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RFLP mapping of three new loci for resistance genes to powdery mildew (Erysiphe graminis f. sp. hordei) in barley

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Abstract Three new major, race-specific, resistance genes to powdery mildew (Erysiphe graminis f. sp. hordei) were identified in three barley lines, 'RS42-6*O', 'RS137-28*E', and 'HSY-78*A', derived from crosses with wild barley (Hordeum vulgare ssp. spontaneum). The resistance gene originating from wild barley in line 'RS42-6*O' showed a recessive mode of inheritance, whereas the other wild barley genes were (semi)-dominant. RFLP mapping of these three genes was performed in segregating F2 populations. The recessive gene in line 'RS42-6*O' was localized on barley chromosome 1S (7HS), while the (semi)-dominant genes in lines 'RS137-28*E', and 'HSY-78*A', were localized on chromosomes 1L (7HL) and 7L (5HL), respectively. Closely linked RFLP clones mapped at distances between 2.6 cM and 5.3 cM. Hitherto, specific loci for powdery mildew resistance in barley had not been located on these chromosomes. Furthermore, tests for linkage to the unlocalized resistance gene Mlp revealed free segregation. Therefore, these genes represent new loci and new designations are suggested: mlt ('RS42-6*O'), Mlf ('RS137-28*E'), and Mlj ('HSY-78*A'). Comparisons with mapped QTLs for mildew resistance were made and are discussed in the context of homoeology among the genomes of barley (H-vulgare), wheat (Triticum aestivum), and rye (Secale cereale). Duplications of RFLP bands detected in the neighbourhood of Mlf and mlt might indicate an evolutionary interrelationship to the Mla locus for mildew resistance.

Key words Hordeum vulgare ssp. spontaneum Erysiphe graminis f. sp. hordei · Mildew resistance · RFLP mapping · Homoeology

Introduction

Powdery mildew caused by Erysiphe graminis D. C. f. sp. hordei is an obligate parasite and one of the most important diseases of barley in temperate climates. Based on the gene-for-gene hypothesis of Flor (1955), which was confirmed for powdery mildew of barley by Moseman (1959), many race-specific powdery mildew resistance genes from different origins have been recognized in barley (Moseman 1955; Wiberg 1974). Mapping studies have localized these genes on chromosomes 4 (4H), 5 (1H), and 6 (6H) (Jørgensen 1993). Recently, the MlA-mildew resistance gene was mapped on chromosome 2 (2H) by means of RFLP markers (Hulbers et al. 1992; Giese et al. 1993).

RFLP (Restriction Fragment Length Polymorphism) mapping is a powerful means to localize genes in plant genomes without knowledge of their function or their sequence (Beckmann and Soller 1983; Tanksley 1983). Many resistance genes of graminaceous species have been marked with RFLP clones, e. g. the complex resistance locus Rpl for resistance to Puccinia sorghi in maize (Hulbert and Bennetzen 1991), the gene Xa21 for resistance to bacterial blight in rice (Ronald et al. 1992), the ym4 gene for resistance to barley yellow mosaic virus or barley mild mosaic virus (Graner and Bauer 1993), the genes Pml, Pro2, Pro3 (Hartl et al. 1993, 1995; Ma et al. 1994), Pro4 (Ma et al. 1994) and Pro12 (Jia et al. 1994) for resistance to E. graminis in wheat.

Loci for resistance to powdery mildew of barley, such as Mla (Schüller et al. 1992), MlA (Hülbers et al. 1992), mlo (Hinze et al. 1991) and Mlg (Görg et al. 1993), which are widely used in barley breeding, have also been marked with RFLP clones. One of the long-term aims is to isolate these genes by map-based cloning (Paterson and Wing 1993). Accessions of H. vulgare ssp. spontaneum lines from Israel have repeatedly been described as a very rich gene pool for powdery mildew resistance (Moseman 1955; Fischbeck et al. 1976). Many resistances were identified,
but allelism or close linkage with already known loci for mildew resistance has been determined for only some of them (Jahoor 1987; Jahoor and Fischbeck 1987a, b).

The objective of the present study was to identify new major genes for powdery mildew in barley lines derived from *H. vulgare* ssp. *spontaneum*, and localize them by the application of molecular markers.

### Materials and methods

#### Plant material

'RS137-28*E', 'RS42-6*O', 'HSY-78*A', and 'D* 1B-87B' are random sampled (RS) barley lines from the F$_1$ bulks between accessions of *H. vulgare* ssp. *spontaneum* ('137-28', '42-6', 'HSY-78', '1B-87') collected in Israel, and barley cultivars ['Elgina; (E), 'Oriol' (O), 'Aramir' (A), 'Diamant' (D)], and are therefore called wild barley derived lines (Jahoor 1987). In each generation from F$_2$ to F$_7$ single-plant selections were made for mildew resistance derived from the original wild barley lines, and the agronomic type of cultivated barley. In the F$_7$, homozygous lines have been extracted and tested crossed with different barley cultivars and barley lines. The test-crosses with the cultivars 'Roland' and 'Koral', possessing the genes *Mlal* or *Mlal3*, respectively, served to study the mode of inheritance and genetic relationship of the wild barley genes to the highly polymorphic *Mla* locus. Test-crosses with the NIL (near-isogenic line) 'P19' (Kolster et al. 1986), carrying the gene *Mlpi*, and with the line 'RS170-35*A', carrying the gene *Mlp2* (Jahoor et al. 1989), were performed to study the genetic relationship to genes of the *Mlp* locus. F$_2$/F$_3$ populations from crosses between 'RS137-28*E', 'RS42-6*O', 'HSY-78*A', and the cultivars 'Pallas' or 'Gitte' served as mapping populations for the localization of the resistance genes derived from wild barley with RFLP markers.

#### Tests with isolates of powdery mildew

The mildew tests were performed at the seedling stage in detached leaves placed upon agar (Aslam and Schwarzbach 1980). In order to prevent contamination, the seedlings were raised in a growth chamber at 18°C with permanent light for 8 days. The detached leaves were placed in plastic plates upon agar (5%) containing 30 mg/l of benzimidazol in order to delay leaf chlorosis and 30 µg/ml of Ampicillin for protection against bacteria. The leaves were inoculated with appropriate isolates derived from single conidia of powdery mildew maintained at the Department of Agronomy and Plant Breeding, TUM Weihenstephan. During the incubation period of 9 and 11 days employed for European and Israeli isolates respectively, the plates were kept under the same controlled conditions as used to raise the seedlings. Mildew infection readings were after 9 or 11 days, respectively, according to the scoring scale 0 (fully resistant) to IV (fully susceptible) described by Torp et al. (1978).

#### RFLP analysis

For RFLP analysis, 80 F$_2$ plants were randomly selected from each cross. The genomic DNA was isolated according to the CTAB procedure described by Saghai-Maroof et al. (1988). The DNA was digested with the restriction enzymes BamHI, EcoRI, HindIII, EcoRV, and XbaI following the manufacturer's recommendations (Pharmacia Uppsala). The digested DNA (12 µg/lane) was subjected to electrophoresis in 0.75% agarose gels (Seakem FMC), and subsequently transferred to a Biodyne B Nylon membrane (Pall, Portsmouth) as described by the supplier (Pall Corporation, Dreieich).

All RFLP clones used in this investigation originated from the MWG collection (Graner et al. 1991; Jahoor et al. 1991). The inserts of the recombinant plasmides from the stock of MWG clones were labelled with $\alpha$-32P-dCTP by random priming (Feinberg and Vogelstein 1983), and subsequently used as probes. Hybridization and further treatments of the membrane were conducted with reference to Jahoor et al. (1991). The exposure time of the X-ray film was 1–2 weeks at $-70^\circ$C.

#### Linkage analysis

The two-point analyses were performed using the LINKAGE-1 program (Suiter et al. 1985). The multipoint analyses of data from the RFLP and powdery mildew loci were performed with MAPMAKER (Version 3.0/Exp) (Lander et al. 1987; Lincoln and Lander 1992). The recombination values in % were converted into centiMorgans (cMs) by applying the Kosambi function (Kosambi 1944). The standard errors of the recombination fractions were only calculated for two-point analyses and are therefore only given in % units.

#### Results

Mode of inheritance and tests for allelism with the *Mla* locus

Two test crosses were performed for an assessment of the inheritance patterns of the wild barley resistance genes involving the *H. vulgare* ssp. *spontaneum*-derived lines 'RS137-28*E' and 'HSY-78*A'. The F$_2$ seedlings were inoculated with selected powdery mildew cultures that were avirulent to these lines and virulent for the genes *Mla7*, *Mla12*, *Mla9*, and *Mla13* present in cultivars 'Elgina', 'Aramir', 'Roland', and 'Koral', respectively. The latter two cultivars were used as test-cross parents. In both test-crosses, a 3:1 segregation was obtained for the wild barley derived mildew resistance (Table 1). Since infection types of the resistant F$_2$ progenies varied constantly between immunity and intermediate reaction types, a clear classification for homozygous and heterozygous progenies was not possible. In both crosses, therefore, all resistant progenies were pooled for segregation analysis. Apparently, the degree of dominance is not complete. The test-cross with line 'RS42-6*O', and 'Gitte' was examined in the same way but with isolates virulent to 'Oriol' (*Mlal7*) and 'Gitte' (*Mlal1*). For this test-cross, a recessive inheritance was established (Table 1). The F$_2$ generation of the fourth test-cross ('D* 1B-87B' * Roland') was tested with 'We-3', avirulent to wild barley resistance and virulent to *Mla9* carried by 'Roland'. An inheritance of two genes segregating in a 13:3 manner was obtained. This segregation is interpreted as an independent segregation of a (semi)-dominant and a recessive gene. For allelism tests with the *Mla* locus, isolates avirulent against both test-cross parents but virulent against the resistance genes derived from the original cultivars of the wild barley derived lines were employed, and free segregation was confirmed in all four cases (Table 1). It was therefore concluded that the resistance genes originating from the *H. vulgare* ssp. *spontaneum* lines, as far as they have been detected in the tests for allelism with the isolates used, are neither allelic nor linked to alleles of the *Mla* locus.