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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.) Shoemark] 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 MILa ("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 MILa 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 F1-derived population of 103 DH lines, obtained from M. Heun (As, Norway), was used in our tests. These DHs were produced by
anthec 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 'Oriace' and 'Rebele' 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 F1, 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 30000 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'-GGTCTTTACGTTACCTGCC-3'] and [5'-CGAGCCTGCTGCGAGG-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 µM dNTPs, 1 U Taq polymerase (Boehringer) and 1.5 mM MgCl₂, 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 F1 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 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 in 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 (h² = σ²/p σ²/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 (h² = 0.88) was found by Delogu et al. (1989) for field resistance to barley leaf stripe in a population of European winter barley varieties.

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**Fig. 1** Frequency distribution of leaf stripe severity (as percentage infected plants) in the barley doubled haploid (DH) population originating from the F1 of the cross 'Proctor' × 'Nudinka'. The DH lines were inoculated with monoconidial *Pyrenophora graminea* isolate I2. Reactions of parents and F1 are indicated by arrows.