening, all individuals were also scored for PBC as described above.

RFLP and PCR analysis

DNA was extracted from leaf tissue using the procedure described by Lagudah et al. (1991). DNAs from the parental genotypes and the F_2 lines were digested with seven restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Nco*I, *Sac*I and *Xba*I). DNA hybridization analysis was conducted according to Seah et al. (1998). PCR was performed in 20 µl volumes using the protocol previously described by Spielmeyer et al. (2003).

Comparative analysis of wheat and rice sequences

The region containing the *Sr2* locus on wheat chromosome 3BS is syntenic with the distal region of chromosome 1S of rice (Munkvold et al. 2004). Seventy-nine wESTs previously mapped to the deletion bin 3BS 0.78–1.0 were downloaded from the Graingenes web site (http://wheat.pw.usda.gov/NSF/progress_mapping.html). BlastN searches (E value $\leq$ e$-$15 and the length of identity greater than 100 bp) were carried out to identify related rice sequences on BAC clones using the NCBI and Gramene databases. A table containing the set of matching wESTs was generated based on the best hit on rice chromosome 1 (Table 1).

Cloning and sequencing of wESTs

Primer pairs designed on the basis of published EST sequences were used to amplify from 'CS' genomic DNA and gel purified (Qiagen, Germany). The amplified product was subsequently cloned into the pGemT Easy Vector system (Promega, USA). Insert identity was confirmed by DNA sequencing and the cloned frag-

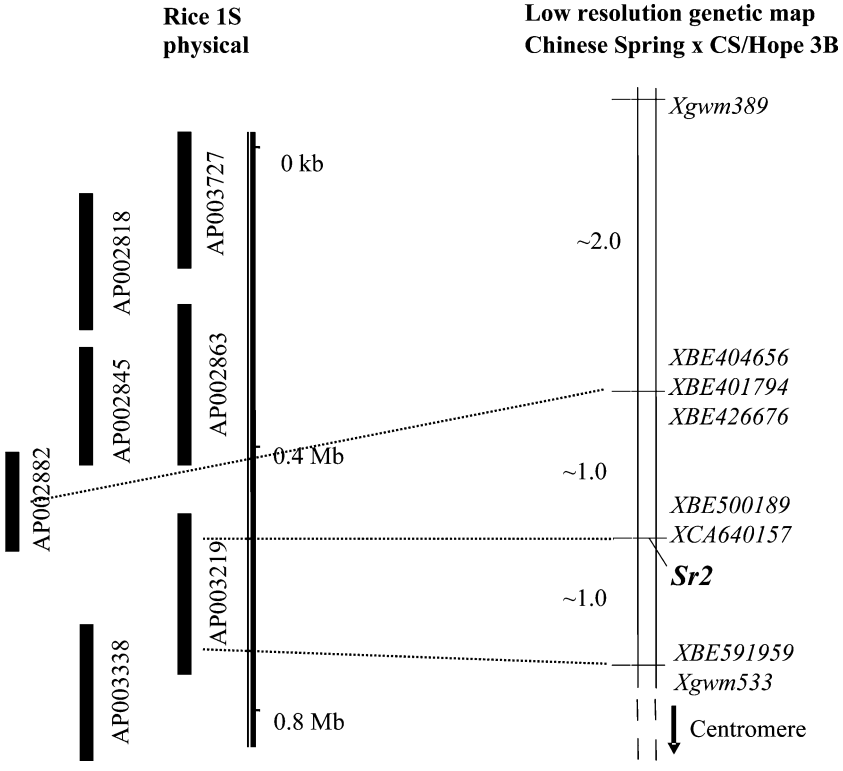

Fig. 2 Comparative maps of wheat chromosome 3BS and rice chromosome 1S. *Left* physical map of the sub-distal portion of rice chromosome 1S constructed from the sequence annotations of BAC/PAC clones with wEST markers connected to their rice orthologs by *dotted lines*. *Right* genetic mapping of wheat chromosome 3BS in the low-resolution mapping family. Distances shown on the *left of the chromosome* are in centiMorgans (cM)

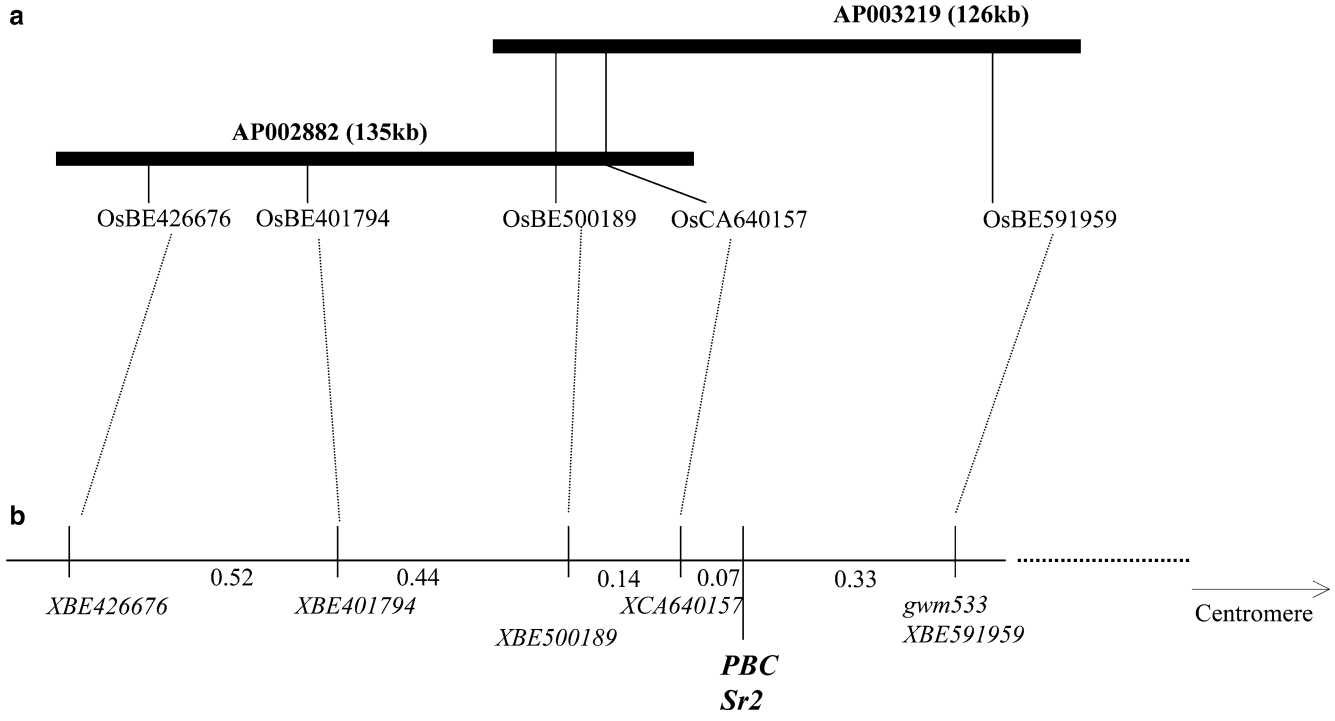

Fig. 3 Comparative mapping of rice chromosome 1 with the distal end of the short arm of chromosome 3B of wheat containing the Sr2 gene. **a** BAC physical contig (AP002882–AP003219) at the distal end of the rice chromosome 1S containing five sequences closely related to wESTs (BE426676, BE401794, BE500189, CA640157 and BE591959). **b** A high-resolution genetic map of the corresponding region of wheat chromosome 3BS

ments were amplified by PCR to generate probes for DNA hybridization analysis.

Results

Wheat EST markers tightly linked to the Sr2 resistance gene

Two SSR markers, *Xgwm389* and *Xgwm533*, had previously been shown to flank the *Sr2* gene and were positioned within the distal deletion bin 3BS 0.78–1.0 of the 3BS chromosome arm (Spielmeyer et al. 2003). As a first step in developing additional markers within the *Xgwm389–Xgwm533* interval, we used 79 wESTs previously positioned within the same deletion bin (Munkvold et al. 2004; http://wheat.pw.usda.gov/NSF/progress_mapping.html). Of the 79 wESTs, at least 18 EST sequences detected closely related genes on chromosome 1 of rice (Table 1) consistent with previous reports of synteny between rice chromosome 1 and wheat chromosome 3 (Ahn et al. 1993; Munkvold et al. 2004). The remaining 61 wESTs failed to detect closely related rice genes or the corresponding rice genes mapped to other chromosomes. Among the 18 wESTs, a subset of 13 wESTs shared significant sequence relatedness to rice genes within a contiguous 2 Mb of sequence at the end of rice chromosome 1S (Table 1). The remaining five sequences detected genes in other regions of rice chromosome 1 (Table 1). A putative order for wESTs was inferred from the position of the corresponding rice genes and used as the basis for selecting a subset of five wESTs for RFLP mapping. From a set of seven restriction enzymes that were used to screen for polymorphism between parental lines, three of the five wESTs (BE404656, BE426676 and BE591959) displayed polymorphism and were subsequently mapped to the *Xgwm389–Xgwm533* interval flanking the *Sr2* gene in a segregating family of 53 F_3 plants (Fig. 2). The corresponding rice sequences for the mapped wheat genes were located on two adjoining BAC clones AP002882 and AP003219 on rice chromosome 1S. The annotated rice genes within these BAC clones were used in turn to search public databases to identify an additional 16 closely related wESTs. Of these ESTs, a subset of 12 sequences were isolated and used as probes to identify bands polymorphic between parental lines. This resulted in the mapping of three additional wESTs (BE401794, BE500189 and CA640157) to the target interval (Fig. 2). Using the segregating family of 53 F_3 plants from mapping family 1, two wESTs (*XBE500189* and *XCA640157*) co-segregated with *Sr2* whereas markers *XBE401794*, XBE404656 and *XBE426676* were separated by one recombinant event from the resistance locus. In summary, we identified six wEST markers that were tightly linked to *Sr2* and collinear with the corresponding rice genes within the syntenic region on rice chromosome 1S.

High-resolution mapping of Sr2 and PBC

To resolve the marker order in the *Sr2* region, a high-resolution mapping family was developed. The SSR markers *Xgwm389* and *Xgwm533*, which flank a genetic interval of approximately 4 cM spanning the *Sr2* region, were used to screen 1,344 F_2 seeds from a cross between 'CS' and 'CS (Hope 3B)'. One hundred and seven F_2 lines incorporated recombination events within the *Xgwm389–Xgwm533* interval. To develop a high-resolution map, the six previously mapped RFLP markers (BE404656, BE426676, BE401794, BE500189, CA640157 and BE591959) were mapped in the 107 recombinants (Fig. 3). The marker order was consistent with previous results except that markers, *XBE500189* and *XCA640157*, which initially co-segregated in the smaller F_3 mapping family, were now separated by four recombination events.

Based on the position of *Sr2* on the low-resolution map and the genotypic scores of flanking markers in the high-resolution mapping family, a subset of 30 F_2 genotypes was phenotyped for *Sr2* and PBC. The selected 30 F_2 lines incorporated all the recombination events between markers *XBE401794* and *Xgwm533* which defined the predicted genetic interval for *Sr2*. PBC was scored by the presence or absence of dark pigmentation on the stems of F_3 progeny from each of the 30 F_2 recombinants. Based on the scores of approximately 24 F_3 plants, 12 of the 30 F_2 lines were classified as homozygous for the absence of PBC (CS type), four lines as homozygous for the presence of PBC (Hope 3B type) and 14 lines were heterozygous. Based on these results, PBC was placed within the predicted genetic interval, separated by two recombination events (0.07 cM) from the nearest marker *XCA640157* (Fig. 3).

To position *Sr2* on this map, F_4 families from selected F_3 plants that were homozygous for flanking markers were evaluated for disease reaction in replicated field trials. These selected F_3 plants were predicted to be homozygous at the resistance gene locus producing either homozygous susceptible or resistant F_4 families. Field data collected from replicated experiments at three sites indicated that all lines with PBC had similar levels of disease to the *Sr2* resistant parent CS (Hope 3B), whereas lines lacking PBC had a higher disease level similar to CS. Given the resolution of this mapping family, we conclude that *Sr2* is either very tightly linked to PBC (with a maximum genetic distance of 0.1 cM between loci ($P \geq 0.05$), Hanson 1959) or that the same gene controls both traits.

Is there an Sr2 ortholog in rice?

At least six wheat EST-derived markers in the *Sr2* region were collinear with the corresponding rice genes on three BAC clones (AP002845, AP002882 and AP003219) and approximately 225 kb of rice sequence. The nearest flanking markers to *Sr2* (*XCA640157* and *XBE591959*) covered a genetic distance of 0.4 cM in wheat whereas the corresponding rice genes were located within a single BAC clone AP003219, separated by approximately 92 kb of sequence. For this interval, a total of seven annotated rice genes were predicted to encode 'hypothetical proteins' (four genes), protein kinases (two genes) and an NBS-LRR disease resistance protein (one gene). Database searches did not yield any wESTs for rice genes that encoded 'hypothetical proteins', but for the two kinase-like genes wESTs with significant matches were identified (Table 2). For the NBS-LRR gene, wEST (CA611132) showed a moderate level of sequence relatedness (65% homology over 89 bp at the nucleotide level). We were unable to map the NBS-LRR sequence in our mapping population due to poor hybridization of the probe to genomic DNA. Further, the kinase-like genes could not be mapped in the mapping population due to multiple hybridization patterns and a lack of polymorphism between parental lines.

Discussion

The fine mapping of the stem rust resistance gene *Sr2* largely depended on the identification of recombinant lines and the development of markers within a small genetic interval that span the *Sr2* locus. In this study, the availability of public, co-dominant SSR markers (*Xgwm533* and *Xgwm389*) assisted in the rapid identification of recombinant lines that comprised the high-resolution mapping family. The wEST deletion bin mapping project provided tightly linked cDNA markers, whereas the rice genome sequence was used to infer the putative gene order for orthologous wheat genes and to provide additional markers once the syntenic interval in

Table 2 Rice genes present in the two BACs between corresponding markers CA640157 and BE591959

Candidate gene	Position on rice chr 1 (kb)	wEST	BlastN
Putative arm/beta-catenin-like repeat	645	*CA640157*	1E-27
NBS-LRR-like sequence	681	CA611132	9E-10
Protein kinase-like gene	715	CA692249	4E-50
Protein kinase-like gene	722	CA666207	2E-33
Receptor serine/threonine kinase PR5K-like gene	737	*BE591959*	3E-20

wESTs mapped in the current work are in *italics*. An equivalent wEST match is provided with their respective *E* value for each listed rice gene

rice was identified. We used this approach to map six wESTs in the *Sr2* region. Further, the mapped wESTs were collinear with the physical order of corresponding rice genes on chromosome 1, suggesting no major rearrangements between wheat and rice in this region. Similar results were obtained from the comparison of the wheat region carrying one of the major vernalization genes *Vrn-A1* on chromosome 5AL and the corresponding region on rice chromosome 3. Perfect collinearity was found between 12 wheat genes spanning approximately 800 kb of wheat sequence with the corresponding genes in rice (Yan et al. 2003).

However, the gene order between rice and other cereals is not always conserved. For instance, at the *Rph7* barley leaf rust resistance gene locus, micro-collinearity was significantly disturbed by an inversion and multiplication of numerous genes in barley compared to rice (Brunner et al. 2003). Similarly, comparative mapping of the wheat leaf rust resistance locus *Lr10* found limited and only partially conserved synteny between wheat and rice (Guyot et al 2004).

The nearest flanking wESTs to *Sr2* spanned a genetic distance of approximately 0.4 cM in wheat, while the corresponding genes on rice chromosome 1 were separated by only 92 kb. This relatively small interval in rice contained three annotated genes including one member of the NBS-LRR class. We were particularly interested in mapping the NBS-LRR sequence, although relatedness of the wEST sequence to the rice NBS-LRR sequence was relatively low. We were unable to map the wheat sequence in our population and therefore cannot exclude this gene as a candidate for *Sr2*. However, given the partial, non-hypersensitive resistance response of *Sr2*, it is unlikely that this kind of resistance is mediated by a NBS-LRR gene. It remains unclear whether the low level of sequence homology between the NBS-LRR genes reflects sequence divergence of syntenic genes or the comparison of non-syntenic members of the NBS-LRR gene family. It is possible that rice does not contain an *Sr2* ortholog despite collinearity of the other genes in this region. For instance, Brueggeman et al. (2002) reported the absence of an ortholog of barley stem rust resistance gene *Rpg1* in an otherwise collinear region in rice.

In this study we were unable to separate *Sr2* from PBC by recombination. In drawing parallels with other broad-spectrum, adult-plant resistance genes in wheat such as *Lr34/Yr18* conferring non-hypersensitive resistance to leaf rust and stripe rust, a delay in the infection process was associated with a build up of cell wall appositions that may impede haustorial formation thus resulting in partial resistance (Singh 1992). In another example, the recessive *mlo* gene in barley confers resistance to all races of the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*, by enhancing the resistance of epidermal cells to penetration by the fungus (Lyngkjær et al. 2000). It is possible that the expression of PBC in resistant plants may be involved in the formation of physical or chemical barriers that delay the infection process. Knowledge of the molecular basis of *Sr2* may enable us to manipulate the expression of PBC while maintaining adequate expression of rust resistance.

Current work is focused on developing a physical map of wheat for the *Sr2* region and the positional cloning of *Sr2*. Given that no durable, adult-plant rust resistance gene has been cloned from wheat, isolation of the *Sr2* gene may provide new insights into the molecular mechanisms governing host–pathogen recognition.

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References

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci 99:9328–9333

Brunner S, Keller B, Feuillet C (2003) A large rearrangement involving genes and low copy DNA interrupts the microcolinearity between rice and barley at the *Rph7* locus. Genetics 164:673–683

Devos KM (2005) Updating the 'Crop Circle'. Curr Opinion Plant Biol 8:155–162

Guyot R, Yahiaoui N, Feuillet C, Keller B (2004) In silico comparative analysis reveals a mosaic conservation of genes within a novel colinear region in wheat chromosome 1AS and rice chromosome 5S. Func Integr Genomics 4:47–58

Hanson WD (1959) Minimum family sizes for the planning of genetic experiments. Agron J 51:711–715

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistances in "Hope" and related cultivars to wheat rusts. Z Pflanzenzuctg 83:350–367

Lagudah ES, Appels R, McNeil D (1991) The *Nor-D3* locus of *Triticum tauschii*: natural variation and linkage to markers in chromosome 5. Genome 34:387–395

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 26:817–823

Lyngkjær MF, Newton AC, Atzema JL, Baker SJ (2000) The barley *mlo*-gene: an important powdery mildew resistance source. Agronomie 20:745–756

McIntosh RA (1988) The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, pp 1–9

McIntosh RA, Park RF, Wellings CR (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publications, East Melbourne

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses, line up and form a circle. Curr Biol 5:737–739

Munkvold JD, Greene RA, Bermundez-Kandianis CE, La Rota CM, Edwards H et al (2004) Group 3 chromosome bin maps of wheat and their relationship to rice chromosome. Genetics 168:639–650

Qi LL, Echalier Chao BS, Lazo GR et al. (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Rajaram S, Singh RP, Torres E (1988) Current CIMMYT approaches in breeding for rust resistance. In: Simmonds NW,

Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, pp 101–118

Roelfs AP (1988) Resistance to leaf and stem rusts in wheat. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, pp 10–22

Seah S, Sivasithamparam K, Karakousis A, Lagudah ES (1998) Cloning and characterization of a family of disease resistance gene analogs from wheat and barley. Theor Appl Genet 97:937–945

Sheen S-J, Ebeltoft DC, Smith GS (1968) Association and inheritance of "black chaff" and stem rust reactions in Conley wheat crosses. Crop Sci 8:477–480

Singh RP (1992) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835–838

Sorrells ME, La Rota M, Bermudez-Kandianis CE et al. (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Sci 43:333–336

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268