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ORIGINAL PAPER

Identification and mapping of the leaf stripe resistance gene *Rdg1a* in *Hordeum spontaneum*

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Abstract Leaf stripe of barley, caused by *Pyrenophora graminea*, is an important seed-borne disease in organically grown as well as in conventionally grown Nordic and Mediterranean barley districts. Two barley segregating populations represented by 103 recombinant inbred lines (RILs) of the cross L94 (susceptible) × Vada (resistant) and 194 RILs of the cross Arta (susceptible) × *Hordeum spontaneum* 41-1 (resistant) were analysed with two highly virulent leaf stripe isolates, Dg2 and Dg5, to identify loci for *P. graminea* resistance. A major gene with its positive allele contributed by Vada and *H. spontaneum* 41-1 was detected in both populations and for both pathogen isolates on chromosome 2HL explaining 44.1 and 91.8% R^2, respectively for Dg2 and Dg5 in L94 × Vada and 97.8 and 96.1% R^2, respectively for Dg2 and Dg5 in Arta × *H. spontaneum* 41-1. Common markers in the gene region of the two populations enabled map comparison and highlighted an overlapping for the region of the resistance locus. Since the map position of the resistance locus identified in this report is the same as that for the leaf stripe resistance gene *Rdg1a*, mapped earlier in Alf and derived from the 'botanical' barley line *H. laevigatum*, we propose that leaf stripe resistance in Vada and *H. spontaneum* 41-1 is governed by the same gene, namely by *Rdg1a*, and that *Rdg1a* resistance could be traced back to *H. spontaneum*, the progenitor of cultivated barley. PCR-based molecular markers that can be used for marker-assisted selection (MAS) of *Rdg1a* were identified. An *Rdg1a* syntenic interval with the rice chromosome arm 4L was identified on the basis of rice orthologs of EST-based barley markers. Analysis of the rice genes annotated into the syntenic interval did not reveal sequences strictly belonging to the major class (nucleotide-binding site plus leucine-rich repeat) of the resistance genes. Nonetheless, four genes coding for domains that are present in the major disease-resistance genes, namely receptor-like protein kinase and ATP/GTP-binding proteins, were identified together with a homolog of the barley powdery mildew resistance gene *mlo*. Three (out of five) homologs of these genes were mapped in the *Rdg1a* region in barley and the *mlo* homolog map position was tightly associated with the LOD score peak in both populations.

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Introduction

Leaf stripe is a widespread seed-borne disease of barley caused by the fungal pathogen *Pyrenophora graminea* (Ito and Kuribayashi). The fungal mycelia survive in seeds between the parenchymatic cells of the pericarp, the hull and the seed coat, but not in the embryo. During seed germination, fungal hyphae grow intercellularly from the coleorhiza up all sides to the roots and scutellar node where they start the infection of the shoot (Haegi et al. 2008). Infection spreads into the young leaves, where it causes

longitudinal dark brown stripes between the leaf veins. Spores produced on the infected leaves of susceptible plants during flowering spread to infect nearby heads. No secondary cycles are present in this disease. The disease is particularly acute in Nordic countries (spring sowing) and in the Mediterranean's winter barley districts, where soil temperatures below 12°C during seed germination promote the infection of the rootlet. The typical symptoms, spike sterility and chlorotic stripes on leaves, which gradually extend to the full length of the leaf and finally become necrotic, lead to severe yield reductions when seed infection is high, especially in organic farming systems (Delogu et al. 1995; Mueller et al. 2003). A variation in pathogenicity among different fungal isolates on the same genetic material has been reported, and the selective pressure by the pathogen strains on the host population may explain the existence of different resistance genes (Boulif and Wilcoxson 1988; Gatti et al. 1992).

In a search for sources of resistance, 1,029 varieties and lines from various Nordic barley collections were tested for their reaction to leaf stripe (Skou and Haahr 1987). Using pedigree analysis, the resistance of about 50–100 barley cultivars was traced to a Gull × *Hordeum laevigatum* hybrid that had been crossed to introduce the *MlLa* resistance gene for powdery mildew carried by *H. laevigatum* into Gull. It was proposed that this leaf stripe resistance gene be referred to as the Vada resistance gene, because it was unconsciously introduced into many spring cultivars, together with *MlLa*, through Vada (Skou and Haahr 1987; Skou et al. 1994). The Vada resistance locus was mapped to the long arm of the chromosome 2H using a doubled haploid population derived from the cross Alf × Vogelsanger Gold (Thomsen et al. 1997) and designated *Rdg1a*. A QTL analysis for resistance against the *P. graminea* isolate Dg2 conducted on RILs derived from the cross L94 × Vada detected one major gene on chromosome 2H at the same location as *Rdg1a* (Arru et al. 2002). Based on common markers it was previously suggested that the resistance of Vada to *P. graminea* is conferred by the *Rdg1a* gene (Arru et al. 2002), which governs resistance to leaf stripe in the barley cultivar Alf (Thomsen et al. 1997). The hypothesis that Vada and Alf carry the same leaf stripe resistance gene is further supported by the observation that the two cultivars share *H. laevigatum* as an ancestor and as the donor of the powdery mildew race-specific resistance gene *MlLa*, which is in linkage with *Rdg1a* on barley chromosome 2HL (Giese et al. 1993). Vada resistance (*Rdg1a* gene) proved to be effective against two highly virulent leaf stripe isolates and against the natural field pathogen population of different barley cultivating countries, thus suggesting that *Rdg1a* may have a very wide range of effectiveness (Skou et al. 1994; Arru et al. 2003a; Mueller et al. 2003).

In addition to *Rdg1a*, other leaf stripe resistance genes have been identified. The major resistance gene *Rdg2a* located distal on the short arm of chromosome 7H (Tacconi et al. 2001) has been mapped to fine genetic resolution (Bulgarelli et al. 2004). *Rdg2a* confers complete resistance (immunity) to at least three Italian isolates of the pathogen, including the most virulent one (Dg2), but it is not effective against the isolate Dg5. The partial resistance of Proctor is conferred by a gene in the centromeric region of chromosome 7H in the spring barley cross Proctor × Nudinka (Pecchioni et al. 1996). This QTL had a major effect on the trait and has been designated as the Proctor resistance gene. Partial resistance of cv Steptoe is governed by major QTLs mapped to the long arm of chromosome 2H and on chromosome 3H (Arru et al. 2003b).

In the course of a barley germplasm screening for leaf stripe resistance sources with a wide range of effectiveness against highly virulent monoconidial isolates and the field pathogen population, *H. spontaneum* 41-1 and the barley cv Vada were among the genotypes with the highest level of resistance, while Arta and L94 were among the most susceptible (Mueller et al. 2003 and unpublished data). These genotypes were verified as being parents in two barley mapping populations (Baum et al. 2003; Marcel et al. 2007a).

In the present work QTL analysis was applied to the RILs progenies of two crosses that included *H. spontaneum* 41-1 and Vada as resistant parents, and were therefore segregating for the resistance against two highly virulent leaf stripe monoconidial isolates. In one of the crosses, L94 × Vada, a dense molecular marker map was used to precisely detect the map position of the Vada resistance. The other population, Arta × *H. spontaneum* 41-1, was used to identify resistance loci contributed by *H. spontaneum*, the wild progenitor of cultivated barley. Markers added to this latter map also enabled comparisons among the resistance loci detected in the two populations. A syntenic interval on the rice chromosome arm 4L was identified on the basis of rice orthologs of EST-based barley markers, allowing a search for possible candidate genes for *Rdg1a* resistance among the annotated rice genes. Mapping of three *Rdg1a* candidates revealed that one of them, a homolog of the powdery mildew resistance gene *mlo*, was tightly associated to the LOD peak of the resistance gene in both the populations.

Materials and methods

Plant material

Two spring barley segregating populations of RILs obtained by single-seed descent were tested with their

respective parents for leaf stripe resistance in an artificial inoculation test. One barley population (L × V) consisted of 103 F9 inbred lines obtained from a cross between L94 (leaf stripe susceptible), a two-rowed line with black and naked seeds, and Vada (leaf stripe resistant), a two-rowed cultivar with white and hulled seeds (Qi et al. 1998). The second population (A × H.sp.) comprised 194 F8 RILs of the cross between Arta (leaf stripe susceptible), a two-rowed pure line selected from the Syrian white-seeded landrace "Arabi Abiad", and *H. spontaneum* 41-1 (leaf stripe resistant), a pure line selected for its adaptation to severe drought stress conditions; this cross was originally developed to study agronomic traits associated with adaptation to Mediterranean environment (Baum et al. 2003). The cvs. Rebelle (six-rowed, winter, highly resistant), Thibaut (six-rowed, winter highly resistant to isolate Dg2 and susceptible to isolate Dg5) and Mirco (six-rowed, highly susceptible) were used as reference lines in the inoculation test. The cv. Gull was tested for resistance to leaf stripe isolates Dg2 and Dg5 to assess derivation of the Vada resistance.

Inoculation test and disease evaluation

The *P. graminea* isolates used (Dg2 and Dg5) are the most virulent in a collection of 12 Italian monoconidial isolates tested on European barley varieties (Gatti et al. 1992; Mueller et al. 2003). In the L × V population, infection scores for the isolate Dg2 were determined in a previous work (Arru et al. 2002), but in the present work 38 lines out of 103 were scored again with this leaf stripe isolate. In addition, 91 lines of L × V were screened with the isolate Dg5. In the A × H.sp. population 122 and 121 lines were, respectively tested with the isolates Dg2 and Dg5. The RILs, parents and test cvs. were inoculated using the "sandwich" method following the procedure described in Pecchioni et al. (1996).

For each line, sixty seeds were surface-sterilized in 70% ethanol for 30 s and 5% NaOCl for 10 min, rinsed thoroughly in deionized water, left to dry and then incubated in three Petri dishes (20 seeds each) between two potato dextrose agar (PDA; Liofilchem, Teramo, Italy) layers colonized by an actively growing mycelium. After 20 days of incubation in the dark at 6°C, the emerged seedlings were transplanted to pots 12 cm in diameter and grown in the greenhouse until heading at 12°C night (10 h dark) and 20°C day (14 h light at a quantum flux density of 28 μE m^{-2} s^{-1}). A randomized, complete-block design with three replications of 20 plants per line was used. At heading, infected (showing leaf stripes) and healthy plants were counted. Resistance was assessed as the incidence of infection, i.e. the percentage of infected plants.

DNA marker analysis

Genomic DNAs of the parents and each RIL was isolated by placing leaf tissues in 96 × 1.2-ml-well microtube plates. Plant material was ground using the Retsch® MM300 Mixer Mill instrument and for DNA purification the Wizard® Magnetic 96 DNA Plant System (Promega) was used following manufacturer's instructions. In order to identify molecular markers, L94, Vada, Arta and *H. spontaneum* 41-1 were screened for polymorphisms. PCRs for STS, CAPS and dCAPS analyses were performed in volumes of 20 μl, 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 60 ng template DNA. The PCR conditions comprised one cycle of 2 min at 94°C, 36 cycles of 40 s at 94°C, 50 s at 60°C (66°C for marker TC163743), 1 min 20 s at 72°C, and a final extension 72°C for 10 min. Gel-purified PCR products were directly sequenced to confirm identity (by comparison to the original sequence), and to identify polymorphisms between the parents of the mapping populations. When amplification products were longer than 1 kb, sequencing primers were used to extend sequence reading to the whole amplified fragments. Restriction sites covering polymorphic sites were identified using the RestrictionMapper V3.0 program (http://www.restrictionmapper.org/), while primers for dCAPS analysis were identified with the program dCAPS Finder V2.0 (http://helix.wustl.edu/dcaps/dcaps.html). For CAPS and dCAPS 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/μl of acetylated BSA. The resulting fragments were size fractionated in 2% agarose gels. For SSR analysis each reverse primer was 5′-tailed with the M13 forward consensus sequence. The M13-tailed reverse primers were then used in combination with the forward primers and with a standard M13 primer dye-labelled at its 5′ end (Boutin-Ganache et al. 2001). PCRs were performed in volumes of 10 μl, containing 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.2 μM of forward primer, 0.02 μM of M13-tailed reverse primers, 0.08 μM of M13 dye-labelled primer, 0.5 U Taq polymerase, and 60 ng template DNA. The PCR conditions comprised one cycle of 2 min at 94°C, 36 cycles of 45 s at 94°C, 45 s at 55°C, 1 min at 72°C, and a final extension 72°C for 7 min. Microsatellites polymorphisms were visualized using an ABI PRISM 3130xl genetic analyzer (Applied Biosystems, Foster City, CA).

The InDel-based HvCSG STS marker applied on the L × V population was obtained with primers reported in Table 1 designed from position 4,500 to position 5,205 of GenBank sequence X58339 coding for the barley chalcone synthase gene (Becker and Heun 1995). Additionally, the CAPS marker MWG2068 (Marcel et al. 2007b) and the

Table 1 STS, dCAPS (d) and CAPS markers tested for linkage to *Rdg1a*

Marker	Primer	Restriction enzyme	
		L × V	A × H.sp.
HVCSG	5′-CCTTCTCGACCGTTTATCTTCGTCATGG		
	5′-CTGCAGGGCTGCTTCAATGAGC		
NP450530(d)	5′-GCAGCGTCAGCGTGTCAAGAACCGTTCCGTCGTCA	*BseLI*	
	5′-TTCCCGGAGGACCAGACCTAC		
TC163743	5′-AAGGAGTTCAACTGGAACTTTGAA	*HinfI*	
	5′-CCAGTCATAGTCGCATACTATC		
FD526114	5′-TCTCTCATCTATGATATGATCCTAGC	*AflII*	*TasI*
	5′-CAACAGGATCAGAGAAACCATGC		

For each marker the sequence of the primers and the restriction enzyme used to detect the polymorphisms are shown

SSR markers GBM1047, GBM1462 and GBM1475 (Varshney et al. 2007) were applied on the A × H.sp. population. Sequences of barley ESTs representing putative orthologs of *Rdg1a* candidates identified on the rice chromosome 4L (see below) were also used for primer design (Table 1) using procedures previously described (Chen et al. 2009) in order to include intron sequences within the amplified genomic fragments.

Linkage analysis

For the L × V, the segregation data of 958 markers were obtained from the L94 × Vada 2006 map data of GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) (Marcel et al. 2007b), and for A × H.sp., the segregation data of 193 markers were obtained from Baum et al. (2003). Map position of the molecular markers added in the present work was established with the software JoinMap 4.0 (van Ooijen 2006) using Kosambi's (1944) mapping function. All mapped markers were tested for the expected 1:1 segregation ratio using a χ^2 goodness-of-fit test and were joined into the corresponding linkage groups using LOD score of 3.0 or higher using the "Second Order" mapping in Join Map 4.0. Mapping of TC163743, NP450530, HVCSG and FD526114 into the chromosome 2H of the L × V population was conducted by adding the order of the microsatellite markers HVM54, GBM1200, GBM1047 GBM1462, Bmag0749 and GBM1475 as a "fixed order file" into Join Map 4.0. "Fixed order files" were not used for mapping of GBM1047, FD526114, GBM1462, MWG2068 and GBM1475 into the A × H.sp. map.

Statistical and QTL analyses

ANOVA and correlation analyses of the resistance data were performed using SYSTAT v.9 software (Systat Software Inc., CA, USA). Broad sense heritabilities ($h^2 = \delta^2 g/\delta^2 p$) were calculated for the four experiments (reaction scores to Dg2 and Dg5 in L × V and A × H.sp populations) on ANOVA results. For QTL analysis, mean data of infection scores were first used for simple interval mapping (SIM) to identify the markers most significantly associated with variation in leaf stripe resistance. To improve the QTL detection capacity, automatic co-factor selection (ACS) analysis was performed to identify markers significantly associated with leaf stripe resistance. Co-factors were then used in a multiple-QTL model (MQM) in MapQTL v. 5. The QTL analysis was repeated by selecting the markers associated with the QTLs as co-factors as described by van Ooijen (2004). Permutation tests (1,000 iterations) were performed for each experiment to determine the threshold at which the LOD score became significant ($P < 0.05$) and highly significant ($P < 0.001$) for QTL identification (van Ooijen 2004). For QTL mapping, co-segregating markers were removed from L × V.

Syntenic relationship with rice

Identification of orthologous rice genes for the markers GBM1498, GBM1462, and GBM1012 is described in Stein et al. (2007), while identification of orthologous rice genes for the marker WBE110 is described in Marcel et al. (2007a). The same procedure has been used in this work to identify a rice ortholog of the barley *Gln2* locus coding for a glutamine synthetase 2 (accession number X53580). Rice genomic sequence from the leaf stripe resistance locus syntenic region of chromosome 4 was scanned for resistance proteins of all classes, as defined in Table 2 of Hammond-Kosack and Parker (2003) by using the release 6.1 of the MSU rice genome annotation project database (http://rice.plantbiology.msu.edu/pseudomolecules/info.shtml; Ouyang et al. 2007). Initial searches were conducted using 20-kb sections and, for sections of interest, additional searches were performed using 10 kb sections. Putative barley orthologs of the rice genes were identified by Blast search in the barley gene indices at DFCI (http://compbio.dfci.harvard.edu/tgi/plant.html).

Results

Phenotypic analysis

The analysis of variance on the percentage of infected plants in the two segregating populations for the two leaf stripe isolates showed a highly significant effect of the genotype ($P < 0.001$) on the incidence of barley leaf stripe, with no significant differences observed in the replications. *H. spontaneum* 41-1 and Vada were resistant to both isolates while Arta and L94 were highly susceptible (Fig. 1). In this work, when L94 was infected with isolate Dg2 the infection score recorded (85%) (Fig. 1a) was higher than that previously observed (38%) by Arru et al. (2002). Because of this difference, resistance tests on L94 were carried out in three additional independent infection experiments though results always confirmed the higher infection score (data not shown). Figure 1 shows the distribution of the leaf stripe resistance to each isolate in the two segregating populations, calculated as percentage of infected plants. The infection score dataset for isolate Dg2 used for QTL mapping in the present work was based on 103 RILs. For 65 of these RILs the infection data for isolate Dg2 were the same as those used in the mapping experiment of Arru et al. (2002), while for the remaining 38 lines new infection experiments with isolate Dg2 were carried out in the present work. For these 38 lines, previous infection scores for isolate Dg2 (Arru et al. 2002) were contrasting with infection scores obtained for isolate Dg5 in the present work, while for the remaining 65 lines there was a substantial agreement for Dg2 and Dg5 infection values. This evidence prompted a new detailed evaluation to be carried out. As previously observed (Arru et al. 2002), the higher infection scores of the population means for Dg5 (at least in L × V) together with the higher values observed for the parents may indicate that Dg5 is a more virulent isolate than Dg2.

In the two RIL populations the distribution of resistance to both isolates deviated significantly from normality, even though fitting a U-shaped (or sigmoid shaped for L × V population infected with isolate Dg2) frequency distribution separated by a region of very low frequencies (Fig. 1). Although the phenotypic distribution could fit the segregation of a single gene (at least for distributions in Fig. 1b–d), lines belonging to intermediate resistance classes were also observed, thus raising the possibility that more than one locus could be involved in the resistance. For this reason data were subsequently processed by means of a QTL analysis.

The estimates of heritability in broad sense for resistance to isolates Dg2 and Dg5, respectively within L × V and A × H.sp populations were 0.998 and 0.997 for L × V, and 0.999 and 0.999 for A × H.sp. These high heritability values indicated that the majority of the phenotypic variance was due to genetic effects. A significant correlation was found between the disease incidence of isolate Dg2 and that of isolate Dg5 in both the L × V ($r = 0.796$; $P < 0.001$) and A × H.sp ($r = 0.941$; $P < 0.001$) populations.

Genetic mapping and QTL analysis

SSR markers GBM1047, GBM1462, GBM1475 and the CAPS marker MWG2068 were mapped on the A × H.sp. population, while the STS marker HVCSG was mapped in the L × V population (Fig. 2). These additional markers were used because, on the basis of preliminary mapping experiments, loci for resistance were shown to be localized in this distal region of chromosome 2HL. Markers cited by several authors as being linked to this region (Marcel et al. 2007b; Varshney et al. 2007) were therefore added to the two maps both to increase the marker density of the gene region and to obtain bridge markers allowing comparison of the locus position. In addition, markers derived from the barley chromosome 2H/rice chromosome 4L syntenic relationship (see below), represented by NP450530 (dCAPS marker), TC163743 and FD526114 (CAPS markers) were mapped in L × V, while FD526114 was mapped in A × H.sp. (Fig. 2; ESM Fig. S1). In this latter population, mapping of NP450530 and TC163743 was not possible because of the absence of polymorphisms between the two parents. An inverted order was observed for the markers GBM1047 and HVCSG in the two maps obtained, which was unexpected, and so segregation data for the two markers were visually scored in the two populations to control for incoherencies with the assigned map position but, even after this analysis, the results of the linkage analysis were confirmed. The different map order of the two markers could therefore depend on a genuine inversion of the two markers, or, more likely, on the lower density of markers within the A × H.sp. map not allowing for a precise map position assignment.

In the L × V population, a resistance gene with major effects for resistance to *P. graminea* isolates Dg2 and Dg5 was detected on chromosome 2HL with LOD scores of 12.9 and 42.7, respectively (Fig. 2; Table 2). The parent Vada contributed the resistance alleles for this gene to both isolates and the percentage of phenotypic variance explained was 44.1% for *Q-Vada-Dg2* and 91.8% for *Q-Vada-Dg5* (Table 2). The region of the locus conferring resistance to the two isolates was completely overlapping (the LOD score peak was included in the same marker interval) and is in agreement with the position of the one previously detected for the isolate Dg2 in Arru et al. (2002), which represents the *Rdg1a* gene. The gene conferring resistance to isolate Dg2 was in fact previously localized (Arru et al. 2002) on the chromosome 2H region

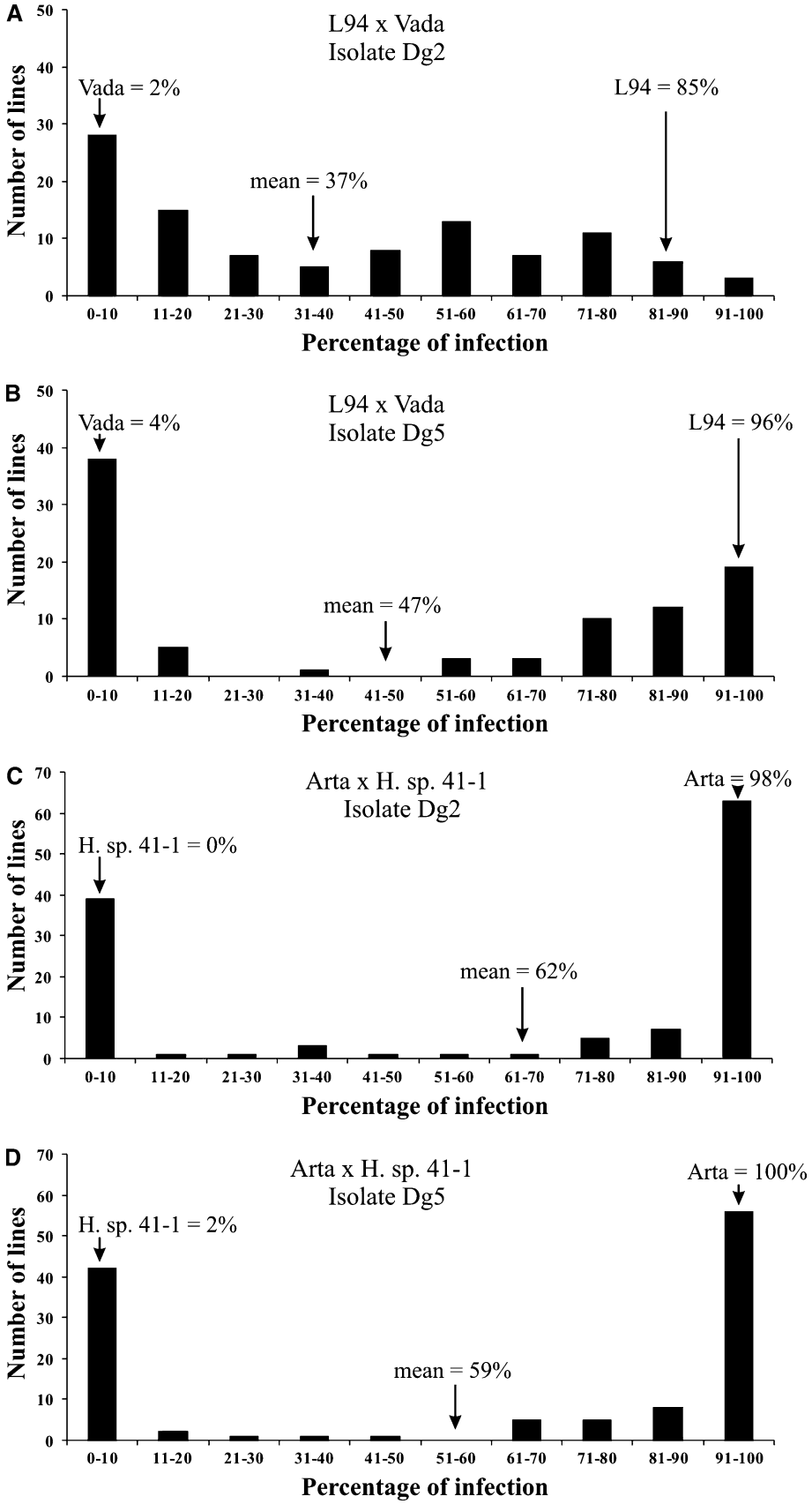

Fig. 1 Frequency distribution of phenotypic reaction to leaf stripe isolates Dg2 and Dg5 expressed as percentage of infection in RILs derived from L94 × Vada (**a**, **b**) and Arta × *H. spontaneum* 41-1 (**c**, **d**). Resistance values of the parents and means of the populations are shown and their position is indicated by arrows

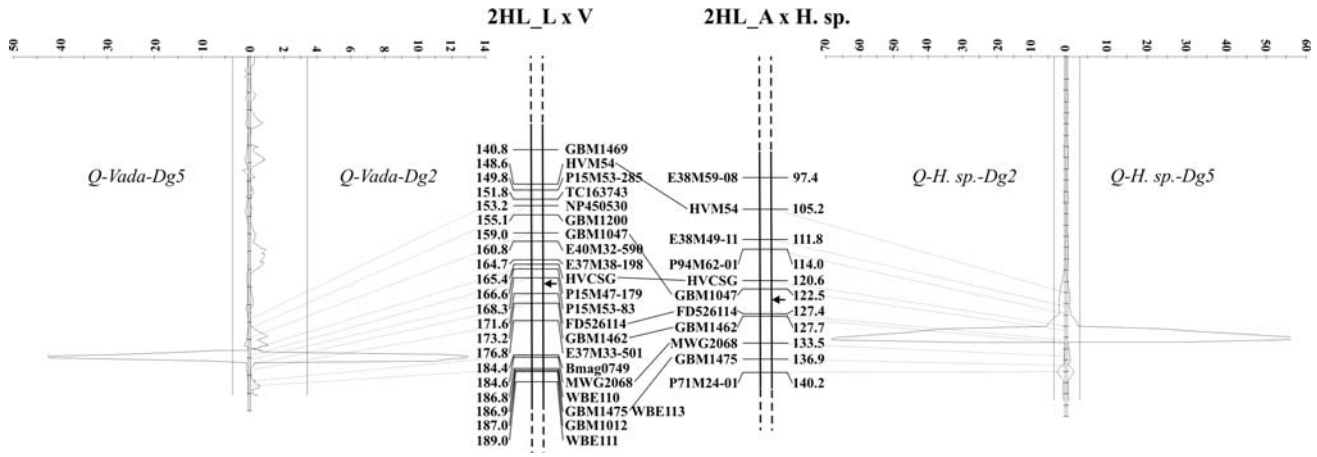

Fig. 2 Multiple-QTL model (MQM) LOD scans of barley chromosome arm 2HL where loci for leaf stripe resistance to isolates Dg2 and Dg5 were detected in the two RIL populations. *Vertical lines* indicate significance threshold for each experiment, estimated from 1,000 permutation of the data. Distances are given in Kosambi cM. Markers in common among the two populations are connected with *continuous lines*. Positions of the markers on the QTL LOD plots are indicated with *dotted lines*. *Arrows* indicate the map position of the locus for resistance to isolates Dg2 and Dg5 in A × H.sp. and for resistance to isolate Dg5 in L × V, as mapped by JoinMap 4.0

Table 2 LOD scores, percentages of phenotypic variance explained (R^2) and estimated additive effects of the detected QTLs in the RIL populations L × V and A × H.sp

Locus	Marker interval[a]	Donor	LOD	R^2 (%)[b]	Additive effect[c]
Q-Vada-Dg2	P15M53-83-FD526114	Vada	12.9	44.1	18.9
Q-Vada-Dg5	P15M53-83-FD526114	Vada	42.7	91.8	40.6
Q-H.sp.-Dg2	GBM1047-FD526114	H. sp.	68.3	97.8	48.0
Q-H.sp.-Dg2	GBM1047-FD526114	H. sp.	55.9	96.1	46.4

[a] The marker interval including the QTL peak position

[b] The amount of total trait variance explained by a QTL at this locus

[c] The positive values indicate that alleles of the gene from Vada or from *H. spontaneum* positively contributed to the resistance or reduced the severity of disease

delimited by AFLP markers E40M32-590 (this marker is presented in Fig. 2) and E42M40-644 (this marker is 1 cM distal to GBM1462). The present work therefore supports that *Q-Vada-Dg2* and *Q-Vada-Dg5* represent effects of the leaf stripe resistance gene *Rdg1a*.

In the A × H.sp. population, MQM mapping detected a resistance gene with major effects for resistance to isolates Dg2 and Dg5 on chromosome 2HL with LOD scores of 68.3 and 55.9, respectively (Fig. 2; Table 2). The resistance allele of this gene was contributed by the parent *H. spontaneum* 41-1 and the percentage of phenotypic variance explained was 97.8% for *Q-H.sp.-Dg2* and 96.1% for *Q-H.sp.-Dg5* (Table 2). Also in this mapping population, the resistance to both isolates mapped to the same location.

No additional loci were found above the threshold using MQM either in L × V or in A × H.sp. populations. The minor QTL detected by Arru et al. (2002) as a subthreshold peak (LOD = 2.04) on chromosome arm 7HL was not detected in any of the experiments in the present work.

In the two populations QTL peaks were defined by an interval of 3 cM between P15M53-83-FD526114 in L × V, and about 5 cM between GBM1047-FD526114 in A × H.sp.(Table 2; Fig. 2). The SSR marker GBM1462 is just distal to the gene region in both populations (Fig. 2). In the two populations, allelic variation at markers FD526114 and GBM1462 was associated to leaf stripe resistance, as demonstrated by the observation that alternate alleles of the two markers were the most predictive for the average level of resistance or susceptibility in the RILs from the L × V and A × H.sp. populations (Fig. 3). This locus–marker relationship was further tested by mapping the resistance gene loci as qualitative traits using JoinMap 4.0. Mapping was carried out for resistance to isolates Dg2 and Dg5 in A × H.sp. and only for resistance to Dg5 in L × V by excluding RILs with intermediate infection values (from 20 to 60%) from the mapping dataset. In A × H.sp., a locus for resistance to both isolates was mapped between markers GBM1047 and FD526114 (1.5 cM distal to GBM1047 and 2 cM proximal to FD526114), while in L × V the resistance

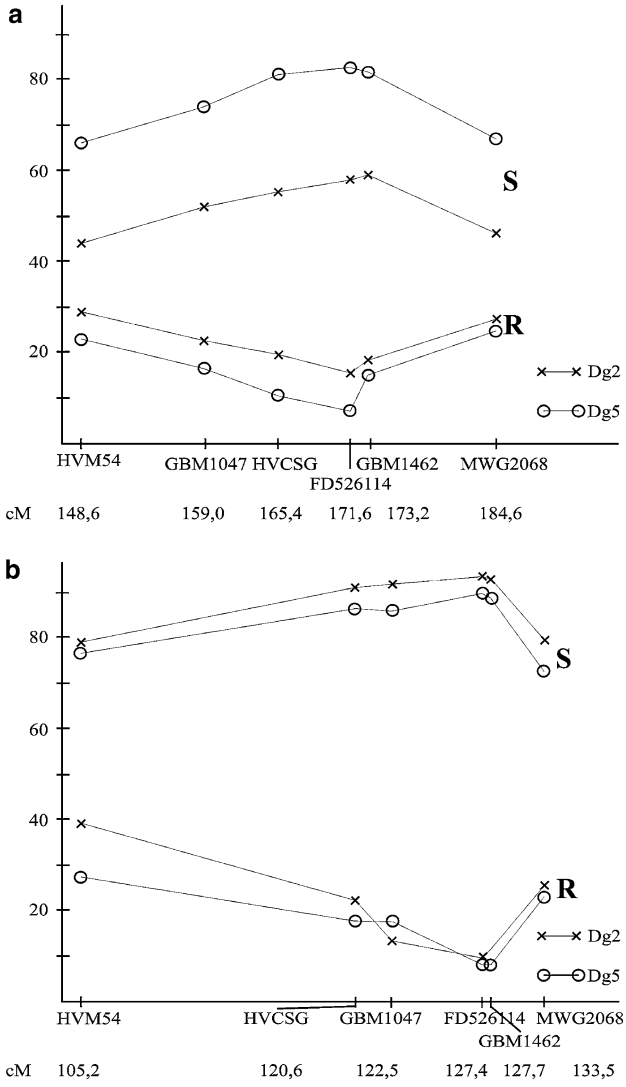

Fig. 3 Average infection response (percentage of infected plants) after inoculation with isolate Dg2 or Dg5 of lines from the L × V (**a**) and A × H.sp (**b**) populations with the resistance (*V* or *H*.sp.) or the susceptibility (L or A) alleles at molecular markers linked to *Rdg1a*. Along the *X*-axis the marker names and their distances in cM are indicated. Different scales were used to define marker distances in **a** and **b**

gene was localized between the markers P15M53-83 and FD526114 (1.0 cM distal to P15M53-83 and 2.2 cM proximal to FD526114) (Fig. 2). These JoinMap 4.0 mapping results were therefore in complete agreement with QTL mapping data.

A hypothetical origin for *Rdg1a*

Leaf stripe resistance test supports that *Rdg1a* gene in Vada can only be derived from *H. laevigatum*, because the other parent (Gull) scored as highly susceptible to the two leaf stripe isolates (Fig. 4). On the basis of the present mapping result, it can be postulated that *Rdg1a* confers resistance to the two leaf stripe isolates tested. The QTL peak for resistance against Dg2 and Dg5 was flanked by the markers HVCSG and GBM1462, common to both the populations used in the study, which corresponded to a genetic interval of 7.8 and 7.1 cM in L × V and A × H.sp., respectively (Fig. 2). This overlapping interval therefore, strongly supports the hypothesis that leaf stripe resistance in Vada and *H. spontaneum* 41-1 is governed by the same gene, namely, by *Rdg1a*, and that *Rdg1a* resistance derived from *H. laevigatum* could be traced back to *H. spontaneum*, the progenitor of cultivated barley (Fig. 4). This hypothesis is further supported by the nearly identical level of resistance of the two barley genotypes (*H spontaneum* 41-1 and Vada) to the two leaf stripe isolates (Figs. 1, 4).

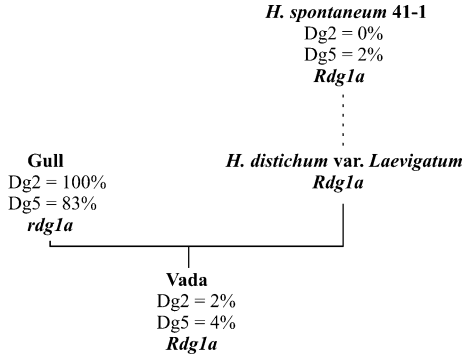

Fig. 4 Diagram depicting the proposed derivation of *Rdg1a* from *H. spontaneum* 41-1

Syntenic relationship with rice

A syntenic relationship between the long arm of the chromosome 2 region bearing the leaf stripe resistance locus identified in this work and the rice chromosome arm 4L was highlighted in previous studies (Marcel et al. 2007a; Stein et al. 2007; Chen et al. 2009). Figure 5 depicts the syntenic relationship between this barley chromosome region and the corresponding region of rice chromosome arm 4L. Rice orthologs of the EST-based SSR markers GBM1498, GBM1462 and GBM1012 were identified by using the MoMaVis program (http://pgrc.ipk-gatersleben.de/transcript_map/momavis.php; Stein et al. 2007), while information on the rice homolog of the barley EST marker WBE110 was taken from Table S1 of Marcel et al. (2007a). For the barley gene *Gln2*, a rice ortholog with a high level of similarity ($E = 2.10^{-213}$) was identified in the TIGR (The Institute for Genomic Research Rice Genome Annotation project, http://rice.tigr.org, release 6.1) locus Os04g47066. Markers Gln2 and GBM1498 were not directly mapped in the L × V population but their map position was extrapolated on the basis of loci shared between two maps: the map position of Gln2 was assigned by comparing L × V with the consensus map of Marcel et al. (2007b), while GBM1498 was assigned by comparing

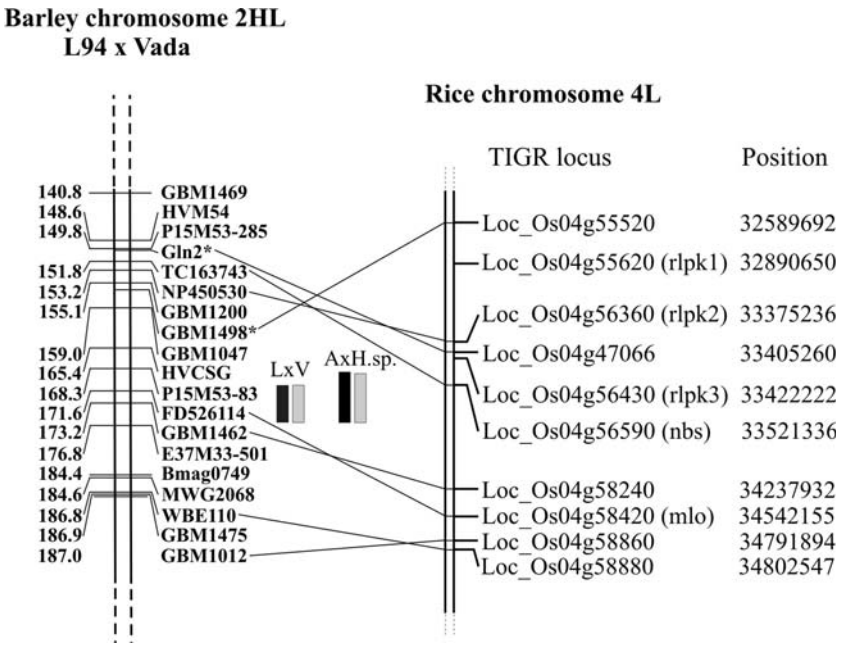

Fig. 5 Alignment of the L94 × Vada genetic linkage map covering the *Rdg1a* region on barley chromosome 2HL with the homologous region on rice chromosome 4L. *Asterisks* denoted markers whose map position was extrapolated on the basis of loci shared between two maps. Distances are given in Kosambi cM. *Black and grey rectangles* indicate the significant LOD interval for resistance, respectively to isolate Dg2 and Dg5 in the two segregating populations. The eight rice orthologs identified are connected with lines to the corresponding barley homologs and define a rice physical contig of 2.219409 Mb. The column position indicate, in bp, the 5′ end of the corresponding rice locus on the Nipponbare chromosome arm 4L. *rlpk1, rlpk2, rlpk3, nbs* and *mlo* are codes for *Rdg1a* candidates in rice, as indicated in Table 3

L × V with the consensus map of Varshney et al. (2007), as hereafter described. Marker Gln2 was mapped 0.022 cM distal to the AFLP marker P15M53-285 in a barley consensus map (Marcel et al. 2007b) and GBM1498 was mapped between markers GBM1200 and GBM1047 (0.9 cM from the first and 4.0 cM from the second marker, respectively) in a microsatellite consensus map of barley (Varshney et al. 2007). We attempted to map these two markers in the two segregating populations used in the study but, for Gln2, no SNP or In/Del polymorphisms were identified after amplification and sequencing of about 3,160 bp of genomic sequences amplified from the four parents using primers designed on the accession number X53580 (data not shown). Similarly, amplification products of the SSR marker GBM1498 were monomorphic between the parents of the two segregating populations.

The five barley EST-based markers for which a rice ortholog was identified, allowed alignment of the L × V map to a rice physical contig of about 2.212 Mb that includes the *Rdg1a* syntenic region of barley (Fig. 5). Inspection of all predicted genes annotated in the leaf stripe resistance syntenic interval in rice revealed the presence of 507 sequences coding for putative expressed proteins, 32 putative retrotransposons and 9 putative transposons. Among the 507 putative proteins genes, four were identified as coding for domains conserved within the major classes of disease-resistance proteins, three genes coding for receptor-like protein kinases and one gene coding for an ATP/GTP-binding (or nucleotide-binding sites, NBSs) protein. In addition, also an *Mlo*-like protein was present in this region (Table 3). Putative barley orthologs of these sequences were identified by searching the barley gene indices (Table 3), thus enabling development of dCAPS/CAPS markers for three of them (TC163743, NP450530 and FD526114) (ESM Fig. S1) and assignment of their map position within the *Rdg1a* genomic region (Fig. 5). For two barley ESTs, TC191004 and BY841818, it was not possible to obtain the corresponding markers. In TC191004, no SNPs were detected even after sequencing approximately 1,300 bp in the four parents, and in BY841818, the presence of multiple amplification fragments, despite the use of five different primer combinations, did not allow sequencing of the amplification products. Among the candidates tested FD526114, a sequence coding for an *Mlo*-like protein, was the most tightly associated with the leaf stripe resistance locus (ESM Fig. S1; Fig. 5) and its map position was under the QTLs LOD plots in both the populations, but not coincident with the map position of the QTL peak.

Discussion

In the present study, two barley populations segregating for leaf stripe resistance were evaluated for their response to

Table 3 Rice genes encoding domains conserved in disease-resistance proteins identified in the *Rdg1a* rice syntenic region

Code[a]	Putative function	Position[b]	Rice locus	Barley homolog[c]	*E* value[d]
rlpk1	Receptor-like protein kinase	32,890,650	Loc_Os04g55620	TC191004	9.6E-86
rlpk2	Receptor-like protein kinase	33,375,236	Loc_Os04g56360	NP450530	9.6E-124
rlpk3	Receptor-like protein kinase	33,422,222	Loc_Os04g56430	BY841818	1.2E-87
nbs	ATP/GTP-binding protein	33,521,336	Loc_Os04g56590	TC163743	2.8E-143
mlo	*Mlo*-like protein	34,542,155	Loc_Os04g58420	FD526114	1.4E-42

[a] Identification code reported in Fig. 5

[b] Position in bp for the 5′ end of the corresponding rice locus on the Nipponbare chromosome arm 4L

[c] Best barley homologs retrieved after Blast search of barley gene indices with the rice EST sequences

[d] *E* value obtained with the Blast alignment of rice sequences against the barley gene indices

two highly virulent *P. graminea* isolates in order to identify resistance loci. In the two populations only one genomic region, defined by a marker interval that included the LOD score peaks, was identified as responsible for leaf stripe resistance. This result is also supported by the high portion of phenotypic variance explained by the loci identified and from a previous study on the Vada resistance to leaf stripe isolate Dg2 in which only one major QTL was detected on chromosome 2H (Arru et al. 2002). The genomic region of resistance loci towards the two isolates was coincident in L × V and A × H.sp. populations; in addition, no recombinant lines for resistance to Dg2 and Dg5 (i.e. highly resistant to one isolate and highly susceptible to the other) were identified in the RILs analysed in either populations, thus supporting that the same gene is responsible for resistance to both isolates.

In this work we demonstrated that *H. spontaneum* accession (*H. spontaeum* 41-1) possesses the leaf stripe resistance gene *Rdg1a*. *H. spontaneum* 41-1 is a line selected from an accession originally collected in Israel, Beit Shean Valley, at an altitude ranging between 225 and 150 m below sea level (S. Grando, personal communication). The presence of the gene in this accession suggests that *Rdg1a* is present with some degree of frequency (currently unknown) within the *H. spontaneum* gene pool, and that, as a consequence, *H. spontaneum* accessions may have contributed the *Rdg1a* resistance allele to *H. distichum laevigatum*. It is known that *H. spontaneum* is a rich source of genes that impart resistance against important barley diseases and a high frequency of resistance (60–98%) to leaf blotch, leaf rust, net blotch and powdery mildew has been found in accessions from Jordan and Israel (Fetch et al. 2003). Although no studies have been performed to evaluate the frequency of leaf stripe resistance genes in *H. spontaneum*, this disease is typical of Mediterranean environments and leaf stripe is definitely present in the fertile crescent where *H. spontaneum* occurs (Yahyaoui 2004; Tunali 1995; Golzar 1995). Since the presence of the pathogen should increase selection for disease resistance, it is likely that an overlapping of the *P. graminea* and *H. spontaneum* areas may have lead to an increased frequency of leaf stripe resistance genes in the *H. spontaneum* gene pool. Very little information is available about the ancestry and provenance of *H. laevigatum* (Skou and Haahr 1987) or about the possibility of having introgressed genes from *H. spontaneum*. However, the fact that *H. laevigatum* is a landrace of *H. vulgare* ssp. *vulgare* suggests that no crossing barriers would have limited gene transfer between *H. spontaneum* and *H. laevigatum* (Asfaw and von Bothmer 1990). It is therefore possible that *Rdg1a* in *H. laevigatum* is derived from *H. spontaneum*.

A QTL for partial resistance to leaf stripe isolates Dg2 and Dg5 derived from the barley cultivar Steptoe was mapped to the long arm of chromosome 2H (Arru et al. 2003b); the peak marker of this QTL was the molecular marker Pcr1 that, on the basis of the barley consensus map of Marcel et al. (2007b), is 4.3 cM distal to GBM1462. This marker relationship therefore excludes that the resistance of Vada and the Steptoe QTL is conferred by alleles of the same *Rdg1a* gene but support the hypothesis that this region of barley chromosome 2H is enriched of sequences conferring resistance to the leaf stripe pathogen *P. graminea*.

In a previous work, only AFLP markers were identified as associated with the *Rdg1a* gene (Arru et al. 2002). In this work, the PCR-based markers HVCSG, FD525114 and GBM1462 demonstrated to efficiently predict the resistant/susceptible phenotype within the RILs analysed. These markers can therefore be used for marker-assisted selection (MAS) of *Rdg1a* in segregating populations when using Vada or *H. spontaneum* 41-1 as the donor of *Rdg1a* leaf stripe resistance. The same is true when using some of the many two-rowed spring varieties that were indicated as possessing the Vada resistance gene (Skou et al. 1994; Kraakman et al. 2006). In addition to their use in MAS, these markers should enable recombinant screening in large F2 segregating populations with the aim of mapping the *Rdg1a* gene at a higher genetic resolution. The region of the

long arm of chromosome 2 belongs to the 2L1.0 section defined as a gene-rich and highly recombinogenic region (Dilbirligi et al. 2005) with physical to genetic distance estimates of 1.1 Mb/cM (Künzel et al. 2000). This should facilitate the fine-mapping procedure through the identification of markers with a tight physical association with the *Rdg1a* gene.

Previous studies highlighted a syntenic relationship between the *Triticeae* chromosome 2 and the rice chromosomes 4 and 7 (Moore et al. 1995; Devos 2005). This syntenic relationship was in agreement with the present study for eight barley EST marker loci in the *Rdg1a* genomic region for which the corresponding rice homologs were identified. Except for two possible discontinuities in the barley–rice colinearity that can be explained by an inversion of segments, the marker order in the barley map was in agreement with the order of the predicted genes on rice chromosome 4. Two possible inversions were observed in the order of the EST-based markers Gln2-GBM1498 and WBE110-GBM1012 (Fig. 5). Since the map position of the first two markers (Gln2 and GBM1498) was extrapolated and not assigned by segregation mapping, the first inversion observed may be due to inaccuracies of their marker-locus assignment. Nonetheless, inversion events in the region that includes the marker HVM54 were previously observed in a barley–rice colinearity analysis for this region of chromosome 2HL (Chen et al. 2009), supporting that the one observed in this work represents a genuine rearrangement. The second possible inversion refers to tightly associated loci (distance between WBE110 and GBM1012 is 0.2 cM) and can therefore represent a small translocation/inversion, like those observed when saturating regions of barley resistance genes *Rph7* and *Rph5* (Brunner et al. 2003; Mammadov et al. 2005). Until now, using the rice genome sequence for the identification of *Triticeae* resistance genes during map-based cloning has been a rather fruitless approach. A homologue of the barley *Rpg1* kinase-encoding stem rust resistance gene, for example, is absent from the rice syntenic region and the whole rice genome (Han et al. 1999; Brueggeman et al. 2002) and similarly, no candidates for the barley leaf stripe resistance gene *Rdg2a*, which maps to chromosome arm 7HS, were identified in the syntenic region of rice chromosome 6 (Bulgarelli et al. 2004). Nonetheless, with the double purpose of identifying additional markers derived from rice for the *Rdg1a* region, together with possible candidates, a search for genes containing domains conserved within the major classes of disease-resistance proteins was conducted for the rice *Rdg1a* syntenic region. Analysis of the Nipponbare rice genes annotated into the 2.219 Mb leaf stripe resistance syntenic interval in rice did not reveal sequences strictly belonging to the major class (nucleotide-binding site plus leucine-rich repeat) of resistance genes. A homolog of the barley powdery mildew resistance gene *mlo*, which maps to barley chromosome 4H (Hinze et al. 1991), was identified within the rice *Rdg1a* syntenic region. This was the only candidate to map under the QTLs LOD plot area. High-resolution genetic mapping of the locus will clarify whether this gene represents an *Rdg1a* candidate, or its map localization is a positional coincidence. Furthermore, other rice EST derived from the interval defined by Loc_Os04g56590 and Loc_Os4g58240 may enable the generation of additional genetic markers in barley that are closer to *Rdg1a*.

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