Theor Appl Genet (2005) 111: 1623–1631
DOI 10.1007/s00122-005-0094-0

ORIGINAL PAPER

Daryl J. Somers · Julian Thomas · Ron DePauw
Stephen Fox · Gavin Humphreys · George Fedak

Assembling complex genotypes to resist *Fusarium* in wheat (*Triticum aestivum* L.)

Received: 7 April 2005 / Accepted: 22 August 2005 / Published online: 30 September 2005

Abstract Fusarium head blight of wheat is a major deterrent to wheat production world-wide. The genetics of FHB resistance in wheat are becoming clear and there is a good understanding of the genome location of FHB resistance QTL from different sources such as Sumai3, Wuhan, Nyubai and Frontana. All the components needed for assembling complex genotypes through large-scale molecular breeding experiments are now available. This experiment used high throughput microsatellite genotyping and half-seed analysis to process four independent crosses through a molecular breeding strategy to introduce multiple pest resistance genes into Canadian wheat. This included two backcrosses and selection for a total of six FHB resistance QTL, orange blossom wheat midge resistance (*Sm1*) and leaf rust resistance (*Lr21*). In addition, the fixation of the elite genetic background was monitored with 45–76 markers to accelerate restoration of the genetic background at each backcross. The strategy resulted in 87% fixation of the elite genetic background on average at the BC_2F_1 generation and successfully introduced all of the chromosome segments containing FHB, *Sm1* and *Lr21* resistance genes. The molecular breeding strategy was completed in 25 months, at an equal pace to conventional crossing and selection of spring wheat.

Communicated by P. Langridge

D. J. Somers (✉) · J. Thomas · S. Fox · G. Humphreys
Agriculture and Agri-Food Canada,
Cereal Research Centre, Winnipeg,
MB R3T-2M9, Canada
E-mail: somersd@agr.gc.ca

G. Fedak
Agriculture and Agri-Food Canada,
Eastern Cereals and Oilseeds Research Centre,
Ottawa, ON K1A 0C6, Canada

R. DePauw
Agriculture and Agri-Food Canada,
Semi-arid Prairie Agriculture Research Centre,
Swift Current, SK S9H 3X2, Canada

Introduction

Fusarium head blight (FHB) of wheat is caused primarily by *Fusarium graminearum* in North America, while other species such as *F. culmorum* are the dominant species causing infection in Europe (Mesterhazy 1983). Resistance to FHB is complex, many genes control different aspects of the disease such as resistance to initial infection, resistance to the spread of the disease in the spike and resistance to the accumulation of mycotoxins such as deoxynivalenol (Mesterhazy 1995; Gilbert and Tekauz 2000). The degree of infection by the *Fusarium* species is influenced by environmental factors requiring specialized wheat breeding nurseries to enable selection of resistant lines. Taken together, resistance to FHB is difficult to select for by conventional breeding and pathology techniques.

There is now extensive evidence about the genetic control of FHB resistance in wheat; several research groups have identified a common set of FHB resistance QTL in different crosses and genetic backgrounds (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Somers et al. 2003; Yang et al. 2003). Although, each QTL identified appears to explain small amounts of the variation in infection, the effects appear to be additive (Somers et al. 2003; Yang et al. 2003; Yang et al. 2005). Thus it seems essential to select for multiple genes in order to provide a sufficient level of resistance to FHB in wheat. The primary FHB resistance QTL occur on chromosomes 2DL, 3BS, 4B, 5AS, 6B (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Somers et al. 2003; Yang et al. 2003; McCartney et al. 2004), and derive from sources such as Sumai 3, Wuhan, Nyubai and Frontana. This suite of FHB resistance QTL has been identified in several wheat mapping populations. There are many other QTL reported as well, but we are less confident in these as they are typically unique to one mapping cross.

In addition to our understanding of the FHB QTL position, high throughput genotyping technology

platforms such as capillary electrophoresis have matured in the last 3–5 years and marker density on wheat maps has improved substantially in the last 1–3 years (Somers et al. 2004). The intention of creating the microsatellite consensus map included the development of a map that represented genetic distances one might expect in typical wheat breeding crosses. This would enable a reasonable prediction of population size required to select for multiple QTL intervals in the genome without knowing the actual genetic distance between flanking QTL markers in a cross. The current research tests the applicability of the microsatellite consensus map (Somers et al. 2004) in this regard.

Therefore, the knowledge and tools required for assembling complex genotypes through molecular breeding in wheat are publicly available and essential biological and genetic information on FHB is available. The strategies for molecular breeding of complex traits such as FHB resistance in wheat can be taken further than only selecting and pyramiding QTL in segregating progeny (Gupta and Varshney 2000; Somers 2004). One strategic improvement is to simultaneously monitor restoration of the genetic background with QTL introgression and select progeny with recombination events in critical chromosome positions. The advantage of using this amount of scrutiny in selecting progeny based only on genotype is the opportunity to reduce linkage drag for deleterious alleles and fix regions of the genome essential for seed quality and environmental adaptation in early generations.

The following research brings together many aspects of molecular breeding for assembling complex genotypes. The research includes the pyramiding of six FHB resistance genes plus resistance genes for orange blossom wheat midge (*Sm1*) (Thomas et al. 2005) and leaf rust (*Lr21*) (Spielmeyer et al. 2000) as well. The intent of the first publication from this project is to describe the molecular breeding approach and progress made from genotype analysis. The phenotypic evaluation of derived lines is in progress and will be presented in a later publication. Data are presented demonstrating the use of: (1) high throughput genotyping; (2) a robust microsatellite map of wheat; (3) FHB resistance QTL introgression; and (4) accelerated restoration of the elite genetic background.

Materials and methods

Plant material

The germplasm is divided into two groups, elite parents and FHB resistance donor parents. The elite parents were spring wheat and included 98B69*L47, BW301, Prodigy and Kanata (BW263). Each of these lines is elite in that they possess many of the characteristics for seed quality, agronomy and disease resistance that are required for production in western Canadian growing conditions. The donor parents were HC374 (Wuhan/Nyubai), HC376 (AC Foremost//Biggar/Sumai 3) and 98B08*A11 (BW252//AC Domain*2/Sumai 3). Each of the donor parents was a doubled haploid (DH) line carrying specific FHB resistance QTL (Somers et al. 2003; Yang et al. 2003) and has demonstrated FHB resistance based on several years of field and greenhouse testing. The line HC374 comes from the cross Wuhan/Nyubai and was erroneously reported as a Wuhan/Maringa derivative in Somers et al. (2003). Details of the parental material are shown in Table 1.

Crossing scheme

The large number of pest resistance genes being pyramided required four crossing streams to facilitate assembling the desired gene/QTL combinations (Fig. 1) with each stream introgressing different FHB resistance QTL into different elite parents. Each crossing stream followed an identical scheme of donor × elite followed by two backcrosses to the elite parent and one selfing generation to derive BC_2F_2 plants . Stream 3 was delayed one generation due to dormancy and BC_2F_1 plants were used for stream intercrossing while BC_2F_2 plants were used for intercrossing streams 1, 2 and 4. Streams 1, 2 and 4 used the same elite parent as was used in the initial cross, stream 3 began with HC376 × 98B69*L47 and used Prodigy as the recurrent parent in the subsequent two backcrosses (Fig. 1).

Genotyping and selection

Genotyping was performed on an ABI 3100 capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA) and used M13-tailed microsatellite markers as described in Somers et al. (2004). Ten seeds of each parental line (Table 1) were genotyped with 12 robust microsatellite markers to identify any heterogeneity in the parental seed lots and to assure that the seeds selected to be used in crossing were representative of the parental line.

Table 1 List of parental material used in the molecular breeding of multiple pest resistance and their attributes

Parent	Pedigree	Attributes
Elite parent		
98B69*L47	Augusta/HW Alpha//3*BW252	Hard red, *Sm1*
BW301	AC Elsa/AC Cora	Hard red,*Lr21*
Prodigy	SWP2242/Stoa	Hard red
BW263	RL4137*6//Thatcher/Poso48/3/AC Domain	Hard white
Donor parent		
HC374	Wuhan/Nyubai FHB QTL – 2DL, 3BS, 3BSc, 4B, 5AS	
HC736	AC Foremost//Biggar/Sumai 3 FHB QTL – 3BS, 4B, 6B	
98B08*A111	BW252//AC Domain*2/Sumai 3 FHB QTL – 3BSc, 5AS, 6B	

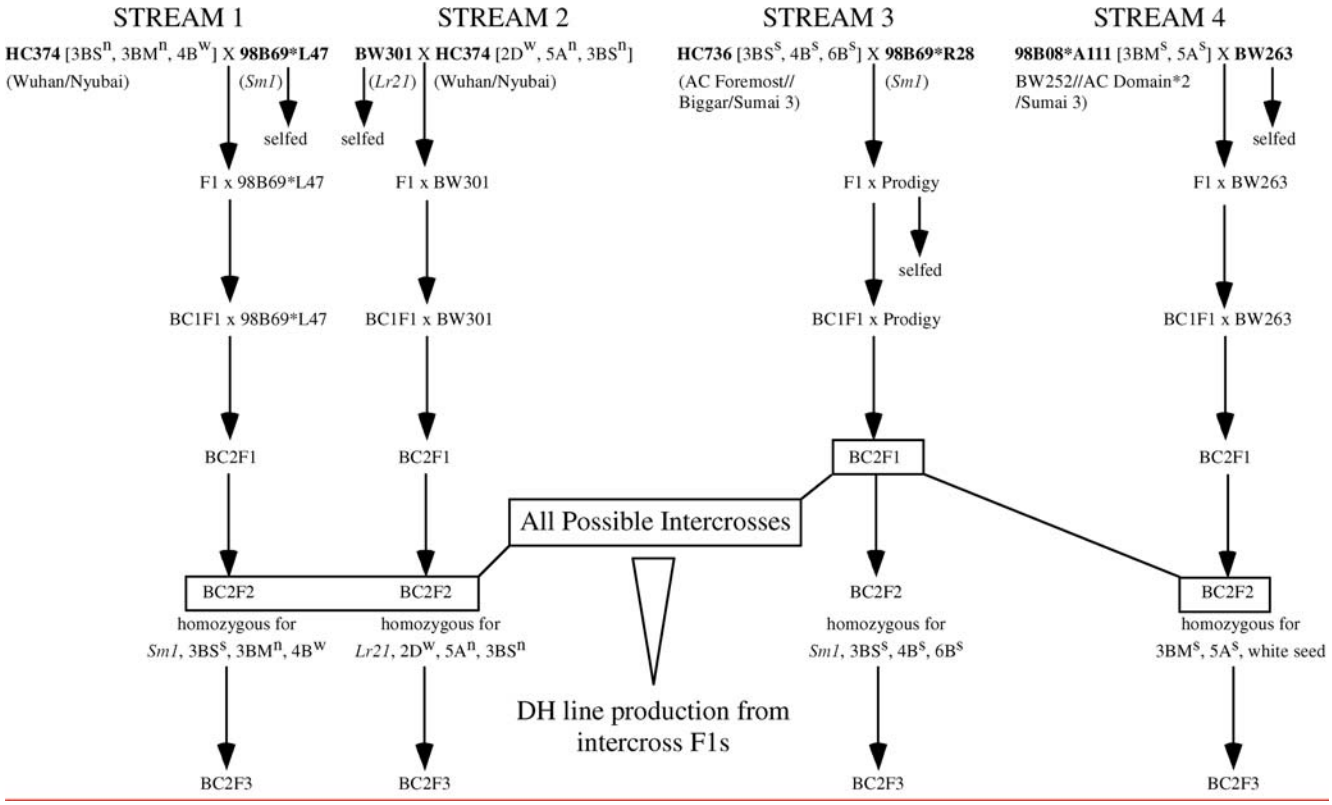

Fig. 1 Schematic diagram showing the crosses made in the four streams of the molecular breeding project for multiple pest resistance. The donor parent is female in streams 1, 3 and 4 and is male in stream 2. The elite parent switches to 'Prodigy' in stream 3 at the first backcross. The chromosomes harbouring an FHB resistance QTL being introgressed in each cross are indicated with the donor parent and the superscript indicates the allele source for the QTL (*w*Wuhan; *n*Nyubai and *s* Sumai 3). The FHB resistance QTL 3BS is near the telomere and 3BSc is near the centromere. The pedigree of the donor parent is indicated. 98B69*L47, 98B69*R28 carry *Sm1* resistance, Prodigy is *Sm1* susceptible, BW301 carries *Lr21* and BW263 carries the white seed characteristic. The final intercrosses used BC_2F_2 homozygous plants from streams 1, 2 and 4 and BC_2F_1 plants from stream 3

The parents were first genotyped with all available microsatellite markers in the six FHB resistance QTL intervals. This determined the parents for each cross by placing emphasis on: (1) maximizing the number of polymorphic markers in the interval; and (2) having microsatellites that were codominant. The list of codominant, polymorphic markers used for the selection of each chromosomal segment carrying FHB resistance QTL or other pest resistance genes is indicated in Table 2. The parents used in the project were then screened with 356 microsatellite markers (gwm–Roder et al 1998; GDM–Pestova et al. 2000; wmc–Somers et al. 2004; and BARC–http://www.ScabUSA.org) to identify microsatellites across the genome in each cross that were codominant and polymorphic to be used for recurrent parent genome selection. Markers were selected to achieve maximum genome coverage without targeting other important genome regions. The segregating populations for each stream in the BC_1F_1 generation consisted of 968–1,152 seeds. Each seed was cut laterally in half to provide the endosperm and embryo halves for further analysis. The endosperm half seed was crushed with pliers and placed into a 96 deep well plate. The half seeds were then ground to a powder using 3 mm glass beads with shaking for 5 min. DNA was extracted using the DNeasy Plant DNA extraction kit (Qiagen, Mississauga, Ontario) and quantified by fluorimetry using the Hoechst 33258 stain.

Three polymorphic markers for each FHB resistance QTL were identified, one in the centre of the QTL and two flanking markers (Table 2). Each BC_1F_1 half seed was first genotyped with the centre FHB resistance QTL marker. half seeds that were heterozygous for the centre marker were then genotyped with the flanking markers. half seeds that were heterozygous across the three marker interval were then genotyped with the suite of background genome markers polymorphic for that cross. The number of background genome loci fixed as homozygous was expressed as a percentage of the total number of background markers. Since full genetic maps were not constructed in this project, the degree of genome coverage using the background loci was based on the wheat microsatellite consensus map (Somers et al. 2004). Half-seed embryos that were heterozygous for the FHB resistance QTL and that had the most fixation of the elite genome were germinated to be advanced as females in the crossing scheme. This same process was performed at the BC_2F_1 and BC_2F_2 generations in all four streams with

Table 2 List of microsatellite markers used for the selection of FHB resistance QTL intervals. The allele size of the FHB resistant donor is shown in parentheses

Crossing stream	FHB[a] QTL	FHB resistance type[b]	Microsatellite markers[c]			Distance[d] (cM)	Reference
			Distal	Centre	Proximal		
1	3BS	II	gwm533 (140)	gwm493 (197)	wmc808 (159)	33	Somers et al. 2003
	3BSc	I	wmc625 (121)	gwm566 (124)	wmc418 (263)	13	Somers et al. 2003
	4B	II	wmc710 (89)	wmc238 (220)	gwm149 (154)	20	Somers et al. 2003
2	2DL	I	wmc245 (152)	gwm608 (133)	gwm301 (214)	40	Somers et al. 2003
	3BS	II	gwm533 (140)	gwm493 (197)	wmc808 (159)	33	Somers et al. 2003
	5AS	I, II	gwm293 (199)	wmc705 (170)	gwm154 (97)	18	Buerstmayr et al. 2002
3	3BS	II	gwm389 (134)	gwm533 (141)	gwm493 (194)	11	Anderson et al. 2001
	4B	II	wmc710 (89)	wmc238 (226)	gwm149 (154)	20	Somers et al. 2003
	6B	I, II	wmc104 (135)	wmc397 (157)	gwm219 (177)	45	Yang et al. 2003
4	3BSc	I	wmc625 (113)	gmw566 (124)	wmc418 (267)	13	Somers et al. 2003
	5AS	I, II	gwm293 (199)	wmc705 (167)	gwm154 (103)	18	Buerstmayr et al. 2002

[a] FHB resistance QTL 3BS is near the telomere, 3BSc is near the centromere

[b] *Type I* resistance to initial infection based on field observations; *Type II* resistance to fungal spread based on single floret injection experiments

[c] Marker order on the chromosome is based on Somers et al. (2004) and sizes include a 19 bp M13 tail

[d] Distance between distal and proximal markers is based on Somers et al. (2004)

the exception that genome analysis was omitted on BC_2F_2 plants. The segregation ratio of genotypes in the BC_1F_1 and BC_2F_1 populations was tested by Chi square analysis. The number of progeny expected to be heterozygous at a QTL centre marker or across QTL intervals was calculated based on the number of QTL being selected in the stream and the size of the QTL intervals.

Background genome loci that were fixed for elite alleles at BC_1 were not regenotyped at BC_2 and thus fewer markers were used in the BC_2 generation. Further, stream 3 was delayed due to seed dormancy in the initial F_1 and intercrossing was performed with BC_2F_1 plants. All possible stream intercrosses were made and included multiple intercrosses between the same streams in order to sample the 14 different BC_1F_1 plants (Table 3). Intercross F1 seed was then used to develop large DH populations. The intercross F_1 seed involving stream 3 was screened with appropriate FHB resistance markers to select seeds fixed for FHB resistance QTL where possible, before DH line production.

Results

Half-seed analysis

Seeds were cut laterally in half to extract DNA from the endosperms and to keep the embryo half in storage for germination later. The primary reason for this process was to facilitate shipment of selected embryo half seeds to coauthors for further backcrossing. The DNA extraction from the endosperm half seed released 2.0–3.0 μg of DNA which was sufficient for 100 PCR using 24 ng of template DNA for each PCR. In cases where the DNA was entirely consumed prior to making the final half-seed selection, the remaining embryo half seeds were germinated in root trainers and 10 days later, leaf tissue was used to extract DNA and complete the genotyping. The four streams were processed in the laboratory by staggering the work. Typically, each stream in each generation required 20 days and two full-time personnel to process from seed cutting to the final selection of elite plants. Seed cutting and DNA extraction required 7 days, centre marker genotyping on three QTL required 5 days, flanking maker genotyping required 2 days, background genome genotyping required 4 days and data curation and analysis required 2 days.

Genotyping

The parental lines were screened with approximately 356 microsatellites distributed across the genome to establish a set of microsatellites that were codominant and polymorphic for each cross. Sets of 45–76 microsatellites were selected to examine the genetic background in each BC population (Table 3). The greatest restriction placed on microsatellite selection was the need to be codominant and amplify alternate alleles that were clearly separated by electrophoresis. In addition, sets of three microsatellites were established to select for each FHB QTL interval in each stream (Table 2, Fig. 2). These QTL intervals and markers were determined based on prior mapping of FHB resistance (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Somers et al. 2003; Yang et al. 2003) and comparative mapping to the Synthetic × Opata maps of Roder et al. (1998) and Somers (Agriculture and Agri-Food Canada, unpublished). These QTL intervals were delimited conservatively with at least a 1.0 LOD drop off from the QTL

Table 3 Details of the FHB resistance molecular breeding half-seed analysis and genome restoration for all four crossing streams in all generations

Generation	Family	No. of half-seeds tested			No. of genome markers[d]	% Fixed elite alleles		No. of seeds selected	Range % fixed elite alleles[e]
		Centre marker	Flanking marker[b]	Genome marker[c]		Range	Average		
Stream 1									
BC_1F_1		1,076	154 (135)**	72 (71)**	76	33–66	49	4	57–66
BC_2F_1	1–0938	264	29 (33)**	12 (13)**	26	74–89	81	6	82–89
	1–0708	272	34 (34)**	21 (16)*	32	63–85	76	4	81–85
	1–0656	248	28 (31)**	11 (13)**	30	69–81	77	1	79
	1–0253	224	32 (28)**	15 (15)**	32	68–85	78	3	82–85
BC_2F_2		1,152	52					14	
Stream 2									
BC_1F_1		1,151	134 (144)**	64 (43)ns	70	20–66	49	2	60–66
BC_2F_1	2–0139	579	55 (72)*	12 (18)**	28	79–100	88	5	89–100
	2–0281	523	63 (65)**	20 (20)**	41	71–91	79	2	88–91
BC_2F_2		1,152	23					8	
Stream 3[a]									
BC_1F_1		1,152	118 (144)*	22 (23)**	68	27–67	46	5	48–67
BC_2F_1	3–0953	180	24 (23)**	8 (4)*	22	84–91	87	4	86–93
	3–0497	260	26 (33)**	5 (5)**	22	80–91	87	3	83–91
	3–0027	269	28 (34)**	4 (6)**	28	79–85	83	0	
	3–0708	247	23 (31)**	2 (5)**	35	74–79	76	0	
	3–0415	181	25 (23)**	8 (5)**	32	70–85	77	2	81–85
BC_2F_2 (no data generated)									
Stream 4									
BC_1F_1		968	236 (242)**	133 (168)ns	45	27–71	51	3	65–71
BC_2F_1	4–0461	134	39 (34)**	31 (28)**	17	73–92	83	2	92
	4–0467	155	51 (39)*	36 (36)**	21	76–93	84	3	89–93
	4–0393	117	38 (29)*	19 (27)*	17	77–91	83	1	91
BC_2F_2		768	64					18	

[a] The BC_2F_2 generation was not examined prior to intercrossing due to a delay in stream 3. A total of nine BC_2F_1 half seeds were advanced to intercrossing

[b] The number of half seeds that were heterozygous at the QTL centre marker and the (expected) number of half seeds based on the number and size of each QTL interval (Table 2). Chi square test significance at 0.05**, 0.1*, non-significant (ns)

[c] The number of half seeds that were heterozygous across the QTL intervals (streams 1, 2 and 4) plus *Sm1* locus (stream 3) and (expected) number of half seeds based on the number and size of QTL intervals (Table 2). Chi square test significance at 0.05**, 0.1*, non-significant (ns)

[d] The number of markers used to examine the genetic background for genome restoration

[e] The percentage of fixed elite alleles within the selected seed

peak to ensure that the FHB resistance gene was introgressed. Further, the QTL intervals were delimited by markers that were necessarily codominant in the specific cross.

BC_1F_1 generation

Streams 1 and 2 each included introgression of three FHB QTL and the recurrent parent possessed *Sm1* and *Lr21* resistance genes, respectively. Stream 3 included introgression of three FHB QTL and *Sm1*. Stream 4 included introgression of two FHB QTL (Fig. 1). The number of genes being introgressed and the size of the selected QTL intervals influenced the size of the BC_1F_1 population. Approximately 1,152 (12 × 96) seeds in streams 1 through 3 and 968 seeds for stream 4 were used. The number of seeds selected as heterozygous with centre FHB QTL markers approximated $1/2^n$ where n = number of loci. The number of seeds remaining after selection with the flanking FHB markers was reduced by 53–83%. This left 72, 64, 22 and 133 half seeds which were in proportion with the number and size of QTL intervals being introgressed for each stream. An example of this genotype selection process is depicted in Fig. 2 showing the selection of heterozygotes at three microsatellite loci on chromosome 4B in both the BC_1F_1 and BC_2F_1 generations.

The analysis of the background genome markers ranked the half seeds based on the percentage of fixed elite alleles and those half seeds with the highest degree of genome fixation were selected to be advanced. The selection of 2–5 BC_1F_1 half seeds in the crossing streams resulted in a selection pressure ranging from 0.17 to 0.43% based on the initial BC_1F_1 seed quantities. The average percentage of fixed elite alleles ranged from 46 to 51% across all streams in the BC_1F_1 generation. The highest level of elite allele fixation achieved across all streams ranged from 66 to 71% (Table 3, Fig. 3).

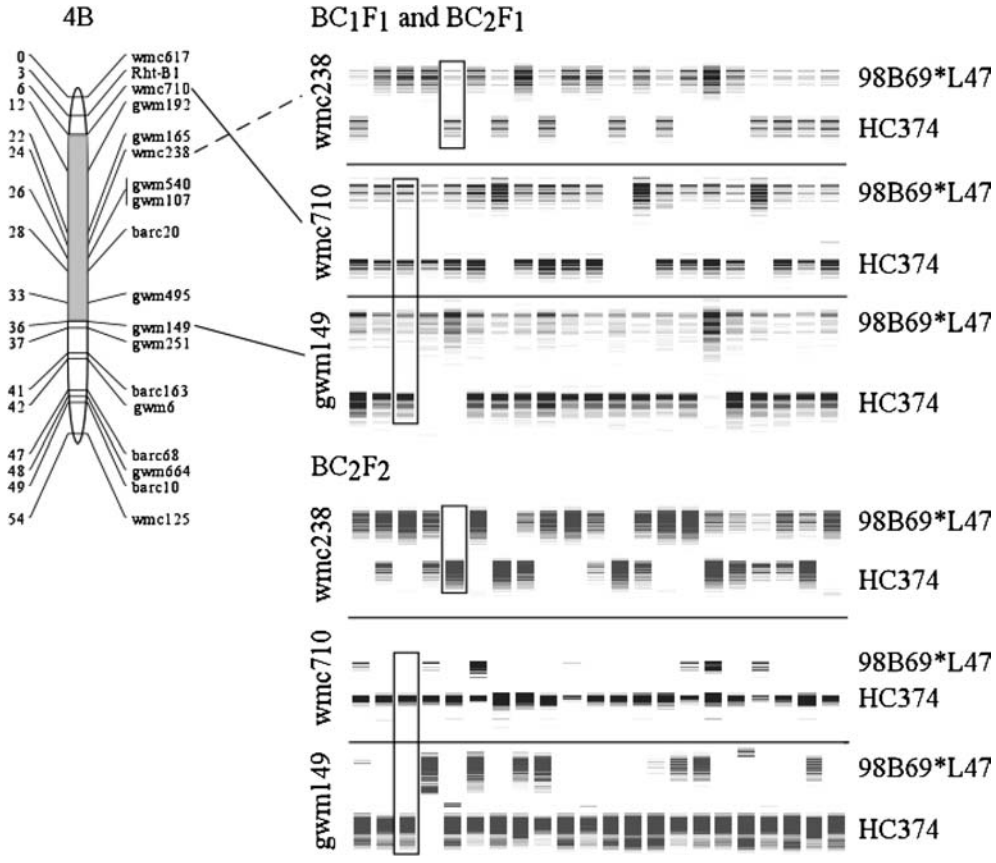

Fig. 2 A genetic map (Somers et al. 2004) and an example microsatellite profile of markers on chromosome 4B used for the selection of a FHB resistance QTL in stream 1 of the project. In this example, wmc238 was used first to select heterozygotes (an example is *boxed*). Heterozygous individuals were then genotyped with wmc710 and gwm149 to complete selection of the heterozygotes (*boxed*) across the interval. This process was the same for BC_1F_1 and BC_2F_1 generations. The same markers and process were used to select BC_2F_2 individuals that were homozygous for HC374 (FHB resistant) alleles (*boxed*)

BC_2F_1 generation

Table 3 shows the BC_2F_1 generation details in all streams separated as individual families derived from separate BC_1F_1 females. A similar number of half seeds was examined from each family within a stream. The number of selected half seeds in each family following selection with the centre FHB QTL marker and flanking markers was similar to that observed in the BC_1F_1 populations (Table 3, Fig. 2).

The BC_2F_1 generation background genome marker genotyping proceeded with loci known to remain heterozygous from the BC_1F_1 generation and thus the number of markers used was dependant on the degree of BC_1F_1 elite parent allele fixation. The average percent fixation of elite alleles in the BC_2F_1 populations ranged from 76 to 88% across all streams. The highest levels of elite allele fixation achieved across all streams ranged from 79 to 100% (Table 3, Fig. 3). The number of selected half seeds ranged from 1 to 6 within a BC_2F_1 population which resulted in a selection pressure of 0.38–2.27% across all streams based on the initial BC_2F_1 seed quantities.

The half seeds with the greatest amount of recurrent parent genome fixation were selected for selfing to create BC_2F_2 populations. The percentage elite allele fixation in these selected BC_2F_1 half seeds ranged from 79–89%, 88–100%, 81–93% and 89–93% in streams 1, 2, 3 and 4, respectively. The BC_2F_2 generations were produced for streams 1, 2 and 4 only due to delays in the stream 3 crossing scheme. Large numbers of BC_2F_2 half seeds were examined with the three markers around each FHB QTL and resulted in 52, 23 and 64 seeds meeting the criteria of being homozygous across each FHB QTL for streams 1, 2 and 4, respectively (Table 3, Fig. 3). Stream 3 half seeds were advanced to intercrossing as heterozygous BC_2F_1. A reduced number of BC_2F_2 (streams 1, 2, 4) or BC_2F_1 (stream 3) seeds was selected for intercrossing that assured that all of the 14 BC_1F_1 generation plants would be represented in the final DH populations. Over the entire project, this resulted in a selection pressure of 0.44% (49 selected seeds from 11,072 seeds examined).

The initial elite × donor crosses were made in March 2002 and the final intercross seed between streams was mature in April 2004. This strategy took 25 months to pass through five generations in four independent crosses and included genotyping of 11,072 seeds in two of the generations. The approximate cost of the genotype data collection was $1.20/data point (excluding labour) using the ABI 5 dye system as described in Somers et al. (2004). The most expensive components of this cost include DNA extraction ($0.70/sample) and Taq polymerase ($0.14/PCR). Further reductions in the cost can be achieved by multiplexing the PCR reactions.

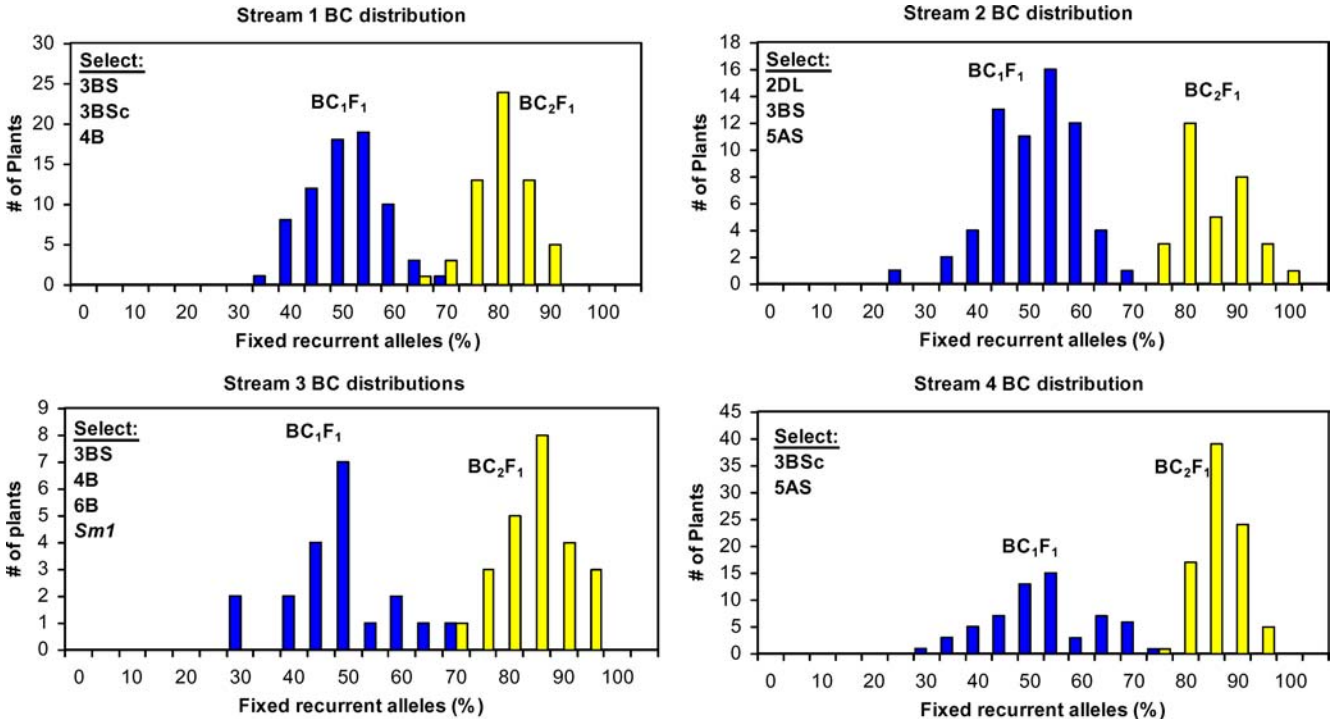

Fig. 3 Histograms showing the BC_1F_1 and BC_2F_1 population distributions from each crossing stream based on the degree of genome restoration following genotyping with markers across the genome. All of the lines represented in these distributions are heterozygous for the FHB resistance QTL. BC_2F_1 populations were derived from BC_1F_1 plants selected with the highest percentage fixed recurrent alleles

Discussion

The design of this research project required that: (1) multiple pest resistance genes should be introgressed into elite germplasm; (2) unique pest resistance gene pyramids should be created; (3) much of the genetic background should be restored in two backcrosses; and (4) the molecular breeding progress should proceed at or faster than the rate used in conventional plant crossing and selection. Numerous sources of FHB resistance have been genotyped and mapped (McCartney et al. 2004) and there is a need to validate the QTL and the markers and implement marker-assisted selection in wheat improvement.

There were six FHB resistance QTL identified in the literature or previous work at Agriculture and Agri-Food Canada that were targeted for introgression into the elite wheat backgrounds (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Somers et al. 2003; Yang et al. 2003). This included a mixture of sources of FHB resistance genes (Sumai 3, Nyubai, Wuhan) and none of the FHB resistance QTL had been tested alone in the elite Canadian wheat backgrounds. The elite wheat backgrounds were selected so that a degree of genetic diversity was represented and contrasting alleles for seed quality and agronomic performance could be pyramided during stream intercrossing (Table 1). The donor FHB resistant lines carried between two and five FHB resistance QTL (Table 1), but a maximum of three FHB resistance QTL were selected in any stream (Fig. 1). This was done to balance the size of the populations and the number of crossing streams that could be analysed against the resources and time available. In addition, BW301, 98B69*R28 and 98B69*L47 carried *Lr21*, *Sm1* and *Sm1*, respectively, which could be restored easily during backcrossing. The background genome marker data for BC_2F_1 and BC_2F_2 seeds were checked to ensure that selected plants carried *Lr21* and *Sm1* alleles. Stream 3 was the exception, where three FHB QTL and *Sm1* were heterozygous in the initial F_1 and all four genes needed to be selected in each BC population using Prodigy as the recurrent parent. Further, stream 4 was used to introgress only two FHB QTL into BW263 that is also homozygous for white seed colour genes on group 3 chromosomes. Therefore, stream 4 is a red × white seed cross and an effort was made to study the genotype of the BC_1F_1 and BC_2F_1 half seeds on linkage group 3 and select for fixation of white seed alleles. These seed colour genes were restored and fixed to the white seed condition in BC_2F_1 as evidenced by the white seed colour in the BC_2F_2 populations.

Overall, the segregation of pest resistance genes/QTL in the BC_1F_1 and BC_2F_1 populations followed Mendelian inheritance and conditions of genetic linkage. For example, selection for three or two FHB loci on different chromosomes should result in selecting 0.125 and 0.25 of the BCF_1, respectively. Further, simultaneous selection for zero recombination across QTL intervals of 33, 13 and 20 cM each, as is the case

in stream 1 (Table 2), should result in selecting 0.47 of the progeny. Following selection for the QTL centre markers in the BC_1F_1 and BC_2F_1 populations, the expected segregation was observed in 13/18 ($P < 0.05$) and 5/18 ($P < 0.10$) populations. Similarly, after selection for multiple loci and QTL intervals in each BC_1F_1 and BC_2F_1 population, the expected segregation was observed in 13/18 ($P < 0.05$) and 3/18 populations ($P < 0.10$). Two populations (stream 2-BC_1F_1, stream 4-BC_1F_1) showed segregation distortion caused by missing data in the QTL interval selection step of the process (Table 3). Table 3 shows the actual numbers of seeds processed and includes seeds where genotype data were missing. These segregation results suggest that the genetic distances on the microsatellite consensus map (Somers et al. 2004) in these QTL intervals approximate the actual distances in the four populations and that the consensus map can be used as a tool to predict a required population size for molecular breeding experiments.

The selection of background genome markers to cover the whole genome was restricted by the level of polymorphism and the density of available markers in distal chromosome regions. As a result, the estimated amount of genome coverage was 71, 71, 67 and 55% for streams 1, 2, 3 and 4, respectively, based on the microsatellite consensus map (Somers et al. 2004). Streams 1–3 have more genome coverage because the crosses are genetically wider and more polymorphic. Stream 4 is genetically a more narrow cross showing less polymorphism. Despite these restrictions, the background genome markers do facilitate tracking restoration of the polymorphic portion of the genome in the crosses.

The initial BC_1F_1 populations were between 968 and 1,152 seeds which were sufficiently large enough to leave 72, 64, 22 and 133 plants (streams 1, 2, 3 and 4, respectively) for the whole genome genotyping after selection of the pest resistance genes/QTL. The combination of the two backcrosses in all streams, each fixing a substantial amount of the recurrent background loci, resulted in an average of 87.4% of the loci being fixed (Table 3, Fig. 3). The expected average amount of genome fixation at BC_2 is 75% and at BC_3 is 87.5%. This experiment achieved the BC_3 level of recurrent parent allele fixation in two backcrosses.

Codominant markers were used for all of the loci under selection and further, only markers with a clear separation of alleles by capillary electrophoresis were used (Fig. 2). This ensured that every locus of every seed that was genotyped was classified correctly into one of the three possible allele configurations. The microsatellite consensus map (Somers et al. 2004) was developed from four independent crosses and showed that 38% of the microsatellite marker alleles in the four pairs of mapping parents differed by 4 bp. This emphasizes the need for: (1) very dense genetic maps to provide greater selection of markers in critical intervals; and (2) capillary electrophoresis which enables clear resolution of alleles at 2 bp or more.

This experiment was effectively replicated four times with independent crosses and achieved a high level of recurrent parent fixation each time (Fig. 3). Presumably, this result could be repeated every time with a similar experimental design. In contrast, if only the FHB resistance QTL were selected and monitoring restoration of the genetic background was ignored, a similar result would be achieved rarely. The average probability of selecting the single most restored plant from the BC_1F_1 and the subsequent four BC_2F_1 populations in stream 1 would be < 0.0008. The probability of selecting, from stream 1, the 14 most restored plants as was done in this experiment would be orders of magnitude less. The 14 BC_1F_1 plants selected from all streams to be advanced to the next generation had a background genome fixation ranging between 48 and 71%. There was a good correlation between the percentage genome fixation at BC_1F_1 to the percentage genome fixation at BC_2F_1 of $r = 0.67$ ($P < 0.01$). The experimental design and techniques of molecular breeding were able to direct the selection of a high amount of genome fixation and not leave this important aspect of breeding to chance.

In summary, it appeared feasible to assemble complex genotypes with microsatellite marker genotyping. This experiment accomplished selecting up to four pest resistance genes/QTL in segregating populations and simultaneously restored the elite genetic background to an adequate level (87% after BC_2) in four separate crosses. The centralized high throughput genotyping resources, half-seed analysis and coordination between collaborating research laboratories enabled the project to keep pace with conventional breeding practices in spring wheat. DH lines from the intercrossed BC_2F_2 and BC_2F_1 plants are being generated for further genotype and phenotype analysis under field and greenhouse conditions.

Acknowledgements The authors wish to thank the AAFC technical staff including Debbie Stern, Wenguang Cao and Devin Dahlman for production of BC seed and plant maintenance. We also thank the molecular breeding technical staff including Leslie Bezte, Monika Eng, Debbie Miranda, Yanfen Zheng, Allison Brown-Hoeppner, Mira Popovic and Zlatko Popovic for the genotypic analysis. This project was supported by the Western Grains Research Foundation Wheat Check-off fund and the Agriculture and Agri-Food Canada Matching Investment Initiative.

References

Anderson JA, Stack RW, Liu S, Waldron BL, Field AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTL in two wheat populations. Theor Appl Genet 102:1164–1168

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbaue P (2002) Molecular mapping of QTL for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Genet 104:84–91

Gilbert J, Tekauz A (2000) Review: recent developments in research on Fusarium head blight in Canada. Can J Plant Path 22:1–8

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

McCartney C, Somers DJ, Fedak G, Cao W (2004) Allele diversity at FHB loci. Theor Appl Genet 109(2):262–271

Mesterhazy A (1983) Breeding wheat for resistance to *Fusarium graminearum* and *Fusarium culmorum*. Z Pflanzenzuchtg 91:295–311

Mesterhazy A (1995) Types and components of resistance to Fusarium head blight. Plant Breed 114:377–386

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Somers DJ (2004) Molecular marker systems and their evaluation for cereal genetics. In: Gupta PK, Varshney RK (eds) Cereal genomics. Kluwer Academic Publishing, pp 19–34

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555–564

Somers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109(6):1105–1114

Spielmeyer W, Huang L, Bariana H, Laroche A, Gill BS, Lagudah E (2000) NBS-LRR sequence family is associated with leaf and stripe rust resistance on the end of homoeologous chromosome group 1S of wheat. Theor Appl Genet 101:1139–1144

Thomas J, Fineberg N, Penner G, McCartney C, Aung T, Wise I, McCallum B (2005) Chromosome location and markers of Sm1: a gene of wheat that conditions antibiotic resistance to orange wheat blossom midge. Molec Breed 15(2):183–192

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805–811

Yang Z-P, Gilbert J, Somers DJ, Fedak G, Procunier JD, McKenzie R (2003) Marker-assisted selection of Fusarium head blight resistance genes in two doubled-haploid populations of wheat. Mol Breeding 12(4):309–317

Yang Z-P, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome 48(2):187–196