Theor Appl Genet (2004) 108:1401–1408
DOI 10.1007/s00122-003-1557-9

ORIGINAL PAPER

D. Bulgarelli · N. C. Collins · G. Tacconi · E. Dellaglio · R. Brueggeman · A. Kleinhofs · A. M. Stanca · G. Valè

High-resolution genetic mapping of the leaf stripe resistance gene *Rdg2a* in barley

Received: 16 July 2003 / Accepted: 24 November 2003 / Published online: 20 December 2003
© Springer-Verlag 2003

Abstract The dominant gene *Rdg2a* of barley conferring resistance to the hemi-biotrophic seed-borne pathogen *Pyrenophora graminea* is located in the distal region of chromosome arm 1 (7H)S. As the first step towards isolating the gene, a high-resolution genetic map of the region was constructed using an F_2 population of 1,400 plants (Thibaut*Rdg2a*×Mirco). The map included six classes of resistance gene analogues (RGAs) tightly associated with *Rdg2a. Rdg2a* was delimited to a genetic interval of 0.14 cM between the RGAs ssCH4 and MWG851. Additional markers were generated using the sequence from the corresponding region on rice chromosome 6, allowing delimitation of the *Rdg2a* syntenic interval in rice to a 115 kbp stretch of sequence. Analysis of the rice sequence failed to reveal any genes with similarity to characterized resistance genes. Therefore, either the rice-barley synteny is disrupted in this region, or *Rdg2a* encodes a novel type of resistance protein.

Communicated by P. Langridge

D. Bulgarelli · G. Tacconi · E. Dellaglio · A. M. Stanca · G. Valè (✉)
Istituto sperimentale per la Cerealicoltura,
Sezione di Fiorenzuola d'Arda,
Via S. Protaso 302, 29017 Fiorenzuola d'Arda, Italy
e-mail: gp.vale@iol.it
Fax: +39-523-983750

N. C. Collins
Sainsbury Laboratory, John Innes Centre,
Norwich, NR4 7UH, UK

R. Brueggeman · A. Kleinhofs
Department of Crop and Soil Sciences,
Washington State University,
Pullman, WA 99164-6420, USA

Present address:
N. C. Collins, Australian Centre for Plant Functional Genomics,
University of Adelaide, SA 5064 Glen Osmond, Australia

Introduction

Leaf stripe, which is caused by the seed-transmitted fungus *Pyrenophora graminea*, is a common disease in barley districts with a cold sowing season. In susceptible cultivars, the disease causes brown stripes on the leaves, stunted growth and severe yield reductions (Porta-Puglia et al. 1986). A serious outbreak of the disease is anticipated under certified organic farming conditions in countries of the European Union where seed treatments are not allowed. Resistance genes are therefore desirable for controlling leaf stripe disease. Both polygenic partial resistance (Skou and Haar 1987; Pecchioni et al. 1996; Arru et al. 2002, 2003a) and race-specific resistance genes have been identified. *Rdg1a*, conferring complete resistance to a subset of *P. graminea* isolates, has been mapped on the long arm of barley chromosome 2 (2H) (Giese et al. 1993; Thomsen et al. 1997). *Rdg2a*, conferring complete resistance to the most virulent Italian isolate of *P. graminea* (Dg2), has been mapped to barley chromosome 1 (7H) (Tacconi et al. 2001). Plants containing *Rdg2a* are almost immune to disease caused by avirulent isolates, exhibiting no brown stripes on the leaves. *Rdg2a*-containing breeding lines selected using Dg2 also appear to be resistant to the natural field populations of the pathogen, suggesting that *Rdg2a* may have a useful range of activity (Arru et al. 2003b); this view is also supported by the fact that when the near-isogenic lines Mirco and Mirco*Rdg2a* are inoculated with the isolates Dg12, Dg19 and Dg10, which are virulent towards Mirco, the resistant NIL shows complete resistance to Dg12 and Dg19 and a partial resistance to Dg10 (G. Valè, A. Haegi, unpublished results). Assessment of resistant barley accessions indicated that *Rdg2a* is widespread throughout the world in both six-rowed and two-rowed genotypes. Molecular markers have been developed to facilitate *Rdg2a* selection in breeding populations (Arru et al. 2003b).

Most of the cloned plant disease resistance genes encode proteins containing nucleotide binding site (NBS) and leucine rich repeat (LRR) domains (Hammond-

Kosack and Parker 2003). In addition to known resistance genes, plant genomes contain hundreds of other NBS-LRR encoding genes, called resistance gene analogues (RGAs) (The Arabidopsis Genome Initiative 2000; Goff et al. 2002). RGAs may represent undiscovered resistance genes of complete or partial effect. Alternatively, they may perform some function unrelated to resistance (Dangl and Jones 2001; Backes et al. 2003). Several classes of RGAs, which are unrelated to one another at the DNA level, have been mapped to the *Rdg2a* region. These include pic20 (Ayliffe et al. 2000; Rostoks et al. 2002), Hvb9 (Leister et al. 1999), ssCH4 (Seah et al. 1998), ABG1019 (Brueggeman et al. 2002), MWG851 (Madsen et al. 2003) and the highly similar BE216309 (Kleinhofs laboratory, http://barleygenomics.wsu.edu/) and S-9202 (Madsen et al. 2003). This region also contains the stem rust resistance gene *Rpg1* encoding a novel receptor-kinase-like resistance protein (Brueggemann et al. 2002). The region immediately surrounding *Rpg1* shows a high level of synteny with a region on the short arm of rice chromosome 6, although a *Rpg1* homologue is absent from this region (and the whole rice genome; Kilian et al. 1997; Han et al. 1999; Rostoks et al. 2002).

In the present work, we performed fine mapping of the *Rdg2a* resistance gene in a large segregating population. RGA markers mapping in the region, representing potential candidates for *Rdg2a*, were tested for co-segregation with *Rdg2a*. Furthermore, we developed additional markers closely linked to *Rdg2a* using the genomic rice sequence from the related region on rice chromosome 6. In this way, we defined a 115 kbp stretch of sequence as the syntenic interval for *Rdg2a* in rice. This work provides a useful advance towards isolating the *Rdg2a* gene.

Materials and methods

Mapping and resistance assays

A Thibaut (resistant)×Mirco (susceptible) cross was used to generate F_2 populations for *Rdg2a* mapping. The genotype for the *Rdg2a* locus was determined for all F_2 individuals (medium-resolution population) or for selected F_2 recombinants (high-resolution population), by performing leaf stripe resistance assays on F_3 progeny, essentially as described by Tacconi et al. (2001). Isolate Dg2, which is avirulent with respect to the resistance gene *Rdg2a*, was used to inoculate germinating seeds artificially. For each F_2 plant, two independent inoculation experiments were performed, each using 60 F_3 seeds (20 per pot). The development of the disease symptoms was followed in a growth chamber, under a regime of 16 h daylight (21°C) and 8 h darkness (16°C).

Map construction was performed using the MAPMAKER computer program (Lander et al. 1987), using the Kosambi mapping function to convert recombination frequencies into centimorgans (cM). Upon checking the marker order against the raw mapping data, a number of individuals were found to have allele combinations only possible as a result of (typically rare) double or triple recombination events within a small genetic interval. This prompted the re-scoring of the markers from re-isolated DNA of these individuals. In almost all cases, this showed the original markers to be mis-scored. However, re-scoring continued to show unexpected allele combinations for two individuals, which were consequently removed from the analysis.

PCR marker development

Procedures used for PCR marker development were essentially those used by Collins et al. (2001). Chromosomal positions of rice BAC and PAC clones sequences were obtained from the physical/genetic rice maps (http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/irgsp-status.cgi). EST and rice genomic sequences were downloaded and used in BLAST searches (Altschul et al. 1997) at NCBI (http://www.ncbi.nlm.nih.gov/). Barley or wheat ESTs with a DNA identity of 85% or greater with the rice sequences were considered as potential orthologues. In cases where a barley EST could not be identified, primers based on a wheat EST were used to amplify the corresponding gene from barley DNA. Genomic DNA to be used as PCR template was isolated from barley leaves according to Stein et al. (2001). PCRs from barley genomic DNA template were performed in volumes of 20 μl (or multiples thereof), containing 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 μM of each primer, 5% DMSO, 0.5 U *Taq* polymerase, and 90 ng template DNA. The PCR program comprised one cycle of 2 min at 94°C; 35 cycles of 40 s at 94°C, 50 s at 65°C, 1 min 20 s at 72°C, and a final extension 72°C for 7 min. Gel-purified PCR products were directly sequenced to verify identity (by comparison to the original sequence), and to identify polymorphisms between the parents of the mapping population. In cases where primers were based on EST sequences, the genomic amplification products often contained additional sequence due to introns. When sequence polymorphisms identified between the mapping parents were not within restriction sites, PCR markers were made by introducing a restriction site into the PCR product using the dCAPS strategy (Neff et al. 1998). For PCR markers, 20 μl of PCR mixture was digested overnight in a volume of 25 μl containing 1× restriction enzyme buffer, 1.5 U of restriction enzyme and 0.5 μg/ul of acetylated BSA. The resulting fragments were size fractionated in 2% agarose gels (or 3% agarose for the marker BV078160).

Conversion of the RAPD marker SCQ-9_{700} to CAPS marker ScOPQ9

Previously, the *Rdg2a*-linked RAPD fragment SCQ-9_{700} was cloned and sequenced, a specific pair of primers made, and the primers used to amplify and sequence the fragment from the mapping population parents, Thibaut and Mirco (Tacconi et al. 2001). Since no sequence polymorphism was identified within the fragment, we recovered additional sequence flanking the original fragment, by using an adapter-mediated PCR method (Siebert et al. 1995). Using a pair of nested primers facing outwards from the SCQ-9_{700} sequence, we obtained a 1.2 kbp amplification product, which was cloned, sequenced, and verified as a SCQ-9_{700}-flanking sequence by virtue of a 90 bp overlap with 100% identity to the original fragment. A pair of specific primers spanning the new sequence was made and used to recover and sequence the interval from the mapping parents. A sequence polymorphism was identified, enabling development of the CAPS marker ScOPQ9 (Table 1; Fig. 1).

RGA clones used to make RFLP probes

The ssCH4 RGA clone (AF052396) was kindly provided by Wolfgang Spielmeyer and Evans Lagudah (CSIRO Plant Industry). The ABG1019 RGA clone (AY339782), briefly described by Brueggeman et al. (2001), was isolated by PCR from barley Morex BAC DNA using degenerate primers based on the conserved P-loop (NBS1-F, GTC/GC/GGGG/AAAGACG/AACC/TCTTG) and GLPL (GLP-R1 (AGA/GGCA/GAGC/GGGA/GAGA/GCCA/TC/TCACATTT) motifs of the resistance protein NBS domain. PCR reactions of 20 μl contained 40 ng of

Table 1 CAPS and dCAPS (d) markers tested for linkage to the *Rdg2a* locus. For each CAPS marker the sequence of the primers and the restriction enzyme used to detect the polymorphisms are shown

Marker	Primer	Restriction enzyme
ScOPQ9	5′-GGCACAGTAACATTACCTTGATTGTG- 5′-CCAGTTGTCCAGCCTAAACAGGCATA-	*Mcr*I
MWG851	5′-TGACAAGCTACACGGCACCAACTACGCC- 5′-CTTGCAATTCTTCGTGCAATACCAGC-	*Acy*I
9202	5′-CCGGCTTGCAGAACTTGAGAGTCG- 5′-TGGAAAATGACTGTGAGACGGTGATCC	*Taq*I
BV078153	5′-GCCTGCAAAATCATGCAGCAGCTCA- 5′-CGCGCCAATCCGGGTTATCAAGAAG-	*Alu*I
BV078155	5′-CGTCAGAGTCGTCTGCATCAATTTCACG- 5′-CGTGAACGCAAGCACTTCTTCATCCAAG-	*Dde*I
BV078160(d)	5′-CCAAGCTAGAAGGAAACCTTCCACTCT- 5′-GCATGATGACGACACGTGGCTT-	*Van*91I
BV078159(d)	5′-CGGTGGACGCTCGGCACGATGGCGA- 5′-GTACTTCCTGGCGATCCTGCCCTGGCG-	*Hha*I
BV078158	5′-GCTGCAGCTGGAGAAGAGCCTAAGC- 5′-GACCAGCTCAGTCTCCACTTCCATG-	*Cla*I

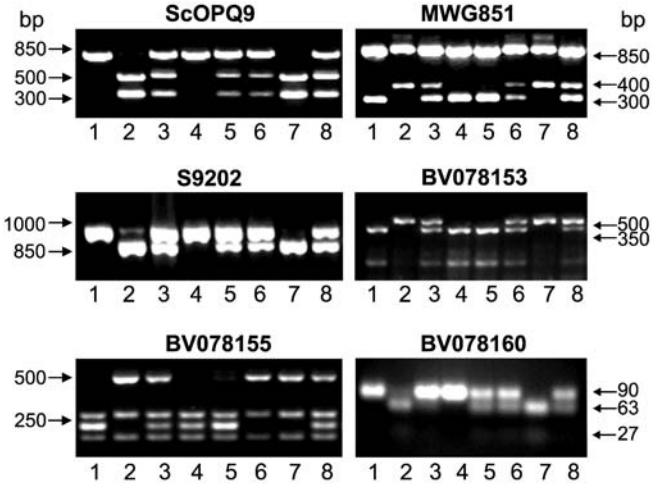

Fig. 1 CAPS markers linked to the *Rdg2a* locus. Ethidium bromide stained gels were used to resolve restriction enzyme digested fragments amplified from Thibaut (*1*), Mirco (*2*) and six F_2 individuals to demonstrate allele segregation (*3–8*). DNA fragment sizes (bp) are indicated

pooled BAC DNA, 0.2 mM dNTP mix, 20 pmol of each primer, 1 U of Red *Taq* DNA polymerase (Sigma), and 2 μl of 10× Red *Taq* reaction buffer. The PCR program consisted of 95°C for 4 min; 35 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min; a final extension step of 72°C for 7 min. A single band was visualized on a 1% agarose gel migrating at ~ 550 bp. ABG1019 showed significant similarity to NBS-LRR resistance proteins in a BLASTX search and was mapped to barley chromosome 7H bin 01 in the Steptoe×Morex mini mapper population (Brueggeman and Kleinhofs, unpublished data). The pic20 probe was derived from the most proximal member of the pic20 RGA gene cluster on 7H (pic20C; Rostoks et al. 2002) by amplifying a 724 bp section of the gene (nucleotides 63 to 786 of AF414173) from Thibaut genomic DNA. The fragment identity was confirmed by sequencing. Likewise, the b9 RGA probe was derived by amplifying a 548 bp segment (156 to 704 of AF032687) from Thibaut. The barley EST BE216309 was identified by searching the Triticeae EST database for homology to the rice Pib-like gene on rice PAC OSJNBa0007O20 (AP003487) (Rostoks, Johnson and Kleinhofs, unpublished, http://barleygenomics.wsu.edu/). The clone S9202 is nearly identical to BE216903 and was independently isolated by Madsen and co-workers (2003).

RFLP analysis

Genomic DNA for RFLP analysis was prepared using a modified CTAB method (Saghai-Maroof et al. 1984). Genomic DNA was subjected to restriction digestion as recommended by the restriction enzyme manufacturers (Promega), separated in 0.8% agarose gels, and blotted as recommended by the manufacturers of the membrane (Immobilon-Ny+, Millipore). αP^{32}-dCTP labelled DNA probes were made by the random hexamer priming method (Feinberg and Vogelstein 1983), using gel-purified PCR products or excised clone inserts as template. Filters were hybridized under standard conditions (Sambrook et al. 1989), washed to medium stringency, and subjected to autoradiography using the Biomax MS (Kodak) system.

Search for resistance gene-like sequences in the rice sequence

Rice genomic sequence from the *Rdg2a* syntenic region of chromosome 6 was used in BLASTX searches of the database to reveal any potential homologies to known resistance proteins. Initial searches were conducted using 4 kb sections. To maximize sensitivity, additional searches were performed after removing regions with strong homologies to multiple entries (e.g. retrovirus polyproteins). Readouts were scanned for resistance proteins of all classes, as defined in Table 2 of Hammond-Kosack and Parker (2003).

Results

Defining an *Rdg2a* interval

With the aim of identifying flanking markers suitable for screening for recombination around *Rdg2a*, we further developed the map of Tacconi et al. (2001), using a slightly expanded segregating population (Fig. 2A). The population, derived from a Thibaut (resistant)×Mirco (susceptible) cross, comprised the 128 previously described F_2 segregants, plus 90 additional F_2 plants. We used the PCR-based markers ABG704, MWG2018 and

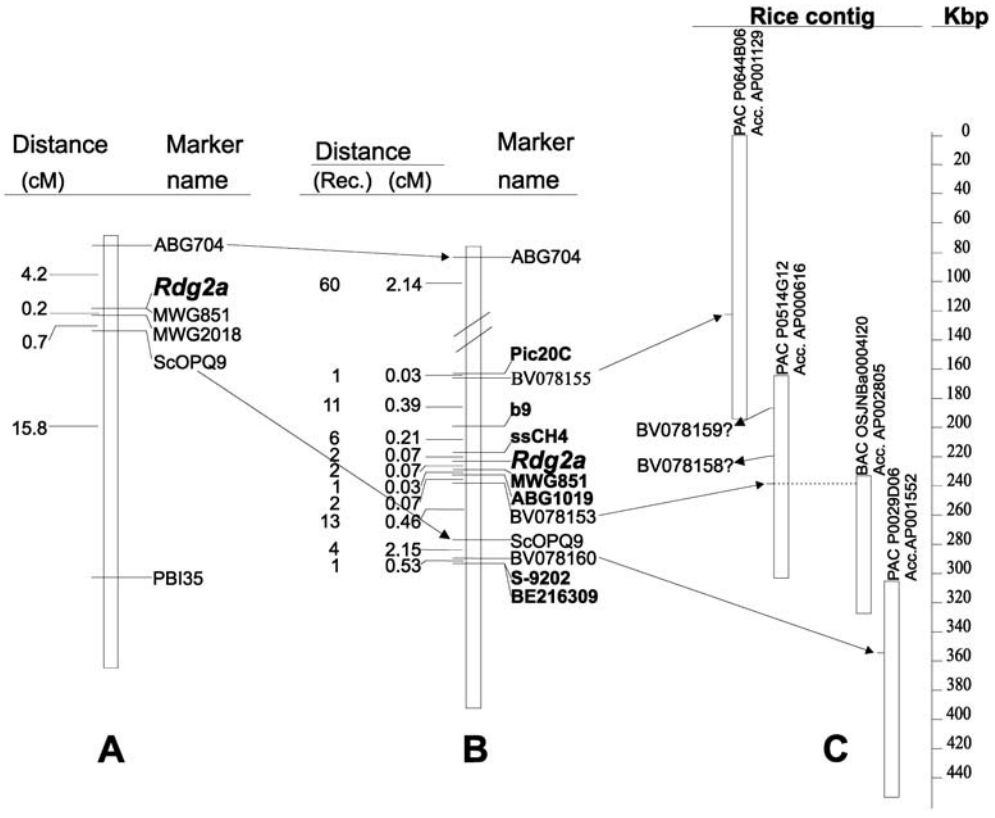

Fig. 2 A–C Consecutive stages of high-resolution mapping at the *Rdg2a* locus. The first map (**A**) was constructed using a population of 218 F_2 plants. Markers ABG704 and ScOPQ9 were used to screen a population of 1,400 F_2 plants, and the resulting 93 recombinants used to construct the second map (**B**). The two barley genetic maps are not drawn to scale. RGAs in **B** are shown in *bold*. The three CAPS markers derived from rice ESTs (BV078155, BV078153, BV078160) enabled alignment to a rice physical contig of 453,648 kb comprising three PAC clones and one BAC clone (**C**). *Arrows* between **B** and **C** indicate the position of homologues present in the rice sequence. *Question marks* indicate loci which mapped to positions unlinked to *Rdg2a* in barley. Genetic distances (cM) as well as number of recombinants observed for specific intervals on the high resolution map are shown. Physical scale in rice is indicated on the *right* in **C**. Note that BE216309/S9202 has a homologue approximately 400 kb proximal to D24970 in rice (not shown). Distances for markers proximal of ScOPQ9 were obtained using the 93 recombinants, although the observed recombination frequency was halved to correct for expected positive interference arising from selected recombination in the ABG704-ScOPQ9 interval

PBI35, previously developed from RFLP markers (Tacconi et al. 2001; Fig. 2A). In addition, the RAPD marker OPQ-9_{700} (Tacconi et al., 2001) was converted into a more robust, co-dominant, CAPS marker (ScOPQ9), and the RFLP probe MWG0851 mapping to the region (Graner et al. 1994) was used to derive a PCR-based marker (see Materials and methods; Table 1, Fig. 1). The molecular markers plus the resistance locus were scored in the population, yielding a map of the region (Fig. 2A). The order of the loci in this map differ from the one previously obtained (Tacconi et al. 2001), and we attribute this to the poor information originally provided by the dominant RAPD marker OPQ-9_{700}. Based on the map, we chose the ABG704 and ScOPQ9 PCR markers which flank *Rdg2a* by 4.2 cM and 0.9 cM, respectively (Fig. 2A), for conducting the recombinant screen.

Selection of recombinants for fine mapping

For fine mapping of the *Rdg2a* gene, 1,400 Thibaut×Mirco F_2 plants were screened for recombination between ABG704 and ScOPQ9. A total of 93 F_2 recombinant plants were identified. Only these recombinants were subjected to additional marker analysis and were *Rdg2a* genotyped by F3 progeny resistance assays.

Testing for co-segregation between *Rdg2a* and resistance gene analogues

A number of RGAs have been previously mapped on the short arm of barley chromosome 7HS. To investigate whether any of these could represent candidates for *Rdg2a*, we tested for co-segregation between these RGAs and *Rdg2a* in the panel of 93 recombinants. MWG851 had previously been shown to be an RGA (Madsen et al. 2003). The RGA S-9202 (Madsen et al. 2003) was developed into a PCR marker (Table 1, Fig. 1). All other

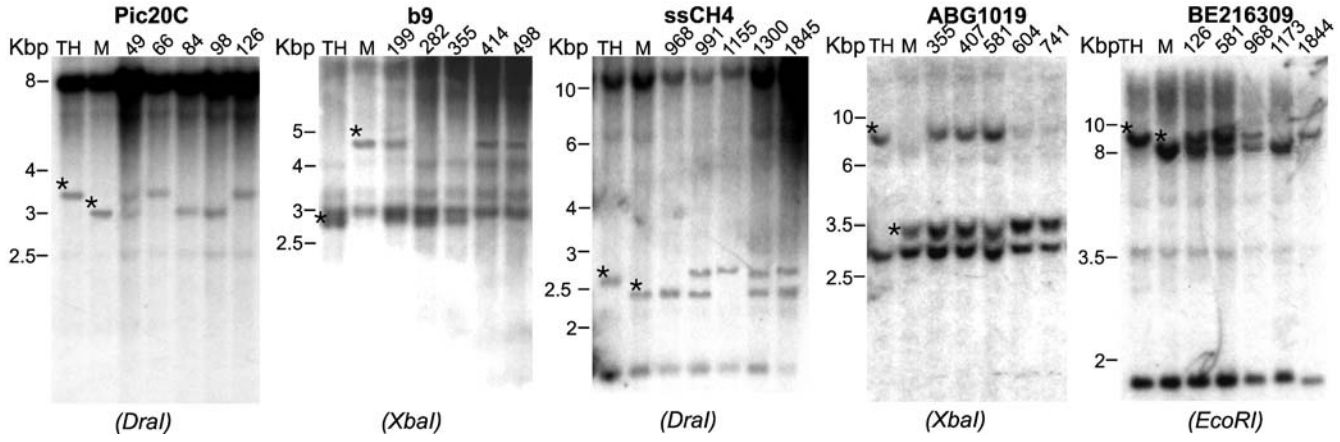

Fig. 3 RFLP analysis of RGA clones linked to the *Rdg2a* locus. Restricted genomic DNA of Thibaut (*TH*), Mirco (*M*) and of five F_2 individuals (to demonstrate allele segregation) was hybridized with the RGAs probes indicated *above*. Polymorphic hybridizing fragments are indicated by an *asterisk* in Thibaut and Mirco. Size markers (kb) are shown

RGAs were mapped using RFLPs (Fig. 3). RGA RFLP probes comprised b9 (Leister et al. 1999), ssCH4 (also called Rlch4; Seah et al. 1998), ABG1019 (Brueggeman et al. 2001), pic20C (as defined by Rostoks et al. 2002), and the barley EST BE216309 which has high homology to S-9202. The relative positions determined for the RGAs (Fig. 2B) were in complete agreement with locus positions obtained previously using other mapping populations (Ayliffe et al. 2000; Brueggeman et al. 2001; Madsen et al. 2003). None of the RGA loci co-segregated with *Rdg2a.* However, the ssCH4 and MWG851 loci tightly flanked the *Rdg2a* locus and were located respectively only 2 recombinants (0.07 cM) distally and 2 recombinants proximally (Fig. 2B). The cloned resistance gene *Rpg1* is positioned between a pic20 gene cluster and ABG704 (Brueggeman et al. 2002; Rostoks et al. 2002), distal of *Rdg2a* (Fig. 2B). All other members of the *Rpg1* gene family present in cv. Morex have also been cloned and mapped (Brueggeman and Kleinhofs, unpublished). These data make it unlikely that *Rdg2a* belongs to the *Rpg1* gene family.

Barley-rice synteny in the *Rdg2a* region

The distal part of the short arm of rice chromosome 6 is the region of the rice genome most related to the distal part of barley chromosome arm 7HS (Kilian et al. 1997). To facilitate exploitation of the available rice genomic sequence for marker generation and candidate gene identification in our program to isolate *Rdg2a*, we set out to more precisely define the conservation between these two chromosome segments in the vicinity of *Rdg2a*. At the time of this study, the sequence of the short arm of rice chromosome 6 generated by the public rice genome sequencing consortium (Delseny 2003) was essentially complete, enabling detection of sequences in this region by BLASTN searching. The ScOPQ9 sequence did not identify any related sequence in BLASTN searches of the available rice genome sequence. Except for BE216309/S9202 (see below), none of the RGA classes from 7HS identified close homologues on rice chromosome 6. Instead, close homologues of these RGAs were identified on other rice chromosomes (data not shown). The poor conservation shown between the RGA locations in barley and rice prompted us to compare locations of other gene types.

Table 2 Accession numbers of the Thibaut STSs and of the rice ESTs anchored to the chromosome 6 genomic contig. In the third column, the accession numbers of barley or wheat ESTs used to design oligonucleotide primers are shown

STS accession no.	Rice EST	Barley (B) or wheat (W) ESTs
BV078155	AU032852	AV920572 (B)
BV078153	AU056781	BJ462078 (B)
BV078160	AF486280 (D24970)	BF267183 (B)
BV078159	AU78644	BE416659 (W)
BV078158	U90344	AJ467364 (B)
BV078156	AC007017	BE214582 (B)
BV078157	U20184	BE193581 (B)
BV078154	X85187	CA001705 (B)

Rice sequences from the interval spanned by rice genomic PAC clones P0644B06, P0514G12 and P0029D06 (Fig. 2C) were used to identify ESTs from barley or wheat, representing potential orthologues of the rice genes. Oligonucleotide primers based on the barley or wheat EST sequences were used to PCR amplify fragments of the corresponding barley genes from the two parents of the *Rdg2a* mapping population. The products were directly sequenced to identify polymorphisms between the mapping parents suitable for PCR marker generation. For 19 rice genes, homologous barley or wheat ESTs were identified, and primers designed for amplification of the barley genes. Fragments from 8 of the barley genes were successfully PCR-amplified from Thibaut and Mirco genomic DNA, leading to the identification of barley STSs (Table 2). Sequence polymor-

phisms were identified for five of the genes, and a PCR marker was developed for each of them (Table 1). Markers corresponding to the STSs BV078159 and BV078158 were not linked to the *Rdg2a* locus, nor were they linked to each other (Fig. 2C). This indicates either an exception to the synteny, or failure to identify the true orthologue of the rice genes. The other three markers, corresponding to the STSs BV078153, BV078155 and BV078160 (Fig. 1), mapped to the *Rdg2a* region in an order colinear with their counterparts in rice (Fig. 2B, C). The fragments for which no sequence polymorphism was identified (STSs BV078156, BV078157 and BV078154) were used to probe blots of DNA of Thibaut and Mirco digested with eight different restriction enzymes, but no RFLPs were identified.

RGA BE216309/S9202 has three close homologues at dispersed locations on rice chromosome 6 (data not shown). The most distal BE216309/S9202 homologue on the short arm (protein BAA90798.1) is located on PAC P0425F02, approximately 400 kb proximal to D24970. However, given the dispersed nature of the BE216309/S9202 family on rice chromosome 6, and the lack of rice-barley conservation in the position of other RGAs in this region, it is uncertain whether the position of the rice gene on PAC P0425F02 corresponds directly to the mapped BE216309/S9202 locus on barley 7HS.

The markers BV078155 and BV078153 define the smallest *Rdg2a* syntenic interval in rice (Fig. 2C). The current annotations for this 115 kbp sequence interval (entries AP001129 and AP000616) did not include any predicted protein with assigned similarity to a known resistance protein. Additionally, our own analysis of this DNA sequence interval (see Materials and methods) did not reveal any potential to encode any resistance protein-like sequence. This indicates that either an orthologue of *Rdg2a* does not occur in this rice interval due to a breakdown in synteny, or that *Rdg2a* encodes a type of resistance protein not yet described.

Discussion

In this work, the *Rdg2a P. graminea* resistance gene on barley chromosome arm 7HS was subjected to high-resolution mapping using an F_2 population representing 2,800 gametes. *Rdg2a* was located to a marker interval defined by ssCH4, located 0.07 cM (2 recombinants) distally, and MWG851, located 0.07 cM proximally. The recombinants and markers identified here will be invaluable resources for the isolation of this gene. Furthermore, the *Rdg2a* syntenic interval established in rice, comprising 115 kbp of completely sequenced genomic DNA, may provide a source of further markers closely linked to *Rdg2a.*

Ideally, the distance between the *Rdg2a* locus and one of the closest flanking markers ssCH4 and MWG851 would be less than about 100 kb, a distance that could be bridged using an average sized BAC clone. Cloning of a gene from greater distances would require a chromosome walk, an endeavour that is likely to be fraught with difficulty in the large genome species barley. Based on the average ratio of physical to genetic distance in the barley genome of 4 Mb/cM, the distances of ssCH4 and MWG851 from *Rdg2a* would be 280 kbp each. However, the ratio of physical to genetic distance varies substantially between different regions of the genome, and is also influenced by genetic background. Most recombination in Triticeae chromosomes occurs close to the telomeres (Künzel et al. 2000), suggesting that the markers may in fact be closer to *Rdg2a*. However, on a finer scale, adjacent chromosome regions may also recombine at vastly different frequencies. In the region spanning the pic20 and *Rpg1* loci (Fig. 2C), the ratio of physical to genetic distance for six marker intervals was found to vary from 350 kb/cM to greater than 25 Mb/cM (Brueggeman et al. 2002; Rostoks et al. 2002). Clearly, the physical distances of ssCH4 and MWG851 from *Rdg2a* can only be determined empirically. The actual distances may be revealed by our current efforts to use these flanking probes to recover BAC clones containing the *Rdg2a* locus.

We used a single high-resolution mapping population to map six classes of RGAs (Fig. 2B) previously reported to map to the distal region of chromosome arm 7HS (Seah et al. 1998; Leister et al. 1999; Ayliffe et al. 2000; Brueggeman et al. 2001; Madsen et al. 2003). Although these RGAs all have predicted products resembling NBS-LRR resistance proteins, they are unrelated at the DNA level. The pic20 gene family has a cluster of five members on 7HS (Rostoks et al. 2002). The b9 locus on 7HS also represents a cluster of RGA gene family members, as determined using a probe made using the b9 maize orthologue pic15 (Collins et al. 2001). BE216309 hybridized to two bands in cv. Morex that co-segregated in a small mapping population, suggesting that there may be two family members. Therefore, this region of 7HS contains a high concentration of RGAs of diverse sequence, in addition to the *Rpg1* resistance gene encoding a receptor-kinase like protein, and the presently uncloned *Rdg2a* resistance gene. Other clusters containing diverse RGAs and resistance genes have been described in plant genomes (Leister et al. 1998; Wei et al. 1999; Huang and Gill 2001; Hulbert et al. 2001; Ling et al. 2003; Madsen et al. 2003). These studies demonstrate that RGAs can provide a useful source of markers closely linked to resistance genes. High-resolution resistance-segregating mapping populations such as the one in our study are essential for eliminating closely linked RGAs as candidates for functional resistance genes.

Curiously, only one of the six classes of RGAs mapped in the vicinity of *Rdg2a* in barley (BE216309/S9202) had a homologue present in the corresponding region of rice chromosome 6. A homologue of the *Rpg1* kinase-encoding resistance gene is also absent from the region and indeed, the whole rice genome (Han et al. 1999; Brueggeman et al. 2002). These results partly support the assertion that RGAs evolve relatively rapidly and that they often provide exceptions to rice-barley synteny

(Leister et al. 1998). However, two genes in the rice syntenic interval that are not RGAs also map to non-syntenic locations in barley (Fig. 2C). The absence of any sequences resembling resistance genes in the *Rdg2a* syntenic interval either means that an *Rdg2a* orthologue is absent from the interval, or that *Rdg2a* encodes an unknown type of resistance protein. In any case, other genes in the interval may enable the generation of further genetic markers in barley that are closer to *Rdg2a*.

Acknowledgements The authors thank Nadia Faccini and Donata Pagani for technical assistance. We would like to thank Wolfgang Spielmeyer and Evans Lagudah for the ssCH4 probe and Lene Madsen and Jens Stougaard (University of Aarhus) for the S-9202 DNA sequence. This work was supported by the Italian MiPAF, Project "Protezione delle piante mediante l'uso di marcatori molecolari (PROMAR)" and scheda 7/90 MiPAF. N.C. was funded by Syngenta.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipmann DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Arru L, Nicks RE, Lindhout P, Valè G, Francia E, Pecchioni N (2002) Genomic regions determining resistance to leaf stripe (*Pyrenophora graminea*) in barley. Genome 45:460–466

Arru L, Francia E, Pecchioni N (2003a) Isolate-specific QTLs of resistance to leaf stripe (*Pyrenophora graminea*) in the Steptoe×Morex spring barley cross. Theor Appl Genet 106:668–675

Arru L, Faccini N, Govoni C, Cattivelli L, Pecchioni N, Delogu G, Stanca AM, Valè G (2003b) The PCR-based marker *MWG2018* linked to the *Rdg2a* leaf stripe resistance gene is a useful tool for assessing barley resistance in breeding programs. Crop Sci 43:1036–1042

Ayliffe MA, Collins NC, Ellis JG, Pryor A (2000) The maize *rp1*rust resistance gene identifies homologues in barley that have been subjected to diversifying selection. Theor Appl Genet 100:1144–1154

Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localisation of genes for resistance against *Blumeria graminis* f. sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor Appl Genet 106:353–362

Brueggeman R, Druka A, Kudrna D, Kleinhofs A (2001) Efficient cloning of resistance gene analogs from barley. Barley Genet Newsl 31:24–27

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 USA 99:9328–9333

Collins N, Park R, Spielmeyer W, Ellis J, Pryor AJ (2001) Resistance gene analogs in barley and their relationship to rust resistance genes. Genome 44:375–381

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Delseny M (2003) Towards an accurate sequence of the rice genome. Curr Opin Plant Biol 6:101–105

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Giese H, Holm-Jensen AG, Jensen HP, Jensen J (1993) Localization of the *Laevigatum* powdery mildew resistance gene to barley chromosome 2 by the use of RFLP markers. Theor Appl Genet 85:897–900

Goff SA, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun W-L, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *Japonica*). Science 296:92–100

Graner A, Bauer E, Kellermann A, Kirchner S, Muraya JK, Jahoor A, Wenzel G (1994) Progress of RFLP-map construction in winter barley. Barley Genet Newsl 23:53–59

Hammond-Kosack KE, Parker JE (2003) Deciphering plant-pathogen communication: fresh perspecitives for molecular resistance breeding. Curr Opin Biotech 14:177–193

Han F, Kilian A, Chen JP, Kudrna D, Steffenson B, Yamamoto K, Matsumoto T, Sasaki T, Kleinhofs A (1999) Sequence analysis of a rice BAC clone covering the syntenous barley *Rpg1* region. Genome 42:1071–1076

Huang L, Gill BS (2001) An *RGA*-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. Theor Appl Genet 103:1007–1013

Hulbert SC, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utlization. Annu Rev Phytopathol 39:285–312

Kilian A, Kudrna DA, Kleinhofs A, Yano M, Kurata N, Steffenson B, Sasaki T (1995) Rice-barley synteny and its application to saturation mapping of the barley *Rpg1* region. Nucleic Acids Res 23:2729–2733

Kilian A, Chen J, Han F, Steffenson B, Kleinhofs A (1997) Towards map-based cloning of the barley stem rust resistance genes *Rpg1*and *Rpg4*using rice as an intergenomic cloning vehicle. Plant Mol Biol 35:187–195

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154:397–412

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze-Lefert P (1998) Rapid re-organization of resistance gene homologs in cereal genomes. Proc Natl Acad Sci USA 95:370–375

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Graner A, Schulze-Lefert P (1999) RFLP and physical mapping of resistance gene homologues in rice (*O. sativa*) and barley (*H. vulgare*). Theor Appl Genet 98:509–520

Ling HQ, Zhu Y, Keller B (2003) High resolution mapping of the leaf rust disease resistance gene *Lr1* in wheat and characterization of BAC clones from the *Lr1* locus. Theor Appl Genet 106:875–882

Madsen LH, Collins NC, Rakwalska M, Backes G, Sandal N, Krusell L, Jensen J, Waterman EH, Jahoor A, Ayliffe M, Pryor AJ, Langridge P, Schulze-Lefert P, Stougaard J (2003) Barley disease resistance gene analogs of the NBS-LRR class: identification and mapping. Mol Gen Genomics 269:150–161

Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. Plant J 14:387–392

Pecchioni N, Faccioli P, Toubia-Rahme H, Valè G, Terzi V (1996) Quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) is dominated by one major locus. Theor Appl Genet 93:97–101

Porta-Puglia A, Delogu G, Vannacci G (1986) *Pyrenophora graminea* on winter barley seed: effect on disease incidence and yield losses. J Phytopathol 117:26–33

Rostoks N, Zale JM, Soule J, Brueggeman R, Druka A, Kudrna D, Steffenson B, Kleinhofs A (2002) A barley gene family homologous to the maize rust resistance gene *Rp1-D*. Theor Appl Genet 104:1298–1306

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

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

Siebert PD, Chenchik A, Kellogg DE, Lukyanov A, Lukyanov SA (1995) An improved method for walking in uncloned genomic DNA. Nucleic Acids Res 23:1087–1088

Skou JP, Haahr V (1987) Screening for and inheritance of resistance to barley leaf stripe (*Drechslera graminea*). Risø report 554, Risø National Laboratory, Roskilde, Denmark

Stein N, Herren G, Keller B (2001) A new DNA extraction method for high-throughput marker analysis in a large-genome species such as *Triticum aestivum*. Plant Breed 120:354–356

Tacconi G., Cattivelli L, Faccini N, Pecchioni N, Stanca AM, Valè G (2001) Identification and mapping of a new leaf stripe resistance gene in barley (*Hordeum vulgare* L.). Theor Appl Genet 102:1286–1291

The Arabidopsis Genome Initiative (2000) Analysis of the genome of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Thomsen SB, Jensen HP, Jensen J, Skou JP, Jørgensen JH (1997) Localization of a resistance gene and identification of sources of resistance to barley leaf stripe. Plant Breed 116:455–459

Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (Powdery Mildew) resistance cluster is associated with three *NBS-LRR* gene families and suppressed recombination within a 240 kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–1948