Theor Appl Genet (2009) 118:695–702
DOI 10.1007/s00122-008-0930-0

ORIGINAL PAPER

Semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to Fusarium head blight

Srinivasachary · N. Gosman · A. Steed · T. W. Hollins · R. Bayles · P. Jennings · P. Nicholson

Received: 19 July 2008 / Accepted: 4 November 2008 / Published online: 26 November 2008

Abstract Fusarium head blight (FHB) is an important disease of wheat worldwide. Soissons is one of the most resistant varieties grown in UK. The current study was undertaken to identify QTL for FHB resistance in Soissons and to determine whether the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* have a similar influence on susceptibility to FHB. A Soissons (*Rht-B1b; Rht-D1a*) × Orvantis (*Rht-B1a*; *Rht-D1b*) doubled haploid (DH) population was assessed for FHB resistance in three trials. Soissons contributed a single, stable major FHB QTL linked to the *Rht-D1* locus. In contrast, the *Rht-B1b* allele (contributed by Soissons) conferred no negative effect on FHB resistance, even conferring a very minor positive effect in one trial. The influence of the *Rht-B1b* and *Rht-D1b* alleles on FHB resistance was further investigated using both Mercia and Maris Huntsman near-isogenic lines. Under high disease pressure both *Rht-B1b* and *Rht-D1b* significantly decreased Type 1 resistance (resistance to initial infection). However, whilst *Rht-D1b* has no effect on Type 2 resistance (resistance to spread of the fungus within the spike), *Rht-B1b* significantly increased Type 2 resistance. Our study demonstrates that the choice of semi-dwarfing gene used in plant breeding programmes may be a significant consideration where resistance to FHB is an important breeding target.

Communicated by B. Keller.

Srinivasachary · N. Gosman · A. Steed · P. Nicholson (✉)
John Innes Centre, Norwich Research Park,
Colney Lane, Norwich NR4 7UH, UK
e-mail: paul.nicholson@bbsrc.ac.uk

Present Address:
N. Gosman · R. Bayles
National Institute of Agricultural Botany (NIAB),
Huntington Road, Cambridge CB3 0LE, UK

T. W. Hollins
RAGT Seeds, Grange Road, Ickleton,
Saffron Walden, Essex CB101TA, UK

P. Jennings
Central Science Laboratory, Sand Hutton,
York YO41 1LZ, UK

Introduction

Fusarium head blight (FHB) is a destructive disease of wheat world wide (Parry et al. 1995; Waldron et al. 1999). The predominant causal agents *Fusarium graminearum* and *F. culmorum,* both reduce yield and can contaminate grain with mycotoxins that render it unsuitable for human and livestock consumption (Gilbert and Tekauz 2000). Thus, FHB can be a major threat for producers, processors and consumers of wheat. Agronomic practices and fungicides are not fully effective in controlling the disease and thus breeding of resistant cultivars has been the strategy adopted to minimise yield and grain quality losses (Shen et al. 2004). Several components of resistance to FHB have been proposed, of which two have been commonly accepted, Type 1 and Type 2 (Schroeder and Christensen 1963). Resistance to initial infection (Type 1) is assessed as disease incidence following natural infection or inoculation by spraying heads at mid-anthesis with conidia. Resistance to spread within the head (Type 2) is assessed by injection of inoculum into single florets within the head.

A number of traits including plant height (PH), presence of awns and heading date have been associated with FHB resistance. Generally, it has been accepted that taller plant varieties show fewer FHB symptoms compared to shorter

ones possibly due to different micro-climates (Miedaner 1997; Steiner et al. 2004). In agreement with this, several QTL studies have found FHB resistance loci associated or coincident with PH QTL (Gervais et al. 2003; Steiner et al. 2004; Draeger et al. 2007; Klahr et al. 2007). However, not all height QTL are coincident with FHB QTL indicating that the relationship between height and FHB is not simply a consequence of disease escape.

Draeger et al. (2007) concluded that FHB susceptibility is associated with the dwarfing allele *Rht-D1b* (formerly termed *Rht2*) resulting from linkage or pleiotropy rather than an effect of height per se. A recent investigation involving lines near-isogenic for *Rht-D1b* and a DH population from a Spark × Rialto cross (Rialto carries the *Rht-D1b* allele) revealed that *Rht-D1b* compromised Type 1 resistance either because of tight linkage to FHB susceptibility genes or due to a pleiotropic effect of the *Rht-D1b* allele itself (Srinivasachary et al. 2008). Most of the winter wheat varieties grown in the UK possess the semi-dwarfing allele *Rht-D1b* on chromosome 4D, and almost all UK varieties are also highly susceptible to FHB (Gosman et al. 2007). Soissons, which carries the homeologous semi-dwarfing allele *Rht-B1b* (formerly termed *Rht1*) was found to be the most resistant of the wheat varieties recommended for growing in the UK (Gosman et al. 2007). The objective of the present study was to identify the FHB resistance QTL carried by Soissons using a population containing both *Rht-B1b* and *Rht-D1b* semi-dwarfing alleles to allow comparison of the effect of these alleles on resistance to FHB. This study was supported by additional investigations using lines near-isogenic for the *Rht-B1b* and *Rht-D1b* alleles to ascertain whether they have similar or divergent effects with regards to FHB.

Materials and methods

Field phenotyping for FHB resistance in the Soissons × Orvantis population

A Soissons (FHB resistant and carrying *Rht-B1b* and *Rht-D1a* alleles) × Orvantis (FHB susceptible and carrying *Rht-B1a* and *Rht-D1b* alleles) F_1 derived doubled haploid (DH) population, generated using the method of Laurie and Reymondie (1991), was provided by RAGT Seeds. Most DH lines carrying both *Rht-B1b* and *Rht-D1b* (double-dwarf) had been removed from the population in earlier trials and 190 lines were used in the current study. The FHB resistance of the DH lines along with their parents was assessed in field trials during the summers of 2005/2006 at the Central Science Laboratory (CSL), Sand Hutton, York, at the National Institute of Agricultural Botany (NIAB), Cambridge and at the JIC, Norwich. Hereafter, the experimental data generated from these experiments are designated as N2005, C2005 and J2006 for the NIAB, CSL and JIC trials, respectively. Field experiments were conducted using a randomised complete block design. To facilitate inoculation, the lines were grouped in each block according to flowering time.

The same inoculum was used in all the trials and comprised of conidia of a highly virulent DON-producing *F. culmorum* isolate (Fu42). The inoculum was produced as described previously by Gosman et al. (2005). Plants were spray inoculated at mid-anthesis (growth stage (GS) 65, Zadoks et al. 1974) with a conidial suspension of 1×10^5 spores mL^{-1} amended with 0.05% Tween 20. In all trials inoculum was applied at 50 mL m^{-2} and the plants were mist irrigated for a minimum of 72 h post inoculation to maintain high humidity. The inoculation was repeated after an interval of 3 or 4 days.

Trials were visually assessed for disease levels in each plot as described by Gosman et al. (2005). Additionally, at N2005, disease incidence (percentage of infected ears per plot) and disease severity (average percentage of disease on ears within each plot) were measured. The area under the disease progress curve (AUDPC) was calculated to provide an integrated measure of disease for N2005, C2005 and J2006 (Buerstmayr et al. 2000). The data from the three sites were also combined to produce a pooled AUDPC data set for analysis. PH to the top of the spike at mid-anthesis was also recorded at the JIC location.

Evaluation of FHB resistance of *Rht* near-isogenic lines of Mercia and Maris Huntsman

Tall (*rht*), *Rht-B1b* and *Rht-D1b* near-isogenic lines of the varieties Maris Huntsman and Mercia (Flintham et al. 1997) were phenotyped for FHB resistance at JIC in the summer of 2005 following inoculation with *F. culmorum* in a randomised complete block design field trial with three replicate plots per variety. Lines were inoculated by spraying with a conidial suspension (1×10^5 mL^{-1}) at GS 65 (Zadoks et al. (1974) using a knapsack sprayer (150 mL m^{-2}). Disease severity was visually assessed several times and data were used to calculate AUDPC.

The resistance of tall (*rht*), *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) near-isogenic lines of Maris Huntsman (Flintham et al. 1997) and Mercia to FHB were assessed in 2005 at JIC in an unheated polytunnel with capillary matting irrigation. Inoculum preparation, inoculation and disease assessment were as described by Gosman et al. (2007). Two deoxynivalenol (DON) producing isolates were used in this study: *F. culmorum* (Fu42) and *F. graminearum* (UK1). Lines of Maris Huntsman were inoculated at GS 65 by spray inoculation with a conidial suspension (1×10^5 mL^{-1}) of *F. culmorum* or by point inoculation, with 10 µl of conidial

suspension (1×10^6 mL^{-1}) injected into a single floret within the central portion of each spike. For the spray inoculation, pots containing individual plants were arranged in a randomised complete block design of three blocks of nine plants per variety and for the point inoculation treatment ten plants per variety were used in each replicate. Following spray inoculation AUDPC was calculated on the basis of the percentage of spikelets infected per head at each score date 7, 14 and 21 days post inoculation (dpi). Following point inoculation, disease was measured as the number of diseased spikelets at 21dpi. Near-isogenic *Rht* lines of Mercia were similarly assessed for FHB resistance following spray and point inoculation with conidia of *F. graminearum*. For each treatment group, pots containing individual plants were arranged in a randomised complete block design of four replicates of seven plants per genotype and disease severity assessed after 350 day degrees (Gervais et al. 2003). A portion of the data from the field trial and the Mercia polytunnel trial relating to the *rht* and *Rht-D1b* genotypes has been reported previously (Srinivasachary et al. 2008).

Statistical analysis

All statistical analyses were performed using GenStat for Windows 9th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Analysis of variance (ANOVA) was carried out using the generalised linear model (GLM) of regression analysis. Broad sense heritability across environments was estimated from the ANOVA using the formula: $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_{GE}/E) + (\sigma^2_e/rE)]$, and experimental repeatability (R) using the formula: $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_e/r)]$, with σ^2_G the genetic variance, σ^2_{GE} the genotype × environment interaction variance, σ^2_e the residual variance, E the number of environments, r the number of replicates per genotype (Nyquist 1991).

Genotyping, map construction and QTL analysis

Soissons, Orvantis and DH lines were genotyped using SSRs and DArT markers. In addition, all lines were genotyped for wild-type (tall) and semi-dwarfing alleles at the *Rht-B1* and *Rht-D1* loci as described in Ellis et al. (2002) with the following modifications. For genotyping *Rht-D1*, a single forward primer (5′-CGCGCAATTATTGGCCA GAGATAG-3′) was used for both *Rht-D1a* (tall) and *Rht-D1b* alleles. For both *Rht-B1* and *Rht-D1,* the reaction mixtures (20 μL) contained 100 ng of DNA, 4 nmol of dNTPs, 10 pmol each of the forward and reverse primers, 1× Hotstar Buffer, 1× Hotstar Q solution and 1 unit of Hotstar *Taq* polymerase (Qiagen). PCR was performed using the following programme: 15 min at 95°C, 38 cycles of 30 s at 94°C, 30 s at 63°C and 30 s at 72°C with a final primer extension for 5 min at 72°C.

A genetic linkage map was constructed using 12 SSRs, 226 DArT markers and perfect markers *Rht-B1* (*Rht1*) and *Rht-D1* (*Rht2*) and the linkage analysis was performed with JoinMap (version 3.0) (Van Ooijen and Voorips 2001), using the Kosambi mapping function (Kosambi 1944). The map derived from 190 DH lines comprised 240 loci organised into 36 linkage groups (LGs). MapQTL® 4.0 programme (Van Ooijen 2004) was used for QTL mapping. The Kruskal-Wallis test was used in a preliminary analysis to detect associations between markers and individual traits, followed by interval mapping (IM) to identify the major QTL. Automatic cofactor selection was used to fit the multiple QTL model (MQM) [backward elimination ($P > 0.02$)] and to detect significantly associated markers as cofactors. For each trait, a permutation test (1,000 permutations) was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5% ($P < 0.05$) and a threshold LOD value of 2.5 was used to declare the presence of QTL. QTL identified in more than one environment which mapped very close to one another on the same linkage group and with alleles derived from the same parent, were considered to represent a single QTL. The QTL that explained >10% of the variance (R^2) in at least one environment were arbitrarily classified as major QTL and those explaining <10% as minor QTL. The linkage map was drawn using MAPCHART (Voorrips 2002).

Results

Soissons × Orvantis population: FHB resistance and PH

Disease intensity differed markedly at the three sites but in all cases Soissons was much less diseased than Orvantis (Table 1). FHB severity was continuously distributed amongst the DH lines in all three trials (data not shown). The mid parent AUDPC values were greater than the population means in C2005 and J2006. The level of FHB resistance amongst DH lines was slightly skewed towards the resistant parent Soissons. Transgressive segregation was observed in all environments being towards greater resistance in J2006 and N2005 and towards greater susceptibility in C2005. The PH of Soissons and Orvantis was 90.3 cm and 96.1 cm, respectively (Table 1) and PH within the DH population was normally distributed about the parents (data not shown).

Correlations, ANOVA and broad sense heritability

Correlations of AUDPC across C2005, N2005 and J2006 were all significant ($P < 0.001$) (data not shown) and the correlation coefficients (r) ranged from 0.43 ($P < 0.001$)

Table 1 Summary statistics for plant height and AUDPC in Soissons/Orvantis DH population

Experimental site	Trait	Soisson	Orvantis	Mid parent value	Range	Population mean
C2005	AUDPC	0	519.2	259.5	743.2	238.7
J2006	AUDPC	208.6	1838.4	1023.5	1670.5	470.6
N2005	Incidence	6.6	45	25.8	95	34.6
	Severity	4.1	13.9	9	58.8	13.7
	AUDPC	34	677	356	5508	659
Across J,N,C	AUDPC	80.9	1011.3	556.1	2382.7	546.1
J2006	Plant height (cm)	90.3	96.1	93.2	70	92.9

between C2005 and N2005 to 0.55 ($P < 0.001$) between N2005 and J2006. Disease severity at N2005 was more highly correlated with AUDPC at C2005 and J2006 than AUDPC being 0.43 ($P < 0.001$) and 0.55 ($P < 0.001$) for C2005 and J2006, respectively. PH was weakly to moderately correlated with AUDPC ($r = -0.22$ to -0.4; $P = 0.003$ to <0.001) and with disease incidence at N2005 ($r = -0.51$; $P < 0.001$). Highly significant differences in AUDPC amongst the genotypes was observed in all the environments ($P = 0.007$ to <0.001) (Table 2). The experimental repeatability for disease resistance (AUDPC) ranged from 42 to 90% and the broad sense heritability (h^2) was 42%.

QTL analysis

The genetic map was constructed using SSRs and Diversity array technology (DArT®) markers, a micro-array based genome analysis tool called 'diversity array technology' (DArT), a relatively cheap, sequence independent, robust and high through put marker system with minimal sample DNA requirement (Jaccoud et al. 2001). The map comprised 36 linkage groups (LGs) spanning approximately 1197 cM. Thirty-four of the LGs could be related to the 21 wheat chromosomes based on the consensus wheat DArT map from the Triticarte website (http://www.wheat-research.com.au/media/Hot%20Topics/TriticartewhtmapalignV1-2.xls) and/or using the marker information from previously published maps. The size of the A, B and D genome maps were 324, 561 and 315 cM, respectively. Because DArT markers formed the bulk of the markers in the LGs, the position of QTL were determined on the basis of either consensus wheat maps published on the Triticarte website or the wheat genetic maps (DArT markers anchored with SSRs) of Semagn et al. (2007).

QTL analysis of AUDPC revealed only a single major FHB QTL *Qfhs.jic-4D* (LOD = 4.0–6.4; $R^2 = 10.6$–16.1) effective in all three trials (Table 3; Fig. 1). *Qfhs.jic-4D* is in the region of the *Rht-D1* locus, although wPt-0710 was the marker closest to the QTL peak. Soissons (*Rht-D1a*) contributed the FHB resistance allele. The *Qfhs.jic-4D* QTL was also effective, but to a lesser extent, in the N2005 trial when the disease incidence and disease severity traits were analysed separately.

Five QTL for PH were detected with alleles contributed by both parents. PH QTL were associated with five linkage groups (1BL-1, 1BL-2, 4BS-1, 4DS and 7AL-2). The QTL on 4BS (LOD = 8.6 and $R^2 = 15.9$) and 4DS (LOD = 11.6 and $R^2 = 21.9$) were the major PH QTL in this population. These correspond to the *Rht-B1* and *Rht-D1*, loci with alleles for PH deriving from Orvantis and Soissons, respectively. Two minor PH QTL were detected on 1B, with one on 1BL-1 contributed by Soissons and one on 1BL-2 from Orvantis.

Table 2 Variance components identified using generalised linear model for FHB-AUDPC and plant height in the Soissons/Orvantis DH population

Source of variation	Disease								Plant height	
	N2005		C2005		J2006		Across J,N,C			
	ms	*F* pr	ms	*F* pr	ms	*F* pr	ms	*F* pr	ms	*F* pr
Genotype (DH line)	3.77×10^8	0.007	11.71×10^5	<0.001	486578	<0.001	631795	<0.001	137.962	<0.001
Genotype × replication	2.85×10^7	0.5	4.33×10^5	0.5	27577	0.5	431372	<0.001	8.384	0.5
Replication or expt. site	1.28×10^8	0.082	38.84×10^5	0.003	2529	0.762	35.52×10^6	0.486	106.381	<0.001
Residual error	2.40×10^7		4.33×10^5		27577		430715		8.384	
	$R = 0.88$		$R = 0.58$		$R = 0.9$		$h^2 = 0.42$		$R = 0.9$	

ms mean square, *F pr* *F*-probability, *R* experimental repeatability and h^2 broad sense heritability

Table 3 Plant height and FHB resistance QTL identified in Soissons/Orvantis DH population using interval and MQM mapping in N2005, C2005 and J2006 trials

FHB trait	QTL	Closest locus	Origin	Chr.	Position	J2006		N2005		C2005		Across J, N, C	
						LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
AUDPC	*Qfhs.jic-4D*	wPt-0710	Soissons	4DS	33.2	6.4	16.1	4.3	11	4	10.6	5	12.4
Incidence	*Qfhs.jic-4D*	wPt-0710	Soissons	4DS	33.2			3.2	8.2				
Severity	*Qfhs.jic-4D*	wPt-0710	Soissons	4DS	33.2			2.5	6.3				
Plant height	*QHt.jic-1B.1*	wPt-5562	Soissons	1BL-1	47	3	5.1						
	QHt.jic-1B.2	wPt-9809	Orvantis	1BL-2	14.6	4.1	7.4						
	QHt.jic-4B.1	*Rht-B1*	Orvantis	4BS-1	0	8.6	15.9						
	QHt.jic-4D	*Rht-D1*	Soissons	4DS	24	11.6	21.9						
	QHt.jic-7A	wPt-2780	Orvantis	7AL-2	0	3.2	5.4						

Chr. chromosome, *LOD* Log of adds ratio and R^2 percentage variance explained

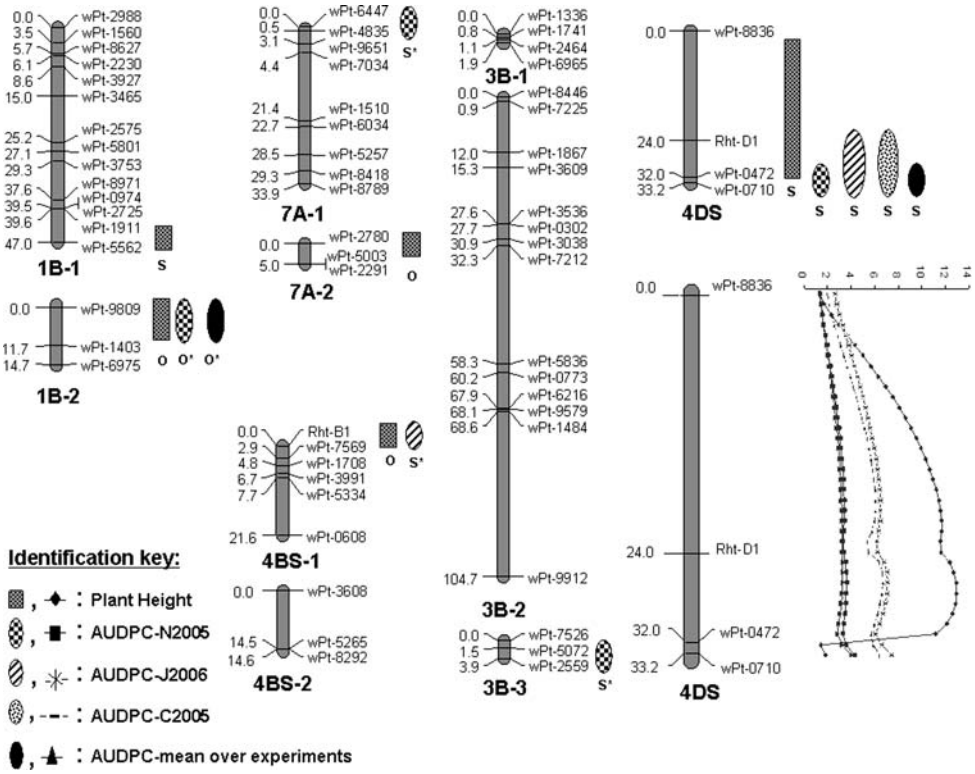

Fig. 1 Linkage maps of chromosome segments constructed from the Soissons/Orvantis doubled haploid population. Putative QTL positions for FHB resistance and plant height are shown on the *right* of each linkage map. Genetic distances are shown in centimorgans to the *left* of each linkage map. *S* and *O* refer to alleles contributed by Soissons and Orvantis, respectively. *Asterisk* indicates QTL detected below LOD threshold value

Field trial of FHB resistance of *Rht-B1b* and *Rht-D1b* near-isogenic lines

In the field trial of lines of Mercia and Maris Huntsman the *Rht* status was found to be highly significant ($P = 0.010$) whilst neither variety nor the *Rht* status by variety interaction were significant (Table 4). The mean AUDPC of the semi-dwarf *Rht-D1b* isogenic lines of the two varieties (13417) was significantly higher ($P < 0.001$) than the AUDPC of the tall (*rht*) parental varieties (8250). The AUDPC of the *Rht-B1b* lines (11083) was intermediate between that of the semi-dwarf *Rht-D1b* and the *rht* lines and no significant differences in AUDPC were found between *Rht-B1b* ($P = 0.109$) and tall (*rht*), and *Rht-B1b* and *Rht-D1b* ($P = 0.180$).

FHB resistance of *Rht-B1b* and *Rht-D1b* near-isogenic lines: spray inoculation

Disease levels differed markedly following spray inoculation of Mercia and Maris Huntsman near-isogenic lines with *F. culmorum* or *F. graminearum.* For this reason, the two trials were analysed separately. For Mercia, the AUDPC of the tall line was 3729 whereas those of *Rht-B1b*

Table 4 Analysis of variance and *t* test results of FHB disease levels on *rht*, *Rht-B1b* and *Rht-D1b* near-isogenic lines of winter wheat varieties Mercia and Maris Huntsman in a field trial and following spray and point inoculation in a polytunnel

Experiment	Analysis of variance					*t* test	
	Source of variation	*df*	ms	*F* value	*P* value	Comparison	Probability
Field trial (Mercia and Huntsman)	Variety	1	1.68×10^6	0.29	0.6	*Rht-B1b* with *rht*	0.109
	Rht status	2	4.02×10^6	6.95	0.01	*Rht-D1b* with *rht*	<0.001
	Rht × variety	2	938889	1.62	0.237	Between *Rht-B1b* and *Rht-D1b*	0.18
	Error	12	5.78×10^6				
Spray inoculation (Mercia)	Block	2	21.82×10^6	0.75	0.524	*Rht-B1b* with *rht*	0.015
	Rht status	3	17.98×10^7	6.21	0.003	*Rht-D1b* with *rht*	0.013
	Error	77	28.96×10^6			Between *Rht-B1b* and *Rht-D1b*	0.853
Spray inoculation (Huntsman)	Block	2	55077	0.53	0.593	*Rht-B1b* with *rht*	0.005
	Rht status	2	575215	5.5	0.006	*Rht-D1b* with *rht*	0.005
	Error	58	104525			Between *Rht-B1b* and *Rht-D1b*	0.573
Point inoculation (Mercia and Huntsman)	Block	3	14.81	2.29	0.081	*Rht-B1b* with *rht*	0.001
	Variety	1	51.51	7.96	0.005	*Rht-D1b* with *rht*	0.269
	Rht status	2	76.59	11.84	<0.001	Between *Rht-B1b* and *Rht-D1b*	0.001
	Rht × variety	2	5.57	0.86	0.425		
	Error	154	6.47				

df degrees of freedom, *ms* mean square

and *Rht-D1b* were 6187 and 6554, respectively. Similarly, the AUDPC of the *rht* (tall) line of Maris Huntsman was 1160 whereas those of *Rht-B1b* and *Rht-D1b* were 1412 and 1472, respectively. ANOVA showed that the effect of *Rht* status (genotype) was highly significant for both varieties (Table 4). The AUDPC of *rht* (tall) lines of both varieties were significantly lower ($P < 0.05$) than those of their respective *Rht-B1b* and *Rht-D1b* near-isogenic counterparts (Table 4). However, for both varieties, following spray inoculation, the AUDPC of the *Rht-B1b* and *Rht-D1b* lines were very similar and no significant difference existed between them (Table 4).

FHB resistance of *Rht-B1b* and *Rht-D1b* near-isogenic lines: point inoculation

Following point inoculation, disease scores in Mercia and Maris Huntsman lines were sufficiently similar to permit the data from the two experiments to be combined prior to analysis. The average number of spikelets infected was 8.2, 6.5 and 8.8 for the *rht* (tall), *Rht-B1b* and *Rht-D1b* lines, respectively. Following point inoculation, the influence of *Rht* status on disease levels was highly significant ($P < 0.001$, Table 4). Disease levels in *Rht-B1b* lines were significantly less ($P < 0.001$) than those in either the *rht* (tall) or *Rht-D1b* lines (Table 4). Although the mean number of diseased spikelets was slightly greater in *Rht-D1b* lines than in *rht* (tall) lines this difference was not significant ($P = 0.269$).

Discussion

Almost all the winter wheat varieties on the UK National List for 2003 have been shown to be highly susceptible to FHB (Gosman et al. 2007). Soissons was one of three varieties that showed significant resistance, the others being Spark and Vector. In the current study, a large DH population of Soissons (*Rht-B1b; Rht-D1a*)/Orvantis (*Rht-B1a*; *Rht-D1b*) was assessed for FHB resistance in three field trials. A single, stable major FHB QTL (*Qfhs.jic-4D*) was identified across trials (Table 3; Fig. 1) and at one site (N2005), FHB resistance was measured in terms of incidence and severity as well as AUDPC and *Qfhs.jic-4D* was detected for both traits, being slightly more pronounced for incidence. In addition to this, the study also detected putative but less significant QTL often appearing in more than one trial (1BL, 3BL, 4BS and 7AL) and Soissons contributed all the alleles for FHB resistance except that on linkage group 1BL (Fig. 1). The majority of FHB QTL detected in this study co-localised with QTL for PH. In most instances the same parent contributed the positive (FHB resistance) allele for both traits.

The major PH QTL associated with *Rht-D1* locus on 4DS co-localised with the major FHB QTL *Qfhs.jic-4D* and Soissons contributed the alleles for both these QTL. The second major PH QTL segregating in this population was associated with the *Rht-B1* locus with Soissons carrying the *Rht-B1b* (semi-dwarf) allele whilst Orvantis possesses the *Rht-B1a* (tall) allele. Surprisingly, considering the large influence of the 4D homeologous locus on PH and FHB no evidence was found to suggest that the *Rht-B1b* allele was associated with reduced resistance to FHB. On the contrary, this allele appeared to have a minor positive effect on FHB resistance in the J2006 trial.

Disease escape has long been considered to be potentially important in relation to resistance to FHB. Genotypes that differ in flowering time and PH may either completely escape from infection or show fewer FHB symptoms. It is therefore, important to consider the potential pleiotropic effects of such traits on FHB infection. Generally, taller genotypes show fewer FHB symptoms (Miedaner 1997; Hilton et al. 1999; Steiner et al. 2004) and it has been postulated that conidia spread more easily to the heads of short varieties because of the reduced distance between leaf layers (Mesterhazy 1995). This view was supported by findings that tall and short varieties expressed similar levels of symptoms following artificial inoculation (Mesterhazy 1995). However, the effect of PH on FHB has been shown to differ markedly in different populations ranging from stable and highly significant (Somers et al. 2003) to moderate or absent depending upon environment (Klahr et al. 2007).

Recent studies have revealed that QTL for FHB resistance often co-localise with those for PH (Gervais et al. 2003; Paillard et al. 2004; Steiner et al. 2004; Schmolke et al. 2005; Draeger et al. 2007). Importantly, however, not all PH QTL appear to be negatively associated with FHB resistance. Whilst QTL for FHB and PH were coincident on 5A in a Renan/Recital population, the PH QTL on 4A was not associated with differences in resistance to FHB (Gervais et al. 2003). Similarly, although some PH QTL overlapped with those for FHB in an Arina/Forno population, the main PH QTL were not coincident with FHB QTL (Paillard et al. 2004). Likewise, Schmolke and colleagues (2005) determined that whilst one of the QTL in their Dream/Lynx population was coincident for FHB and PH, other FHB and PH QTL were independent in this cross. They concluded that the coincidence of FHB and PH QTL has a genetic basis, linkage or pleiotropy, rather than being due to escape.

A previous report comparing the semi-dwarf (*Rht-D1b)* and tall (*Rht-D1a*) lines revealed that *Rht-D1b* lines appear to be more susceptible to initial infection (Type 1 resistance) but are unaffected in resistance to spread of the fungus in the head (Srinivasachary et al. 2008). In the present study, with two independent sets of lines that are near-isogenic for the *Rht-B1* and *Rht-D1* loci, data clearly showed that the semi-dwarfing *Rht-B1b* and *Rht-D1b* alleles differ in their effect on FHB (Table 4). Under field conditions, with moderate disease pressure, *Rht-D1b* is associated with a very significant increase in disease levels whereas *Rht-B1b* has only a very limited effect on FHB. Under severe disease pressure, such as that present in the polytunnel trials carried out under conditions of high temperature and humidity, both *Rht-B1b* and *Rht-D1b* are associated with greater susceptibility to FHB than tall (*rht*) lines. This latter finding is in agreement with Hilton et al. (1999) who found no evidence for differences in the effect of the *Rht-B1b* and *Rht-D1b* alleles. However, significantly and unexpectedly, following point inoculation, symptoms in *Rht-B1b* lines were significantly less than those of either the *Rht-D1b* or the tall (*rht*) lines. Our observations suggest that this effect, although statistically significant, is relatively modest compared to the Type 2 resistance conferred by the *Fhb1* gene of Sumai 3. Overall, the results from the near-isogenic lines indicate that both *Rht-B1b* and *Rht-D1b* exhibit reduced resistance to initial infection (Type 1) but that, whereas *Rht-B1b* has greater resistance to spread (Type 2) than the tall (*rht*) lines, the resistance of *Rht-D1b* is similar to (or may be even slightly less) than that of tall (*rht*) lines. The consequence of this difference is that in field conditions under moderate disease pressure where few spikelets on each head become infected, the enhanced Type 2 resistance associated with *Rht-B1b* may lead to an overall reduction in FHB symptoms in plots despite the greater susceptibility to infection of both *Rht-B1b* and *Rht-D1b* relative to the tall (*rht*) lines.

Previous studies have identified an association between the *Rht-D1b* allele and increased susceptibility to FHB of other semi-dwarf varieties including Riband, in a Riband/Arina population (Draeger et al. 2007) and Rialto in a Rialto/Spark population (Srinivasachary et al. 2008). In both reports, the association between *Rht-D1b* and greater susceptibility to FHB was shown not to be due to differences in height per se but these authors were not able to determine whether the effect was due to pleiotropy or linkage. *Rht-B1* and *Rht-D1* are homoeoloci on chromosomes 4B and 4D, respectively. It is possible that the *Rht-B1b* and *Rht-D1b* alleles at these homoeoloci function differentially in respect to FHB resistance. However, it is also possible that the differences in response are due to linkage to nearby genes that differ in their impact on FHB resistance rather than to differential pleiotropic effects of these alleles. In support of this view was our finding that, although FHB resistance was associated with the *Rht-D1* locus, interval mapping and MQM analysis consistently place the QTL peak at DArT marker wPt-0710 that mapped a short distance away (Fig. 1). However, further work is required to fine map this area and resolve this question.

The majority of UK winter wheat varieties are highly susceptible to FHB and almost all these carry the semi-dwarfing *Rht-D1b* allele. Neither Soissons nor Spark carry *Rht-D1b*: Soissons possesses *Rht-B1b* and Spark has a tall (*rht*) genotype with its reduced height being due to non-*Rht* genes. It appears that the difference in FHB resistance between these two varieties and the others on UK National List of 2003 may, in large part, be simply a reflection of the presence or absence of *Rht-D1b*. Under conditions of moderate disease pressure, use of the *Rht-B1b* semi-dwarfing allele may provide the desired crop height without compromising resistance to FHB to the same extent as lines carrying *Rht-D1b*.

Acknowledgments We wish to express our sincere gratitude to UK Home-Grown Cereals Authority (HGCA) and the Department of Environment, Food and Rural Affairs (Defra) through a Defra-LINK project (No. LK0932). All the authors would like to thank collaborating commercial partners Nickerson (UK) Ltd., Elsoms Seeds Ltd., Advanta Seeds (UK) Ltd. and RAGT Seeds Ltd. (collaboration initiated with Monsanto (UK) Ltd., who subsequently transferred their wheat breeding programme to RAGT) for their invaluable contributions and for all those involved in field and polytunnel related work. We also thank Dr. Marc Ellis, CSIRO, Australia, for providing unpublished protocols for genotyping *Rht1* and *Rht2*.

References

Buerstmayr H, Steiner B, Lemmens M, Ruckenbauer P (2000) Resistance to Fusarium head blight in winter wheat: heritability and trait associations. Crop Sci 40:1012–1018

Draeger R, Gosman N, Steed A, Chandler E, Thomsett M, Srinivasachary Schondelmaier J, Buerstmayr H, Lemmens M, Schmolke M, Mesterhazy A, Nicholson P (2007) Identification of QTL for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor Appl Genet 115:617–625

Ellis MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theor Appl Genet 105:1038–1042

Flintham JE, Borner A, Worland AJ, Gale MD (1997) Optimising grain yield: effects of Rht (Gibberllin-insensitive) dwarfing genes. J Agric Sci 128:11–25

Gervais L, Dedryver F, Morlais JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003) Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theor Appl Genet 106:961–970

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

Gosman N, Chandler E, Thomsett M, Draeger R, Nicholson P (2005) Analysis of the relationship between parameters of resistance to Fusarium head blight and in vitro tolerance to deoxynivalenol of the winter wheat cultivar Wek0609 (R). Eur J Plant Pathol 111:57–66

Gosman N, Bayles R, Jennings P, Kirby J, Nicholson P (2007) Evaluation and characterization of resistance to Fusarium head blight caused by *Fusarium culmorum* in UK winter wheat cultivars. Plant Pathol 56(2):264–276

Hilton AJ, Jenkinson P, Hollins TW, Parry DW (1999) Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathol 48:202–208

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucl Acids Res 29:e25

Klahr A, Zimmermann G, Wenzel G, Mohler V (2007) Effects of environment, disease progress, plant height and heading date on the detection of QTL for resistance to Fusarium head blight in an European winter wheat cross. Euphytica 154:17–28

Kosambi DD (1944) The estimation of map values from recombination value. Ann Eugen 12:172–175

Laurie DA, Reymondie S (1991) High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. Plant Breed 106:182–189

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

Miedaner T (1997) Breeding wheat and rye for resistance to Fusarium diseases. Plant Breed 116:201–220

Nyquist WE (1991) Estimation of heritability and prediction of selection response in plant populations. CRC Crit Rev Plant Sci 10(3):235–322

Paillard S, Schnurbusch T, Tiwari R, Messmer M, Winzeler M, Keller B, Schachermayr G (2004) QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.). Theor Appl Genet 109:323–332

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol 44:207–238

Schmolke M, Zimmermann G, Buerstmayr H, Schweizer G, Miedaner T, Korzun V, Ebmeyer E, Hartl L (2005) Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. Theor Appl Genet 111:747–756

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831–838

Semagn K, Skinnes H, Bjornstad A, Maroy AG, Tarkegne Y (2007) Quantitative trait loci controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid wheat population from 'Arina' and NK93604. Crop Sci 47:294–303

Shen R, Kong LR, Ohm H (2004) Fusarium head blight resistance in hexaploid wheat (*Triticum aestivum*)–*Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. Theor Appl Genet 108:808–813

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(4):555–564

Srinivasachary, Gosman N, Steed A, Simmonds J, Leverington-Waite M, Wang Y, Snape J, Nicholson P (2008) Susceptibility to Fusarium head blight is associated with the Rht-D1b semi-dwarfing allele in wheat. Theor Appl Genet 116:1145–1153

Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor Appl Genet 109:215–224

Van Ooijen JW (2004) MapQTL Version 5.0, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V, Wageningen

Van Ooijen JW, Voorips RE (2001) JoinMap 3.0. Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Voorrips RE (2002) MAPCHART: Software for the graphical presentation of linkage maps and QTL. Heredity 93:77–78

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

Zadoks JC, Chang TT, Konzak CF (1974) Decimal code for growth stages of cereals. Weed Res 14:415–421