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Quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) is dominated by one major locus

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Abstract A major gene underlying quantitative resistance of barley against *Pyrenophora graminea*, a seed-borne pathogen causing leaf stripe, was mapped with molecular markers in a barley doubled haploid (DH) population derived from the cross 'Proctor' × 'Nudinka'. This quantitative trait locus (QTL) accounts for $r^2 = 58.5\%$ and was mapped on barley chromosome 1, tightly linked to the "naked" gene. A second resistance QTL accounting for 29.3% of the variation in the trait was identified on the P arm of barley chromosome 2. Another two minor QTLs were detected by further analysis. None of the QTLs was found in the barley chromosome 2 "Vada" region studied by Giese et al. (1993).

Key words Barley · RFLPs · QTL mapping · Quantitative resistance · *Pyrenophora graminea*

Introduction

Pyrenophora graminea Ito and Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech.) Schoemarker] is a seed-borne pathogen and the causal agent of leaf stripe in barley (*Hordeum vulgare* L.). The brown stripes on the barley leaves drastically reduce the photosynthetic area and cause reductions in yield (Porta-Puglia et al. 1986). The fungus survives on kernels as mycelium inside the parenchymatical cells of the pericarp. When the barley seeds germinate, the pathogen enters the plantlets via the coleorhiza (Platenkamp 1976).

It is well-known that genetic differences exist between barley cultivars as a continuum way ranging from high susceptibility to high resistance (Takauz 1983; Skou and Haahr 1987; Delogu et al. 1989). Variability in the infectiveness of different *Pyrenophora* isolates and an interaction between cultivars and fungus strains has also been recognized (Knudsen 1986; Gatti et al. 1992). Resistance reactions governed by polygenic systems were first assumed by Knudsen (1981) and Smedegaard-Petersen and Jørgensen (1982). Later, Boulif and Wilcoxson (1988) found that segregations can be explained either by the presence of a single dominant gene, by two genes with epistatic effects or by two recessive genes with additive effects. Skou and Haahr (1987) postulated a single genetic factor controlling the complete resistance to *Pyrenophora graminea* that was introduced into many North European barley cultivars derived from *Hordeum laevigatum* via cv 'Vada'. This "Vada-resistance" is still effective in Denmark (Skou et al. 1994). It was probably introgressed into the barley genome along with the *MlLa* ("*Laevigatum*") powdery mildew resistance as the two factors have been found to be tightly linked (Haahr et al. 1989). Giese et al. (1993) mapped the *MlLa* locus as the 'Vada'-resistance factor on the M arm of barley chromosome 2. However, little is known about either leaf stripe quantitative resistance, which is widespread in barley European germplasm, or the genetic basis of other complete resistances found in non-European cultivars (Skou et al. 1994).

Since the parents used for constructing a restriction fragment length polymorphism (RFLP) map (Heun et al. 1991) are different with respect to their leaf stripe resistance, we used this doubled haploid (DH) population to study the genetic basis of barley leaf stripe quantitative resistance. Our aim was to map putative resistance loci with a highly virulent *Pyrenophora graminea* isolate and to verify eventual linkages of quantitative trait loci (QTLs) to the qualitative "Vada-resistance" factor.

Materials and methods

Plant material

An F_1-derived population of 103 DH lines, obtained from M. Heun (Ås, Norway), was used in our tests. These DHs were produced by

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anther culture from the cross between the resistant cultivar 'Proctor' and the susceptible cultivar 'Nudinka', in 1989, by M. Jäger-Gussen and M. Heun. An RFLP map had been constructed utilizing this DH population (Heun et al. 1991), and the mapping data were made available to us.

Other barley genotypes, the highly resistant 'Onice' and 'Rebelle' and the highly susceptible 'CI6944', were used as standards in the experiments. The line 'CI6944' was kindly provided by J.P. Skou (Roskilde, Denmark).

Disease reaction

The *P. graminea* isolate used (I2) is the most virulent of the collection of 12 monoconidial isolates tested on European barley varieties by Gatti et al. (1992).

The DHs, the two parents, the F_1 and the three test cultivars were artificially inoculated using the "sandwich method" technique (Houston and Oswald 1948; Skou and Haahr 1987). A randomized complete block design with four replications of six pots of five plants each was utilized; the plants were grown in the greenhouse in 1993. After initial disease scoring, the ten most susceptible hulled and ten most resistant naked lines were inoculated again in a second experiment.

Owing to the "sandwich" technique, 30 seeds of each repetition were sterilized in 70% ethanol for 30 s and 5% NaClO for 5 min, rinsed well in three changes of deionized water and then incubated in Petri dishes between two PDA layers colonized by actively growing mycelium. Thirty control seeds of each line were sterilized and grown under the same conditions between two layers of non-infected PDA medium. After 20 days of incubation in the dark at 6°C, the emerged seedlings were transplanted into 12-cm-diameter pots and grown until heading in the greenhouse at 12°C night (10 h dark) and 20°C day (14 h light at about 30 000 lux).

Plants were treated with the foliar fungicide Triadimefon (25% a.i.) to control powdery mildew. At heading, plants were harvested and examined for leaf stripe symptoms. The diffusion of the disease was expressed as percentage of infected plants. QTL analyses were carried out after arcsin transformation of the data for a better fit to a normal distribution.

Polymerase chain reaction (PCR) mapping

Giese et al. (1993) demonstrated that the xMSU21 RFLP locus is tightly linked (approximately 3–7 cM) to the barley leaf stripe "Vada-resistance" factor on the M arm of chromosome 2. The two primers [5'-GGTCTTTCATGTACCTACC-3'] and [5'-CGAGCTCCTGT-CGAGG-3'] developed by Shin et al. (1990) were used to map xMSU21 as sequence-tagged site (STS) with the DHs derived from 'Proctor' × 'Nudinka'. Plant DNA was extracted according to Murray and Thompson (1980). Samples of each genomic DNA (100 μg) were amplified in a 40-μl reaction volume containing 250 ng of each primer, 250 μ*M* dNTPs, 1 U *Taq* polymerase (Boehringer) and 1.5 m*M* $MgCl_2$, at an annealing temperature of 55°C. Amplification products were analyzed by ethidium bromide-stained 1% agarose gels.

Linkage and QTL analysis

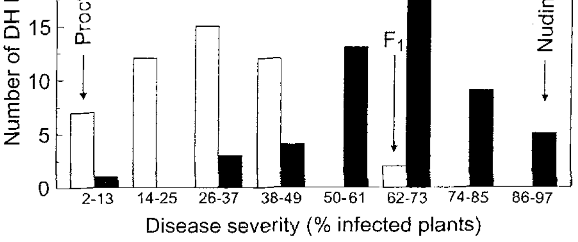
The RFLP mapping data underlying the map (Heun et al. 1991) were converted into an MS-DOS format. The STS marker *aMSU21* was added to the RFLP map using the MAPMAKER/Exp 3.0 package, DOS version (Lander et al. 1987). The same program was used to construct the ten (129 markers) chromosome frameworks for the QTL analysis; these frameworks excluded perfectly cosegregating markers and regions of chromosomes 2 and 4 not covered by RFLP markers (Heun et al. 1991). The total length of the barley map used was computed to be 1312.3 (Haldane) cM long. The computer software MAPMAKER/QTL 1.1, DOS version, (Lincoln et al., 1992) was used for QTL analyses of the DH population. Taking into account the number of chromosomes, the length and the mean density (10.4 cM) of the barley RFLP map (Heun et al. 1991; Heun 1992), we considered a LOD (Log-likelihood) threshold of 2.5 as evidence for the existence of a QTL.

Results and discussion

The cultivar 'Proctor' is quantitatively resistant (12.0% diseased plants) while 'Nudinka' is highly susceptible (94.8% diseased plants) to isolate I2. The reaction of the 103 DH lines analyzed ranged from 5.8% infected plants (PN32, hulled) to a maximum of 95.0% (PN129, naked), with a mean disease severity of 50.2% (Fig. 1). The F_1 plants were susceptible (69.2% infected plants) but not to the same degree as the susceptible parent 'Nudinka'. The distribution of naked and hulled genotypes indicates the presence of two distinct groups: naked/susceptible lines and hulled/resistant ones. The mean of the 48 hulled DHs was 31.2% diseased plants; that of the 55 naked ones was 64.9%. This separation suggests a strong correlation between the naked trait and resistance. However, the presence of recombinant genotypes is worth noting: PN130 and PN77, for example, are naked, but with a disease score of 12.5% and 29.0%, respectively; PN39 and PN115 are hulled and have values of 71.0% and 69.8%, respectively. These naked/resistant and hulled/susceptible lines, the connection confirmed by the second experiment, demonstrated that the basis of this relationship is genetic rather than physiological.

After arcsin transformation, the infection data fitted a normal distribution as requested by the MAPMAKER/QTL algorithm, with a skewness near to zero and a slightly negative kurtosis (−0.66%). Broad-sense heritability ($h^2 = \sigma^2 g/\sigma^2 p$) calculated on the 103 DH reaction scores was 0.89, but this value may be overestimated because of the lack of effects due to locations and years. However, a similar value ($h^2 = 0.88$) was found by Delogu et al. (1989) for field resistance to barley leaf stripe in a population of European winter barley varieties.

Fig. 1 Frequency distribution of leaf stripe severity (as percentage infected plants) in the barley doubled haploid (DH) population originating from the F_1 of the cross 'Proctor' × 'Nudinka'. The DH lines were inoculated with monoconidial *Pyrenophora graminea* isolate I2. Reactions of parents and F_1 are indicated by *arrows*



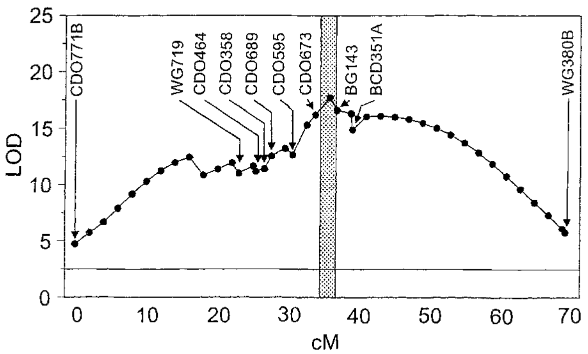
The interval mapping approach of the MAPMAKER/QTL package algorithm (Lander and Botstein 1989; Lincoln et al. 1992) was applied to this DH barley population. The use of DH lines is necessary because of the possibility of escapes from the pathogen attack (Skou and Haahr 1987). Moreover, this approach has already resulted in the mapping of resistance QTLs in barley (Heun 1992; Chen et al. 1994) and rice (Wang et al. 1994).

The MAPMAKER/QTL package was originally not designed for DH or RI populations but despite this, it appears to be robust to violations of the originally used population structures, as evidenced by the similarity of results obtained from linear regression models and MAPMAKER/QTL interval analysis (Knott and Haley 1992; Wang et al. 1994). Wang et al. (1994) performed a point analysis using the SAS/GLM linear procedure (SAS 1988) and MAPMAKER/QTL (by setting the distances between markers to 0 cM) on the same population of rice recombinant inbreds and found the percentage of phenotypic variance explained at the marker loci to be almost identical.

Using all of the mapping data, we detected two putative resistance loci: the first on the M arm of chromosome 1, accounting for 58.5% of the variation in the trait (variance explained: r^2); and the second, on the P arm of barley chromosome 2, accounting for 29.3% of the variation in disease severity (Figs. 2 and 3 respectively). The variance explained at the first resistance locus, when compared to the calculated heritability of the trait, demonstrated that barley leaf stripe quantitative resistance to isolate I2 is dominated by a major locus on chromosome 1. As this accounted for most of the genetic variation in the trait, we suggest this locus to be the "Proctor-resistance" factor.

The major resistance QTL occurred in a region of chromosome 1 spanning more than 70 cM and had a LOD score peak of 18.18. It was positioned between RFLP markers *CD0673* and *BG143* (co-mapping with the "naked" locus), 2.1 cM from the first and 1.0 cM from the second one. The ΔLOD 1.0 confidence interval (Lander and Botstein 1989) is shown in Fig. 2 and covers a narrow region (≈ 2 cM) preceding the "naked" locus. The tightly linked markers *CDO673* and *BG143*, which flank the peak in a 3.1-cM segment, may be useful for the scoring of quantitatively resistant genotypes. The resistant allele, as expected, was provided by the 'Proctor' parent.

Fig. 2 Plot of LOD (Log-likelihood) scores for leaf stripe severity over a 70-cM (Haldane) portion of barley chromosome 1 around the "naked" locus. The RFLP marker *BG143* showed complete cosegregation with the "naked" trait. The *grey column* indicates one LOD support interval for the position of the QTL



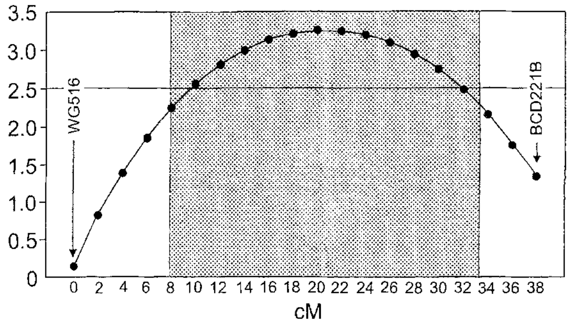


Fig. 3 Plot of LOD (Log-likelihood) scores for leaf stripe severity over a 37.8-cM (Haldane) portion of the P arm of barley chromosome 2. The *grey column* indicates one LOD support interval for the position of the QTL

The log-likelihood map of the second QTL on chromosome 2 is depicted in Fig. 3. This minor resistance locus resided in a large confidence interval spanning approximately 24 cM, between RFLP markers *WG516* and *BCD221B*. The LOD score peak of 3.27 was located 20.4 cM from *WG516*. The resistant allele again came from 'Proctor'. The resolution of this second QTL is far less than ideal because it is located in a large interval (37.8 cM) and is not close to either of the flanking markers.

A two-loci model involving both chromosomes 1 and 2 was computed by MAPMAKER/QTL, and this gave a higher LOD score (19.77) and explained more variation (62.7%) than the single major QTL model. However, a significant reduction in LOD score and r^2 was observed when compared to the sum of the individual locus effects (LOD = 21.45, $r^2 = 87.8$). This reduction suggests that the two loci do not function additively, which could be due to a poor resolution of the chromosome 2 interval, to epistatic effects between the two loci or to other unknown factors. This "1 major + 1 minor" model explained a consistent part of the variation in the trait (62.7%), which, compared with the calculated heritability (0.89), excluded the presence of other major-effect loci in the genomic regions not covered by RFLP markers (for which the log-likelihood map was not computed).

To clarify if the large region of chromosome 1 with LOD values exceeding the threshold of 2.5 was due to one or to more than one linked QTLs, a genome scan keeping "fix" as highly significant the QTL in the interval *CDO673-BG143* was performed by MAPMAKER/QTL. No linked QT effects were found in this region and thus no more than a single locus is presumably responsible for the high LOD values on the chromosome 1 portion (Fig. 2). Nevertheless, two other minor QTLs with LOD scores exceeding 2.5 were elucidated by this further analysis; these loci were covered initially by the "Proctor-resistance" effect.

The genome regions of interest are on the M arm of barley chromosome 1 between RFLPs *WG338B* and *WG420* in a region spanning 32.4 cM and on the P arm of chromosome 4 between markers *WG622* and *CDO669* (23.9 cM). The respective LOD peaks, 2.55 and 4.28, were situated 28 cM from *WG338B* and 0.4 cM from *WG622*. When added to the two-QTL model, these regions resulted in an increase in the LOD score to 1.82 and 4.28 (+3% and +13% r^2, respectively). Thus, only the minor QTL on chromosome 4 behaved totally additively; the other on chromosome 1 showed some interaction with the two-QTL model. These two additional QTLs carried the favourable allele from the 'Proctor' parent.

The complete four-loci model, arising from the multiple QTL analysis performed by MAPMAKER/QTL, had a LOD value of 26.21 and explained 76.8% of the variation in the trait. It is remarkable that if a genotype would carry the 'Proctor' alleles at all the marker loci flanking QTLs, it would have an expected resistance level of 19.6%, while in the opposite case ('Nudinka' alleles), 79.2% of the plants would be infected. No resistance alleles were found to be carried by the susceptible parent 'Nudinka', as opposed to the results obtained in other systems (Dirlewanger et al. 1994; Kreike et al. 1994).

Because we examined a DH and not a F_2 population, we could not determine the relative importance of dominance and additive effects at each locus. Nevertheless, the artificial inoculation of the F_1 seedlings with *P. graminea* isolate I2 demonstrated that the "Proctor resistance" is not dominant (Fig. 1). This is in agreement with the findings of Skou and Haahr (1987) regarding the "Vada-resistance" factor and with the behaviour of other Italian barley resistant breeding material (G. Delogu, personal communication).

Robertson (1985) postulated that at least some of the quantitative resistance factors could be "mild" alleles of qualitatively acting genes, and some of these loci have been found in regions carrying qualitative resistance factors (Leonards-Schippers et al. 1994; Wang et al. 1994). Despite this, Heun (1992) showed that the *Mla12* qualitative locus was not involved in a barley quantitative resistance to powdery mildew. Also, in the barley/*P. graminea* isolate I2 interaction studied here, no QT effects were found in the genomic region following the *aMSU21* STS marker, putatively linked to the major qualitative resistance factor called "Vada-resistance", since neither LOD score in this region arose above significant levels (Table 1). In a scan of the intervals 24 cM after and before the *aMSU21* marker, LOD values did not rise above 0.45, which is very far from the threshold of 2.5. Nevertheless, the map position of *aMSU21*, the marker for which all of the mapping population was scored, on the M arm of barley chromosome 2 (between the markers *DG.F.15* and *DG.F.41*) was consistent with that found by Shin et al. (1990) and Giese et al. (1993).

Table 1 LOD values around the two identified QTL-related loci for barley leaf stripe partial resistance, *BG143* and *BCD221B*, and around marker locus *aMSU21*, which has been demonstrated to be in linkage (4 cM) with the "Vada-resistance" factor (Giese et al. 1993)

Position (cM)	*BG143*	*BCD221B*	*aMSU21*
−24	10.290*	3.005*	0.129
−20	11.966*	3.226*	0.095
−16	10.856*	3.259*	0.045
−12	11.952*	3.111*	0.020
−8	12.545*	2.760*	0.328
−4	15.292*	2.164	0.391
Locus	16.604*	1.329	0.394
+4	16.034*	0.896	0.412
+8	15.995*	0.941	0.439
+12	15.451*	1.720	0.433
+16	14.424*	0.974	0.409
+20	12.854*	0.899	0.364
+24	10.768*	0.778	0.274

* Positions above the LOD threshold of 2.5

The "Proctor-resistance" identified here accounted for 58.5% of the variation in disease resistance, indicating it is a major factor, and had a high peak LOD score (18.18). Three (one plus two) minor loci contributed to resistance expression. This type of model is often detected when studying plant quantitative resistance. The results of Chen et al. (1994) with barley/*Puccinia striiformis* f. sp. *hordei* are surprisingly similar to our own. Quantitative resistances to nematodes in potato (Kreike et al. 1994), to anthracnose stalk rot in maize (Jung et al. 1994) and to *Ascochyta pisi* race C in pea (Dirlewanger et al. 1994) have also been explained by similar major/minor loci models. A possible explanation of this fact could be that the quantitative resistances described are not quantitative traits in the true classical concept of many loci with small additive effects on the trait, but only mime these kinds of traits. Some "polygenic" resistances to barley leaf stripe hypothesized, for example, by Knudsen (1981) or Boulif and Wilcoxson (1988), without the aid of molecular markers, should be verified by QTL mapping.

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