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Transfer of a dominant gene for powdery mildew resistance and DNA from *Hordeum bulbosum* into cultivated barley (*H. vulgare*)

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Summary. In an attempt to transfer traits of agronomic importance from *H. bulbosum* into *H. vulgare* we carried out crosses between four diploid barley cultivars and a tetraploid *H. bulbosum*. Eleven viable triploid F_1 plants were produced by means of embryo rescue techniques. Meiotic pairing between *H. vulgare* and *H. bulbosum* chromosomes was evidenced by the formation of trivalents at a mean frequency of 1.3 with a maximum of five per cell. The resulting triploid hybrids were backcrossed to diploid barley, and nine BC_1 plants were obtained. Three of the BC_1 plants exhibited *H. bulbosum* DNA or disease resistance. A species specific 611-bp DNA probe, pSc119.2, located in telomeres of the *H. bulbosum* genome, clearly detected five *H. bulbosum* DNA fragments of about 2.1, 2.4, 3.4, 4.0 and 4.8 kb in size present in one of the BC_1 plants (BC_1-5) in *Bam*HI-digested genomic Southern blots. Plant BC_1-5 also contained a heterozygous chromosomal interchange involving chromosomes 3 and 4 as identified by N-banding. One of the two translocated chromosomes had the *H. bulbosum* sequence in the telomeric region as detected using in situ hybridization with pSc119.2. Two other BC_1 plants (BC_1-1 and BC_1-2) were resistant to the powdery mildew isolates to which the barley cultivars were susceptible. Seventy-nine BC_2 plants from plant BC_1-2 segregated 32 mildew resistant to 47 susceptible, which fits a ratio of 1:1, indicating that the transferred resistance was conditioned by a single dominant gene. Reciprocal crosses showed a tendency towards gametoselection that was relative to the resistance. Mildew resistant plant BC_1-2 also had a 1-kb *H. bulbosum* DNA fragment identified with a ten-base random primer using polymerase chain reaction (PCR). Forty-three BC_2 plants, randomly sampled from the 79 BC_2 plants, also segregated 23:20 for the presence versus absence of this 1-kb *H. bulbosum* DNA fragment, thereby fitting a 1:1 ratio and indicating that the PCR product originated from a single locus. The 1-kb DNA fragment and disease resistance were independently inherited as detected by PCR analysis of bulked DNA from 17 resistant and 17 susceptible plants as well as by trait segregation in the 43 individual plants. The progenies produced could serve as an important resistant source in plant breeding. This is the first conclusive report of the stable transfer of disease resistance and DNA from *H. bulbosum* to *H. vulgare*.

Key words: *Hordeum vulgare* – *H. bulbosum* – Powdery mildew resistance gene transfer – Genomic Southern blots – RAPD detection

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Introduction

Hordeum bulbosum L., a wild relative of cultivated barley, has many useful agronomic traits, including (1) haploid production by the preferential elimination of *H. bulbosum* chromosomes in the hybrids with cultivated barley (Kasha and Kao 1970); (2) winter hardiness and drought tolerance; (3) anther extrusion and self-incompatibility; (4) pest resistance. The latter includes resistance to powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* MARCHAL), yellow rust (*Puccinia striiforms* WEST), brown rust (*Puccinia hordei* OTTH), scab (*Fuarium* spp.), yellow mosaic virus, net blotch (*Pyrenophre teres*), scald (*Rhynchosporium secalis*) and Russian wheat aphid (*Diuraphis noxia*) (Xu and Snape 1989; S Wang personal communication; Pickering 1991 b; Kindler and Springer 1991). This makes *H. bulbosum* germ plasm attractive for incorporation and utilization in commerical barley breeding.

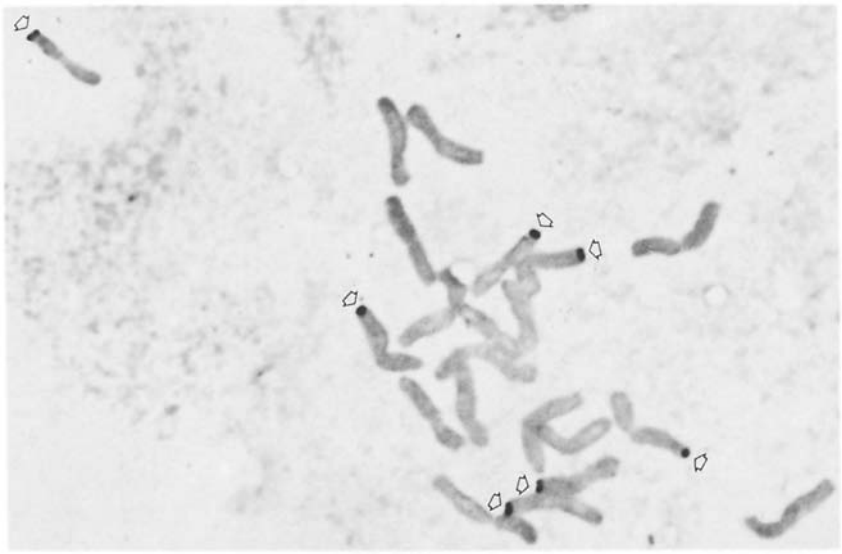

Fig. 1. In situ hybridization of a *H. bulbosum* species-specific rye DNA sequence, pSc119.2, to chromosomes of a triploid F_1 hybrid (2n = 3x = 21) between barley cv. 'Su Pie' and tetraploid *H. bulbosum* GBC141. Seven *H. bulbosum* chromosomes have hybridization sites in telomeres as indicated with *arrows*

Attempts to transfer agronomic traits from *H. bulbosum* to *H. vulgare* date back to 1934 when Kuckuck obtained the first interspecific hybrid by crossing *H. vulgare* (2×) with *H. bulbosum* (4×). Since then, a great number of papers have been published regarding the mechanism of chromosome elimination in the hybrids (Kasha 1974; Ho and Kasha 1975), applications of the *H. bulbosum* method for doubled haploid production in plant breeding and genetic analysis (Jensen 1976; Kasha and Reinbergs 1980; Choo et al. 1985; Snape 1987) and genome relationships between the two species (Kasha and Sadasivaiah 1971; Xu and Snape 1988). Homoeologous allosyndesis in diploid or triploid F_1 hybrids between the two species has been observed by many reseachers and has led to the belief that *H. bulbosum* and *H. vulgare* are the closest *Hordeum* relatives and share the same I genome (Bothmer et al. 1991). This homoeology could be expected to give rise to interspecific recombination. Although many BC_1 plants have been produced over the years in many laboratories, there is little evidence of the stable transfer of agronomic traits except for reports by Pohler and Szigat (1982) on the presence of *H. bulbosum* characters in cross progenies and by Pickering (1991 a) who proposed that he had obtained substitutions of *H. bulbosum* chromosomes into *H. vulgare*.

Among the reasons for the lack of success in obtaining the gene transfer from this distant hybridization, one in particular should be considered. Barley is a diploid crop plant and has less tolerance to genetic disturbance than polyploid species. Thus, alien chromatin segments, whenever transferred into cultivated barley, may need to be very small in size in order to be stably inherited. This transfer is usually beyond the power of the cytogenetic and morphological detection techniques that have been used in all previous studies. However, recently developed genetic markers at the DNA level, such as species-specific DNA probes, RFLPs, RAPDs, VNTRs or in situ hybridization, provide more sensitive approaches to the characterization of genetic polymorphism. In this paper we present conclusive evidence for the transfer of powdery mildew resistance and DNA from *H. bulbosum* into *H. vulgare* using disease tests and molecular cytogenetic techniques.

Materials and methods

Crosses

A tetraploid (2n = 4x = 28) *H. bulbosum* accession, GBC 141, was used as the male parent and four *H. vulgare* cvs: 'Su Pie', 'Chapais', 'Leger' and 'Bonanza', were used as female parents. 'Su Pie' is a two-rowed Chinese winter cultivar, and the other three are all six-rowed Canadian spring cultivars. The crossing procedures and embryo rescue techniques of Kasha and Kao (1970) were followed. The barley cultivars were used as the female to produce F_1 hybrids and BC_1 progenies, while reciprocal crosses with 'Su Pie' were used to produce BC_2 progenies.

Powdery mildew disease tests

Disease tests on parental materials, F_1 hybrids, BC_1 and BC_2 progenies were conducted using two powdery mildew isolates that are considered to be common in Ontario, Canada. The isolates were tentatively identified as having virulence against resistance genes *Ml-h* and *Ml-a* + *Ml-h*, respectively, based on their reactions on barley isogenic lines 'Algerian' (*Ml-a*), 'Hanna' (*Ml-h*), 'Kwan' (*Ml-k*), and 'Psaknon' (*Ml-p*) (J. Louter personal communication). In addition, the resistant BC_2 plants were tested against a mixture of isolates that were collected from naturally infected plants in a growth cabinet. All plants were tested at the five-to-eight leaf stage using the detached leaf method described by Wolfe et al. (1975). 'Bonanza' was used as the control in all tests since it is susceptible to all isolates of powdery mildew. Four replicates, two each from two separate tests, were used. Infection types were scored about 2 weeks after inoculation. The plants with a few growing myceliums were scored as

susceptible, while plants with necrosis and without growing myceliums were scored as resistant.

Southern blotting and in situ hybridization

Two rye repetitive DNA probes, pSc119.1 and pSc119.2, a gift from Dr. C. L. McIntyre of the Department of Agronomy, University of Missouri, USA, were used in genomic Southern blotting and in situ hybridization following the procedures described in our previous experiments (Xu et al. 1990). It was found that the two probes hybridized strongly to DNA of *H. bulbosum* and very weakly to DNA of the barley cultivars, thereby providing *H. bulbosum* species-specific DNA probes. The two probes showed different physical locations in the *H. bulbosum* genome (Xu et al. 1990) with pSc119.1 distributed predominantly in centromere regions with some interstitial sites and pSc119.2 located in only a few telomeric regions (Fig. 1). This apparent heterozygosity for pSc119.2 might be expected in this cross-pollinated species.

Random amplified polymorphic DNA (RAPD)

Eight ten-nucleotide random primers were used for amplification of genomic DNAs of 'Su Pie', GBC141, the F_1 hybrid and subsequent backcross plants. Genomic DNA for the amplifications was extracted from leaf disks using the techniques of Edwards et al. (1991), with the modification that two isopropanol precipitations were performed. The amplification conditions of Williams et al. (1990) were followed except that the concentration of primers was increased to 0.4 m*M*.

Results

Crossability, cytology and morphology

F_1 hybrids. The effect of barley cultivar genotype on the frequency of production of F_1 hybrids was clearly seen (Table 1). All 11 F_1 hybrid plants had an *H. bulbosum*-like appearance with a somatic chromosome number of 2n = 3x = 21, except for 1 plant with 2n = 3x = 20. Chromosome pairing at metaphase I was studied in 10 F_1 hybrids (Table 2). Homoeologous allosyndesis in the form of trivalents was observed in the cross 'Su Pie' × GBC 141 at a mean frequency of 1.2 trivalents and a maximum of 5 per cell and at a mean frequency of 1.5 trivalents and a maximum of 4 per cell in the cross 'Bonanza' × GBC 141. This homoeologous pairing provided a basis for the occurrence of interspecific recombination. Anthers of all F_1 hybrids looked shrivelled with little pollen shedding except for 1 plant. The latter F_1 hybrid plant (2n = 3x = 21) of 'Su Pie' × GBC 141 had large anthers that were extruded from the glumes at anthesis and shed pollen similar to its *H. bulbosum* parent. When used as pollinator in backcrosses with the 'Su Pie' parent, this plant gave rise to 94 BC_1 seeds. The seeds were classified into three groups: highly shrivelled, partially shrivelled and plump, their frequencies being 17.2%, 44.1% and 38.7%, respectively. Seed plumpness reflected variation in the degree of endosperm and embryo development in individual seeds. Only 7 out of the 94 BC_1 seeds germinated into plants, all from the plump seeds, two other BC_1 plants were produced from 5 BC_1 seeds derived from the backcross of 'Bonanza' × GBC 141 to 'Bonanza' (Table 1).

Table 1. Crossability of barley cultivars with tetraploid *H. bulbosum* GBC141 and subsequent backcross results

Diploid	Cross			Backcross		
Barley cultivar	Seeds/ florets	%	F_1 plants	Seeds/ florets	%	BC_1 plants
Su Pie	51/342	14.9	6	94/800	11.8	7
Leger	8/523	1.5	0			
Chapais	47/556	8.5	1 (died)			
Bonanza	341/624	54.7	5	5/1200	0.4	2

BC_1 plants. All nine BC_1 plants were morphologically similar to barley with a somatic chromosome number of 2n = 14. The seedlings had many tillers, and at the six-to-eight leaf stage each plant was split into eight pots. Five of the pots for each of the BC_1 plants were put in a growth room with uniform management to observe their growth and development, the other three were placed in a vernalization cabinet at 4 °C for long-term maintenance. No differences were found between barley cv 'Bonanza' and its two BC_1 plants. However, deviations were clearly observed between 'Su Pie' and three of its derived BC_1 plants. It took 94 days from sowing to heading of the first spikes in plants BC_1-1 and BC_1-5 in comparison with 80 days in the barley parent and 72–80 days in the remainder of the BC_1 plants. The percentages of tillers

Table 2. Meiotic chromosome pairing configurations in triploid hybrids produced from *H. vulgare* (2x) by *H. bulbosum* (4x) cross

Cross	Number of plants studied	Number of PMCs somatic number[a]	I	II			III	IV
				Total	Rods	Rings		
Su Pie × GBC141	6	330 M 20.3	5.3	5.7	0.8	4.9	1.2	0.01
		R 17–24	1–10	2–8	0–4	1–8	0–5	0–1
Bonanza × GBC141	4	168 M 20.9	5.5	5.3	0.7	4.7	1.5	0.01
		R 18–23	3–9	3–7	0–4	1–7	0–4	0–1

[a] M and R indicate mean and range, respectively.

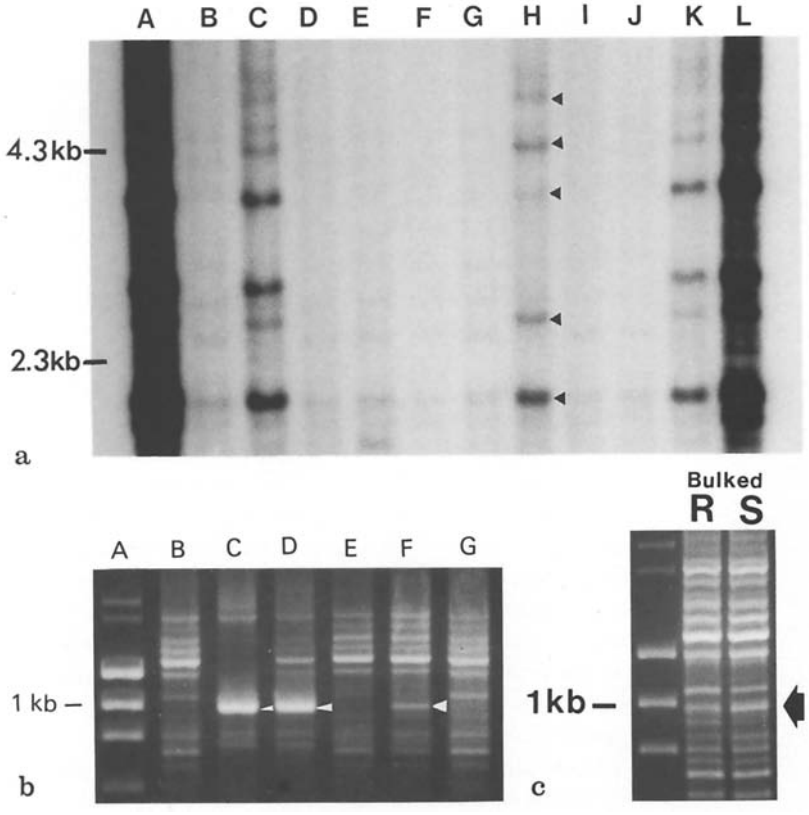

Fig. 2. a Southern blot hybridization of *Bam*HI-digested genomic DNA with a rye DNA sequence, pSc119.2. The order of the genotypes for *lanes A–C* are *H. bulbosum* GBC141, 'Su Pie' and the F_1 hybrid, respectively; lanes D–J are plants BC_1-1, -2, -3, -4, -5, -6 and -7, respectively; *lanes K* and *L* are the F_1 hybrid and *H. bulbosum* GBC141, respectively. Five *H. bulbosum* DNA bands (marked with *triangles*) of about 2.1, 2.4, 3.4, 4.0 and 4.8 kb in size are present in plant BC_1-5 (*lane H*). **b** and **c** Amplification of genomic DNA with a 10-nucleotide-random primer. **b** *Lane A* DNA marker, *lanes B–D*, 'Su Pie', *H. bulbosum* and the F_1 hybrid, respectively. *Lanes E–G* plants BC_1-1, -2 and -5. The *arrows* point to a polymorphic 1-kb band present in *H. bulbosum*, F_1 and plant BC_1-2 (*lane F*). **c** Amplification of bulked DNA from 17 resistant (*R*) or 17 susceptible (*S*) BC_2 plants. The *arrow* points to the 1-kb band present in both amplifications, indicative of no close linkage between the 1-kb DNA band and disease resistance

with headed spikes at 106 days after sowing in plants BC_1-1 , BC_1-2 and BC_1-5 were 1.3%, 10.7% and 2.6%, respectively, all clearly lower than in the barley parent (55.8%) and in the rest of BC_1 plants (26.1%–53.1%). The three BC_1 plants that showed a delay in their growth and development were identified as having *H. bulbosum* DNA or disease resistance in subsequent experiments.

All BC_1 plants but plant BC_1-5 showed the normal meiotic pairing configuration of seven bivalents at metaphase I as did in barley parent (data not shown). Plant BC_1-5 showed one quadrivalent in 97.8% of 92 PMCs examined, indicating that it had a reciprocal translocation in the heterozygous condition (Xu and Kasha 1992).

Genomic Southern blots and in situ hybridization

With probe pSc119.1, no *H. bulbosum* DNA was detected in all nine BC_1 plants in *Bam*HI-digested genomic Southern blots. However, probe pSc119.2 clearly hybridized to DNA of plant BC_1-5 (Fig. 2a). At least five DNA bands of about 2.1, 2.4, 3.4, 4.0 and 4.8 kb were seen in the Southern blotting experiment using this *H. bulbosum* species-specific probe. This probe was also in situ hybridized to both mitotic root-tip and meiotic cells of plant BC_1-5. Of the mitotic cells at interphase 30.3% (277/914) exhibited one hybridization site (Fig. 3a), while 18.7% (14/75) of the mitotic cells at metaphase showed one hybridization site in the telomeric region of one chromosome (Fig. 3b). The hybridization signals appeared to be stronger in less condensed chromosomes. In no case was more than one hybridization site seen in the mitotic cells studied, which indicates that one chromosome has a *H. bulbosum* DNA sequence in the telomeric region. When meiotic cells were studied, 11.3% (7/62) of the cells had one hybridization site, which was always in the quadrivalent, an indication that one of the translocated chromosomes carried this *H. bulbosum* sequence.

Mildew disease tests in parents, F_1, BC_1 and BC_2 plants

All four barley parents showed a high susceptibility to the mildew isolates used, whereas the *H. bulbosum* accession and the F_1 hybrids were highly resistant as has been previously reported for similar crosses (Xu and Snape 1989). This result suggests that resistance in *H. bulbosum* is conditioned by a dominant gene(s). Both of the BC_1 plants derived from the cross with 'Bonanza' were like the Bonanza parent, susceptible to all isolates. However, two of the seven BC_1 plants derived from the cross using 'Su Pie' exhibited resistance to powdery mildew. Plant BC_1-1 showed a resistance to the isolate with virulence against *Ml-h* but was susceptible to the isolate with virulence against *Ml-a* + *Ml-h*. Plant BC_1-2 was resistant to both of these isolates. In addition, all of the BC_1 plants

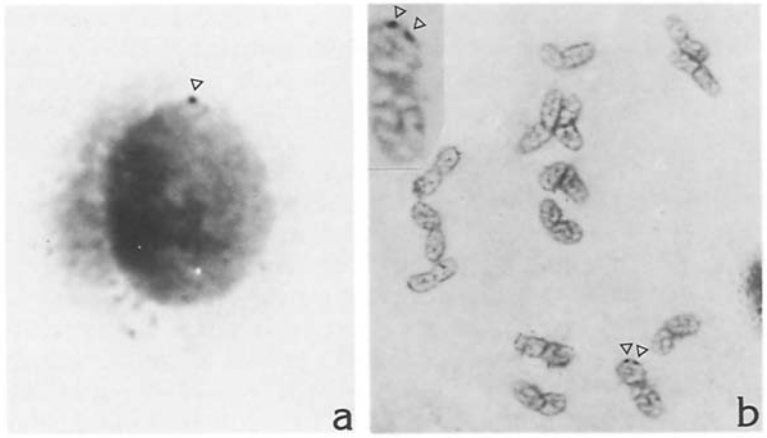

Fig. 3. In situ hybridization of probe pSc119.2 to chromosomes of a BC_1 plant (BC_1-5) with reciprocal translocation between chromosomes 3 and 4 and with *H. bulbosum* DNA sequence identified using pSc119.2 in genomic Southern blotting. All hybridization sites are indicated with *triangles*. **a** One site is in a somatic cell at interphase. **b** One chromosome has a hybridization site in one telomeric region, and the chromosome was enlarged and shown in the insert at *upper left-hand corner*

Table 3. Chi-square tests on reciprocal BC_2 segregation populations

Reciprocal crosses	Number of plants		Expected ratio [b]			
			1R:1S		3R:1S	
	R [a]	S [a]	χ^2	P	χ^2	P
Su Pie × BC_1-2	14	26	3.7	0.10–0.05	34.1	<0.005
BC_1-2 × Su Pie	18	21	0.2	0.75–0.50	17.3	<0.005
Total	32	47	2.9	0.10–0.05	50.1	<0.005

[a] R and S indicate resistant and susceptible, respectively
[b] One gene is expected to segregate 1:1 and two genes 3:1

but BC_1-2, which were kept at 4 °C for 11 months for long-term maintenance, showed severe disease symptoms on their leaves and stems as a result of infection by a mixture of isolates. Plant BC_1-2 showed brown necrotic spots, a typical hypersensitive reaction, on its leaves and stems but no mycelium growth. This observation agreed with the replicated results of disease tests on detached leaves.

In order to study the inheritance of the transferred *H. bulbosum* resistance, plant BC_1-2 was reciprocally backcrossed to its barley parent to produce BC_2 progenies. Seventy-nine BC_2 plants segregated 32 resistant to 47 susceptible, providing a good fit to a 1:1 ratio but not to a 3:1 ratio based on Chi-square tests (Table 3). This indicates that the resistance was probably conditioned by a single dominant gene. The results were consistent for both of the reciprocal BC_2 populations.

Amplification of genomic DNA using PCR

Genomic DNA of 'Su Pie', GBC141, the F_1 hybrids and the three BC_1 plants that were presumed to have *H. bulbosum* germ plasm were amplified using eight random primers. Amplifications were achieved for seven primers. Generally, five-to-ten amplified DNA bands were observed with each primer, these ranged from 0.5 to 1.3 kb in each of the experiments. Polymorphism in band pattern between barley and *H. bulbosum* was seen in all of the tests. The strong bands observed in both parents were always found in F_1 hybrids. A total of 57 scoreable bands were studied for the presence of *H. bulbosum* variation in the BC_1 plants. Amplification with primer ATC TGC GAG C showed that an 1-kb *H. bulbosum*-specific DNA band was present in the F_1 hybrid and backcross plant BC_1-2 (Fig. 2b). Since the BC_1 plant was disease resistant, the inheritance of the 1-kb *H. bulbosum*-specific band and its relationship with the resistance were examined. Forty-three BC_2 plants that were randomly sampled from the 79 disease-tested BC_2 plants segregated 23:20 for the presence or absence of the 1-kb *H. bulbosum* DNA band. This segregation fits a 1:1 ratio ($\chi^2=0.21$, P: 0.75–0.50) but not a 3:1 ratio ($\chi^2=10.61$, $P<0.005$), which proves that the DNA band originated from a single locus.

Linkage relationships were examined using two procedures. Bulked DNA from 17 resistant or 17 susceptible plants was amplified using this primer. The 1-kb DNA band was seen to haves the same intensity in both of the amplications (Fig. 2c), indicating that there was no detectable linkage between the DNA band and the transferred resistance. This was confirmed by segregation analysis of the DNA band (B+, B−) and the resistance (R, S) in 43 individual plants. The frequencies in the four classes were: 9 RB+ : 8 RB− : 14 SB+ : 12 SB−, which provides a good fit to a ratio of 1: 1 : 1: 1 ($\chi^2=2.12$, P:0.75–0.50). The conclusion is that the two transferred *H. bulbosum* 'traits' are independently inherited and that they both segregated normally.

Discussion

Our results unequivocally demonstrate for the first time the stable transfer of mildew resistance and *H. bulbosum*

DNA into barley. The success was largely due to two factors. First, a fertile triploid hybrid obtained from cross involving 'Su Pie' gave rise to 94 BC_1 seeds and seven viable BC_1 plants. One common feature about the interspecific cross is that most of the F_1 hybrid plants are male sterile and produce few seeds when selfing. Fertile hybrids with partially or fully dehiscent anthers have been occasionally encountered, but the derived progeny were without stably inherited *H. bulbosum* characters (Konzak et al. 1951; Lange and Jochemsen 1976; Pickering 1991 b). A variation in fertility among F_1 hybrids is expected because *H. bulbosum* is a cross-pollinated species and produces genetically heterozygous gametes. Again, gametes of high heterozygosity are expected in the F_1 hybrid plants as a consequence of genetic reshuffling at the chromosomal or DNA level. That 90% (90/99) of BC_1 seeds failed to germinate and produce viable plants is probably due to genetic disturbances that resulted from the addition of alien chromosomes and/or the replacement of barley chromatin. It seems reasonable to assume that *H. bulbosum* chromatin present in the viable progenies is either very small in size, functionally neutral, or functionally compensating for missing barley chromatin. Second, the methods used in this experiment – including disease tests, in situ hybridization, Southern blotting and PCR – made it possible to detect the presence of small amounts of *H. bulbosum* germ plasm. Two *H. bulbosum* species-specific probes with different physical locations generally cover the whole *H. bulbosum* genome (Xu et al. 1990). If we assume that each of the amplified DNA bands originated from a different locus and that all of the loci are randomly distributed throughout the genome, a total of 57 amplified bands spread over the seven barley chromosomes should average about eight loci per chromosome. The application of these molecular techniques should provide a good chance of identifying foreign DNA in barley chromosomes.

The utilization of germ plasm from wild *Hordeum* species has been largely limited in barley breeding for two main reasons, as pointed out by Bothmar et al. (1991). First, most of the wild *Hordeum* species are distantly related to cultivated barley, which makes the interspecific cross difficult to achieve. Second, diploid cultivated barley is very sensitive to genetic imbalance. Our success in the transfer of a gene for powdery mildew resistance and DNA from *H. bulbosum* to cultivated barley leads us to conclude that pest resistances in the wild *Hordeum* species have the potential to be transferred and utilized in plant breeding at a high success rate since most of the resistances are controlled by single genes.

The question of whether or not the transferred resistance will be stable was tested by producing reciprocal BC_2 progeny. While the trend was in the direction of gametoselection when BC_1-2 was used as the male parent, the deviation was not significant (Table 3). Therefore, there should be no difficulty in using those materials in breeding studies since recombination should continue to reduce the extent of linkage drag around the transferred mildew resistance gene. Subsequent generations will be grown to test this theory and also to test the expression of the mildew resistance.

Differences in resistance types between the *H. bulbosum* parent and the derived resistant barley plants were noticed. *H. bulbosum* showed no infection symptoms while resistant barley plants showed necrotic spots, a typical hypersensitive reaction. It is most likely that different defense mechanisms operate in the two genetic constitutions. Resistance in *H. bulbosum* prevents the sporulation of conidia at a very early stage of the infection, whereas the derived resistant plants allowed the initial development of the pathogen. This initial development includes germination of conidia, formation of appressoria and penetration of the host cell wall, all of which trigger the defense system into forming necrotic areas that stop pathogen development. There are two possible genetic reasons for the differences. First, there could be many mildew resistance genes or a gene family acting in cooperative manner in *H. bulbosum* that prevents the pathogen from development at all stages. In contrast, when only one of these resistance genes was transferred into cultivated barley, its action was not as strong as the gene family. Second, this one dominant gene from *H. bulbosum* reacts differently in the resistant plants because of the new genetic background.

At least 15 barley powdery mildew resistance gene loci have been identified and, 12 of them have been mapped on chromosomes. There are eight loci (*Ml-a, Ml-k, Ml-at, Ml-p, Ml-nn, ml-d, Ml-ra* and *Ml-(Ga)*) on chromosome 5 (Jensen 1988; Doll and Jensen 1986; Hossain and Sparrow 1991), three loci (*Ml-g, Ml-(cp)* and *ml-o*) on chromosome 4 (Jensen 1987; Wiberg 1974), and one locus (*Ml-h*) on chromosome 6 (Hayashi and Heta 1985). Most of the identified resistance genes were effective for only a few years when used on a commercial basis before being overcome by mutant isolates of the pathogen (Hossain and Sparrow 1991). The *H. bulbosum* accession used here has shown resistance for many years to many powdery mildew isolates in Europe, China and North America, which indicates that it carries a highly durable type of the resistance. It is most likely that the transferred resistance gene is a new one in barley and might be expected to be more durable than previously identified resistance genes, although this needs to be confirmed.

Our results using PCR amplification with bulked DNA for the detection of linkage illustrate the time-saving aspect of this technique for identifying DNA markers linked to disease resistance genes in comparison to individual plant DNA studies, both here and as previously reported by Martin et al. (1991). Efforts will continue to

find DNA markers tightly linked to the transferred mildew resistance gene.

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