Molecular Marker Assisted Introgression of Loose and Covered Smut Resistance into CDC McGwire Hulless Barley

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Abstract

CDC McGwire is a high yielding hulless barley cultivar from the Crop Development Centre (CDC), University of Saskatchewan which is susceptible to both true loose and covered smut. Screening for these diseases is time, labour and space consuming and escapes are very frequent making it necessary to screen putative resistant lines several times to confirm resistance. In addition, both the diseases are expressed in the inflorescence, simultaneous screening for them is not possible. Molecular Marker Assisted Selection (MAS) is a good alternative to combine the resistance to both diseases in the same line. Sequence characterized amplified region (SCAR) markers linked to the loose smut resistance gene *Run8* (Eckstein et al. 2002) and covered smut resistance gene *Ruhq* (Ardiel et al. 2002, Grewal et al. 2004) have been developed at the CDC and a program was initiated to introgress loose smut resistance (*Run8*) and covered smut resistance (*Ruhq*) into CDC McGwire using MAS. Loose smut resistance was transferred from TR251 (*Run8*) and covered smut resistance from either Q21861 or TR640 (*Ruhq*).

Two strategies (doubled haploidy and marker-assisted backcrossing) were used to introgress both resistance genes. Screening 35 microspore culture derived doubled haploid (DH) lines against covered and loose smut in the field and greenhouse showed that in most lines, the phenotype defined by disease reactions and genotype defined by the SCAR markers agreed. Screening putative resistant lines three times for covered and loose smut identified 12 DH lines resistant to both smuts and positive to molecular markers of both the diseases. In the marker-assisted backcross program, plants were genotyped in each generation and plants positive for both markers were backcrossed to CDC McGwire. The genotyping of BC_1F_1, BC_2F_1 and BC_3F_1 plants with SCAR markers (UhR450 and $Un8_{700}R$) showed a 1:2:2:1 segregation indicating the presence of two major genes. Twenty BC_3F_3 lines (10 lines selected with the covered smut resistance from Q21861 and 10 lines selected with the covered smut resistance from TR640) were evaluated for covered smut reaction in the field in 2004 and in the greenhouse in the fall of 2004. All lines showed resistance to covered smut. BC_3F_3 lines were inoculated with loose smut in the field in 2004 and evaluated in the greenhouse. Eighteen of 20 lines showed loose smut resistance. These putative double resistant lines will be phenotyped in BC_3F_5 generations to confirm disease reactions.

These results confirm that molecular markers can assist in rapid introgression of simply inherited disease resistance genes into elite lines with considerable savings in time and cost.

Introduction

True loose smut (*Ustilago nuda* (Jens.) Rostr.) and covered smut (*U. hordei* (Pers.) Lagerh.) of barley (*Hordeum vulgare* L.) occur in all barley growing regions of the world (Mathre 1997). The diseases result 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). The smuts can be effectively controlled by fungicidal seed treatments, sowing disease-free seed and 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 (*U. nuda*) have been reported on winter barley crops in France (Leroux and Berthier 1988). Furthermore, seed treatment with fungicides is not an option for organic production. Resistant cultivars are generally recognized as the most economical and preferred method of control. However, breeding for smut resistance in barley is expensive. Screening for these diseases is time, labour and space consuming and escapes are frequent making it necessary to screen putative resistant lines several times to confirm resistance. As both diseases infect the inflorescence, screening for them simultaneously is not possible. Molecular Marker Assisted Selection is a good alternative to combine the resistance to both the diseases.

CDC McGwire (a barley cultivar from Crop Development Centre (CDC), University of Saskatchewan, Saskatoon feed barley program) is a very high yielding hulless barley cultivar but it is susceptible to true loose and covered smut. Loose smut resistant lines in a CDC McGwire background with resistance from TR251 (having the *Run8* gene) were developed. *Run8* confers resistance to most known races of *U. nuda* in western Canada and is present in the majority of resistant barley cultivars (Thomas and Menzies 1997). Similarly, covered smut resistant lines (having the *Ruhq* gene) in a CDC McGwire background were available with resistance from Q21861 and TR640. *Ruhq* shows resistance to western Canadian isolates of *U. hordei* (Grewal et al. 2004). Each of these lines thus had 50% of their background from CDC McGwire.

A sequence characterized amplified region (SCAR) marker linked to the loose smut resistance gene *Run8* has been developed and is routinely used to select loose smut resistant lines in the CDC barley breeding program (Eckstein et al. 2002). Similarly, a SCAR marker linked to covered smut resistance in Q21861 and TR640 has been developed (Ardiel et al. 2002). This project has initiated to introgress the loose smut resistance (*Run8*) and covered smut resistance (*Ruhq*) into CDC McGwire using molecular markers.

Materials and Methods

A loose smut resistant line in the CDC McGwire background of was crossed with a covered smut resistant line with a CDC McGwire background. Breeding line SH00752 (CDC McGwire/TR251) was crossed with breeding line SH01470 (CDC McGwire/Q21861). Two strategies were used to introgress covered and loose smut into CDC McGwire i.e. Doubled haploidy and marker-assisted backcrossing.

Doubled haploidy

F_1 seeds from the cross SH00752 X SH01470 were used to produce doubled haploids. Fifty one DH plants were produced using microspore culture and were tested with UhR450 and $Un8_{700}R$ SCAR markers using the methods described by Ardiel et al. (2002) and Eckstein et al. (2002), respectively. Seeds were obtained from 38 cultured-derived plants. Seeds from the 38 DH lines were planted in the greenhouse and were also sent to New Zealand for increase. Three DH lines viz. MC0181- 6, 12, 38 were polyploids and were excluded for further testing leaving 35 DH lines. These lines were tested against covered smut and loose smut.

Covered smut screening: For inoculation, disease screening and evaluation, the techniques used were as reported earlier (Ardiel et al. 2002, Grewal et al. 2004). The 35 DH lines (population MC0181) were inoculated with a mixture of isolates of *U. hordei* (inoculum source 2000) along with the original parents (Q21861, CDC McGwire, TR251) and susceptible check, CDC Candle. All lines were screened in the field in the summer of 2003 at the Preston Plots, University of Saskatchewan, Saskatoon. The plots were planted on May 26, 2003 and the experiment was arranged in a randomized complete block design with four replications of each line. Each replication consisted of 4-m single row per line. Parents and check were widely distributed throughout the trial. At every 56 rows, there were two rows of each parent and one row of the susceptible check. The level of covered smut infection was evaluated as percent infected heads. Two 1 m sections were measured within each row. In each 1 m section, counts of the total number of heads and number of infected heads were conducted. The two counts were averaged for each replication and the percentage of infected heads calculated.

In the fall of 2003, 21 putative resistant lines (those that showed less than 3% infected heads in the field) were re-screened in the greenhouse along with parents and check. There were four replications for each line. Each replication consisted of one pot, with five seeds per pot. 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 infected. In each pot, the number of infected plants and total plants were counted and percentage of infected plants calculated. Putative resistant lines were re-tested again in the field in the summer of 2004.

Loose Smut screening: All DH lines, parents and the check were inoculated in the field in the summer of 2003 using the syringe inoculation technique described by Eckstein et al. (2002). Five spikes from each line were inoculated with *U. nuda* teliospores just prior to anthesis. Mature inoculated spikes were harvested and inoculated seeds were planted in the greenhouse in the fall of 2003 to evaluate for resistance to loose smut. There were three replications for each line. Each replication consisted of one pot, with 20 seeds per pot. A line with any smutted head

in any replication was rated as susceptible and a line with no smutted head as resistant. Twenty one lines showing no infection along with the parents and check were re-inoculated with loose smut as described above and inoculated seeds were grown in the field in the summer of 2004. There was one 4-m row for each line. The lines were evaluated as resistant or susceptible. All lines were inoculated again in the field in the summer of 2004 and inoculated seeds were grown in the greenhouse in the fall of 2004 for evaluation.

Marker-assisted backcrossing

The project began with crossed seeds from the cross SH00752 X SH01470. Ten F_1 plants were grown in the greenhouse and tested with both SCAR markers. Nine of 10 plants were positive for both markers and five plants were backcrossed to CDC McGwire. A new cross of SH00752 X SH01477 (CDC McGwire/TR640) was also made. In the summer of 2002, F_1 and BC_1F_1 plants were seeded in the greenhouse and tested with both the markers. For molecular marker screening, a quick, simple and effective method reported by Eckstein et al. 2004 was used. All F_1 plants were positive for both markers indicating they were crossed seeds. Ten F_1 and 14 BC_1F_1 plants positive for both markers were backcrossed to CDC McGwire. In the fall of 2002, BC_2F_1 plants were screened with both markers and plants positive to both were identified (Table 2). A few plants positive for both markers were backcrossed to CDC McGwire. Similarly, BC_3F_1 plants were screened and those positive for both markers were transplanted in the greenhouse for selfing. In the BC_3F_2 generation, a large number of plants were screened with the markers and the plants positive for both were also screened with SCAR marker $Un8_{700}S$ (linked to susceptible allele of *Run8* gene - Eckstein et al. 2002) and RAPD marker $OPJ10_{450}$ (linked to susceptible allele of *Ruhq* gene - Ardiel et al. 2002) to identify the plants homozygous for the markers. Sixty-two BC_3F_2 plants positive for both markers were transplanted in the greenhouse for selfing. Twenty BC_3F_3 lines (10 lines with covered smut resistance from Q21861 and 10 with covered smut resistance from TR640) were evaluated for covered smut reaction in the field in 2004 along with the parents and check. These lines were re-tested again in the greenhouse in the fall of 2004. All lines were inoculated with loose smut in the field in the summer of 2004 and grown out in the greenhouse in the fall of 2004 for evaluation. These lines were again inoculated with loose smut in the greenhouse and will be grown to evaluate their resistance to loose smut in 2005.

Results

Doubled haploidy

Of the 35 DH lines, 14 were positive for both the markers and 10 were negative. The UhR450 marker was positive in 18 lines and the Un8 was positive in 21 (Table 1). Screening of all the lines along with the parents and check in the field in the summer of 2003 against covered smut showed that Q21861 was resistant whereas CDC McGwire and TR251 were susceptible. Twenty lines showed resistance to covered smut. Many lines showed complete resistance to covered smut indicating that minor resistance genes from CDC McGwire and/or TR251 may have been combined with the major resistance gene from Q21861. In the majority of the lines, the phenotype defined by the covered smut reaction and genotype defined by the covered smut

marker UhR450 agreed. Lines showing putative resistance (showing <3% infected heads) were screened in the greenhouse and again in the field to confirm their reaction.

Screening all lines against loose smut showed that TR251 was resistant (no infected head) and Q21861 and CDC McGwire were susceptible. Twenty-one DH lines showed resistance to loose smut and for 33/35 lines the phenotype and genotype data agreed. Resistant lines were screened twice to confirm their resistance. All but one were resistant in the two subsequent tests.

Table 1. Phenotype and Genotype Data of 35 Doubled-Haploid Lines

Barley lines	Test	Covered smut reaction*			UhR450	Un8	Loose smut reaction**		
		Field 2003 % infected heads	Gh 2003 % infected plants	Field 2004 % infected heads	covered smut marker	Loose smut marker	Gh 2003	Field 2004	Gh 2004
CDC Candle	check	48.5	75.0	65.1	No	No	S	S	S
Q21861	parent	0.4	0.0	0.1	Yes	No	S	S	S
TR251	parent	8.1	17.6	2.5	No	Yes	R	R	R
CDC McGwire	parent	10.5	16.7	4.4	No	No	S	S	S
MC0181-01	SH00752/SH 01470	**1.1**	**8.3**	**0.2**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-02		0.6	0.0	0.1	Yes	Yes	S		
MC0181-03		15.8			No	No	S		
MC0181-04		4.1			No	Yes	R	R	R
MC0181-05		0.0	0.0	0.0	Yes	No	S		
MC0181-07		6.5			No	No	S		
MC0181-08		**0.0**	**0.0**	**0.3**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-09		***0.0***	***0.0***	***0.0***	***No***	***No***	***R***	***R***	***R***
MC0181-10		3.6			No	Yes	R	R	R
MC0181-11		4.7			No	Yes	R	R	R
MC0181-14		0.0	0.0	0.0	Yes	Yes	R	S	R
MC0181-15		**0.9**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-18		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-21		19.9			No	Yes	R	R	R
MC0181-22		18.7			No	Yes	R	R	R
MC0181-23		3.2			No	No	S		
MC0181-24		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-25		11.7			No	No	S		
MC0181-26		8.8			No	No	S		
MC0181-27		13.7			No	Yes	R	R	R
MC0181-28		**0.0**	**7.1**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-29		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-30		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-31		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-32		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-33		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-34		3.3			No	No	S		
MC0181-37		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-40		3.0			No	No	S		
MC0181-45		1.3	0.0	0.2	No	No	S		
MC0181-46		0.6	0.0	0.1	No	No	S		
MC0181-47		14.0			No	Yes	R	R	R
MC0181-48		2.6	5.5	0.0	Yes	No	S		
MC0181-49		0.0	0.0	0.0	Yes	No	S		
MC0181-50		0.0	0.0	0.0	Yes	No	S		

*In field, covered smut was evaluated as % infected heads whereas in the greenhouse, it was evaluated as % infected plants.

**R - no infected head; S - any infected head. Loose smut inoculations were performed in the field and inoculated seeds were grown in the greenhouse for disease development and vice versa.

After testing of putative resistant lines three times against covered smut and loose smut, 12 lines showed resistance to both the diseases and positive to both the markers (Table 1 - bold). There is one line (MC0181-09) resistant to both the diseases but negative to both the markers. All 12 lines will be tested for agronomic and quality traits and may be released as new cultivars or used as a bridging material to transfer smut resistance into high yielding elite lines.

Marker-assisted Backcrossing

Plants were genotyped in each generation and plants positive to both the markers were backcrossed to CDC McGwire. The number of BC_1F_1, BC_2F_1 and BC_3F_1 plants genotyped are shown in Table 2 and plants segregated in a 1:2:2:1 ratio for the markers indicating the presence of two major genes. An unexpected ratio in the BC_3F_1 generation (with covered smut resistance from TR640) was probably due to the fact that only a few plants were tested. In the BC_3F_2 generation, a high number of plants were positive to either Un8 and/or UhR450 markers because these are dominant markers thus we were unable to distinguish between homozygous and heterozygous plants. To identify the homozygous plants, these plants were screened with SCAR marker $Un8_{700}S$ (linked to susceptible allele of *Run8* gene - Eckstein et al. 2002) and RAPD marker $OPJ10_{450}$ (linked to susceptible allele of *Ruhq* gene - Ardiel et al. 2002) resulting in the identification of many homozygous plants.

Table 2. Genotyping of Backcrossed Plants with Un8 and UhR450 Markers

Generation	Total plants screened	Positive to both markers	Run8	UhR450	No marker
Covered Smut resistance from Q21861					
BC_1F_1	166	27	79	68	46
BC_2F_1	240	61	119	115	67
BC_3F_1	103	22	51	52	21
BC_3F_2	186	99	136	131	18
Covered smut resistance from TR640					
BC_1F_1	47	9	27	16	13
BC_2F_1	59	8	27	25	15
BC_3F_1	20	4	8	8	9
BC_3F_2	181	65	98	122	27

Evaluation of 10 BC_3F_3 lines each having covered smut resistance from Q21861 and TR640 against covered smut in the field in the summer of 2004 indicated that all these lines were resistant (Table 3). A few lines showed some infected heads but percentage infection was not significantly higher than that of the resistant parent, Q21861. These lines (BC_3F_4) were tested in the greenhouse to confirm their reaction and majority showed no infection. Five lines showed one infected plant each and in those instances there was only one infected head/plant.

Table 3. Screening of Backcrossed Lines against Loose Smut and Covered Smut

Barley lines	Test	Loose smut*	Covered smut reaction**	
			Field 2004	Gh winter 2004
			% infected heads	% infected plants
CDC Candle	check	S	65.1	87.5
Q21861	parent	S	0.1	0.0
TR251	parent	R	2.5	35.7
CDC McGwire	parent	S	4.4	50.0
TR640	parent	S	0.0	0.0
SH041241	BC_3F_4	R	0.0	6.7
SH041242		R	0.0	0.0
SH041243		R	0.0	5.9
SH041244		R	0.0	0.0
SH041245		R	0.0	0.0
SH041246		R	0.0	0.0
SH041247		R	0.0	0.0
SH041248		R	0.4	0.0
SH041249		R	0.0	0.0
SH041250		R	0.0	0.0
SH041251		R	0.0	0.0
SH041252		S	0.0	0.0
SH041253		R	0.5	5.9
SH041254		R	0.0	5.9
SH041255		R	0.0	0.0
SH041256		R	0.0	0.0
SH041257		R	0.0	5.6
SH041258		S	0.0	0.0
SH041259		R	0.0	0.0
SH041260		R	0.0	0.0

*R - Resistant, no infected head; S - Susceptible, one or two infected heads/pot. The plants were inoculated with loose smut in the field in summer 2004 and evaluated in the greenhouse in Fall 2004.
**In field, BC_3F_3 lines were screened against covered smut and in the greenhouse, BC_3F_4 lines were screened.

These 20 lines, along with the parents and the check, were inoculated against loose smut in the field and evaluated in the greenhouse in the fall of 2004. The 18/20 lines were resistant to loose smut. These lines will be tested again in 2005 for loose and covered smut to exclude the possibility of any escape. These results confirm that molecular markers can assist in rapid introgression of disease resistance genes into elite lines with considerable savings in terms of time and cost.

Discussion

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. This problem is further aggravated in loose smut due to the fact that the plants must be grown to anthesis, the individual florets inoculated by syringe, and the inoculated mature seed must be harvested. Plants from this seed need to be grown to the heading stage to assess the disease reaction. In addition, infection is inconsistent and escapes occur, making it necessary to screen lines several times to confirm resistance. As both the diseases infect the inflorescence, simultaneous screening is not possible. Thus both the diseases are good candidates for MAS. The MAS strategy is a way to capitalize on available markers and to introgress valuable traits into elite lines.

The backcross method is used in plant breeding to transfer desirable alleles from a donor parent, which has mostly poor agronomic traits, into a recipient elite genotype. This is also known as defect-elimination as the recipient genotype has desirable agronomic and quality traits except one or two defects e.g. CDC McGwire is a high yielding cultivar with desirable quality traits but it is susceptible to loose and covered smut. As an alternative to time-consuming and unreliable screening methods, molecular markers can be used as a diagnostic tool to trace the presence of target gene (s) in successive backcross generations (Frisch et al. 2001).

High susceptibility of the susceptible check, CDC Candle to both the diseases in all tests showed that inoculation techniques used were effective. Testing of DH putative resistant lines three times against covered smut and loose smut, showed 12 lines resistant to both the diseases and positive for both the markers, proving indirect selection using molecular markers is feasible. Of note, one line (MC0181-09) is resistant to both the diseases but negative for both markers. Both markers are about 5 cM away from their respective genes and a few recombinants are expected, however, there is a 95 % chance of selecting the desirable lines based on the markers.

In the marker-assisted backcrossing program, blind selection based on the markers was conducted until BC_3F_2 generation. In every generation, the plants for backcrossing were selected based only on their genotype. We were fortunate to have markers linked to susceptible alleles, thus were able to identify homozygous plants for resistance to both diseases in BC_3F_2 generation. The resistance of BC_3F_3 and BC_3F_4 lines to both covered and loose smut proves MAS is practical. These lines are more than 93% similar to CDC McGwire as we started with 50% CDC McGwire in each parent. Phenotypically, they appear very similar to CDC McGwire in the field. These lines will be further tested in BC_3F_5 generation against loose and covered smut to confirm their reactions. Lines showing resistance to both the diseases will be evaluated in yield trials and for quality in 2005. As these lines are very similar to CDC McGwire limited testing will be required to detail overall performance. This material may be released as a new cultivar - fully smut resistant hulless barley! Release of these MAS-improved cultivars will demonstrate the power of this technology.

These results confirm that molecular markers can assist in rapid introgression of disease resistance genes into elite lines with considerable savings in time and cost. In addition to trace

the presence of target genes, molecular markers with a good coverage of the entire genome can be used to select for rapid recovery of the recurrent parent genome.

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