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The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L. to barley (*H. vulgare* L.) chromosome 2 (2I)

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Abstract *Hordeum bulbosum* L. is a source of disease resistance genes that would be worthwhile transferring to barley (*H. vulgare* L.). To achieve this objective, selfed seed from a tetraploid *H. vulgare* × *H. bulbosum* hybrid was irradiated. Subsequently, a powdery mildew-resistant selection of barley phenotype (81882/83) was identified among field-grown progeny. Using molecular analyses, we have established that the *H. bulbosum* DNA containing the powdery mildew resistance gene had been introgressed into 81882/83 and is located on chromosome 2 (2I). Resistant plants have been backcrossed to barley to remove the adverse effects of a linked factor conditioning triploid seed formation, but there remains an association between powdery mildew resistance and non-pathogenic necrotic leaf blotching. The dominant resistance gene is allelic to a gene transferred from *H. bulbosum* by co-workers in Germany, but non-allelic to all other known powdery mildew resistance genes in barley. We propose *Mlhb* as a gene symbol for this resistance.

Key words *Hordeum vulgare* · *Hordeum bulbosum* · Powdery mildew resistance · Gene introgression · Irradiation

Introduction

By improved tissue-culture techniques such as embryo rescue, plant breeders can now produce hybrids from a wide range of interspecific and intergeneric crosses in the Triticeae involving *Hordeum vulgare* as one of the parents (Fedak 1992). These hybrids are necessary pre-requisites for introducing new sources of useful agronomic traits, for example durable disease resistances, into cultivated crop plants (Jiang et al. 1994). Successful gene transfer from wild species into barley (*H. vulgare* L.) has, until recently, been restricted to crosses between *H. vulgare* and *H. spontaneum* (Lehmann 1991). A more distantly related species, *H. bulbosum* L., also possesses desirable disease resistances that would be worthwhile transferring to barley (Xu and Snape 1989). However, in this case there are several problems associated with successful gene transfer. These include partial interspecific incompatibility, hybrid chromosome instability, low inter-genomic chromosome recombination, and hybrid infertility (Pickering et al. 1995). Despite these problems, a plant with resistance to the powdery mildew fungus, *Erysiphe graminis* DC. f.sp. *hordei* Em. Marchal, has been obtained from *H. vulgare* × *H. bulbosum* crosses (Xu and Kasha 1992). Prior to this report, a powdery mildew-resistant barley (code 81882/83) was identified among selfed progeny derived from an *H. vulgare* × *H. bulbosum* tetraploid hybrid (Pickering et al. 1987) although the introgression of *H. bulbosum* DNA into 81882/83 was not confirmed. In the present paper, we present molecular data that establish the chromosomal location of a single dominant powdery mildew resistance gene transferred from *H. bulbosum* into barley selection 81882/83.

Materials and methods

Pickering et al. (1987) described the origin of the powdery mildew resistant selection 81882/83. Briefly, 2087 selfed seeds were obtained from the tetraploid (2n=28) hybrid Vada VB1, produced by the colchicine treatment of a powdery mildew-resistant diploid hybrid plant from *H. vulgare* cv. 'Vada' × *H. bulbosum* genotype S1 (2n=14) (Thomas and Pickering 1983). The seeds were stabilized to 15% moisture content and irradiated with 120 Gy of γ rays from a ^{60}Co source at Risø National Laboratory, Roskilde, Denmark, in June 1980. The treated seeds were sown in a glasshouse and plants that resembled barley were retained. Selfed seeds were sown in 0.75-m

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rows in the field for agronomic assessment. Selections were made from two rows that contained plants differing morphologically and pathologically from the 'Vada' control. Pollen mother cells (PMCs) of one of these selections (81882/83), which was more resistant to powdery mildew than 'Vada', were cytologically analyzed using the methods of Thomas and Pickering (1983).

Disease assessments

Powdery mildew susceptibility tests were carried out on 81882/83 by inoculating seedlings in isolation or by placing detached first and/or second leaves on a benzimidazole gel (Bennett 1981) using races and isolates present in Great Britain, New Zealand and Germany. Results were recorded 10–14 days later with reaction types based on those of Moseman (1968).

For genetic analyses of the powdery mildew resistance, a homozygous plant of 81882/83 was crossed with 'Vada' and an F_2 population screened. The data were analyzed with the χ^2 test to confirm the mode of inheritance. Intercrosses were also made between a homozygous powdery mildew-resistant backcross selection (877G) produced from 81882/83 × 'Vada'/'Emir'/'Domen', and two barley lines 14/1/30 (Michel et al. 1994) and BC_1-2/6 (Xu and Kasha 1992), which possess single dominant powdery mildew resistance genes derived from *H. bulbosum*. None of the three powdery mildew resistant lines shared any common ancestry. To detect allelism among the lines, F_2 populations were inoculated in New Zealand and Germany with powdery mildew races and isolates virulent on the parental barley cultivars. Joint segregation of powdery mildew resistance and RFLPs observed using probe cMWG682 were performed on an F_2 population produced by crossing 877G with *H. vulgare* genotype 907-12, a homozygous powdery mildew susceptible breeder's selection.

Molecular analyses

RFLP analysis. The DNA probes used for analysis were two rye repetitive DNA sequences derived from plasmids pSc119.1 and pSc119.2 (McIntyre et al. 1990), the barley repetitive DNA sequence pHv7161 (Vershinin et al. 1990), and cDNA clone cMWG682, described by Graner et al. (1991). The insert fragments were obtained by PCR amplification and labelled with (α-^{32}P)dCTP as previously described (Timmerman et al. 1993).

Plant DNA extractions and RFLP analyses were undertaken according to the methods of Timmerman et al. (1993) with the following modifications – 5 µg of DNA was digested and transferred to Hybond-N$^+$ nylon membrane (Amersham) by alkaline blotting according to the manufacturer's recommendations. All membrane washes were carried out at 65°C. The membrane was rinsed, washed for 15 min, then 30 min, in 2 × SSC, 0.1% SDS. A final wash was carried out in 0.5 × SSC, 1% SDS for 10 min.

AFLP analysis. The protocol used was described by Zabeau (1992) in example 2 (pp 13–16) of the patent 'Selective restriction fragment amplification of tomato DNA with two restriction enzymes'. The same restriction enzymes and primers P3, P4, P5 and P7 were used. AFLP products were separated by electrophoresis on a 6% acrylamide, 8 M urea, 1 × TBE sequencing gel at 70 W constant power for about 3.0 h.

Results

Out of 2087 irradiated seeds of Vada VB1, 1167 (56.2%) germinated to produce 1004 hybrid-like plants and 163 plants (14% of the total) that resembled *H. vulgare*. Two selections were made in the field, one of which was uniculm. This will not be described further here. The second selection (81882/83) had less spike glaucosity and greater resistance to powdery mildew than 'Vada'. A seed sample was grown on in the glasshouse for inoculation with race AB14, which is virulent on 'Vada'. Reaction types (RT) of 4 (large pustules without chlorosis or necrosis) were recorded on 'Vada' and two plants from the bulk sample of 81882/83. Nineteen other plants from 81882/83 responded with RT 0c and 0n (small chlorotic and necrotic areas, respectively) on leaves 1 to 4, and RT 2 (moderate-sized pustules with chlorosis and/or necrosis) and 4 on the tips of leaves 1 and 2 only. When resistant plants were grown in pathogen-free environments, necrotic blotches on the leaves were observed. In contrast, susceptible plants appeared normal. Seeds set on the resistant plants were of two sizes – small (12% of the total), 0.020 mg mean seed weight; and normal, 0.053 mg. The small seeds developed into powdery mildew-resistant plants that had a C-banding pattern identical to that of triploid *H. vulgare* (Linde-Laursen personal communication), whereas the normal-sized seeds produced plants that resembled diploid *H. vulgare*. Forty-one PMCs from spikes of diploid 81882/83 analyzed at meiotic metaphase-I contained seven bivalents, 78% of which were closed rings. All other stages of meiosis were normal.

Inheritance studies

Reciprocal crosses were made between 81882/83 and 'Vada'. Triploid seeds were only observed on spikes of F_1 plants when 81882/83 was the female parent, but occurred in all F_2 populations regardless of the direction of the cross. An F_2 population derived from a sample of diploid seeds was screened for powdery mildew-resistance, and 216 resistant (RT 0n): 58 susceptible (RT 2,3; where 3=large pustules with some chlorosis or necrosis) were recorded ('Vada'=RT 2,3; Vada VB1=RT 0, immune). These proportions do not differ significantly from a 3 resistant : 1 susceptible ratio (χ^2=2.15; 1 *df*), and are consistent with a single dominant gene inheritance. F_2 populations from crosses between the three lines 14/1/30, BC_1-2/6 and 877G, the backcross selection from 81882/83, were screened for powdery mildew response using two races and two isolates virulent on the *H. vulgare* parents. From the results, we concluded that the mildew-resistance genes in 81882/83 and 14/1/30 are allelic or closely linked, since no fully susceptible progeny were observed in a population of 394 plants. The gene is, however, independently inherited from that in BC_1-2/6 as the numbers of resistant (156) and susceptible (12) plants did not differ significantly from the expected 15 resistant: 1 susceptible ratio (χ^2=0.214; 1 *df*).

Molecular analyses

Using repeat-sequence RFLP probes, there was no evidence for *H. bulbosum* DNA introgression into 81882/83. The probes pSc119.1 and pSc119.2 were used on blots containing plant DNA digested with the restriction endonucleases *Bam*HI and *Bgl*II; pHv7161 was used to probe blots

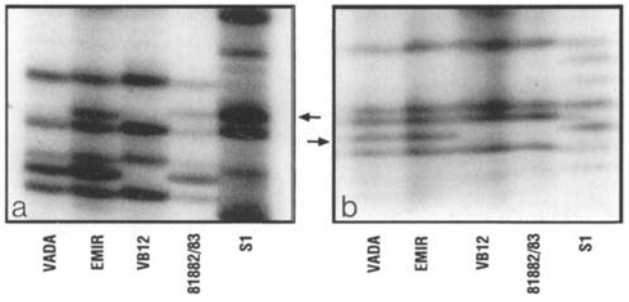

Fig. 1a, b AFLP analysis of powdery mildew-resistant barley selection 81882/83 compared with its parents, *H. vulgare* cv 'Vada' and *H. bulbosum* genotype S1. (*H. vulgare* cv 'Emir' and VB12, a putative recombinant from a cross involving 'Vada', 'Emir', and S1, are also included in the figure). **a** The *arrow* indicates the presence of an *H. bulbosum* band in 81882/83, but its absence in cv 'Vada', when using primer P3. **b** The *arrow* indicates a band associated with 'Vada', but absent in 81882/83 and *H. bulbosum* S1, when using primer P4

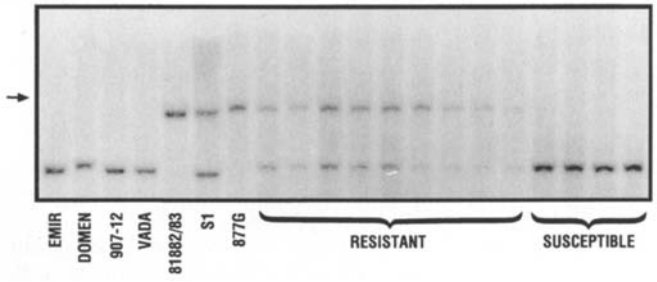

Fig. 2 DNA hybridization analysis of powdery mildew-resistant selections 81882/83 and 877G and pooled samples of DNA from resistant (*lanes 8 to 16*) and susceptible plants (*lanes 17 to 20*) from an F_2 population derived from 877G×907-12. DNA from the *H. vulgare*, *H. bulbosum* (S1), and recombinant parents occupy *lanes 1 to 7*. Total plant DNA was digested with *Eco*RI and the chromosome 2 (2I), specific probe cMWG682 was used to probe the Southern blot. The *arrow* indicates the *H. bulbosum* band (10.7 kb) that is present in 81882/83, 877G, and resistant F_2 plants, but absent in the *H. vulgare* genotypes and the susceptible F_2 plants. Note that the diagnostic *H. vulgare* bands are missing in 81882/83 and 877G, indicating that the *H. bulbosum* DNA introgression is in a homozygous form

containing plant DNA digested with *Bgl*II (data not presented).

After performing AFLP analyses, one *H. bulbosum*-specific band was identified in 81882/83 with primer P3 (Fig. 1 a). The loss of one 'Vada' band was observed in each of the 81882/83 profiles produced using primers P4 (Fig. 1 b) and P7 (data not shown), suggesting the deletion or substitution of barley DNA by *H. bulbosum* DNA. There were no apparent differences between 'Vada' and 81882/83 using primer P5. On the autoradiograms the number of AFLP bands per primer ranged from 142 to 175 for 'Vada' and 81882/83, and 146 to 188 for *H. bulbosum* S1. Among the four primers there were 2.7–11.5% more bands associated with the *H. bulbosum* lane compared with the *H. vulgare* lane.

During the course of this investigation, the location of the powdery mildew-resistance gene in 14/1/30 was established on the short arm of chromosome 2 (2I) using probe cMWG682 (Michel 1995). Co-segregation of cMWG682 and the powdery mildew-resistance gene that originated in 81882/83 was shown by RFLP analysis as follows, and confirmed that the resistance genes in lines 14/1/30 and 81882/83 are allelic or closely linked. A segregating F_2 population was developed by crossing line 877G with line 907-12. F_2 plants were screened for powdery mildew-resistance, giving 53 resistant (RT 0n) and 18 susceptible (RT 4) plants. A Southern blot was prepared using *Eco*R1 DNA from 45 resistant and all 18 susceptible plants, as well as parental DNA samples, and probed with cMWG682 (Fig. 2). Each lane containing progeny DNA consisted of up to five pooled DNA samples. The *H. bulbosum* allele found in 877G was only present in the resistant progeny. In contrast, the susceptible progeny carried only the barley allele of cMWG682, confirming the location of the powdery mildew-resistance gene from *H. bulbosum* to the short arm of chromosome 2 (2I).

Discussion

Successful gene introgression from *H. bulbosum* into *H. vulgare* has been achieved using triploid and tetraploid interspecific *H. vulgare* - *H. bulbosum* hybrids. First, by backcrossing partially fertile triploid 'VBB' hybrids [*H. vulgare* (2n=14) × *H. bulbosum* (2n=28)] to barley, single dominant genes have been transferred from the wild species into barley (Xu and Kasha 1992; Pickering et al. 1994). The use of similar crosses has also enabled the production of a partial series of single monosomic substitutions of *H. bulbosum* chromosomes into barley (Pickering 1992). Second, by screening progeny from a tetraploid 'VVBB' hybrid, Michel et al. (1994) identified two barley plants containing DNA introgressed from *H. bulbosum*. One of the plants was resistant to barley mild mosaic virus (BaMMV). The other plant (14/1/30) had powdery mildew-resistance conferred by a single dominant gene located on chromosome 2 (2I) (Michel 1995). We have now established that the powdery mildew-resistance genes transferred from *H. bulbosum* into 81882/83 (Pickering et al. 1987) and 14/1/30 are allelic, or else closely linked, whereas the genes in 81882/83 and BC_1-2/6 (Xu and Kasha 1992) are non-allelic. The RFLP data presented in the present paper (Fig. 2) using probe cMWG682 confirm the location of the gene in 81882/83 on the short arm of chromosome 2 (2I).

The negative results obtained using the dispersed (pSc119.1) and telomeric (pSc119.2) rye repeat sequences that hybridize with DNA of *H. bulbosum*, but not with DNA of *H. vulgare*, were not unexpected. Xu and Kasha (1992) did not detect an *H. bulbosum* introgression in the powdery mildew-resistant selection BC_1-2 using these two probes. Xu et al. (1990) also reported that pSc119.2 does not hybridize with the telomeric sites on every *H. bulbosum* chromosome arm and that two out of four *H. bulbosum* genotypes tested were heterozygous for the hybridizing sequence on chromosome 6 (6I). As the *H. bulbosum* introgressed DNA is located distally on chromosome 2 (2I)

in 81882/83, the utility of pSc119.1 for identifying recombinants (apart from the rare occasions when large alien DNA segments are transferred) will be limited. Therefore, AFLP analysis, which we used effectively in the present research, is an option for screening small numbers of putative recombinants.

Triploid seed formation associated with 81882/83 was overcome following backcrossing to barley. Triploidy appeared to be determined by the constitution of the egg since all stages of meiosis in PMCs after metaphase-I were normal, and triploid seeds were only observed on spikes of F_1 plants when 81882/83 was the female parent. Thus, 14-chromosome eggs probably arose from restitution nuclei which, after fertilization by 7-chromosome male gametes, resulted in triploid embryo formation. A similar phenomenon has been described previously in cv 'Paavo' (Finch and Bennett 1979).

Necrotic leaf blotching is, however, still associated with powdery mildew-resistance in 81882/83 despite making backcrosses to barley. If the necrosis is a pleiotropic effect of the transferred gene, comparable with the effects of *Mlo* resistance (Jørgensen 1992), then the severity may be reduced in a different genetic background (Jørgensen 1992). There is some evidence that this is the case since no necrosis was observed in the powdery mildew-resistant line 14/1/30.

Regarding the origin of 81882/83, we cannot say whether the introgression was effected by irradiation or normal homoeologous recombination. Successful gene transfer has been achieved using γ-irradiated seeds of a disomic addition line in which a pair of *Avena barbata* chromosomes were added to the complement of *A. sativa* (Aung et al. 1977). No recombinants were obtained using conventional screening procedures since chromosome pairing between the *A. barbata* and the *A. sativa* chromosomes was very low. In contrast, although homoeologous pairing between *H. vulgare* and *H. bulbosum* chromosomes can be relatively high (Thomas and Pickering 1985), recombinants have only rarely been obtained (e.g. Xu and Kasha 1992). Thus, the question of how the powdery mildew-resistance was introgressed into 81882/83 remains unanswered.

81882/83 was the first reported example of a powdery mildew-resistance gene being transferred from *H. bulbosum* into *H. vulgare* (Pickering et al. 1987). It has been regularly inoculated in the field and glasshouse with powdery mildew in Europe and New Zealand since its selection in 1983 and still shows good resistance. However, until a barley cultivar containing this '*H. bulbosum*' resistance is marketed, we can only speculate whether the resistance will be more durable than the genes presently available. As the gene has now been characterized, we propose that it is given the symbol *Mlhb* according to the rules for nomenclature. Seed of 81882/83 and backcrossed selections are available to interested breeders and researchers from the senior author.

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