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Inheritance of resistance to covered smut in barley and development of a tightly linked SCAR marker

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Abstract Inheritance of resistance to covered smut in the barley line Q21861 was studied using a doubled-haploid population produced by crossing Q21861 with the line SM89010. Based on 3 years of screening in the field and two seasons in the greenhouse, segregation for resistance/susceptibility fits a one-gene ratio, indicating a single major gene for resistance in Q21861. Of 440 random 10-mer primers tested using bulked segregant analysis, one primer (OPJ10) resulted in a reproducible polymorphic band. RAPD marker $OPJ10_{450}$ co-segregated in repulsion with the covered smut resistance. This marker was converted to a sequence-characterized amplified region (SCAR) marker linked in coupling (5.5 cM) with the covered smut resistant gene in Q21861. The SCAR marker was amplified in the line TR640 which is also resistant to covered smut, but not in the other resistant lines. The SCAR marker will be useful for marker-assisted selection for covered smut in barley breeding programs.

Keywords Barley · *Ustilago hordei* · Disease resistance · RAPD · SCAR

Introduction

Covered smut of barley (*Hordeum vulgare* L.) caused by *Ustilago hordei* (Pers.) Lagerh. occurs everywhere that barley is grown (Mathre 1997). The disease results in yield reductions ranging from 0.2 to 0.8% in Western Canada (Thomas and Menzies 1997). Economic loss is not only due to decreased yield but also to contamination of healthy seeds with black teliospores (Mathre 1997). Covered smut can be effectively controlled by fungicidal seed treatments, by sowing disease-free seed and by growing resistant cultivars. Seed treatment with fungicides is very effective but the producer must incur additional costs. In addition, the pathogen may become resistant to fungicides (Ben-yephet et al. 1975). Induced mutants of *U. hordei* tolerant to four different fungicides have been reported (Ben-yephet et al. 1975; Henry et al. 1987). Similarly, carboxin- and fenfuram-resistant strains of true loose smut (*Ustilago nuda* (Jens.) Rostr.) have been reported on winter barley crops in France (Leroux and Berthier 1988). Resistant cultivars are generally recognized as the most-economical and preferred method of control.

Understanding the inheritance of resistance to disease is valuable for planning crosses in breeding programs, identifying resistant genes and developing genetic markers to assist in selection. There are few reports on the inheritance of resistance to covered smut (Johnston 1934; Shands 1956; Wells 1958; Metcalfe 1962; Cherewick and Buchannon 1969). Johnston (1934) studied the inheritance of resistance in a cross between cultivars Trebi (moderately susceptible) and Glabron (resistant) but could not explain the genetics of resistance because of unsatisfactory infections from artificial inoculations. Other studies have reported a single dominant gene, two dominant genes or a recessive gene in different resistant cultivars/lines (Shands 1956; Wells 1958; Metcalfe 1962; Cherewick and Buchannon 1969). The different sources of resistance have different levels of expression and the level of expression may vary under different environmental conditions (Thomas 1988). Most of these early studies used segregating early generations (F_2 plants or F_3 families) to study the inheritance of resistance. However, it is often very difficult to interpret results in such genotypes because of heterozygosity and segregation, the inability to replicate such genotypes and the possibility of disease escapes. The use of homozygous lines removes heterozygosity as a source of variation and repeat testing can be carried out to confirm disease reaction (Knox et al. 1998).

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Breeding for resistance to covered smut may not be routinely performed because screening is time consuming and requires considerable resources. Plants need to be grown almost to maturity before symptom development occurs which is space and labor consuming, limiting the size of a breeding program. In addition, infection is inconsistent and even in highly susceptible lines some disease-free plants will occur (Willits and Sherwood 1999). Techniques that aid in selection for resistance, such as molecular markers, would be of value to the breeding program.

Molecular markers facilitate breeding for disease resistance as they can be used to screen material at any stage of growth and are not influenced by the environment. The use of the random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland 1990; Williams et al. 1990) combined with the bulked segregant analysis (BSA) technique (Michelmore et al. 1991) has been successfully used to tag major resistance genes in barley (Barua et al. 1993; Poulsen et al. 1995; Kutcher et al. 1996; Eckstein et al. 2000b) and several other crops (Kelly 1995). For extensive and efficient use in marker-assisted selection (MAS) programs RAPD markers need to be further developed. The short random primers used in RAPD analysis usually anneal with multiple sites in different regions of the genome and thus may amplify several genetic loci. In addition, the RAPD technique is sensitive to reaction conditions, which results in poor reproducibility. To overcome the problems associated with RAPD markers and to improve their utility in MAS, longer primers have been developed from RAPD fragments (Paran and Michelmore 1993). These longer primers generate a sequence-characterized amplified region (SCAR), which can be particularly useful to follow the inheritance of the marked region of the genome. SCAR markers are preferred over RAPD markers as they detect only a single locus, their amplification is less sensitive to reaction conditions, and they can potentially be converted into allele-specific markers. SCAR markers have been developed for many crops including barley (Deng et al. 1997; Hernandez et al. 1999; Eckstein et al. 2000a, b).

In this study, we report on the inheritance of resistance to covered smut in a doubled-haploid barley population. We describe the identification of a RAPD marker linked to covered smut resistance using bulked segregant analysis and its conversion into a SCAR marker.

Material and methods

Plant material

One hundred and twenty seven anther-culture derived doubled-haploid lines from the cross Q21861 x SM89010 were used for this study. Q21861, a barley accession of unknown pedigree from CIMMYT, Mexico, is resistant to covered smut and SM89010 (a barley line from the Crop Development Centre, University of Saskatchewan, Saskatoon, malting barley program) is moderately susceptible. CDC Candle, a hulless cultivar, was used as a susceptible check in all experiments.

The parents of the following populations from the Crop Development Centre Feed Barley breeding program were screened against *U. hordei* in the greenhouse and field: HB328/TR244, CDC Dolly/RFLP Harrington, Baronesse/TR336, SB93763/TR246, CDC McGwire/TR640, HB334/TR640, HB348/TR255, CDC Candle/Q21861, CDC McGwire/Q21861.

Inoculations, field and greenhouse experiments

For inoculation with covered smut the spore suspension technique of Tapke and Bever (1942) was used with some modifications. Thirty grams of seed of each line were inoculated with a mixture of isolates of *U. hordei* (supplied by Dr J. Menzies, Agriculture and Agri-Food Canada, Winnipeg). The barley seeds were shaken vigorously for 30 s with a spore suspension consisting of 1 g of teliospores/l of distilled water, followed by incubation at room temperature for 15 min. The suspension was decanted, the seeds were dried on paper towels at room temperature and either planted immediately or stored at 4°C for up to 2 weeks and planted at convenience.

Field experiments were conducted in the summers of 1998, 1999 and 2000 at the Preston Plots, University of Saskatchewan, Saskatoon. In 1998 and 1999, all 127 lines of the Q21861 x SM89010 cross and parents were screened against covered smut. A highly susceptible cultivar CDC Candle was used as a check. Seeding was done at the end of May and there were four replications of each line. Each replicate consisted of one row (4 m in length) of each line, and five to eight rows of each parent and check in a randomized complete block design. In the summer of 2000, only 72 putative resistant lines (those that had shown less than 2% infection in 1999) were screened in the field along with the parents and check. The same 72 lines and also seven susceptible lines, along with the parents and check were screened in the greenhouse during the spring of 2000. Sixty nine lines that exhibited less than 12% infected plants in the first greenhouse test were screened again in the greenhouse in summer of 2000. There were three replicates of each genotype. Each replicate consisted of one pot, and five seeds were planted in each pot.

Disease evaluation

In the field, the level of covered smut infection was evaluated as the percentage of infected heads. Two 1-m sections were measured within each row. In each 1-m section, counts of the total number of heads (average of 100 heads per 1-m section, with no less than 50) and number of infected heads were conducted. The two counts were averaged for each replicate and the percentage of infected heads was calculated. A cut-off of 2% covered smut-infected heads was used to classify the population (less than 2% infected heads=resistant; greater than 2% infected heads=susceptible). The cut-off was established after reviewing the disease score of the parents and the population.

In the greenhouse, the level of covered smut infection was evaluated as the percentage of infected plants. A plant showing one or more smutted heads was considered to be infected. In each pot, the numbers of infected plants and total plants were counted and the percentage of infected plants was calculated. A cut-off of 12% covered smut-infected plants was used to classify the population (less than 12% infected plants=resistant; greater than 12% infected plants=susceptible). This cut-off was established after reviewing the disease score of the parents and the population.

Bulked segregant analysis with RAPD

DNA was extracted using the CTAB method of Procunier et al. (1990) with minor modifications. The extraction was scaled down 15-fold and the tissue was ground directly in CTAB extraction buffer in a 1.5-ml Eppendorf tube without liquid nitrogen. Based on disease evaluation, covered smut resistant- and susceptible-

bulks were created each consisting of equal amounts of DNA from six individuals from resistant (Q/SM- 29, 36, 38, 47, 84 and 99) and susceptible (Q/SM- 48, 67, 68, 79, 136 and 158) lines. Four hundred and forty 10-mer random primers obtained either from the Biotechnology Laboratory, University of British Columbia, Canada, or Operon Technologies Inc., California, USA, were used for RAPD analysis of the two DNA bulks. Each reaction was performed in a total volume of 25 µl, containing 100 µM each of dATP, dTTP, dCTP and dGTP (Gibco BRL, Bethesda, USA), 0.6 µM of primer, 50 ng of genomic DNA, 2 mM of $MgCl_2$, 1 unit of *Taq* DNA polymerase (Gibco BRL) and 1×PCR buffer (Gibco BRL). Amplification was performed with a GeneAmp thermocycler (Perkin Elmer, USA) and consisted of an initial denaturation step at 94°C for 3 min, followed by 40 cycles as follows: a denaturation step at 94°C for 1 min, an annealing step at 32°C for 1 min and an extension step at 72°C for 1.5 min; ending with an extension period at 72°C for 10 min. All amplification products were separated on a 1% agarose, 0.5×TBE gel, stained with ethidium bromide and viewed under ultra-violet light.

Any primers which amplified a polymorphic DNA fragment between the bulks were then used to amplify DNA from the parents and the individual genotypes constituting the bulks. The one primer (OPJ10) that resulted in a reproducible polymorphism was used to screen the entire Q/SM population.

Cloning and sequencing of RAPD products

The polymorphic amplified product of OPJ10 produced in the susceptible genotype was excised from the agarose gel and a TA Cloning Kit (Invitrogen corporation, USA) was used for cloning, as described by the manufacturer. The recombinant plasmids were transformed into *Escherichia coli* and plated on selective media containing ampicillin and X-gal. White colonies were picked from the plate and cultured overnight. The Wizard Miniprep Kit (Promega, USA) was used for plasmid DNA extraction. To confirm the presence of the insert, PCR and restriction enzyme digestion were performed.

Sequencing of the DNA insert was performed at the Plant Biotechnology Institute, Saskatoon, SK, Canada. The sequences were aligned with the aid of the software program DNASTAR (Lasergene, USA).

SCAR design and analysis

Two primers were developed from the OPJ10 amplified sequence but there was no polymorphism between resistant and susceptible genotypes. A major band was produced in all susceptible and resistant genotypes. The bands produced in the resistant and in the susceptible genotypes were cloned and sequenced. The sequences from resistant and susceptible genotypes were aligned and compared with the aid of the computer program DNAMAN (Lynnon-Biosoft, USA), and two SCAR primers were synthesized to amplify a major band only from the resistant genotype. The sequences of the two SCAR primers were:

ULR1-F GATAAGGATGTTCCGCC, and
ULR-R CCCGAGGTCCAAAATCAG.

Amplification was performed in a total volume of 25 µl, containing 100 µM of each of dATP, dTTP, dCTP and dGTP (Gibco BRL, Bethesda, USA), 200 nM of each primer (ULR1-F and ULR-R), 50 ng of genomic DNA, 2 mM of $MgCl_2$ (Gibco BRL), 1 unit of *Taq* polymerase (Gibco BRL) and 1× Gibco BRL buffer (50 mM KCL, 20 mM Tris-HCL, pH 8.4). Amplification was performed with an Amplitron II thermocycler (Barnstead/Thermolyne, USA) and consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of: denaturation at 94°C for 1 min., annealing at 64°C for 1 min and an extension at 72°C for 1.5 min; ending with an extension at 72°C for 10 min. Amplified products were separated on a 1.25% agarose, 0.5×TBE gel, stained with ethidium bromide and viewed under ultra-violet light.

The SCAR primers were used to screen the entire Q/SM population and compared to the results obtained with OPJ10. These primers were also used to screen the parents of other crosses segregating for covered smut resistance (see earlier). With a view to mapping the marker and resistance, parents of four barley map-

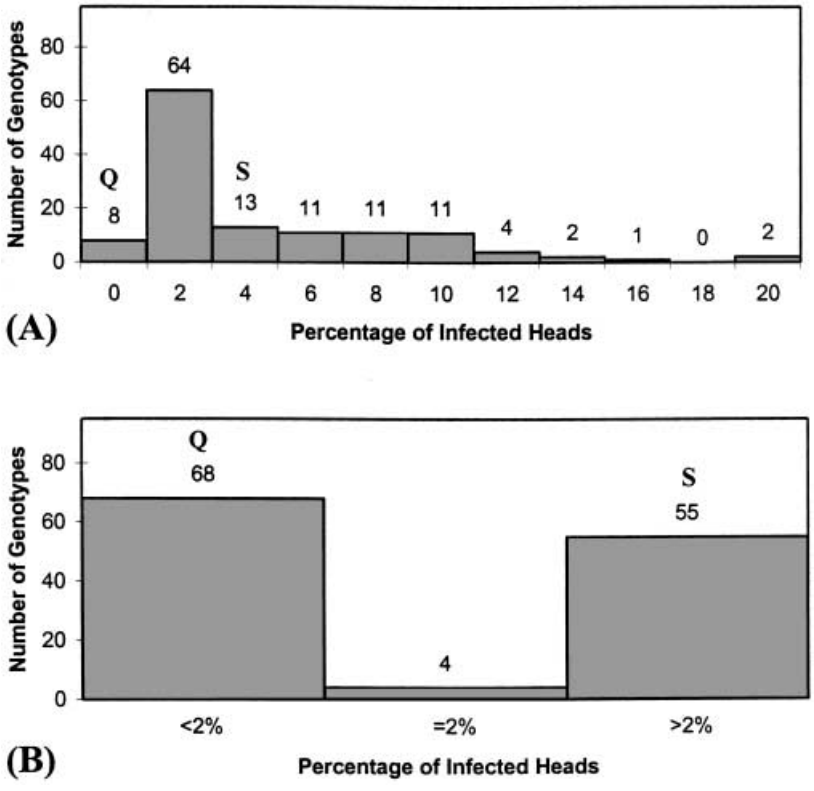

Fig. 1A, B Frequency distribution of the percentage of covered smut infected heads for the 127 lines of the doubled-haploid Q21861×SM89010 population (Q=Q21861; S=SM89010) evaluated at Preston Plots, University of Saskatchewan, August 1998, 1999. (**A**) Lines grouped at intervals of 2 for the percentage of infected heads. (**B**) Lines grouped at less than 2% infected heads, 2% infected heads and greater than 2% infected heads

Table 1 Covered smut incidence of parents, the check and the 72 putative resistant lines in field and greenhouse tests, and the presence/absence of the SCAR marker

Line	% Infected heads in field			% Infected plants in greenhouse		SCAR marker[a]	Line	% Infected heads in field			% Infected plants in greenhouse		SCAR marker[a]
	1998	1999	2000	Spring 2000	Summer 2000			1998	1999	2000	Spring 2000	Summer 2000	
CDC Candle	NT[b]	46.15	33.02	62.50	54.54	No	Q/SM-78	2.30	1.25	0.00	0.00	11.11	No
Q21861	0.40	0.25	0.10	0.00	7.14	Yes	Q/SM-84	0.00	1.21	0.17	0.00	0.00	Yes
SM89010	4.78	2.17	1.49	36.36	20.00	No	Q/SM-85	0.00	0.00	0.00	0.00	0.00	Yes
Q/SM-1	0.00	0.13	0.14	0.00	0.00	Yes	Q/SM-89	0.00	0.23	0.00	0.00	0.00	Yes
Q/SM-2	0.26	0.65	0.10	0.00	0.00	Yes	Q/SM-91	0.00	0.24	0.12	0.00	0.00	Yes
Q/SM-4	0.00	0.48	0.00	0.00	0.00	Yes	Q/SM-93	1.38	0.07	0.11	9.09	0.00	Yes
Q/SM-5	0.00	0.00	0.00	0.00	0.00	Yes	Q/SM-97	0.30	0.00	0.77	0.00	0.00	Yes
Q/SM-10	0.79	0.52	0.33	28.57	NT	No	Q/SM-99	0.00	0.19	0.00	0.00	0.00	Yes
Q/SM-11	0.48	0.08	0.00	0.00	0.00	Yes	Q/SM-101	1.14	0.00	0.00	0.00	0.00	Yes
Q/SM-12	0.44	0.43	0.25	0.00	0.00	No	Q/SM-105	0.00	0.00	0.00	0.00	0.00	Yes
Q/SM-13	0.12	1.61	1.09	10.00	0.00	Yes	Q/SM-108	0.57	0.00	0.11	0.00	0.00	Yes
Q/SM-14	0.54	0.65	0.46	0.00	0.00	Yes	Q/SM-110	0.18	0.00	1.84	0.00	0.00	Yes
Q/SM-15	0.10	1.81	0.00	9.09	0.00	Yes	Q/SM-111	0.27	0.56	0.12	0.00	0.00	Yes
Q/SM-16	0.30	0.33	0.08	0.00	0.00	Yes	Q/SM-113	1.28	0.11	1.95	0.00	0.00	Yes
Q/SM-17	0.00	0.00	0.09	0.00	0.00	Yes	Q/SM-115	1.63	1.65	0.00	0.00	0.00	Yes
Q/SM-18	0.49	0.00	0.23	0.00	0.00	Yes	Q/SM-116	0.00	0.44	0.40	0.00	0.00	Yes
Q/SM-19	0.29	0.33	0.00	0.00	0.00	Yes	Q/SM-117	0.15	0.00	0.07	0.00	0.00	Yes
Q/SM-24	1.34	0.52	1.04	9.09	0.00	Yes	Q/SM-118	0.00	0.00	1.12	9.09	0.00	Yes
Q/SM-28	1.59	1.10	0.58	20.00	NT	Yes	Q/SM-119	0.00	0.14	0.23	0.00	0.00	Yes
Q/SM-29	0.00	0.60	0.36	0.00	0.00	Yes	Q/SM-120	0.00	0.00	0.28	0.00	0.00	Yes
Q/SM-30	2.97	0.89	0.39	0.00	0.00	Yes	Q/SM-122	0.83	0.00	0.21	0.00	0.00	Yes
Q/SM-31	0.00	0.08	0.00	0.00	0.00	Yes	Q/SM-123	0.20	0.00	0.00	0.00	0.00	Yes
Q/SM-35	0.00	0.00	0.00	0.00	0.00	Yes	Q/SM-125	0.81	0.19	0.64	0.00	0.00	Yes
Q/SM-36	0.00	0.47	0.00	0.00	0.00	Yes	Q/SM-126	0.12	0.00	0.24	0.00	0.00	Yes
Q/SM-38	0.00	0.00	0.00	0.00	0.00	Yes	Q/SM-128	0.00	0.00	0.17	0.00	0.00	Yes
Q/SM-39	0.50	0.13	0.00	0.00	0.00	No	Q/SM-129	0.86	0.16	0.00	11.11	0.00	Yes
Q/SM-42	0.00	0.07	0.00	0.00	0.00	Yes	Q/SM-134	8.02	0.00	0.37	0.00	0.00	Yes
Q/SM-45	0.14	0.00	0.10	0.00	0.00	Yes	Q/SM-137	0.00	0.32	0.00	0.00	0.00	Yes
Q/SM-47	0.00	0.42	0.00	0.00	0.00	Yes	Q/SM-138	0.29	0.00	0.18	0.00	0.00	Yes
Q/SM-49	0.94	0.15	0.10	0.00	0.00	Yes	Q/SM-139	0.68	0.63	0.47	0.00	0.00	Yes
Q/SM-52	0.49	0.00	0.15	0.00	0.00	Yes	Q/SM-141	0.10	0.79	0.00	18.18	NT	Yes
Q/SM-56	0.70	1.05	0.13	0.00	0.00	Yes	Q/SM-142	1.63	0.00	0.10	0.00	0.00	Yes
Q/SM-57	1.56	1.19	1.81	16.67	NT	No	Q/SM-143	0.24	0.00	0.11	0.00	0.00	Yes
Q/SM-58	0.78	0.10	0.25	8.33	0.00	Yes	Q/SM-146	0.00	0.46	0.14	0.00	0.00	Yes
Q/SM-63	1.21	0.22	0.71	0.00	0.00	Yes	Q/SM-149	0.13	0.42	1.20	10.00	0.00	Yes
Q/SM-71	0.25	0.00	0.00	0.00	0.00	Yes	Q/SM-156	0.37	0.00	0.00	0.00	6.67	Yes
Q/SM-76	0.52	0.47	0.00	0.00	0.00	Yes	Q/SM-157	0.15	0.00	0.00	0.00	0.00	Yes
Q/SM-77	0.14	0.18	0.24	0.00	0.00	Yes							

[a] Yes=amplified band of 442 bp was present; No=band not present [b] NT=not tested

ping populations (Steptoe/Morex, TR306/Harrington, Igri/Franka, Proctor/Nudinka) were also screened with the primers.

Analysis of data

A chi-square test was used to check the fit of segregation classes. The linkage distance between the markers and the covered smut resistance gene was calculated, using percent recombination, and expressed as a recombination percentage.

Results

Inheritance study

Based on the 1998 and 1999 field data, 72 of the 127 lines in the Q/SM population were putatively classified as resistant (with less than 2% covered smut infected heads) (Fig. 1). Lines that could be clearly designated as susceptible after the first 2 years of testing were not re-tested. To confirm the resistance of the 72 lines they were tested in the greenhouse in the spring of 2000 and again in the field in the summer of 2000. Those lines that exhibited less than 12% infected plants in the greenhouse screening were re-tested in the greenhouse during the summer of 2000. The results of five separate tests on these 72 lines are summarized in Table 1. On the basis of field and greenhouse tests, 5 of the 72 lines (Q/SM-10, 28, 57, 78 and 141) previously classified as resistant were declared susceptible. Based on this classification, the resistant and susceptible lines segregated in the ratio 67:60, not differing significantly from a 1:1 ratio (χ^2=0.38; P=0.50–0.75), indicating segregation for a single major gene.

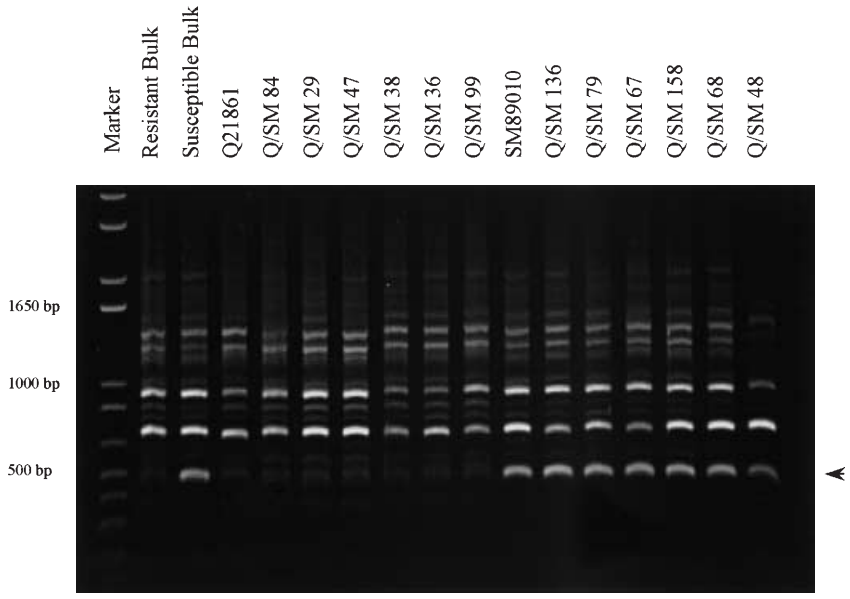

Fig. 2 DNA amplification from the covered smut-resistant bulk, susceptible bulk, resistant parent (Q21861), individuals of the resistant bulk, susceptible parent (SM89010) and individuals of the susceptible bulk with the RAPD OPJ 10 primer (*arrow* indicates polymorphic band)

The reaction of the parents of different populations to *U. hordei* (Table 2) indicated that most of the populations would be segregating for covered smut resistance. TR244, CDC Dolly, TR246, TR640 and TR255 exhibited good levels of resistance to covered smut in the field and greenhouse.

Table 2 Covered smut incidence of parents of different breeding populations in field and greenhouse tests and presence/absence of SCAR marker

Cultivar/Line	% Infected heads in field	% Infected plants in greenhouse	Scar marker[a]
TR244	1.21	11.01	No
HB328	14.60	82.37	No
CDC Dolly	0.86	5.49	No
RFLP Harrington	13.10	67.78	No
Baronesse	7.31	65.38	No
TR336	1.20	26.08	No
SB93763	3.24	60.76	No
TR246	1.72	7.40	No
CDC McGwire	2.52	28.57	No
TR640	0.00	0.00	Yes
HB334	0.69	45.45	No
HB348	3.50	5.00	No
TR255	0.00	NT[b]	No
Q21861	0.25	3.57	Yes
CDC Candle	36.60	80.97	No

[a] Yes=amplified band of 442 bp was present; No=band not present
[b] NT=not tested

RAPD analysis

Of the 440 10-mer random primers tested, 97 (22%) produced no amplification product or unscoreable products seen as a smear or weak banding. A number of primers resulted in polymorphism between the bulks; however, when utilized to screen the parents and individuals constituting the bulks the polymorphic bands were either non-reproducible or not present in the parents or in a majority of the individuals in the bulks. However, the OPJ10 primer (AAGCCCGAGG) resulted in a reproducible bright polymorphic band of about 450 bp present in the susceptible bulk, the susceptible parent and each individual of the susceptible bulk (Fig. 2). The entire population was screened with the OPJ10 primer. Seven of 127 individuals (Table 3) were recombinants between the marker and the resistance gene, suggesting a linkage distance of 5.5 cM.

Development of the SCAR marker

The polymorphic band amplified by the primer OPJ10 was excised, cloned and sequenced. Two primers were developed from the amplified sequence but the same-sized band was amplified in both the susceptible and resistant parent. The band present in the resistant and susceptible genotypes was cloned and sequenced. Several primers complementary to the ends of the fragments

Table 3 The relationship between the OPJ10 RAPD polymorphism and covered smut rating

		Disease rating		
		Resistant	Susceptible	Total
DNA polymorphism Pattern	Resistant	65	5	70
	Susceptible	2	55	57
	Total	67	60	127

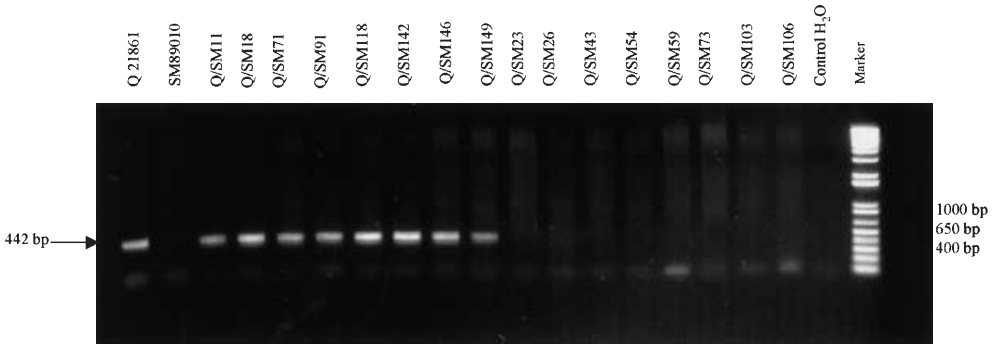

Fig. 3 Amplification product (442 bp) obtained with SCAR primers ULR1-F and ULR-R; present in resistant parent (Q21861) and resistant lines, and absent in susceptible parent (SM89010) and susceptible lines

were designed based on nucleotide differences between the two sequences. These were synthesized and tested in various combinations until a single band was produced in the resistant genotype only. The primer ULR1-F (GATAAGGATGTTCCGCC) was designed based around three nucleotide differences between the sequences of fragments from the resistant and susceptible genotypes, including the two 3′ Cs. This primer, in combination with primer ULR-R (CCCGAGGTCCAAAATCAG) which was based on an area of sequence homology between fragments of resistant and susceptible genotypes, yielded a major band of expected size (442 bp) in the resistant genotypes but not in the susceptible genotypes (Fig. 3). The polymorphic banding pattern was reproducible and the entire population was screened with these SCAR primers. The SCAR marker was found to co-segregate fully in repulsion with the OPJ10 RAPD marker.

The parents of other populations segregating for covered smut resistance and of many mapping populations of barley were screened with these SCAR primers. The 442-bp band was amplified in only Q21861 and the line TR640 and was absent in all other parents. TR640 was observed to be resistant to *U. hordei* in the field and greenhouse (Table 2).

Discussion

Screening for covered smut resistance in a breeding program is difficult, as infection by artificial inoculation is inconsistent (Willits and Sherwood 1999). The occurrence of escapes, variable disease incidence and absence of complete resistance in the resistant parent may cause difficulty in classifying segregating populations with respect to disease reaction, particularly in early segregating generations. The doubled-haploid population used in this study helped to overcome these problems in that the homozygous lines could be repeatedly tested without concern for variation arising through segregation.

In the greenhouse, infection ratings were based on the percentage of infected plants, while ratings in the field were based on the percentage of infected heads in a length of row, as individual plants could not be distinguished. A Pearson correlation was used to analyze the relationship between the percentage of infected heads and the percentage of infected plants. The percentage of infected heads and the percentage of infected plants were significantly correlated (r=0.73, P=0.000). Resource considerations did not allow single plant spacing to be used in the field. Woodward and Tingey (1941) found that when disease ratings of covered smut of barley were based on plant counts as opposed to head counts, a much higher percentage rating was obtained. This was usually the case in the present experiments (Tables 1 and 2) because often the first heads to emerge were free of infection even in susceptible plants. As every head was not infected, plant counts resulted in a higher percentage infection compared to head counts. Single-plant evaluation would be preferable to row evaluation if adequate plant numbers could be grown. However, in resistant lines, although no infected plants were found among the few plants that could be grown in the greenhouse, a few infected plants were observed among the much larger population grown in the field. As rating the disease infection as the percentage of infected plants (as opposed to the percentage of infected heads) resulted in higher levels of infection, this also resulted in a clearer delineation between resistant and susceptible lines.

Resistance in the resistant parent (Q21861) was not complete. Very low levels of infection were observed in both field and greenhouse studies (Table 1). Person and Cherewick (1964) also reported that resistance to *U. hordei* in barley was incomplete. The susceptible parent of the cross (SM89010) showed much less infection (1.49–4.78% in the field and 20–36% in the greenhouse) than the susceptible check CDC Candle (33.02–46.15% in the field and 55–63% in the greenhouse) possibly indicating some minor gene(s) for covered smut resistance in SM89010. However, the susceptible check was a hulless cultivar and hulless cultivars have been found to be more susceptible to covered smut (Ardiel 2000). CDC Candle was the most-susceptible cultivar to the source of inoculum used in this study. The high level of infection served to indicate the effectiveness of the inoculation technique.

A cut-off point of 2% was used to distinguish the resistant and susceptible classes based on the disease reaction of the susceptible parent in the 1998 and 1999 field experiments. After further testing of these putative resistant lines in the field and the greenhouse, ten lines were

moved to the susceptible category as they had exhibited a high score in the field and/or in the greenhouse tests.

The good fit of segregation classes to a 1:1 ratio would suggest that Q21861 carries a major gene for resistance to covered smut. A single dominant gene governing resistance to covered smut has been reported in many barley cultivars (Shands 1956; Wells 1958; Metcalfe 1962; Cherewick and Buchannon 1969). Some lines, viz. Q/SM- 5, 35, 38, 85 and 105, showed complete resistance (more resistant than the resistant parent) in all the tests. This may be due to the complementary effect of minor genes in SM89010 with the major gene of Q21861. The line Q/SM-134 had a very high disease score (8.02%) in only one test (field 1998), otherwise it was highly resistant in all other tests. This may be due to the carry over of seeds during seeding in the 1998 field test.

Of the 440 random primers used in the bulked segregant analysis, only one primer amplified a reproducible polymorphic banding pattern with close linkage to the covered smut resistance gene. Similarly, 540 primers were screened to identify one marker linked to the *Rpg1* gene in barley (Horvarth et al. 1995). However, Poulsen et al. (1995) screened only eight primers and identified a RAPD marker $OPU02_{2700}$ linked with a leaf rust resistance gene (*RphQ*) in Q21861 using bulked segregant analysis. On the other hand, some workers have been unable to find any reproducible polymorphisms with random primers using bulked segregant analysis in barley (Xu and Kasha 1991; Krasichynska 1996).

To overcome problems associated with using the RAPD technique for marker-assisted selection, the RAPD marker was converted into a SCAR marker. Primers were developed based on the sequence of the fragment amplified by the RAPD technique in the susceptible genotype but not the resistant genotype. These amplified a fragment of the expected length in both resistant and susceptible lines. Loss of polymorphism when primers were extended based on the sequence of a cloned RAPD fragment has been reported in other plants (Paran and Michelmore 1993; Deng et al. 1997; Hernandez et al. 1999). RAPD polymorphisms may result from mismatch at primer binding sites due to sequence divergence but also from structural changes between primer sites (Paran and Michelmore 1993). It is likely that the OPJ10 RAPD polymorphism resulted from mismatch at the priming sites, rather than structural changes. Extending primers beyond the RAPD binding site into regions of homology between the two genotypes would result in the loss of the polymorphism. Comparison of the sequences of amplified products from resistant and susceptible genotypes allowed allele-specific primers to be designed and the polymorphism between the susceptible and resistant genotypes to be recovered.

The SCAR marker was found to co-segregate with the RAPD marker. The marker was 5.5 cM from the covered smut resistant gene in Q21861. This level of linkage is generally considered to be adequate for the marker to be utilized in a breeding program for MAS (Burr et al. 1983; Mohan et al. 1997). Similarly, Eckstein et al. (2000b) identified a SCAR marker in the Q21861× SM89010 population linked with the scald resistance gene in SM89010. SCAR markers are now effectively used for MAS in routine breeding programs (Gu et al. 1995; Eckstein et al. 2000a). Mapping of the marker was not possible as the SCAR primers were unable to amplify any band in the parents of mapping populations. Attempts to develop a polymorphism (by adjusting the annealing temperature or making primers for the negative allele) for the SCAR marker in mapping populations will continue.

This is the first report of the development of a molecular marker linked with covered smut resistance in barley. The SCAR marker is closely linked to the gene of interest and can be effectively used in marker-assisted selection. This SCAR marker is also present in TR640 which is resistant to covered smut. It will be used to screen other populations involving TR640 and Q21861 as parents.

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