Fusarium head blight QTL mapping in durum wheat and *Triticum carthlicum* sources of resistance

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ABSTRACT

Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is susceptible to *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schw.) Petch], causal agent of *Fusarium* head blight (FHB), which reduces grain yield, quality and economic value of the crop. This study was conducted to map quantitative trait loci (QTL) for FHB resistance from two crosses. Doubled haploid (DH) lines were developed from the cross (DH1) 'Strongfield' x 'Blackbird' (a *Triticum turgidum* subsp. *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve genotype), and the cross (DH2) 'DT707' x 'DT696' (both advanced Canadian durum breeding lines). Both populations were evaluated in replicated FHB nurseries utilizing artificial inoculations under field conditions. The populations were rated for FHB incidence (Type I resistance) and severity (Type II resistance) and on a 1-9 disease rating scale. The two populations were genotyped with microsatellite markers to construct linkage maps and for QTL mapping. The multiple QTL mapping analyses revealed a QTL peak in DH1 at *cfa2153* on chromosome 1AS (Blackbird as the source of resistance) explaining up to 24% of the phenotypic variation (LOD 5.4) for FHB incidence, up to 15% (LOD 3.1) for FHB severity, and up to 15% (LOD 3.2) for the 1-9 rating scale. This marker is reported to be linked to Hessian fly resistance genes. In DH2, unlinked markers *gwm156* and *wmc110* on chromosome 5A were significantly linked to FHB incidence, severity and 1-9 scale. Significant QTLs on 5A have been previously reported (in hexaploid wheat). The *cfa2153* locus seems to a good candidate for marker-assisted selection because it is putatively novel and is linked to FHB and Hessian fly resistance.

INTRODUCTION

Durum wheat is susceptible to *F. graminearum*, causal agent of *Fusarium* head blight, which reduces grain yield, quality and economic value of the crop. *Fusarium graminearum* produces mycotoxins (vomitoxins), which are harmful to human and animal health. Deployment of FHB-resistant cultivars is considered the most effective and cost-efficient strategy to combat this disease. Type 1–resistance to initial infection or incidence and Type 2–resistance to spread or severity, have been identified in hexaploid wheat. Due to differences in quality parameters for durum and bread wheat, transfer of FHB resistance on the A and B genomes is not straight forward. Compared to hexaploid wheat fewer sources of resistance have been derived from durum wheat (Rudd et al. 2001; Oliver et al. 2008) although we have identified a line, DT696, with moderate resistance to FHB. *Triticum turgidum* L. subsp. *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve and *T. turgidum* L. subsp. *dicoccum* (Schrank ex Schübler) Thell. accessions have been reported to exhibit moderate resistance to FHB (Otto et al. 2002; Somers et al. 2006; Chen et al. 2007; Oliver et al. 2008). The objective of this study was to identify and map FHB QTLs from DT696 (*T. turgidum* L. subsp. *durum*) and Blackbird (*T. turgidum* L. subsp. *carthlicum*).

MATERIALS AND METHODS

Two doubled-haploid (DH) populations were studied. A DH population (DH1) of 90 lines was produced from F_1 hybrids of 'Strongfield' x 'Blackbird', and a second DH population (DH2) of 124 lines was produced from 'DT707' x 'DT696'. Both populations were developed using the maize pollination technique. Line REB68421 ('Blackbird') was obtained from Dr. Maxime Trottet of INRA, Centre de Recherches de Rennes, in France. Molecular marker screening could only be completed on 89 DH1 lines and 121 DH2 lines.

The populations were evaluated for reaction to FHB in nurseries at Portage La Prairie, MB and Carman, MB. DH1 was evaluated in 2006 at Carman and Portage La Prairie and in 2007 at Portage La Prairie; while DH2 was evaluated in 2005 at Carman, 2006 at Carman and Portage La Prairie, and in 2007 at Portage La Prairie. Both were planted as an alpha-lattice with two replicates (DH1 had 12 and DH2 had 17 entries/incomplete block). Plots at Portage La Prairie and Carman were single rows 1 m long. Sowing density was approximately 60 seeds per row. At Carman, the spikes of the entire row were spray-inoculated at 50% anthesis with a 50 ml inoculum solution of virulent strains of *F. graminearum* using a CO_2 powered backpack sprayer calibrated at 2 kPa. Re-inoculation of the same rows was performed 4 d following the first inoculation. The inoculum was a suspension of 50,000 conidia mL^{-1} in water and Tween 20. The nursery was mist irrigated the evening of, and the morning after each inoculation. At the Portage La Prairie nursery, FHB corn spawn was broadcast approximately 2-3 wk prior to heading at 40 g m^{-2} of corn. Immediately upon completion of corn spawn distribution, mist irrigation was applied, and optimum humidity maintained for disease development.

Each plot was visually rated for Type 1 (incidence) and Type 2 (severity) resistance (Engle et al. 2003) and also rated on a 1 to 9 disease scale (1= no infection and 9= >90% infection). FHB Index [(incidence/100) * (severity/100)] * 100, was calculated. Analysis of variance (ANOVA) and Pearson correlations were performed in SAS v 9.1.

DH1 was genotyped with 426 microsatellites, and DH2 was genotyped with 83 microsatellites polymorphic on the parents. The genetic maps were constructed in JoinMap v. 3.0 using the Kosambi mapping function. The Kruskal–Wallis (KW) test ($P<0.005$) and the interval mapping procedure in MapQTL (Van Ooijen and Maliepaard 1996) was performed to identify molecular markers significantly associated with FHB resistance. Logarithm of the odds (LOD) thresholds for significance was obtained by MapQTL's permutation test option (1000 permutations). Genome-wide threshold levels were used to declare significant QTLs based at the 5% significance level. Automatic co-factor detection based on backward elimination was used to identify co-factor markers. Subsequently Multiple QTL Mapping (MQM) was performed using the co-factor markers. Unless otherwise indicated, results are presented from MQM.

RESULTS AND DISCUSSION

Strongfield x Blackbird (DH1): Entries were a significant source of variation in all nurseries. Parents were significantly different in the 2006 Carman nursery for all traits, were not significantly different for severity and index in 2006 at Portage La Prairie, were not significantly different in 2007 for incidence, severity and index at Portage La Prairie but were significantly different for the 1-9 rating (Table 1). Transgressive segregants were present (Table 1). Pearson correlations among traits in the 2006 Carman nursery were significant. In the 2006 and 2007 Portage La Prairie nursery, correlations among incidence, severity, index and the 1-9 rating were significant. Twenty-seven linkage groups were constructed covering 1615 cM distance spanning all 14 chromosomes. The analysis revealed a strong QTL that peaked near marker *cfa2153* on chromosome 1A (Fig. 1). This QTL was significant for FHB incidence, severity and index at Portage La Prairie in 2006 (Table 2 for LOD scores). The same QTL was significant in 2006 and 2007 Portage La Prairie nursery for the 1 – 9 rating scale (Table 2). In all cases, 'Blackbird' was the donor parent for reduced infection and spread. The means for each parental-type molecular variant for significant markers and amount of phenotypic variation explained is given in Table 2. The *gdm33* was previously reported as close to *cfa2153* (Paillard et al. 2003). The KW non-parametric test confirmed the MQM results at 0.001 significance level.

DT707 x DT696 (DH2): Entries were not a significant source of variation in the 2005 and 2006 Carman nurseries. Therefore, no further analysis was done for these two nurseries. Entries were a significant source of variation in 2006 and 2007 at Portage La Prairie. DT696 was the resistant parent in both nurseries (Table 1). In the 2006 and 2007 Portage La Prairie nurseries, only incidence and 1-9 rating were not significantly different for the two parental genotypes (Table 1). Incidence, Severity, Index and 1-9 rating were significantly correlated among each other. Sixty-eight of the 83 polymorphic SSR markers were mapped to linkage groups. Twenty-one linkage groups were constructed covering 369 cM map distance. Except for 1A, 1B, 5A, 5B, 6B and 7A, none of the other linkage groups had more than two markers. Two significant QTL were detected on two linkage groups, both representing chromosome 5A. DT696 was the donor of FHB resistance in both environments. In the 2006 nursery at Portage La Prairie, *gwm156* was significantly associated with incidence, severity, index and the 1-9 scale. In the 2007 nursery at Portage La Prairie, *wmc110* was significantly associated with incidence, severity, index and the 1-9 scale. The QTL on chromosome 5A has been previously reported in hexaploid wheat and is likely the *Qfhs.ifa-5A* FHB QTL reported by Buerstmayr et al. 2003. More markers are needed for this population to develop a more comprehensive map. The KW non-parametric test confirmed the MQM results at 0.001 significance level.

Somers et al. (2006) studied the Strongfield/Blackbird population in controlled environments and mapped FHB Type 2 resistance QTL derived from Strongfield on 2BL and from Blackbird on 6BS. To our knowledge, no FHB QTL has been previously reported on chromosome 1A. Presence of common QTL for incidence and severity is one of the first reports of the same QTL for Type 1 and Type 2 FHB resistance. However, more detailed greenhouse experiments need to be conducted to confirm these two types of resistance coming from a single Blackbird allele. Recently, hessian fly resistance genes H9, H10, H11, H16 and H17 and *Hdic* (from *T. turgidum* subsp. *dicoccum*) were mapped with SSR markers on chromosome 1AS, and were tightly linked to *cfa2153* (Liu et al. 2005a and 2005b; Kong et al. 2008). These results have been backed with spring wheat deletion line bin mapping to the distal 14% of chromosome 1AS and Hessian fly resistance genes along with the powdery mildew resistance gene *Pm3* and leaf rust resistance gene *Lr10* seem to form a resistance gene cluster in the distal region of chromosome 1AS in wheat near *cfa2153* (Liu et al. 2005a; Kong et al. 2008). FHB resistance from 'Blackbird', a *T. turgidum* subsp. *carthlicum* line, also localized in the same gene rich cluster is an excellent candidate for marker assisted selection because it provides opportunity to combine FHB, leaf rust, powdery mildew and hessian fly resistance genes together. However, it is essential to determine the linkage phase for these genes to develop effective marker-assisted-selection strategies. Opportunity for improving the FHB resistance in durum wheat could come from recombining genes found in Blackbird with those found in DT696.

Table 1. Parental reaction for FHB resistance in doubled haploid populations (Strongfield x Blackbird; and DT707 x DT696) evaluated in FHB nurseries at Carman and Portage la Prairie, MB in 2006 and 2007.

Env[1]	T[2]	Range[3]	μ_{P1}, μ_{P2} (sig.)[4]	Pr > F[5]
Strongfield x Blackbird				
C06	Inc	23.1-96.5	49.7, 94.3 (*)	0.0014
	Sev	12.5-95.0	50.0, 90.0 (*)	<0.0001
	Ind	4.9-86.8	25.9, 84.4 (**)	<0.0001
P06	Inc	0.01-97.1	63.2, 6.0 (**)	<0.0001
	Sev	5.5-63.9	27.1, 6.5 (n.s.)	0.0002
	Ind	0.01-57.6	18.4, 2.1 (n.s.)	0.0006
	1-9	34.9-80.3	64.9, 29.5 (**)	<0.0001
P07	Inc	80.5-100	100, 97.1 (n.s.)	0.0087
	Sev	15.0-64.6	32.9, 26.9 (n.s.)	<0.0001
	Ind	11.8-64.8	33.1, 26.1 (n.s.)	<0.0001
	1-9	30.1-70.9	60.0, 34.4 (**)	<0.0001
DT707 x DT696				
P06	Inc	7.5-97.4	58.0, 25.9 (n.s.)	<0.0001
	Sev	9.9-55.2	54.9, 15.0 (**)	0.0005
	Ind	1.6-46.0	37.7, 4.3 (**)	0.0002
	1-9	39.9-75.1	60.1 49.7 (n.s.)	<0.0001
P07	Inc	85.0-100	100.0,85.0 (**)	<0.0001
	Sev	17.5-75.0	70.0, 25.0 (**)	<0.0001
	Ind	15.8-75.0	70.0, 21.5 (**)	<0.0001
	1-9	40.0-70.0	65.0, 45.0 (**)	<0.0001

[1]Environment: C06=2006 Carman nursery, P06=2006 Portage La Prairie nursery, P07=2007 Portage La Prairie nursery; [2]FHB: Sev=severity, Inc=incidence, Ind=index, 1-9=visual rating scale; [3]Range of least square means of DH lines; [4]Mean based on parents P1, Strongfield or DT707, and P2, Blackbird or DT696 and significance at 5%(*), 1%(**) or not significant (n.s.); [5]ANOVA P-value for genotype source of variation.

Table 2: FHB resistance QTL identified in the doubled haploid populations (Strongfield x Blackbird; and DT707 x DT696) evaluated in FHB nurseries at Carman and Portage La Prairie, MB in 2006 and 2007.

Env[1]	T[2]	Locus	lod[3]	μ^4_R	μ^5_S	%[6]
Strongfield x Blackbird						
C06	Sev	*cfa2153*	3.0	54.9	69.7	14.4
P06	Inc	*cfa2153*	5.4	36.9	63.2	24.3
	Sev	*cfa2153*	3.1	23.1	34.4	14.6
	Ind	*cfa2153*	3.6	13.0	24.8	17.1
	1-9	*cfa2153*	3.5	55.8	64.7	16.6
P07	1-9	*cfa2153*	3.2	54.0	61.1	15.1
	1-9	*gdm33*	3.7	53.7	61.3	17.6
DT707 x DT696						
P06	Inc	*gwm156*	3.2	59.7	72.4	11.7
	Sev	*gwm156*	3.4	23.3	29.5	12.4
	Ind	*gwm156*	4.3	15.1	22.3	15.2
	1-9	*gwm156*	6.3	53.7	59.8	21.6
P07	Inc	*wmc110*	2.7	97.1	99.1	9.9
	Sev	wmc110	4.5	38.0	48.1	13.9
	Ind	wmc110	4.3	37.4	47.8	13.7
	1-9	wmc110	2.4	52.8	57.0	8.9

[1]environment: C06=2006 Carman nursery, P06=2006 Portage La Prairie nursery, P07=2007 Portage La Prairie nursery; [2]FHB: Sev=severity, Inc=incidence, Ind=index, 1-9= visual rating scale; [3]Except Strongfield x Blackbird in 2006 Carman severity (LOD significance 3.2) and DT707 x DT696 in 2007 Portage La Prairie 1-9 rating (LOD significance 2.5), all traits were significant for their respective genome wide LOD significance levels; [4]Mean based on resistant parent, Blackbird or DT696, molecular variant; [5] Mean based on susceptible parent, Strongfield or DT707, molecular variant; [6] phenotypic variance explained by the QTL.

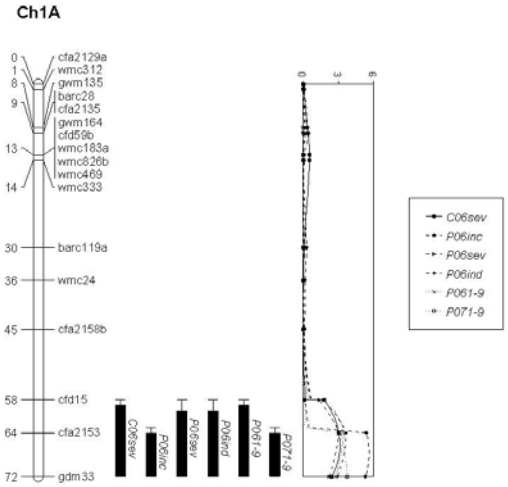

Figure 1. Location of significant FHB resistance QTLs in Strongfield x Blackbird population, supported by their 1-LOD (bars) and 2-LOD (lines) support interval. The LOD profile of each trait with significant QTL are shown on the right with description in the box.

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