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Identification and mapping of a new leaf stripe resistance gene in barley (*Hordeum vulgare* L.)

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Abstract *Pyrenophora graminea* is the seed-borne pathogen causal agent of barley leaf stripe disease. Near-isogenic lines (NILs) carrying resistance of the cv "Thibaut" against the highly virulent isolate Dg2 were obtained by introgressing the resistance into the genetic background of the susceptible cv "Mirco". The segregation of the resistance gene was followed in a F_2 population of 128 plants as well as on the F_3 lines derived from the F_2 plants; the segregation fitted the 1:2:1 ratio for a single gene. By using NILs, a RAPD marker associated with the resistance gene was identified; sequence-specific (STS) primers were designed on the basis of the amplicon sequence and a RILs mapping population with an AFLP-based map were used to position this molecular marker to barley chromosome 1 S (7HS). STS and CAPS markers were developed from RFLPs mapped to the telomeric region of barley chromosome 7HS and three polymorphic PCR-based markers were developed. The segregation of these markers was followed in the F_2 population and their map position with respect to the resistance gene was determined. Our results indicate that the Thibaut resistance gene, which we designated as *Rdg2a*, maps to the telomeric region of barley chromosome 7HS and is flanked by the markers OPQ-9_{700} and MWG 2018 at distances of 3.1 and 2.5 cM respectively. The suitability of the PCR-based marker MWG2018 in selection-assisted barley breeding programs is discussed.

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Introduction

Barley leaf stripe is caused by the fungal seed-borne pathogen *Pyrenophora graminea* (Ito and Kurib.) [anamorph *Dreschlera graminea* (Rabenh. ex. Schlech.) Shoemaker]. In susceptible plants the disease usually results in severe stunting, premature death and complete loss of grain (Tekauz and Chiko 1980). Both field tests and inoculation experiments performed with monoconidial isolates suggest a broad variability in plant response to *P. graminea*, ranging from high resistance (percent of infected plants below 2%) to high susceptibility (percent of infected plants above 80%) (Tekauz 1983; Delogu et al. 1989; Gatti et al. 1992). This variation could be due either to genetic variability for resistance in the host or genetic variability for pathogenicity in the pathogen. In an extensive study conducted in the field with more than 1,000 cvs and lines it has been observed that 42.4% of the barley cvs had 0–10% diseased plants; highly susceptible cvs (near 100% diseased plants) were also observed (Skou and Haahr 1987; Skou et al. 1994). Delogu et al. (1989), analysing a set of winter barley cvs of different origin for leaf stripe resistance in a replicated field trial, identified genotypes with levels of resistance ranging from highly resistant to highly suceptible.

A considerable variation in pathogenicity among different isolates was first reported by Knudsen (1986). Later, the level of virulence of a set of monoconidial isolates collected in different Italian barley growing areas was investigated by inoculation on 19 barley cvs; the isolates were then classified as avirulent, intermediate and virulent (Gatti et al. 1992). In this screening the isolate Dg2 was identified as the most virulent among those tested. Isolate Dg2 has been used during recent years as a tool to select for resistance to *P. graminea* in breeding programs.

The use of resistant varieties is the most economic method for the control of leaf stripe. A single genetic factor controlling complete resistance to *P. graminea*, derived from *Hordeum laevigatum* via cv "Vada", has been introduced into most resistant North-European two-rowed spring barley cvs (Skou and Haahr 1987; Skou et al. 1994). This "Vada resistance" was probably introgressed into the barley genome along with the *MlLa* (*Laevigatum*) powdery mildew resistance, because the two factors have been found to be linked; this resistance, named as *Rdg*1*a*, has been mapped on the long arm of barley chromosome 2 (Giese et al. 1993; Thomsen et al. 1997). It is also known that cvs quantitatively resistant to leaf stripe are widespread in Europe (Skou et al. 1994). A major QTL conferring resistance to barley leaf stripe was identified in the cv "Proctor" by means of QTL analysis; this QTL accounted for 58.5% of the variation in the trait and was mapped on the centromeric region of barley chromosome 1 (Pecchioni et al. 1996).

The cv "Thibaut" was identified as highly resistant to the most virulent Italian isolate, isolate Dg2 (Gatti et al. 1992). In this paper we describe the characterization of the genetic basis of the cv "Thibaut" resistance, the mapping of the locus harbouring this resistance gene and the development of PCR-based molecular markers associated with the resistance, and useful for marker-assisted selection.

Materials and methods

Plant materials and tests for resistance

The *P. graminea* isolate used, Dg2 (previously named I2), is the most virulent of a collection of 12 monoconidial isolates tested on European barley cvs (Gatti et al. 1992).

Near-isogenic lines (NILs) carrying resistance against the isolate Dg2 of *P. graminea* were generated from a cross between the resistant cv "Thibaut" and the highly susceptible cv "Mirco", followed by six backcrosses with the susceptible parent. To select the resistant phenotype we had assumed that the gene was dominant and selection was done on F_1 plants of the backcross generations; the gene was confirmed to be dominant already from the second backross generation. Subsequent analyses were on the BC5 resistant NIL Fo 3819.1 (chosen among the other resistant BC5 lines) and on the BC6 resistant NILs Fo 3877.2, Fo 3878.3, Fo 3879.1 and Fo 3879.2.

Assessment of the resistant phenotype (immune response, absence of leaf stripes) during the backcross generations, as well as on the F_2 plants of the segregating population obtained from a cross between "Thibaut" and "Mirco" (see below) and on the F_3 lines originated from the F_2 plants (see below), was performed by artificial inoculation with the isolate Dg2, using the "sandwich method" technique (Pecchioni et al. 1996).

A total of 134 F_2 plants derived from a cross between cvs "Thibaut" and "Mirco" were inoculated and scored at heading stage for their disease reaction against the isolate Dg2. Leaves of the F_2 plants were then individually harvested and stored at –80°C.

A total of 60 seedlings per F_3 line (three replications of 20 seedlings each) were subsequently inoculated with the isolate Dg2 and scored for their reaction to the disease. Three classes of the percentages of diseased plants were identified that classified the F_3 lines: 0–5% the highly resistant, 20–50% the intermediate phenotype, arising from resistant heterozygote F_2 plants, and above 80% the highly susceptibles. Because homozygous F_2 susceptible plants were usually sterile, the test on the F_3 lines was mainly performed to discriminate between resistant homozygotes and resistant heterozygotes. With respect to the reaction of the F_3 lines, 128 F_2 individual plants were unequivocally classified as resistant homozygotes, resistant heterozygotes and susceptible homozygotes.

A recombinant inbred (RI) population (F_8) containing 117 lines derived from the cross between the barley lines L94×116–5, was generously provided by R.E. Niks. An AFLP linkage map had been constructed based on this RI population (Qi et al. 2000) and the mapping data were kindly made available to us. Parents of other experimental mapping populations were also utilized: "Proctor", "Nudinka", "Steptoe", "Morex", "Dicktoo", "Vada" and C123.

RAPD, STS, CAPS analyses and molecular biology procedures

Genomic DNA for marker analysis was isolated from barley leaves as described in the standard protocol of the CTAB method (Murray and Thompson 1980).

RAPD analysis was conducted on 20 ng of genomic DNA from the cvs "Mirco" and "Thibaut", from the BC5-resistant NIL Fo 3819.1 (chosen among the other resistant BC5 lines), and from the BC6 resistant NILs Fo 3877.2, Fo 3878.3, Fo 3879.1 and Fo 3879.2. A total of 358 RAPD primers (Operon Technologies Inc., Alameda, Calif.) were used in this analysis. PCR amplification was performed in a 20-µl reaction volume with a 1× reaction buffer supplied by the manufacturer [20 mM Tris-HCl (pH 8.4), 50 mM KCl], 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.2 µM of decamer primer, 0.8 units of *Taq* DNA polymerase (GIBCO BRL). The following program was used for RAPD amplification: one cycle for 2 min at 94°C; 45 cycles for 1 min at 94°C, 1 min at 36°C, 2 min at 72°C, with a final extension of 6 min at 72°C.

STS and CAPS analysis was performed in a volume of 20 µl (1× reaction buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 µM of each primer, 0.4 units of *Taq* polymerase) using 90 ng of barley genomic template DNA. Primer sequences and the respective restriction enzymes used for CAPS are shown in Table 1.

Table 1 STS and CAPS markers linked to the *Rdg2a* locus. For each marker the sequence of the primers and the restriction enzyme used to detect the polymorphism are shown. The sequence corresponding to the RAPD primer OPQ-9 is underlined in the STS primer SCQ 9_{700}

Marker	Primer	Restriction enzyme
MWG2018	5′-CACCATGCTCCCCGTCACCTACCTC 5′-CGTGAACCCGTCGCCCAAGTCTAAG	–
ABG704	5′-TCGCCTTCTTCAGATTCCTACCA 5′-ATTACCATGTCGATATTGTTTCCACTG	*Bam*HI
PBI35	5′-CTACGTCAGTTAAGCCAGTCC 5′-TTAAAGCAAAAGCGTCTCATC	*Nla*III
SCQ-9_{700}	5′-GGCTAACCGAGAAAAGTCGA 5′-GGCTAACCGATACATCATCT	–

The following program was used for STS and CAPS analyses: one cycle for 2 min at 94°C; 35 cycles for 40 s at 94°C, 50 s at 65°C (60°C for the primers of SCQ-9_{700}), 1 min and 20 s at 72°C with a final extension of 6 min at 72°C. For CAPS analysis, 10 µl of the amplification products were then digested for 2 h in a final volume of 20 µl with 1× restriction enzyme buffer, 5 units of restriction enzyme and 0.5 µg/µl of acetylated BSA.

Amplification products were subsequently size-fractionated on 1.0% agarose gels (for RAPD amplification products only), 2.5% agarose gels or on 5% acrylamide gels run in 1× TBE buffer (100 mM Tris-HCl, 100 mM boric acid, 2 mM EDTA, pH 8.3).

RAPD amplification products were cloned into the PCRII vector using the TA cloning kit following the manufacturer's instructions (Invitrogen, San Diego, Calif.). Sequences of the amplification products were performed on both strands with a ABI PRISM 310 automated DNA sequencer (Perkin Elmer Applied Biosystems). Southern-blotting and filter-hybridization procedures were as previously described (Sambrook et al. 1989).

Linkage analysis

Segregation of the markers and *Rdg2a* genotypes was tested by a chi-square test against the expected 1:2:1 or 3:1 ratio in the F_2 generation. Linkage analyses and map construction for markers and the resistance locus were performed with the MAPMAKER computer program, DOS version 3.0 (Lander et al. 1987). The recombination values between the markers and *Rdg*2a were estimated by the maximum-likelihood method. A LOD score of 3.0 or greater was established for linkage and the Kosambi mapping function was used to convert recombination frequencies into centimorgans (cM).

Results

Screening for resistance

The cv "Thibaut" is the resistant parent (1–2% diseased plants), whereas "Mirco" is the susceptible parent (85–90% diseased plants). The resistance was introgressed into the genetic background of the susceptible parent by means of six backcross generations to produce NILs. The segregation of the total resistant/suceptible phenotypes for the last four backcross generations fitted a 1:1 ratio (χ^2=0.08, P=0.70–0.90); this segregation also suggested that the resistance gene is dominant.

An F_2 population of 134 plants derived from the cross "Thibaut"×"Mirco" was screened for disease reaction against the isolate Dg2 of *P. graminea*; on the basis of this test, 98 F_2 plants were classified as resistant (absence of leaf stripes) and 36 as susceptible (presence of striped leaves). After selfing, the segregation was verified on the resultant F_3 lines. This eliminated the high rate of escape observed for the disease if the discrimination is made on a single plant basis (Skou and Haahr 1987). Usually on susceptible homozygote plants there is no grain production. Out of 36 F_2 plants classified as susceptibles only three were able to set some seeds. Nevertheless the corresponding F_3 lines showed a percentage of diseased plants above 85%, confirming that the F_2 plants were homozygous susceptibles. On the basis of the reaction of each F_3 line, we clearly assigned the allelic composition at the resistance locus for 128 F_2 plants; this population consisted of 29 resistant homozygotes, 63 resistant heterozygotes and 36 susceptible homozygotes. The segregation fits a 1:2:1 ratio (χ^2=0.79, P=0.50–0.70), indicating that the resistance of "Thibaut" against the isolate Dg2 of *P. graminea* is governed by a single gene.

Identification of a RAPD marker associated with the resistance gene

DNA from the parents and from the resistant NILs was used as the template for amplification of RAPD markers, with a total of 358 10-mer oligonucleotide primers. One product, OPQ-9_{700} was reproducibly generated from the resistant NILs and from the resistant parent "Thibaut", but not from the susceptible parent "Mirco". The amplification product was 726-bp long; after cloning of the amplicon from cv "Thibaut", the insert was used to hybridize a Southern blot of the PCR DNAs obtained for the screening (Fig. 1A), which confirmed the absence of any amplification product in the suceptible recurrent parent. The segregation of the RAPD polymorphism was followed on a set of resistant and susceptible F_2 plants (Fig. 2D) and the results confirmed the association of this marker with the resistance gene.

Data from sequencing of the cloned OPQ-9_{700} fragment were used to synthesize two 20-bp long primers (SCQ-9_{700}, Table 1) and these STS primers were subsequently used to amplify sequences from the same set of samples used for the RAPD screening. The amplification generated a monomorphic product of 726-bp present in all the samples, both resistant and susceptible lines (data not shown). Amplification products obtained from "Mirco", "Thibaut" and from the two NILs Fo3878.3 and 3879.1 were subsequently cloned and sequenced.

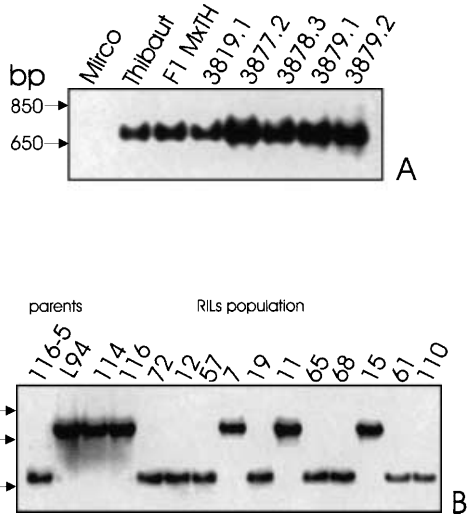

Fig. 1A, B The amplification products obtained with the RAPD primer OPQ-9 on the cvs "Mirco", "Thibaut", F_1 "Mirco"×"Thibaut" and the NILs lines were hybridized with the OPQ-9_{700} probe (**A**). The amplification products obtained with the STS primers SCQ-9_{700} on the RILs obtained from the cross 116–5×L94; the Southern blots were probed with the cloned OPQ-9_{700} probe (**B**). Size markers (1 kb plus DNA ladder, Gibco BRL) are shown at the left of the figures

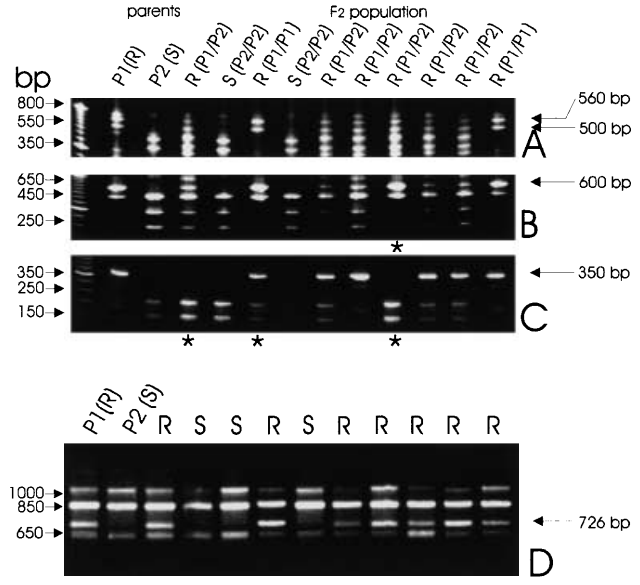

Fig. 2A–D PCR-based markers linked to the *Rdg2a* locus. Ethidium bromide-stained 5% acrylamide (**A, B, C**) or 2.5% agarose gels (**D**) displaying amplification products using "Thibaut" (P1), "Mirco" (P2), and resistant/susceptibles individuals from the F_2 population as template DNAs. The displayed markers correspond to RFLP loci MWG2018 (**A**), ABG704 (**B**), PBI35 (**C**) and to the locus identified by the RAPD marker OPQ-9_{700} (**D**). PCR-primers for locus MWG2018 (**A**) allowed allele-specific discrimination without subsequent restriction digestion. In (**B**) and (**C**), lanes marked with an *asterisk* represent F_2 plants where a recombination between the *Rdg2a* gene and the marker has occurred. Polymorphic amplification product sizes are indicated on the right by an *arrow*. Size markers (50-bp DNA ladder – **A, B, C** – and 1-kb plus DNA ladder – **D** –, Gibco BRL) are shown at the left of the figures

Sequence comparison indicated that there was a perfect identity of the sequences among all the amplification products. This result prompted us to conclude that the only polymorphism present between susceptible and resistant genotypes was in the region of the decamer annealing, and that the longer 20-bp STS primer allowed for PCR annealing in the susceptible parent also.

Genetic mapping

To allow the mapping of the recovered molecular marker associated with the resistance gene, the SCQ-9_{700} STS primers were used to amplify genomic sequences from parents of the mapping populations ("Proctor", "Nudinka", "Steptoe", "Morex", "Dicktoo", "Vada", L94, 116–5 and C123, data not shown). A clear polymorphism was detected between L94 and 116–5, the parents of a segregating population of 117 RILs where an AFLP-based linkage map has been constructed (Qi et al. 2000). The segregation of the marker SCQ-9_{700} was followed on 100 RILs. The identity of the amplification product with that obtained from the resistant NILs was verified by Southern blotting of the RIL amplification product, followed by hybridization with the OPQ-9 amplification product cloned from the cv "Thibaut" (Fig. 1B). The segregation of SCQ-9_{700} in the RILs population fitted the expected 1:1 ratio (χ^2=0.64, P=0.30–0.50). The STS marker was mapped, with respect to the AFLP markers, to the telomeric region of barley chromosome 7HS, between the first two AFLP markers E39M61–574 and E35M48–228 at 5.4 cM from the first and 1.3 cM from the second (data not shown).

Given these results, we referred to consensus maps of barley chromosome 7HS (Grain Genes, http:/wheat.pw.usda.gov) (Qi et al. 1996) to synthesize sequence-specific primers for the RFLP markers MWG2018, MWG2074, PBI35, MWG807, MWG799, ABG704 and MWG530 which are situated in the sub-telomeric and telomeric region of chromosome 7HS. Furthermore, gene-specific primers were synthesized also from the resistance gene analog Hv-b9 mapped to the sub-telomeric region of chromosome 7HS (Leister et al. 1999). These primer combinations were used to amplify genomic DNA from the resistant and susceptible parents, "Thibaut" and "Mirco". Some primers did not yield suitable amplification products (MWG807, MWG530); three primer combinations (MWG2074, MWG799, Hv-b9) yielded amplification products of the expected size, but even after sequencing of the amplicons no polymorphisms were detected. The primer combination corresponding to the RFLP marker MWG2018 directly yielded polymorphic amplification products. The allele of "Thibaut" is represented by two bands of about 500 and 560 bp, while the allele of "Mirco" is represented by two bands of about 350 and 400 bp; the four amplification products are present in the heterozygotes (Fig. 2A). For the amplicons of ABG704 and PBI35, polymorphic restriction enzymes sites were detected after sequencing (Table 1). An STS corresponding to RFLP locus MWG2108, and a CAPS corresponding to RFLP loci ABG704 and PBI35 generated co-dominant molecular markers that allowed the discrimination between resistant homozygous and resistant heterozygous F_2 genotypes (Fig. 2A, B, C).

The segregation of the polymorphisms generated by the STS and CAPS markers obtained from the RFLP markers MWG2018, ABG704 and PBI35, as well as the segregation of the RAPD amplification product OPQ-9_{700}, was followed in the segregating population of 128 F_2 plants (Fig. 2A, B, C, D). The four loci identified by these markers had alleles segregating in the expected ratio (1:2:1 for MWG2018, ABG704 and PBI35, and 3:1 for OPQ-9_{700}) within the F_2 population (Table 2).

Each of the four markers was mapped relative to the "Thibaut resistance" locus in the "Thibaut"×"Mirco" F_2 population. This resistance gene locus is flanked by OPQ-9_{700} and MWG2018 at map distances of 3.1 and 2.5 cM respectively, whereas ABG704 and PBI35 were positioned proximally with respect to MWG2018 (Fig. 3). The order of the markers is the same as that observed for the RFLP maps, and the distances between markers corresponded with those observed in a barley consensus map (Qi et al. 1996). Therefore the "Thibaut

Table 2 Segregation of STS, CAPS and RAPD markers in the F_2 population derived from the cross "Thibaut"×"Mirco". Genotypes of F_2 plants are P1/P1 of the "Thibaut" type, P1/P2 of the heterozygous type, and P2/P2 of the "Mirco" type. For each marker, χ^2 and *P* values are given

Marker	Segregation in F_2 population					
	P1/P1	P1/P2	P2/P2	Total	χ^2	*P* values
MWG 2018	29	63	36	128	0.68	0.70–0.80
ABG704	32	64	32	128	0.00	>0.99
PBI35	27	61	40	120	2.29	0.30–0.50
OPQ-9_{700}	94	–	34	128	0.16	0.50–0.70

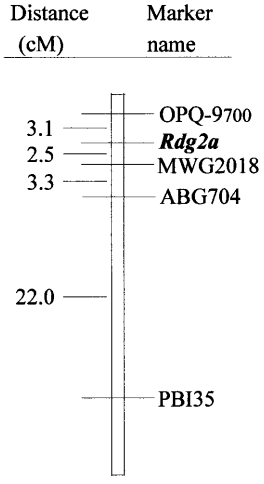

Fig. 3 A map of the telomeric region of barley chromosome 7HS showing the linkage relationship of the leaf stripe resistance gene *Rdg2a* to the RAPD marker OPQ-9_{700}, the STS marker corresponding to the RFLP locus MWG2018, and the CAPS markers corresponding to the RFLP loci ABG704 and PBI35. OPQ-9_{700} is the most-telomeric marker and the PBI35 marker is oriented towards the centromere. The map length, obtained by the MAPMAKER 3.0 package, has been calculated in Kosambi centimorgans (cM) (Kosambi 1944)

resistance" gene locus maps to the telomeric region of barley chromosome 1 S (7HS).

Because the only other identified qualitative resistance to barley leaf stripe, the "Vada resistance", was named as *Rdg1a*, referring to Reaction to *Drechslera graminea*, locus 1, allele a (Thomsen et al. 1997), we propose the designation *Rdg2a* for the "Thibaut resistance" and here after this will be used to designate "Thibaut resistance".

Discussion

In this study we have located a RAPD marker associated with the *Rdg2a* resistance gene on an AFLP-based map (Qi et al. 2000). This enabled us to obtain other sequence-specific primers that generate markers linked to the resistance gene locus. We have chosen RFLP markers mapped to the telomeric region of the barley chromosome 7HS on the basis, either of the SCQ-9_{700} mapping position, or from the identification of an AFLP marker, E42M32–231, in common between the AFLP map of Qi et al. (2000) and a combined RFLP-AFLP map of Becker et al. (1995). These two approaches allowed the recovery of another three polymorphic molecular markers associated with the resistance locus. The loci identified by the RAPD marker OPQ-9_{700} and the SCAR marker MWG2018 define a 5.6-cM interval harbouring the *Rdg2a* gene. The closely linked marker MWG2018 lies 2.5 cM from the resistance gene and thus represents a useful tool for indirect selection of the resistance trait in a breeding program. This source of resistance is valuable because of its effectiveness against the isolate Dg2, the most-virulent Italian isolate among 12 tested (Gatti et al. 1992). Inoculation with the isolate Dg2 is being used to select for *P. graminea* resistance in barley breeding programs in Italy. Lines resistant to this isolate are also resistant to the natural field population of the pathogen, spread by a naturally infected susceptible cultivar (Delogu G, personal communication).

The resistant parent "Thibaut" is resistant to all 12 isolates of the pathogen (percent of infection 0–2%), with the exception of the isolate Dg5 (Gatti et al. 1992). This raises the possibility that the resistance against the other isolates is conferred by the same gene, *Rdg2a*. Alternatively different genes in "Thibaut" could provide the resistance against the other isolates. To test these possibilities we need to screen the *Rdg2a* NILs for their disease reaction against the other isolates and to perform allelism tests.

Other sources of barley resistance to *P. graminea* have been mapped. The "Vada-resistance" gene, designated as *Rdg1a*, has been mapped on barley chromosome 2L (2HL), at about 20% recombination from the powdery mildew resistance gene *MlLa* (Thomsen et al. 1997), and a major QTL, derived from the cv "Proctor" and accounting for 58.5% of the variation in the trait, was positioned near to the centromere of chromosome 7H (Pecchioni et al. 1996). The resistant phenotype conferred by *Rdg2a*, however, appears more effective than those previously described ("Vada" and "Proctor" resistances). The level of diseased plants of "Thibaut" inoculated with the isolate Dg2 was always below 2%, while plants carrying "Vada" or "Proctor" resistances, infected with the same isolate, showed a percentage of infected plants above 10% (Pecchioni et al. 1999). Therefore, *Rdg2a* identifies a new source of leaf stripe resistance and represents an effective qualitative resistance gene against *P. graminea*.

Interestingly, in the chromosomal region to which *Rdg2a* maps, at least three other resistance genes have been identified. The *Rpg1* gene, conferring resistance to the stem rust pathogen *Puccinia graminis* f. sp. *tritici*, was mapped to the subtelomeric region of barley chromosome 7HS, 0.3 cM proximal from the molecular marker ABG704 (Kilian et al. 1994, 1995); the same marker has been posi-

tionated about 6-cM distal from *Rdg2a*. The recessive gene *mlt*, conferring race-specific resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*), has been mapped at about 6-cM distal from the molecular marker MWG851a, which also lies in the telomeric region of chromosome 7HS (Schönfeld et al. 1996). The *Rh2* gene locus conferring resistance to scald (*Rhynchosporium secalis*) also maps to the telomeric region of barley chromosome 7HS (Schweizer et al. 1995). Given these findings, this region of the barley chromosome 7H appears to be a chromosomal region where a clustering of pathogen resistance genes has occurred, as already suggested (Graner 1996).

The closest marker to *Rdg2a* is MWG2018, which lies within 2.5-cM from the gene. To reduce the distance between molecular markers and the *Rdg2a* locus we are currently testing other PCR-based markers obtained from RFLPs mapped in the telomeric region of barley chromosome 7HS. From the mapping of SCQ-9_{700} on the AFLP map (Qi et al. 2000) we have verified that four AFLP markers map proximal to the OPQ-9_{700} locus, which in turn is 3.1-cM distal to *Rdg2a*; these AFLP markers could provide molecular markers closer to the resistance locus. Furthermore, for this region of barley chromosome 7H, a high degree of synteny has been observed with the corresponding region of rice chromosome 6 (Kilian et al. 1995; Han et al. 1999); this could allow the utilization of rice probes mapped in this region to be used as molecular markers for barley chromosome 7H. In addition, the markers flanking *Rdg2a* could be used to identify candidate BACs to reveal new closely associated markers. A relationship between physical and genetic distances based on translocation breakpoints for this chromosomal region has provided an estimation of a value of about 1.3 Mb/cM, which corresponds to a region with a high level of recombination (Künzel et al. 2000). This favourable ratio between physical and genetic distances together with the availability of other molecular markers should allow a high-resolution genetic mapping of the *Rdg2a* chromosomal region in a large F_2 segregating population.

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