Genetic bases of barley resistance to the leaf stripe agent *Pyrenophora graminea*

GIAMPIERO VALÈ1*, NICOLA PECCHIONI2, DAVIDE BULGARELLI1, GIANNI TACCONI1, ELENA DALL’AGLIO1, GIOVANNI DELOGU1 AND MICHELE A. STANCA1

1Agricultural Institute for Cereal Research, Via S.Protaso 302, 29017 Fiorenzuola d’Arda (PC), Italy
2Faculty of Agriculture, University of Modena and Reggio Emilia, Via J.F. Kennedy 17, 42100 Reggio Emilia, Italy

*Corresponding author: gp.vale@popmail.iol.it

Abstract

Leaf stripe, caused by *Pyrenophora graminea*, is a serious disease of barley in many production areas. Genetic mapping of major genes and quantitative trait loci (QTLs) for this disease has revealed resistance loci on chromosomes 1 (7H), 2 (2H) and 3 (3H). QTLs for partial resistance have been identified in segregating populations derived from the crosses between Proctor and Nudinka, L94 and Vada, L94 and C123, and Steptoe and Morex. Major genes conferring a useful range of activity have been identified in the barley cultivars Vada and Thibaut. The Thibaut resistance gene, *Rdg2a*, has been subjected to high resolution mapping and a syntenic relationship of the resistance gene locus with rice chromosome 6 has been established. In the course of mapping major and quantitative disease resistance loci, molecular markers for resistance breeding for disease control using gene technology have been identified and validated for utilization in marker-assisted selection of disease resistance.

Introduction

Leaf stripe is a common disease in barley districts characterized by a cold climate during the sowing season. In susceptible cultivars, the disease causes brown stripes on the leaves, stunted growth and severe yield reductions (Tekauz 1983; Porta-Puglia et al. 1986). The fungus is unable to cause secondary infection through leaf-to-leaf transmission and therefore behaves as a true, seed-borne disease. Seed-borne diseases in barley are caused by both fungal and viral pathogens. The first class includes spot blotch, loose smut and leaf stripe, caused by *Cochliobolus sativus*, *Ustilago nuda* and
Pyrenophora graminea, respectively, while the second class includes a viral disease caused by the Barley Stripe Mosaic Virus (BSMV). Mapping studies have led to the identification of disease resistance loci to seed-borne diseases on several barley chromosomes (Table 1). Even if the infection biology of these pathogens often shares common features, disease resistance is governed by different genes, as suggested by the different map positions of the resistance loci (Table 1). The focus of this review is to analyse the molecular genetics of leaf stripe disease resistance of barley for an integrated programme of resistance breeding and the potential for disease control using gene technologies.

Table 1. Mapped genes for resistance to seed-borne diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Chrom.</th>
<th>Reference marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot blotch</td>
<td>Rs5</td>
<td>7HS</td>
<td>ABC167A</td>
<td>Steffenson et al. 1996</td>
</tr>
<tr>
<td>Spot blotch</td>
<td>Rs6</td>
<td>1HS</td>
<td>Hor2</td>
<td>Bilgić et al. 2004</td>
</tr>
<tr>
<td>Loose smut</td>
<td>Un8</td>
<td>1HL</td>
<td>ABC261</td>
<td>Eckstein et al. 2002</td>
</tr>
<tr>
<td>Leaf stripe</td>
<td>Rdg1a</td>
<td>2HL</td>
<td>MSU21</td>
<td>Thomsen et al. 1997</td>
</tr>
<tr>
<td>Leaf stripe</td>
<td>Rdg2a</td>
<td>7HS</td>
<td>MWG2018</td>
<td>Tacconi et al. 2001</td>
</tr>
<tr>
<td>Barley stripe mosaic</td>
<td>Rom</td>
<td>7H</td>
<td>ABC455</td>
<td>Edwards and Steffenson 1996</td>
</tr>
</tbody>
</table>

Barley resistance genes against leaf stripe

A serious outbreak of the disease is anticipated under certified organic farming conditions where seed treatments with fungicides are not allowed. On the other hand, because cool and humid weather conditions required for infection during germination can occur in Europe, North America, as well as North Africa, Russia, India and China, this disease can represent a threat for barley cultivation. Resistance genes are therefore desirable for controlling leaf stripe disease. For *P. graminea*, both race-specific resistance (Thomsen et al. 1997; Tacconi et al. 2001) and polygenically-based partial resistance (Pecchioni et al. 1996; Arru et al. 2002, 2003b) genes are known and mapped.

Quantitative resistance to leaf stripe

Cultivars quantitatively resistant to leaf stripe have been commonly found in spring but also in winter barley varieties and the construction of genetic maps of the barley genome made it possible to identify genomic regions affecting leaf stripe resistance. A major QTL effect accounting for more than 58% in trait variation and controlling the partial resistance of the two-rowed spring barley Proctor to isolate Dg2, was mapped to the centromeric region of chromosome 1(7H) in the Proctor × Nudinka DH population (Figure 1 and Pecchioni et al. 1996). In this population
derived from Proctor (hulled) × Nudinka (naked) cross, the genetic relationship between the naked trait and susceptibility to leaf stripe was highlighted. In fact, after disease inoculation and testing of the segregating population, the distribution of naked and hulled doubled haploids indicated two clearly distinct groups: naked/susceptible and hulled/resistant lines. The identification, in subsequent inoculation experiments, of highly resistant barley cultivars with naked caryopsis has however demonstrated that the covered kernel does not affect the level of resistance of barley genotypes to the disease (Arru et al. 2003a). Besides the major resistance factor, proposed as “Proctor resistance”, a second QTL accounting for 29% of the trait variation, was identified on chromosome 2(2H).

![Figure 1](image)

**Figure 1.** Schematic representation of the leaf stripe resistance major genes and QTLs found in the barley genome. Overlapping positions with known *P. graminea* resistance genes are indicated by boxes. P × N: Proctor × Nudinka; S × M: Steptoe × Morex.
The marker order in the different barley maps is highly conserved and major differences in the genetic length of the homologous intervals are rare; map colinearity between the leaf stripe resistance QTLs and other disease resistance loci has been investigated using molecular markers (Pecchioni et al. 1999; Arru et al. 2002, 2003b). Colinearity studies between the “Proctor resistance” and the resistance loci RsmMx (resistance to the seed-borne virus BSMV) demonstrated association with common markers (Pecchioni et al. 1999). These results could indicate that the centromeric region of barley chromosome 7H represents a chromosomal region where clustering of resistance genes has occurred.

QTL analysis was also applied on two barley populations derived from two- and six-rowed genotypes with different levels of partial resistance to barley leaf stripe: the L94 × Vada and the L94 × C123 recombinant inbred line populations (Figure 1, Arru et al. 2002). Quantitative trait loci for partial resistance were identified using the composite interval mapping (CIM) method, with the putative QTL markers used as cofactors. In the L94 × Vada mapping population, one QTL for resistance was detected on chromosome 2(2H), the same location as the leaf-stripe resistance gene Rdg1 of cv. Alf (Thomsen et al. 1997), where it confers complete resistance to a Danish isolate of the pathogen. An additional minor-effect QTL was identified by further analyses in this segregating population on chromosome 1(7H) and overlapped with the “Proctor resistance” (Figure 1). In L94 × C123, two QTLs for resistance were mapped (Figure 1, Arru et al. 2002), one on chromosome 1(7H) (according with the “bridge” AFLP-RFLP map of Becker et al. (1995) it coincided with the Proctor major effect QTL) and one on 2(2H), which did not coincide with the chromosome 2 QTL of Proctor being located at a distance of 24 cM proximal from this QTL.

In order to investigate isolate-specificity of partial resistance to P. graminea (Figure 1, Arru et al. 2003b), the Steptoe × Morex segregating population was inoculated with the two highly virulent isolates, Dg2 and Dg5. Partial resistance to leaf stripe (of cv. Steptoe) was found to be governed in part by common loci and in part by isolate-specific ones. One QTL, mapped on the long arm of chromosome 2(2H), accounted for resistance to both isolates and had a major effect on the resistance (R’ values for Dg2 and Dg5 resistance of 18.3 and 30.9%, respectively); this QTL maps to about 27-34 cM distal from the major gene of resistance to leaf stripe Rdg1a. Two tightly-linked QTLs effective against both isolates are localized on chromosome 3(3H) (Figure 1); because their support interval overlaps, their coincidence cannot be excluded. Two other QTLs were identified to be isolate-specific, for isolate Dg2 on chromosome 2(2H) short arm, and for isolate Dg5 on chromosome 7(5H), respectively. These results, together with previous findings where isolate-specific QTLs for resistance were identified (in potato/Phytophthora infestans, in pepper/Potyvirus and in barley/Puccinia hordei interactions - Leonards-Schippers et al. 1994; Caranta et al. 1997; Qi et al. 1999) support the view of isolate-specificity of partial resistance (Parlevliet and Zadoks 1977) rather than Van der Plank’s (1968) horizontal resistance theory.
Collinearities exist between the leaf stripe QTLs found and other QTLs of partial resistance to pathogens in barley. For example, in the chromosome 2(2H) region around ABG459 marker, at least four QTLs mapped in the same cross coincide with the QTL of resistance to leaf stripe: a stripe rust QTL (Toojinda et al. 1998), two *Fusarium* head blight QTLs (De la Pena et al. 1999) and a net blotch QTL (Steffenson et al. 1996). It could be interesting to speculate whether the observed clustering implies functional significance or whether it is only a consequence of genome organization.

**Qualitative resistance to leaf stripe and Rdg2a fine mapping**

The most widespread source of resistance in northern European two-rowed spring barleys was found to be the “Vada resistance”, a major gene with semi-dominant behaviour that was introgressed into cultivated barleys from *Hordeum laevigatum*, together with *MILa* mildew resistance, mainly via cv. Vada (Skou and Haahr 1987). Further studies conducted by Giese et al. (1993) localized the “Vada resistance” gene on barley chromosome 2(2H). Thomsen et al (1997) mapped this qualitative resistance gene in the cv. Alf on the long arm of chromosome 2(2H) and proposed *Rdg1a* as its designation (Figure 1). The *Rdg1a* genotype (“Alf” allele) confers resistance to all European isolates of *P. graminea* tested, including the Italian isolates Dg2 and Dg5 with a low percentage of infected plants.

Recently, a new qualitative resistance gene to *P. graminea* has been identified in the six-rowed winter barley cultivar Thibaut. When tested in NILs, this gene confers complete resistance to the most virulent isolate of *P. graminea*, the isolate Dg2, and increase the resistance to several other tested Italian isolates with the exception of Dg5. Because lines resistant to this isolate are also resistant to the natural field population of the pathogen spread by a naturally susceptible cv., it is likely that resistance provided by this gene has a useful range of activity. The Thibaut resistance gene, designated as *Rdg2a*, maps to the telomeric region of barley chromosome 1(7H) (Figure 1) and it is distally linked to the marker MWG2018 (Tacconi et al. 2001).

This *P. graminea* resistance gene was then subjected to high-resolution mapping using an F2 population representing 2,800 gametes (Figure 2). *Rdg2a* was located to a marker interval defined by ssCH4, located 0.07 cM distally, and MWG851, located 0.07 cM proximally. A single high-resolution mapping population was used to map six classes of RGAs (in bold in Figure 2; Bulgarelli et al. 2004) previously reported to map to the distal region of chromosome arm 7HS (Seah et al. 1998; Leister et al. 1999; Ayliffe et al. 2000; Rostoks et al. 2002; Madsen et al. 2003) to investigate whether any of these could represent candidates for *Rdg2a*; most of these RGAs were found to be closely linked to *Rdg2a* although none co-segregated with the resistance gene locus. 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 conferring stem rust resistance (Brueggeman et al. 2002), and the
presently uncloned *Rdg2a* resistance gene. 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 those in this study are essential for eliminating closely linked RGAs as candidates for functional resistance genes. Rice sequences from the interval spanned by rice genomic PAC clones P0644B06, P0514G12 and P0029D06 (Figure 2, right) were used to identify ESTs from barley or wheat, representing potential orthologs of the rice genes and PCR markers were developed. The markers BV078155 and BV078153 define the smallest *Rdg2a* syntenic interval in rice. The current annotations for this 115 kbp sequence interval did not include any predicted protein with assigned similarity to a known resistance protein (Bulgarelli et al. 2004). This indicates that either an ortholog 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.

**Figure 2.** High-resolution mapping at the *Rdg2a* locus. The map is based on a population of 1,400 F2 plants. RGAs (Resistance Gene Analogues) are shown in bold. Three CAPS markers derived from rice ESTs BV078155, BV078153 and BV078160 enabled alignment to a rice physical contig of 453,648 kbp comprising three PAC clones and one BAC clone (on the right). Arrows between indicate the position of homologs present in the rice sequence (redrawn from Bulgarelli et al. 2004).
Marker-assisted selection (MAS)

MAS represents a valuable tool for leaf stripe resistance breeding compared to field infection and artificial infection (“sandwich method”, Pecchioni et al. 1996). Both procedures have substantial defects ascribable to the high rate of escape to the disease of F₂ field-infected plants (Skou and Haahr 1987) and to the time-consuming assay for resistance screening performed with the “sandwich method”.

The six-rowed winter cv. Rebelle carries resistance to the Italian field population of *P. graminea* and is being used as a source of resistance in winter barley breeding programs. During the *Rdg2a* mapping, an STS marker closely linked to the resistance gene was developed starting from the sequence of the RFLP MWG2018 (Tacconi et al. 2001). We verified that the allelic composition of cv. Rebelle to the *Rdg2*-linked MWG2018 locus is the same as that of the resistant cv. Thibaut. MAS effectiveness using the *Rdg2a*-linked marker MWG2018 was investigated in lines derived from five crosses, where the donor parents of leaf stripe resistance were either Rebelle-derived lines, namely F3494 and F3505 (Figure 3a, b) or the cv. Rebelle itself (Figure 3c). In all the resistant lines examined, the resistant phenotype was always associated to the resistant allele of the marker. In the last cross, three sister lines were analysed; two (F3510.A and F3510.B) were susceptible to leaf stripe,

![Figure 3a-c. STS analysis of the marker validation lines derived from five crosses (redrawn from Arru et al. 2003a).](image-url)
while the third line, F3510.C, was found to be resistant to the disease. Therefore, also in this case the marker correctly predicted the resistant phenotype.

Additional evidence of the suitability of MWG2018 for MAS has been obtained from analysis of the marker allele in F2 plants derived from two crosses (Fo4388 and Fo4389), where Rebelle was the donor of the resistance (Figure 4).

The resistant/susceptible phenotype of the F2 plants genotyped was verified by artificial inoculation of the F3 progenies. The results showed that the resistant phenotype (with one exception for one F3 line of the cross Fo4389) was always associated with the resistant allele of the molecular marker (Table 2).

Table 2. Correspondance between the Rdg2a allele, as detected by artificial inoculation of F3 families, and MWG2018 allele of the F2 plants.

<table>
<thead>
<tr>
<th>Fo4388</th>
<th>Fo4389</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>Rdg2a allele</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
</tr>
<tr>
<td>10</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>Rdg2a allele</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
</tr>
</tbody>
</table>

Figure 4. Pedigree of the two crosses in which Rebelle acted as donor of leaf stripe resistance gene Rdg2a.
The diffusion of this resistance gene among leaf stripe resistant barley genotypes was assessed by verifying the resistant/susceptible phenotype and the allelic composition at the MWG2018 locus of 19 barley cultivars and of 150 barley accessions belonging to the Barley Core Collection (BCC; http://barley.ipk-gatersleben.de) (Arru et al. 2003a). Among the resistant cultivars tested in this screening only five were demonstrated to possess the same MWG2018 allele of Thibaut. Four of these (Thibaut, Haruna Nijo, Galleon and Acuario) showed the same pattern of resistance against the two isolates of the pathogen, raising the possibility that resistance to leaf stripe in these genotypes is governed by the same resistance gene. These barley genotypes (Thibaut, Rebelle, Haruna Nijo, Galleon and the BCC accession 803 cv. Acuario) represent very different barley genetic backgrounds: Thibaut and Rebelle are French six-rowed winter cultivars and the BCC accession 803 was derived from Chile, Haruna Nijo from Japan and Galleon from Australia. Therefore, it is suggested that Rdg2a is widespread in different regions around the world and is carried by both six-rowed (Thibaut and Rebelle) and two-rowed (Acuario, Haruna Nijo and Galleon) genotypes (Arru et al. 2003a). The potential availability of different barley genotypes carrying the same resistance gene may thus help to counter the narrowing of genetic diversity that would result in breeding programs when only a few resistant parents for which either markers and/or polymorphic markers are available (Barr et al. 2000).

Accumulation of QTLs for partial resistance in breeding programs is one of the best ways to improve crops in modern agriculture, especially in organic agriculture. QTLs with the highest effects on the resistance could be suitable candidates for MAS. Markers flanking the genomic regions where major QTLs for leaf stripe resistance lie have been identified and after conversion into simple PCR markers and validation of the amplified products, they can be used in a MAS scheme to introduce tolerance to both isolates into elite breeding lines. Moreover, at least in the case of net blotch and leaf stripe, the introgression from cultivar Steptoe of the same interval on chromosome 2(2H) by means of marker-assisted selection (MAS) would lead to an increase of tolerance to both diseases.

Conclusions

The genetic of resistance to leaf stripe in barley is being elucidated through the mapping of major genes and QTLs. High-resolution mapping of the leaf stripe resistance gene Rdg2a has highlighted a high concentration of RGAs closely linked to the resistance gene locus on 7HS. Only one of the six classes of RGAs mapped in the vicinity of Rdg2a in barley (BE216309/S9202) had a homolog present in the corresponding region of rice chromosome 6. This region of the barley genome also contains the stem rust resistance gene Rpg1; interestingly no homologs of the Rpg1 kinase-encoding resistance gene are present in the region and indeed, in the whole rice genome (Han et al. 1999; Brueggeman et al. 2002). These results further sup-
port the assertion that RGAs evolve relatively rapidly and that they often provide exceptions to rice-barley synteny (Leister et al. 1998).

For some of the QTLs identified and major genes as well, a useful range of activity against different isolates of the pathogen has been demonstrated and molecular markers useful for marker assisted selection have been identified. The durability of disease resistance genes against the evolution of counter-resistance in the pathogens has been demonstrated to be enhanced by combining qualitative and quantitative resistances (Dangl and Jones 2001). The identification of molecular markers tagging leaf stripe-qualitative and quantitative sources of resistance allows the utilization of both the resistances, separately or combined, for a durable resistance in barley breeding.

Acknowledgements

The authors thank Nadia Faccini for technical assistance. This work was supported by the Italian MiPAF Project “Protezione delle piante mediante l’uso di marcatori molecolari (PROMAR)”.

References

Ayliffe MA, Collins NC, Ellis JG, Pryor A (2000) The maize rpl rust resistance gene identifies homologues in barley that have been subjected to diversifying selection. Theor Appl Genet 100:1144-1154
Caranta C, Lefebvre V, Palloix A (1997) Polygenic resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. Mol Plant Microbe In 10:828-878
family of disease resistance gene analogs from wheat and barley. Theor Appl Genet 97:937–945
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
blotch (Pyrenophora teres f. teres) and spot blotch (Cochliobolus sativus) in barley. Theor Appl
Genet 92:552-558
ing of a new leaf stripe resistance gene in barley (Hordeum vulgare L.). Theor Appl Genet
102:1286-1291
Tekauz A (1983) Reaction of Canadian barley cultivars to Pyrenophora graminea, the incitant of leaf
stripe. Can J Plant Pathol 5:294-301
(1998) Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in bar-
ley: an example of marker-assisted line development. Theor Appl Genet 96:123 131