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ORIGINAL PAPER

Analysis of the chromosome 2(2H) region of barley associated with the correlated traits Fusarium head blight resistance and heading date

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Abstract Fusarium head blight (FHB) is a major disease of barley (*Hordeum vulgare* L.) that results in reduced grain yield and quality through the accumulation of the mycotoxin deoxynivalenol (DON). Coincident QTL for FHB severity, DON concentration, and heading date (HD) map to a region of chromosome 2(2H) designated Qrgz-2H-8. It is unclear whether disease resistance at this locus is due to a pleiotropic effect of late HD by delaying the host exposure to the pathogen or a tightly linked resistance gene. The objectives of this study were to develop a set of near isogenic lines (NILs) for the Qrgz-2H-8 region and to genetically dissect the QTL region containing the coincident traits. Two NIL populations were developed consisting of F_2- and F_4-derived recombinants from a cross between a BC_5 line carrying the donor parent (Chevron) alleles in the Qrgz-2H-8 region and the recurrent parent M69. Analysis of field and marker data from these NILs revealed that the Chevron alleles conditioning FHB resistance, late HD, and low DON concentration were successfully introgressed into the BC_5 parent line and were segregating among NILs. QTL analysis of the F_4-derived population showed that the HD QTL is adjacent to the FHB QTL. Furthermore, a single NIL was identified that was similar to the resistant BC_5 parent for FHB severity and the early flowering parent M69 for HD. These results indicate that the relationship between FHB and HD at the Qrgz-2H-8 region is likely due to tight linkage rather than pleiotropy.

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Introduction

Fusarium head blight (FHB), or scab, is a major disease of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) in many temperate environments worldwide. FHB re-emerged in the 1990s within the small grains production region of the Northern Great Plains (North Dakota, Minnesota, and Manitoba) and caused major losses in revenue to barley producers (Windels 2000; Nganje et al. 2001). The disease is caused primarily by the fungal pathogen *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch]. During infection, *F. graminearum* produces trichothecene mycotoxins including deoxynivalenol (DON), which lowers quality of the grain for use in malting, brewing, and as animal feed (Schwartz 2003). Barley producers currently attempt to manage the disease through crop rotation and fungicide application. However, these measures alone are not sufficient to reduce the risk of the disease. The most cost effective approach would be the deployment of resistant cultivars as part of an integrated disease management strategy (McMullen et al. 1997).

Developing FHB resistant barley cultivars is a challenging endeavor for several reasons (reviewed by Steffenson 2003). One, FHB screening experiments are labor-intensive and extremely expensive. Two, disease resistance is quantitatively inherited and highly influenced by the environment. Three, disease severity is strongly correlated with heading date (HD) and other agronomic and spike morphology traits. Since infection can only occur after the spike emerges from the boot, differences in HD make it difficult to distinguish 'true' disease resistance from 'apparent' resistance that is due to host escape from the pathogen. In addition, since disease expression is strongly influenced by the environment, comparisons among barley genotypes that

differ in HD are themselves confounded by the effect of the environment on disease development.

To gain a genetic understanding of FHB resistance in barley, multiple sources of resistance including Chevron (de la Peña et al. 1999; Ma et al. 2000), Gobernadora (Zhu et al. 1999), Fredrickson (Mesfin et al. 2003), Zhedar 2 (Dahleen et al. 2003), and CIho 4196 (Horsley et al. 2006) have been used in quantitative trait locus (QTL) mapping studies. In all of these studies except the one using Gobernadora, the bin 8 region of the long arm of chromosome 2H designated by Horsley et al. (2006) as Qrgz-2H-8 was consistently associated with FHB severity, HD, and DON concentration. The approximate size for the overlapping QTL region ranged from 22 cM in Fredrickson/Stander population (Mesfin et al. 2003) to 45 cM in Chevron/M69 (de la Peña et al. 1999) and CIho 4196/Foster (Horsley et al. 2006) populations. Depending on the population and the environment, Qrgz-2H-8 explained 7–60% of the variation in FHB resistance, 10–30% of the variation in DON concentration, and 12–30% of the variation in HD. In all of the studies, FHB severity and DON concentration were negatively correlated with HD. In a validation study of this QTL, the Chevron introgression at the Qrgz-2H-8 region reduced FHB by 42% and increased HD by 3.8 days. (Canci et al. 2004)

Steffenson (2003) suggested that the association between lower FHB severity and late heading may be due to shorter inoculum exposure (pleiotropy) or tight linkage of separate genes for flowering time and disease resistance. To determine if the association between late HD and FHB resistance is due to linkage or pleiotropy, high resolution mapping of the coincident QTL region is necessary. However, the resolution of the previous primary QTL studies is low due to the limited number of meiotic events during the development of F_2-derived, recombinant inbred, or doubled haploid populations used. Moreover, these mapping studies previously reported QTL simultaneously segregating at multiple locations in the genome (effects at one QTL are affected by segregation at other QTL elsewhere in the genome), generally utilized small population sizes, and were likely characterized by high rates of type-I errors (Fasoula et al. 2004); thus making them inadequate for estimating precise positions of desired QTL. To achieve high resolution and assess the utility of these QTL for marker assisted selection (MAS), fine mapping is necessary.

Several methods exist for fine mapping QTL regions, but the near-isogenic line (NIL) approach described by Paterson et al. (1990) is widely used in plants. This approach targets one QTL region by generating a NIL by backcrossing to a recurrent parent and using markers to select for a donor parent segment from backcross progeny. A set of overlapping recombinant near-isogenic lines (rNILs) with complimentary introgressions at the target QTL region are then developed from the cross between the BC-derived NIL and the recurrent parent. These rNILs are often self pollinated one or more generations to increase seed supply for replicated trials. The NIL approach has been used in plants to attempt to distinguish between pleiotropy and linkage (Han et al. 1997; Meksem et al. 1999; Brouwer and St. Clair 2004; Nichols et al. 2006).

In this study, we report the construction of a fine map for the chromosome 2(2H) QTL region using rNILs derived from a cross between a BC_5 line carrying the Chevron alleles for markers at the Qrgz-2H-8 region and the recurrent parent M69. The specific objectives of the study were (1) to Mendelize the Qrgz-2H-8 QTL region by developing a NIL that carries the Chevron alleles for the region in a M69 genetic background; and (2) to genetically dissect the QTL region by examining contrasting rNILs with different marker haplotypes.

Materials and methods

Development of the parental (pNIL) and recombinant (rNIL) near isogenic lines

To develop the pNIL, a resistant $F_{4:7}$ progeny designated CM62 from the Chevron/M69 mapping population (de la Peña et al. 1999) with the Chevron haplotype for the Qrgz-2H-8 region was crossed with the susceptible mapping parent M69. After the initial cross, segregating progeny were genotyped with RFLP markers *ABG619, ABG14, ABC306*, and *MWG887* flanking the target QTL region. An F_2 line homozygous for the Chevron alleles at the target QTL region was selected and backcrossed to M69. Using the same approach, four subsequent backcross cycles were conducted using flanking SSR markers *EBmac0521a*, *Bmag0140*, and *Bmag0378* to advance lines to the BC_5F_2 generation. A single BC_5F_2 line homozygous for the Chevron haplotype at the *EBmac0521a–Bmag0140* marker interval spanning the target QTL region was selected as the pNIL.

Subsequently, we developed two populations of rNILs for this study. The first population hereafter referred to as Gen1a was developed with a goal of isolating FHB resistant and susceptible alleles for the coincident QTL region in a common genetic background. An F_2 population of 532 plants from a cross between the pNIL and M69 were screened with SSR markers *EBmac0521a* and *Bmag0140* flanking the target QTL region to identify recombinants. Recombinants were further screened with six additional SSR markers (Fig. 1) previously mapped at the *EBmac0521a–Bmag0140* interval (Ramsay et al. 2000; Canci et al. 2004; Thiel et al. 2003) to identify the location of recombination break points. Homozygous recombinants

were further advanced by self pollination to the $F_{2:4}$ generation and evaluated in the field for HD, FHB severity, and DON concentration.

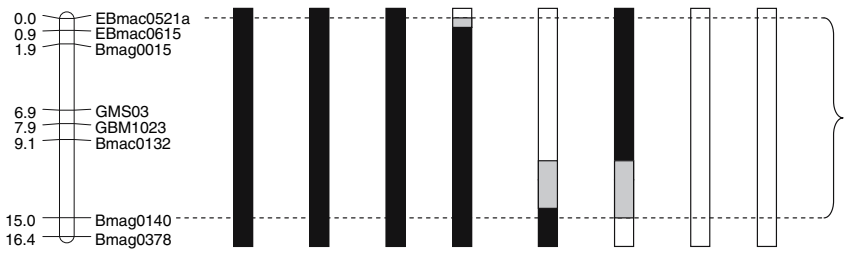
A second population of rNILs hereafter referred to as Gen1b was developed for use in fine mapping the target QTL region and to examine the genetic relationship between FHB and HD. A total of 1,152 F_2 plants developed from a cross between pNIL and M69 were advanced by single seed descent to the F_4 generation and genotyped with flanking markers *EBmac0521a* and *Bmag0140.* Homozygous recombinants were identified and further genotyped with all six SSR markers previously mapped within the QTL region (Fig. 1) to locate the position of the recombination break points. Selected homozygous recombinants were advanced to the $F_{4:5}$ generation and used for field testing.

DNA extraction and marker analysis

The DNA extraction followed the procedure of Edwards et al. (1991). Gel blot analyses and RFLP marker genotyping followed the procedure described by de la Peña et al. (1996). DNA amplifications for simple sequence repeat (SSR) markers were performed in a thermocycler (Gene Amp PCR System model 9700). Each 10-µL reaction mix contained 50 ng genomic DNA, 10 ng of forward and reverse primer, 1.5 mM $MgCL_2$, 0.1 mM dNTPs, and 1 U *Taq* DNA polymerase. The PCR program for different SSR primers varied considerably, but the majority were amplified following a 60 s initial denaturation at 94°C followed by 35 cycles of 10 s at 94°C, 20 s at 55°C, 45 s at 72°C, and a final extension of 2 min 30 s at 72°C. PCR products were separated by electrophoresis in 6% polyacrylamide gels and detected using silver staining as described by Bassam et al. (1991). DNA bands were scored manually either as pNIL (A), M69 (B), heterozygous (H), or missing data (−). We generated a linkage map for the target QTL region using marker data from the Gen1b population of 111 recombinant F_4 plants and the computer package JoinMap Version 3.0 (Van Ooijen and Voorrips 2001).

Field evaluations of rNILs and parental lines

The Gen1a rNILs, Chevron, and the parental lines M69 and pNIL were evaluated at St. Paul and Crookston, MN, in the summers of 2003, 2004, and 2005, and in Hangzhou, China in 2004. The Gen1b rNILs were evaluated at St. Paul and Crookston in 2005 and randomized with the Gen1a population using the same parent and check plots. The Gen1b rNILs were also evaluated in China in 2005. Field experiments were planted in 2.4 m long single-row plots, spaced at 30 cm apart in a randomized complete block with two replicates. At St. Paul, the macroconidia inoculation technique described by Steffenson (2003) was used to inoculate plants with a mixture of 12–18 local isolates of *F. graminearum*. Plots were placed into two inoculation groups according to HD and each group was inoculated twice; the first inoculation 2–3 days after heading and a repeat inoculation about 3 days later. At Crookston and China, a grain-spawn inoculation technique was used by spreading *F. graminearum* infected maize or barley grain in the nursery



		Genotypes							
		Chevron[a]	pNIL[b]	NIL-AC	rNIL-A1	rNIL-A2	rNIL-A3	NIL-AM	M69[c]
Trait	**Env[d]**			15[e]	1	1	1	4	
	CR03	3.8	**2.3***	3.5	**1.9***	9.5	5.4	10.3	7.2
	SP04	**2.0***	**3.3***	7.4	12.7	8.6	10.2	10.0	12.3
	CR04	**8.5***	**6.6***	**8.2***	**10.2***	48.9	**12.3***	41.0	21.5
	HZ04	**1.3***	10.3	4.4	4.3	7.7	**1.8***	9.0	5.9
FHB	**CR05**	**0.3***	**2.8***	4.3	8.0	7.6	3.3	5.6	4.2
	SP03	**60***	**59***	**60***	**60***	55	**60***	55	55
	CR03	**61***	**59***	**60***	**61***	54	**60***	55	57
	SP04	**56***	**59***	**56***	**55***	49	**55***	50	49
	SP05	**59***	**60***	**60***	**60***	57	**61***	57	57
HD	**CR05**	**64***	**65***	**65***	**64***	59	**64***	59	59
	SP03	**1.3***	**1.1***	2.9	2.0	2.5	3.3	2.6	2.3
DON	**CR03**	**7.3***	**7.3***	24.3	37.0	32.1	23.5	27.4	26.8

= Homozygous Chevron DNA; = Homozygous M69 DNA; = Interval in chromosome where crossover took place

Fig. 1 Mean Fusarium head blight (*FHB*) severity, heading date (*HD*) and deoxynivalenol (*DON*) concentration for Chevron (donor parent), M69 (recurrent parent), parental near-isogenic line (*pNIL*) and sets of homozygous recombinant near-isogenic lines (*rNILs*) with the same marker genotypes from Gen1a mapping population. rNILs were labeled AC, A1, A2, A3, and AM. *Significantly different from AM using least significant difference ($P = 0.05$). *a* Donor parent, *b* Parental near-isogenic line, *c* Recurrent parent, *d* Environments: Crookston 2003 (CR03), St. Paul 2003 (SP03), Crookston 2004 (CR04), St. Paul 2004 (SP04), China 2004 (HZ04), Crookston 2005 (CR05), and St. Paul 2005 (SP05), *e* Number of rNILs in genotype class

as described by Steffenson (2003). In all the environments, nurseries were mist-irrigated daily to enhance disease. Plots were assessed for disease in groups based on HD on a fixed number of days after heading or inoculation. Entries were scored for FHB severity by examining 20 arbitrary spikes from each plot. The number of infected spikelets from each spike was counted and the severity expressed as a percent of the total spikelets present. HD was scored as the number of days after planting when 50% of the spikes had emerged half way or more from the boot. DON concentration (μg/g) was measured on grain harvested at St. Paul (2003 and 2005) and Crookston (2003 and 2005). Grain was not harvested from the 2004 nurseries or the China nursery. The procedure described by Mirocha et al. (1998) was used to measure DON concentration.

Statistical analysis

The Gen1a and Gen1b populations were analyzed separately. For each population, analyses of variance were conducted using Proc GLM (SAS Institute 2000) on a per environment basis. In the environments where no significant variation among rNILs was observed for a particular trait, the trait data for the environment were excluded from further analysis. Trait means for parental lines and groups of homozygous rNIL representing different marker genotype classes were compared using protected LSD ($P < 0.05$). A genomic region was declared to be associated with a trait when the mean of the rNIL class carrying the Chevron allele at that region was significantly different from the mean of the NIL class carrying the M69 allele for the entire QTL region.

Results

Chevron/M69 map versus pNIL/M69 map

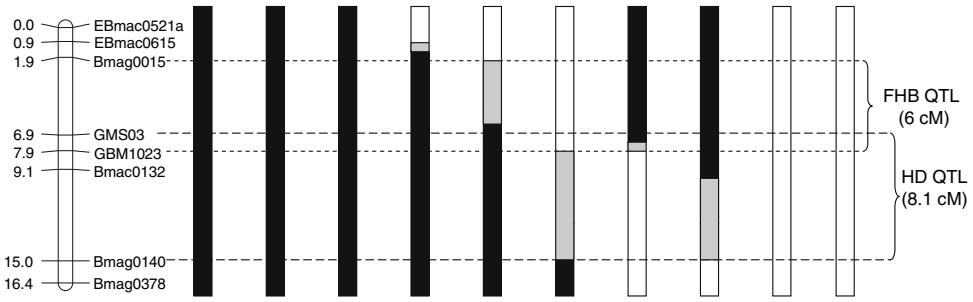
To generate the pNIL/M69 fine map, a total of 13 SSR markers; eight from the Chevron/M69 linkage map of Canci et al. (2004), one from a map by Thiel et al. (2003), and an additional four from a map by Ramsay et al. (2000) were used to genotype 111 F_4-derived rNILs from Gen1b. Of these markers, *EBmac0849*, *EBmac0558*, *EBmac0521b*, *EBmac0521c*, and *Bmac0093* co-segregated with *Bmag0015* and were therefore dropped from the fine map (Fig. 1). The marker order in the fine map was consistent with the Chevron/M69 map of Canci et al. (2004). In the Chevron/M69 map, the target QTL region is flanked by markers *EBmac0521a* and *Bmag0140* and spans about 34 cM (Canci et al. 2004). However, in the validation populations presented in that study the distance was 18.2 and 8.5 cM for the MM and SMN populations, respectively (Canci et al. 2004). In the pNIL/M69 map the same marker region spans 15.0 cM. The region between Ebmac0615 and Bmag0378 spans 15.5 cM in the pNIL/M69 map and 11.0 cM in a recently published consensus map (Varshney et al. 2007).

NIL development

In the development of the Gen1a population, we identified three homozygous recombinants and 19 homozygous non-recombinants (Fig. 1) for use in determining if the coincident QTL was successfully introgressed into the NIL population. We designated the 15 non-recombinants homozygous for Chevron alleles at the entire QTL region as NIL-AC, the rNIL homozygous for Chevron alleles at the *Bmag0378–EBmac0615* interval as rNIL-A1, the rNIL homozygous for Chevron alleles at the *Bmag0378–Bmag0140* interval as rNIL-A2, the rNIL with Chevron alleles as the *Bmac0132–EBmac0521a* as rNIL-A3, and the four homozygous non-recombinants carrying the M69 alleles at the entire QTL region as NIL-AM. During the development of the Gen1b, we identified 15 homozygous recombinants representing five recombinant genotypic classes (Fig. 2). The rNIL homozygous Chevron for the *Bmag0378–Bmag0015, Bmag0378–GMS03, Bmag0378–Bmag0140, GMS03–EBmac0521a*, and *Bmac0132–EBmac0521a* intervals were designated rNIL-B1 to rNIL-B5, respectively. There were five non-recombinants that were homozygous for the M69 allele across the entire region (NIL-BM) and one non-recombinant homozygous for the Chevron allele across the entire region (NIL-BC).

Performance of rNILs and parental lines

The pNIL was not significantly different from Chevron in FHB severity in six of eight environments tested (Tables 1, 2). Compared to the recurrent parent M69, the pNIL was significantly lower in FHB severity in all environments tested except at China in 2004. The pNIL was also similar to Chevron for DON concentration except at Crookston 2003 and 2005 and lower than M69 for all but one environment (Crookston 2005). The pNIL flowered about the same time as Chevron in all environments except for St. Paul and Crookston in 2003. The pNIL also generally flowered 2–5 days later than M69 in all environments. These results show that the pNIL carrying the Chevron allele for markers spanning the QTL region also displayed the Chevron phenotype for FHB severity, DON concentration, and HD as predicted by the previous mapping studies (de la Peña et al. 1999; Canci et al. 2004). There was significant variation among the rNILs evaluated for FHB severity, DON concentration and HD in all environments tested with the exception of FHB severity in St. Paul 2003 and 2005, and DON accumulation at St. Paul and Crookston (Gen1a only) in 2005.



		Genotypes									
		Chevron[a]	pNIL[b]	rNIL-BC	rNIL-B1	rNIL-B2	rNIL-B3	rNIL-B4	rNIL-B5	rNIL-BM	M69[c]
Trait	**Env[d]**			1[e]	3	7	2	1	2	5	
FHB	**CR05**	**0.3***	2.8	2.8	3.1	**2.6***	**2.6***	**1.8***	**1.7***	5.1	4.2
	HZ05	**0.0***	**0.3***	**0.3***	**0.4***	**0.7***	24.6	**0.9***	**0.1***	7.6	9.5
HD	**SP05**	**59***	**60***	**60***	**60***	**61***	54	55	**59***	57	57
	CR05	**64***	**65***	**65***	**65***	**66***	58	59	**63***	59	59
DON	**CR05**	**1.1***	**4.3***	**3.6***	5.3	**4.3***	4.7	5.4	**4.3***	7.2	5.1

Fig. 2 Mean Fusarium head blight (*FHB*) severity, heading date (*HD*) and deoxynivalenol concentration for Chevron (donor parent), M69 (recurrent parent), parental near-isogenic line (*pNIL*) and sets of homozygous recombinant near-isogenic lines (*rNILs*) with the same marker genotypes from Gen1b mapping population. rNILs were labeled B1, B2, B3, B4, B5, and BM. *Significantly different from BM using least significant difference ($P = 0.05$). *a* Donor parent, *b* Parental near-isogenic line, *c* Recurrent parent, *d* Environments: St. Paul (SP05),Crookston (CR05), and China at (HZ05) in 2005, *e* Number of rNILs in genotype class

Table 1 Means and ranges for *Fusarium* head blight (FHB) severity, heading date (HD), and deoxynivalenol (DON) concentration for Chevron (donor parent), M69 (recurrent parent), parental near-isogenic line (pNIL) and recombinant near-isogenic lines (rNILs) in population Gen1a

Trait	Environments	Chevron	pNIL	M69	rNILs	rNILs range	*P*-value[a]
FHB[b]	St. Paul 2003	1.3a	1.8a	3.7b	2.2	0.6–6.8	ns
	Crookston 2003	3.8a	2.3a	7.2b	4.8	0.6–19.4	<0.0017
	St. Paul 2004	2.0a	3.3a	12.3b	7.6	1.6–24.5	<0.0001
	Crookston 2004	8.5a	6.6a	21.5b	15.1	0.7–61.5	<0.0001
	China 2004	1.3a	10.3c	5.9b	5.0	1.1–11.9	<0.0114
	St. Paul 2005	5.8a	5.5a	9.6b	10.8	2.7–26.5	ns
	Crookston 2005	0.3a	2.8b	4.2c	5.1	0.5–17.0	0.0221
HD[c]	St. Paul 2003	60.0c	58.5b	55.0a	58.8	54–61	<0.0178
	Crookston 2003	61.0c	58.5b	56.5a	58.6	54–61	<0.0001
	St. Paul 2004	59.0b	58.5b	57.0a	58.1	52–61	<0.0001
	St. Paul 2005	59.0b	60.3b	57.0a	54.7	51–58	<0.0001
	Crookston 2005	64.0b	65.0b	59.5a	63.4	58–67	<0.0001
DON[d]	St. Paul 2003	1.3a	1.1a	2.3b	2.8	0.6–6.9	<0.0011
	Crookston 2003	7.3a	15.8b	26.8c	25.8	14.5–42.7	<0.0020
	St. Paul 2005	1.8a	1.5a	2.8b	1.8	0.5–4.4	ns
	Crookston 2005	1.1a	4.3b	5.1b	6.9	2.9–12.3	ns

Means within the same row followed by the same letter are not significantly different based on least significant difference ($P = 0.05$)

[a] Test for significant variation among rNILs, ns is non-significant ($P > 0.05$)

[b] FHB severity (% diseased kernels)

[c] Days to heading

[d] Concentration of deoxynivalenol in harvested grain

Mapping FHB, DON, and HD in Gen1a

Mean values for different haplotypes (sets of homozygous rNIL with the same marker genotype) and parental lines are summarized in Fig. 1. The rNIL-A1 and rNIL-A3 haplotypes have a Chevron allele in the *Bmac0132–EBmac0615* interval. The mean FHB severity for the rNIL-A1 and rNIL-A3 haplotype was significantly lower than the control (rNIL-AM) in four of ten treatment combinations (haplotype by environment). In three of the remaining treatment combinations, FHB severity for rNIL-A1 and rNIL-A3 was numerically lower (41–52%) than the rNIL-AM control.

Table 2 Means and ranges for *Fusarium* head blight (FHB) severity, heading date (HD), and deoxynivalenol (DON) concentration for Chevron (donor parent), M69 (recurrent parent), parental near-isogenic line (pNIL) and recombinant near-isogenic lines (rNILs) in population Gen1b

Trait	Environments	Chevron	pNIL	M69	rNILs	rNILs range	*P*-value[a]
FHB[b]	St. Paul 2005	5.8a	5.5a	9.6b	7.0	0.7–16.8	ns
	Crookston 2005	0.3a	2.8b	4.2c	3.1	0.3–9.6	0.0396
	China 2005	0.0a	0.3a	9.5b	4.7	0.0–44.6	<0.0001
HD[c]	St. Paul 2005	59.0b	60.3b	57.0a	53.8	48–58	<0.0001
	Crookston 2005	64.0b	65.0b	59.5a	63.2	57–68	<0.0001
DON[d]	St. Paul 2005	1.8a	1.5a	2.8b	1.7	0.5–4.3	ns
	Crookston 2005	1.1a	4.3b	5.1b	5.2	1.6–10.2	0.0056

Means within the same row followed by the same letter are not significantly different based on least significant difference ($P = 0.05$)

[a] Test for significant variation among rNILs, ns is non-significant ($P > 0.05$)

[b] FHB severity (% diseased kernels)

[c] Days to heading

[d] Concentration of deoxynivalenol in harvested grain

The rNIL-A1 and rNIL-A3 haplotypes were also significantly later in flowering by 5–6 days than rNIL-AM in all environments and had numerically lower DON levels in two of four treatment combinations tested. The rNIL-A2 haplotype was not significantly different from the rNIL-AM control for FHB, DON or HD. These results suggest that FHB, DON and HD QTL map to the 15 cM region flanked by markers *Bmag0140* and *EBmac0521a*. In general, the Gen1a rNILs did not provide the opportunity to compare recombinants within that 15 cM region.

Mapping FHB, DON, and HD in Gen1b

Figure 2 displays graphical genotypes for five rNIL haplotypes and their parents and compares the mean performance of each haplotype to rNIL-BM, carrying the M69 allele across the target QTL region. The mean FHB severity for rNIL-B1, rNIL-B2, rNIL-B4, and rNIL-B5 were significantly lower by 49–99% compared to rNIL-BM (control) in seven of eight treatment combinations. In contrast, the mean FHB severity for rNIL-B3 was lower than the control in one of two treatment combinations. Since the common feature among the haplotypes rNIL-B1, rNIL-B2, rNIL-B4, and rNIL-B5 is the presence of the Chevron allele at the marker locus *GMS03*, these results show that FHB resistance maps within a 6 cM region containing the *GMS03* marker. For HD, the haplotypes rNIL-B1, rNIL-B2, and rNIL-B5 (which have a common Chevron introgression at the *GBM1023–Bmac0132* interval) were significantly later by 2–7 days than rNIL-BM in all environments tested. The rNIL-B3 and rNIL-B4 (which did not carry the Chevron introgression at the *GBM1023–Bmac0132* interval) flowered about the same time or earlier than the rNIL-BM control. These HD results show the HD QTL maps in the 8.1 cM region flanked by markers *GMS03* and *Bmag0140*. For DON accumulation, the Crookston 2005 environment is the only environment of two tested where significant variation among rNILs was observed. In this environment, rNIL-B2 and rNIL-B5 showed significantly lower DON levels than the control. Although these two haplotypes carry the Chevron introgression at the *GMB1023–Bmac0132* interval, so does rNIL-B1 and it was not significantly lower in DON compared to the control. Therefore, we cannot map the DON QTL with certainty. The data strongly suggest that the co-localization of QTL for FHB severity and HD at the Qrgz-2H-8 region is due to tightly linked but separate loci.

Frequency distribution patterns for FHB and HD in Gen1b rNILs

The mean FHB severity among the Gen1b rNILs across three environments (St. Paul 2005; Crookston 2005; China 2005) ranged from 1.4 to 13.8% and the segregation distribution pattern consisted of two distinct groups (Fig. 3a). One group, comprised mostly of rNILs with the Chevron allele at the *GMS03* locus, had similar disease severities as the pNIL. The rNIL group with the M69 allele at the same *GMS03* locus had similar disease severities as the recurrent M69 parent. For HD, the mean rNIL value across all tested environments ranged from 56 to 65 days and the distribution pattern was similarly bimodial (Fig. 3b). rNILs carrying the M69 allele at the GBM1023–Bmac0132 region were similar to M69, while rNILs carrying the Chevron allele for that region were similar to the pNIL.

Discussion

We used a set of rNIL to fine map FHB severity and HD at Qrgz-2H-8 and conclude that previously observed correlations between these traits were likely due to close linkage

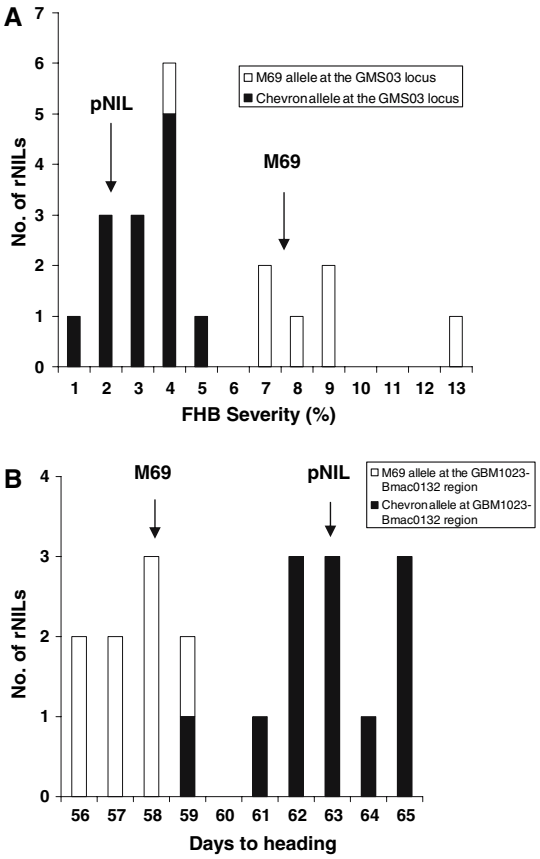


Fig. 3 Frequency distribution of **a** Fusarium head blight severity (*FHB*) and **b** heading date (*HD*) of homozygous recombinant near-isogenic lines (*rNILs*) from Gen1b mapping population. FHB severity and HD were each averaged across three environments. *Arrows* indicate the recurrent parent (M69) and parental near isogenic line (*pNIL*) mean values

rather than pleiotropy. We observed substantially fewer homozygous recombinants than would be expected based on the map that was constructed from the F4-derived population (Gen1a). We are uncertain why we observed segregation distortion other than proximity to the centromere which is commonly observed. Nevertheless, we were able to identify a set of recombinant NIL that were informative for the region of interest. A major advantage of using the NIL approach is that very few individuals are selected and field tested and therefore numerous environments can be used to test for even small QTL effects. This is particularly important for such traits as FHB severity, which are difficult to measure accurately and often associated with high QTL × environment (Q × E) interaction (de la Peña et al. 1999; Mesfin et al. 2003; Dahleen et al. 2003; Canci et al. 2004; Horsley et al. 2006). The tremendous resources required to measure FHB severity also preclude evaluating all of the many lines needed to identify rare recombination events. The only risk of using NILs is that they may carry small donor segments referred to as 'relics' (Paterson et al. 1990). These may continue to segregate independently of the target QTL region and may interact with the QTL within the target region. However, increasing the number of backcrosses during the development of NILs and using independent rNILs to represent haplotypes can effectively eliminate the risk of 'relics.' Since resistance did not segregate perfectly with lines carrying the Chevron allele at *GMS03*, we cannot eliminate this possibility. The increasing availability of SSR markers, Diversity Array Technology (DArT) markers (Wenzl et al. 2006) and single nucleotide polymorphism (SNP) markers (Rafalski 2002; Rostoks et al. 2006) will permit high resolution and complete genome coverage graphical genotyping to investigate this possibility.

Linkage versus pleiotropy

Determining whether the correlation among unrelated traits is due to close linkage or pleiotropy is challenging with quantitative traits. Typical mapping studies using bi-parental populations of moderate sizes tend to estimate the position of QTL with relatively large errors. Simulations studies representing typical QTL experiments indicate that estimates of a QTL position generally fall within a 20 cM or greater region that encompasses the true location of a QTL (Beavis 1998). Resolving the positions of several tightly linked QTL with fairly large errors further complicates tests of pleiotropy. Evaluating NIL derived from recombination events within a QTL region is a powerful approach for fine mapping. Gao et al. (2004) used NILs to fine map the barley malting quality trait, diastatic power, to a genomic region of 2.1 cM. Paterson et al. (1990) used this method to map fruit mass and fruit size in tomatoes (*Lycopersicon esculentum* L.) to genomic regions as little as 3 cM. Frary et al. (2003) fine mapped a tomato scar QTL to a region of 5.7 cM and a Brix QTL to a genomic region of 14.2 cM using NIL mapping. In wheat, Liu et al. (2006) used NILs to fine map a major QTL (*Fhb1*) for type-II FHB resistance to a 1.2 cM genomic region.

Using fine mapping approaches to distinguish between linkage and pleiotropy for co-located QTL has been met with mixed success. Nichols et al. (2006) mapped a protein QTL in soybean (*Glycine Max* L.)to a 3 cM region, but were unable to unambiguously separate it from the negatively correlated traits of seed oil concentration and yield. Frary et al. (2003) were able to determine that the QTL controlling fruit color and soluble solids content in wild tomato (*Lycopersicon chmielewskii* L.) were separate linked loci. Brouwer and St. Clair (2004) used fine-mapping and subNILs to separate disease resistance alleles in wild tomato from genes conditioning undesirable horticultural traits. While they were able to use this method to reduce 'linkage drag,' many of the horticultural traits co-segregated in the same subNIL.

FHB resistance at the Qrgz-2H-8 locus has been consistently association with late heading in mapping populations utilizing four different sources of resistance (de la Peña et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Dahleen et al. 2003; Horsley et al. 2006). Two markers mapped in the Qrgz-2H-8 region in this study (*Bmag0140* and *Bmag0378*) were also included in the previous studies confirming the same location in all four sources of resistance. Because late heading plants can escape infection by the pathogen and therefore appear to be resistant, it was possible that reduced severity was due to a pleiotropic effect of a HD gene. We used rNILs to refine the previous estimate of the location of the coincident QTL from a 34 cM region to a 8 cM region for HD QTL and a 6 cM region for FHB severity QTL. The FHB QTL is closely associated with marker *GMS03* while the HD QTL is closely associated with the marker interval *GBM1023–Bmac0132*. Moreover, when the rNIL are classified by the HD and FHB severity QTL genotypes, they appear to segregate as single Mendelian factors. Several previous fine mapping studies using NILs observed that some QTL behaved as single Mendelian factors (Alpert and Tanksley 1996; Yamamoto et al. 1998). Furthermore, we identified a single rNIL (rNIL-B4) that carries the Chevron allele at *GMS03* and the M69 allele at the adjacent marker *GBM1023*. This rNIL was similar in HD to the early flowering control rNIL-BM, but was at about 25% the level of FHB severity of the same control. To our knowledge, these results are the first to demonstrate that the association between FHB and HD can be explained by linkage.

Effect of the Chevron allele at Qrgz-2H-8

The value of an identified QTL in a mapping population is dependent on the effect of the donor allele in relevant breeding background. For some traits, the estimates of the allelic effect are different in the mapping population compared to their effects in NIL (Glover et al. 2004; Gao et al. 2004). In other cases, the mapping population accurately predicts the effect of the allele in NIL or breeding germplasm (e.g., Han et al. 1997; Chaib et al. 2006). The pNIL in our study carried one of the ten QTL regions that was detected in the original Chevron × M69 mapping population, but behaved similarly to Chevron suggesting that this QTL is the major contributor to resistance among those discovered in the original mapping population. This is consistent with our previous work where we were only able to validate the QTL identified on chromosome 2(2H), which is the subject of this study, and another QTL on chromosome 6(6H). In our study, the pNIL carrying the Chevron alleles at the Qrgz-2H-8 region reduced FHB by 70% and increased HD by 4.5 days. In the original study first identifying this QTL, the Chevron allele at the same QTL region reduced FHB by 46% and increased HD by 3.8 days (de la Peña et al. 1999). In validation experiments, where Chevron resistance was segregating in more elite breeding populations, the Chevron allele at Qrgz-2H-8 reduced FHB by about 42% and increased HD by 4 days (Canci et al. 2004). From these comparisons, it appears that the effects of the Chevron allele at this Qrgz-2H-8 region predicted by the original mapping study is generally consistent with what we observed in the rNIL study.

Implications for breeding

Correlation between FHB severity and other spike-related traits has presented a major barrier to breeding for FHB resistance in barley. Using MAS for the Chevron allele at the Qrgz-2H-8 locus should help breeders surpass this barrier. Conventional breeding for FHB resistance in barley has relied on field-based evaluation of resistance in specialized disease nurseries replicated in multiple locations. Resistant lines in these nurseries are often late heading and/or tall in height, raising the possibility of apparent resistance through escape from the pathogen. Typically, lines that are late heading are selected against. Therefore, any linked resistance genes are discarded as well. Given that the sizes of breeding populations evaluated at the F4–F5 generation are generally small (less than 200) it is unlikely to find recombinants that separate tightly linked loci using typical breeding procedures. In this study, we evaluated over 1,500 F_2 or F_4 lines to identify one recombinant in which resistance was uncoupled with late heading. MAS using this recombinant should permit the use of this gene to enhance resistance without delaying heading. In addition, the precise location of these QTL and the availability of rNILs with defined haplotypes in this region will provide the tools necessary to conduct allelism tests with lines carrying alleles in this region from other sources of resistance.

MAS offers promise to deal with another major barrier to progress in breeding for FHB resistance, namely genotype by environment (G × E) interaction. Most studies that have investigated FHB resistance have found high G × E interaction and many of the QTL for FHB severity are inconsistently detected across environments. Even Qrgz-2H-8, one of the more consistent QTL, was detected in 17 of 22 environments across four mapping studies (de la Peña et al. 1999; Mesfin et al. 2003; Dahleen et al. 2003; Horsley et al. 2006). In many of these cases, Qrgz-2H-8 was detected near the threshold for detection or detected using only simple interval mapping or only composite interval mapping methods. In our rNIL study, two of four rNIL classes that carry resistance alleles at the identified FHB QTL region did not significantly differ from the susceptible rNIL control in one of the environments. This presents a problem for phenotypic selection for disease resistance since breeding

lines carrying resistance alleles at QTL that are expressed or detected inconsistently across environments will not be consistently selected. Often in disease screening, the first round of selection is based on a single trial with little or no replication, further increasing the chance of discarding lines that carry resistance genes. Use of MAS will permit breeders to fix this resistance allele in breeding populations and insure that this gene is not lost in advanced cycles of breeding aimed at pyramiding multiple resistance genes.

Some of the same aspects of FHB resistance in barley that make it difficult to map also make it an excellent candidate for MAS. Moderate to low heritability, costly screening requirements, and strong environmental effect on trait expression contribute to a 'catch 22'; those traits that are the most difficult to map are the traits for which MAS could provide the most benefit (Holland 2004). For FHB and other traits that present similar challenges, the role of MAS may be to manipulate those genes not easily identified using phenotypic selection. Evidence to date in barley suggests that useful levels of resistance to FHB will need to be built from multiple genes with relatively small effects that are not consistently expressed or accurately measured across environments. In fact, the major benefit of MAS for traits of this type could be the ability to unambiguously and consistently select for a segment of the genome that itself has an inconsistent phenotype. Genes such as these will be the most prone to be lost from breeding populations if their maintenance is based solely on phenotypic selection. Pyramiding and maintaining these less than perfect genes through MAS may prove to be the best way to apply QTL mapping to improve this trait.

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