

## Susceptibility to *Fusarium* head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat

Srinivasachary · N. Gosman · A. Steed ·  
J. Simmonds · M. Leverington-Waite ·  
Y. Wang · J. Snape · P. Nicholson

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**Abstract** *Fusarium* head blight (FHB) is an important disease of wheat worldwide. The cultivar Spark is more resistant than most other UK winter wheat varieties but the genetic basis for this is not known. A mapping population from a cross between Spark and the FHB susceptible variety Rialto was used to identify quantitative trait loci (QTL) associated with resistance. QTL analysis across environments revealed nine QTL for FHB resistance and four QTL for plant height (PH). One FHB QTL was coincident with the *Rht-1D* locus and accounted for up to 51% of the phenotypic variance. The enhanced FHB susceptibility associated with *Rht-D1b* is not an effect of PH per se as other QTL for height segregating in this population have no influence on susceptibility. Experiments with near-isogenic lines supported the association between susceptibility and the *Rht-D1b* allele conferring the semi-dwarf habit. Our results demonstrate that lines carrying the *Rht-1Db* semi-dwarfing allele are compromised in resistance to initial infection (type I resistance) while being unaffected in resistance to spread within the spike (type II resistance).

**Keywords** *Fusarium* head blight · Semi-dwarf · *Fusarium culmorum* · *Fusarium graminearum* · Plant height · *Rht2*

### Introduction

*Fusarium* head blight (FHB) is a serious disease of wheat in many temperate regions of the world (Bai and Shaner 1994; Parry et al. 1995; Waldron et al. 1999). Although a number of species have been associated with FHB, *Fusarium graminearum* and *Fusarium culmorum* are the predominant pathogens in most regions (Parry et al. 1995). These species are of particular concern because they contaminate grain with trichothecene mycotoxins such as deoxynivalenol (DON) that are harmful to human and animal consumers.

Several studies have shown that resistance to FHB is horizontal and non-species specific and non-strain specific (Van Eeuwijk et al. 1995; Mesterhazy et al. 1999). The development and deployment of FHB-resistant cultivars is generally accepted as the most cost effective and environmentally benign way to minimise the infection (Parry et al. 1995). Several types of resistance to FHB have been proposed (Schroeder and Christensen 1963). Resistance to initial infection (type I) 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 II) is assessed by the injection of inoculum into single florets within the head. Resistance to FHB is quantitatively inherited (Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Gervais et al. 2003) and the influence of environment (Snijders and Van Eeuwijk 1991; Bai and Shaner 1994) on disease makes reliable phenotyping difficult.

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Srinivasachary · N. Gosman · A. Steed · J. Simmonds ·  
M. Leverington-Waite · Y. Wang · J. Snape · P. Nicholson (✉)  
Crop Genetics, John Innes Centre, Norwich Research Park,  
Colney Lane, Norwich NR4 7UH, UK  
e-mail: paul.nicholson@bbsrc.ac.uk

#### Present Address:

N. Gosman  
National Institute of Agricultural Botany (NIAB),  
Huntington Road, Cambridge CB3 0LE, UK

Advances in phenotyping combined with statistical methods to detect quantitative trait loci (QTL) have led to the identification of numerous QTL for FHB resistance in spring wheat varieties (Anderson et al. 2001; Shen et al. 2003; Steiner et al. 2004; Zhou et al. 2004). The resistance of some of these varieties, notably FHB resistant lines related to the cultivar Sumai-3, appears to be controlled by a few genes of major effect and hence may be amenable for use in marker-assisted breeding programmes (Cuthbert et al. 2006, 2007). In contrast, studies of winter wheat germplasm indicate that, in most cases, the FHB resistance is due to numerous QTL of moderate to small effect (Gervais et al. 2003; Paillard et al. 2004; Schmolke et al. 2005).

Several authors have reported a negative relationship between plant height (PH) and FHB resistance (Hilton et al. 1999; Buerstmayr et al. 2000; Somers et al. 2003). More recently, study of a doubled haploid population developed from Arina (moderately resistant) crossed with Riband (susceptible) identified a potent FHB resistance QTL coincident with the *Rht-D1* PH locus in wheat (Draeger et al. 2007), while Arina has the wild type allele at this locus (*Rht-D1a*), Riband, like most UK winter wheat varieties has the *Rht-D1b* allele, also known as *Rht2*. Results from the Arina × Riband population suggested that the relationship between PH and FHB susceptibility is not due to the PH per se but, rather to either linked genes conferring FHB susceptibility and/or a pleiotropic physiological effect of the *Rht-D1b* allele enhancing susceptibility. The majority of UK winter wheat varieties possess the *Rht-D1b* semi-dwarfing allele and are highly susceptible to FHB, while Spark is moderately resistant and possesses the wild type allele, *Rht-D1a* (Gosman et al. 2007). The current study was undertaken to evaluate the effect of the *Rht-D1* locus in conferring the difference between the FHB resistance of Spark (*Rht-D1a*) with that of a FHB susceptible UK winter wheat, Rialto (*Rht-D1b*). The effect of the *Rht-D1* locus on FHB susceptibility was further examined in a range of experiments involving near-isogenic lines differing at the *Rht-D1* locus.

## Materials and methods

### Plant materials and assessment of FHB resistance in the Spark × Rialto population

A Spark (moderately resistant) × Rialto (susceptible) F<sub>1</sub> derived doubled haploid (DH) population of 129 lines was generated using the method of Laurie and Reymondie (1991). The pedigree of Spark is CB306Y 70(CIMMYT)/MW//Hobbit/Tonic (Singh et al. 2001) and that of Rialto is Haven/Fresco ([http://genbank.vurv.cz/ewdb/asp/ewdb\\_d2.asp?accn=5165](http://genbank.vurv.cz/ewdb/asp/ewdb_d2.asp?accn=5165)).

The parents and DH lines were phenotyped in field plots (double rows of 1 m length with 17 cm row spacing) and hill-plot (hand sown 30 cm<sup>2</sup> plots) environments during the summer of 2005 and in a separate field environment in 2006. Field experiments were conducted using a randomized complete block design (with two blocks in each experiment) grouped according to flowering date (early, medium and late). Field trials were conducted in 2005 at Nickerson Seeds Ltd (N2005), Woolpit, Suffolk and in 2006 at the John Innes Centre (J2006), Norwich, Norfolk. The hill-plot trial was conducted in 2005 at Advanta Seeds (UK) Ltd, (A2005) Docking, Norfolk.

The same inoculum was used in the field and hill-plot trials consisting of conidia of a highly virulent DON producing *F. culmorum* isolate (Fu42), and produced as described previously by Gosman et al. (2007). Groups (early, medium and late flowering) were spray inoculated at mid-anthesis [growth stage 65, Zadoks et al. (1974)], with a conidial suspension ( $1 \times 10^5$  spores ml<sup>-1</sup>) amended with 0.05% Tween 20 detergent. Both in N2005 and J2006 trials, inoculum was applied at 50 ml m<sup>-2</sup> and 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. Inoculation at A2005 was similar except that the inoculum was amended with 0.01% Tween 20 and heads were covered in cellophane bags for 3 days following inoculation.

Disease was visually assessed several times as the percentage (0–100%) of visually infected spikelets for each plot as described in Gosman et al. (2005). Plant height (cm) to the top of the spike is a stable trait and therefore it was only recorded at the JIC location during mid-anthesis. The area under the disease progress curve (AUDPC) was calculated to provide an integrated measure of disease severity for A2005, N2005 and J2006 (Buerstmayr et al. 2000).

### Influence of *Rht-D1a* (tall) and *Rht-D1b* (*Rht2*) on resistance to FHB

Inoculum preparation, inoculation and disease assessment were as described by Gosman et al. (2007). Two DON producing isolates were used in the current study (Chandler et al. 2003; Jennings et al. 2004): *F. culmorum* (Fu42) and *F. graminearum* (UK1).

### Experiment 1

*Rht-D1a* (tall) and *Rht-D1b* (*Rht2*) near-isogenic lines of Maris Huntsman (Flintham et al. 1997) were phenotyped in 2004 at JIC for resistance to FHB in a heated glasshouse (25°C day, 20°C night) where a 16 h photoperiod was maintained with supplementary lighting. Treatment groups

consisted of spray or point inoculation with *F. culmorum*. For the spray inoculation, pots containing individual plants were arranged in a randomised complete block design of three replicates of nine plants per variety and for the point inoculation treatment ten plants per variety were used in each replicate.

Plants were inoculated at GS 65 (Zadoks et al. 1974) by spray inoculation with conidial suspension ( $1 \times 10^5 \text{ ml}^{-1}$ ) or by point inoculation, with 10  $\mu\text{l}$  of conidial suspension ( $1 \times 10^6 \text{ ml}^{-1}$ ) injected into a single floret within the central portion of each spike. High humidity was maintained for 72 h post-inoculation by misting and reducing the ventilation. Following spray inoculation, disease was measured three times at an interval of 7 days and AUDPC was calculated as described in Buerstmayr et al. (2002). Following point inoculation, disease was estimated as the number of diseased spikelets at 21 days post-inoculation (dpi).

### Experiment 2

*Rht-D1a* (tall) and *Rht-D1b* (*Rht2*) near-isogenic lines of the varieties Maris Huntsman and Mercia were phenotyped for FHB resistance at JIC in summer of 2005 following spray inoculation with *F. culmorum* in a randomised complete block design field trial with three replicate plots per variety. Lines were inoculated with conidial suspension ( $1 \times 10^5 \text{ ml}^{-1}$ ) at GS 65 using a knapsack sprayer ( $150 \text{ ml m}^{-2}$ ). Disease severity was visually assessed three times at an interval of 7 days and data used to calculate AUDPC (Buerstmayr et al. 2002).

### Experiment 3

During the summer of 2005, *Rht-D1a* (tall) and *Rht-D1b* (*Rht2*) near-isogenic lines of Mercia were assessed for resistance to FHB in an unheated polytunnel with capillary matting irrigation. Trials with spray and point inoculation were performed as per “Experiment 1”. Within each trial, plants were inoculated at GS 65 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. High humidity was maintained for 72 h post-inoculation by reducing ventilation and disease severity assessed as above.

### Statistical analysis

All statistical analyses were performed using software within GenStat for Windows ninth edition (copy right Lawes Agricultural Trust, Rothamsted Experimental

Station, UK). Analysis of variance (ANOVA) was carried out using the generalised linear model (GLM) of regression analysis. Heritability across environments was estimated from the ANOVA using the formula:  $h^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_{GE}^2 / E) + (\sigma_e^2 / rE)]$ , and the repeatability was calculated, using the formula:  $h^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_e^2 / r)]$ , with  $\sigma_G^2$ , the genetic variance;  $\sigma_{GE}^2$ , the genotype  $\times$  environment interaction variance;  $\sigma_e^2$ , the residual variance;  $E$ , the number of environments;  $r$ , the number of replicates per genotype (Nyquist 1991).

### Map construction and QTL analysis

A genetic linkage map was constructed using simple sequence repeat (SSR) markers, diversity Arrays Technology (DArT<sup>TM</sup>) markers, sequence tagged microsatellite (STM) markers and glutenin markers. All lines were genotyped for the tall, wild type (*Rht-D1a*) and the semi-dwarfing allele (*Rht-D1b*) at the *Rht-D1* locus on chromosome 4D as described in Ellis et al. (2002) and used in the map construction. Linkage analysis was performed with the JoinMap program (version 3.0) (Van Ooijen and Voorrips 2001), using the Kosambi mapping function (Kosambi 1944). The linkage map derived from 129 DH lines comprised 259 loci organised into 39 linkage groups (LGs). Of these 31 could be mapped onto the 21 wheat chromosomes using consensus maps (Somers et al. 2004). QTL analysis was performed using MapQTL<sup>®</sup> 5.0 (Van Ooijen 2004). The Kruskal–Wallis test (a non-parametric equivalent of the one-way ANOVA) 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 was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5% ( $P < 0.05$ ). Based on the permutation tests (1,000 permutations), a threshold LOD value of 2.8 was used to declare the presence of QTL in the interval and MQM analysis. 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 detected above the LOD threshold ( $\text{LOD} \geq 2.8$ ) that explained more than 10% of the variance ( $R^2$ ) in at least one environment were arbitrarily classified as major QTL and those explaining less than 10% as minor QTL. The linkage map was drawn using MAPCHART (Voorrips 2002).

## Results

### Trait analysis of Spark × Rialto DH population

Spark, Rialto and the DH lines were phenotyped for FHB across three environments. The frequency distribution for AUDPC in the A2005, N2005 and J2006 trials was continuous with transgressive segregation and slightly skewed towards greater susceptibility, with the population mean being greater than the mid-parent mean (Table 1).

The analysis of variance (ANOVA) was estimated for AUDPC and PH. Using ANOVA, the repeatability for AUDPC in single environments and heritability ( $h^2$ ) for AUDPC across environments were calculated (Table 2). The repeatability ranged from 0.48 to 0.88 and was highest at J2006. The heritability across environments was 0.49. The correlation coefficient for AUDPC was moderate but significant ( $P < 0.001$ ) ranging from 0.24 to 0.38.

### Association between height and disease

The parental lines had very similar plant heights; Spark (103.3 cm) and Rialto (101.1 cm). The frequency distribution for height within the DH population was continuous, ranging from 82 to 130 cm, and followed a normal distribution pattern centred on the two parents with a population mean of 101.7 cm. Analysis indicated that most of the differences in height within this population have a genetic basis with a high repeatability ( $h^2 = 0.85$ ) (Table 2). A weak/moderate negative correlation was detected between the plant height at J2006 and AUDPC with a correlation coefficient  $r = 0.39$  ( $P = <0.001$ ), indicating that PH is associated with resistance.

### The map and FHB QTL

Genetic maps comprised of a total of 259 markers organised into 39 LGs with 31 major and 8 minor groups. The total map spanned about 1446 cM with 597 cM for the A

genome, 479 cM for the B genome and about 160 cM for the D genome.

The non-parametric (Kruskal–Wallis) test identified a number of putative QTL for FHB resistance on linkage groups 1B, 2A, 3A, 4D, 5A, 6A and 7A. The QTL identified using MQM analysis for FHB resistance derived from individual field assessments and across environments are summarised in Table 3. Nine QTL for FHB resistance were detected with three of these being observed in more than one environment. The total variance explained for AUDPC by all the QTL across environments varied, being greatest at JIC (71.4%). One major QTL for FHB resistance was observed on each of the three chromosomes, 1B, 4D and 6A. The locations of all QTL are shown in Fig. 1.

The QTL of greatest effect was detected on 4D (*Qfhs.jic-4d.1*) which accounted for at least 21% of the phenotypic variance (LOD value 6.8–28.2). The closest locus to this QTL was *Rht-D1* in the J2006 and A2005 trials whereas for the combined data set, the closest locus to the QTL peak was *Xpsp3103* in the N2005 trial (Table 3; Fig. 1). The FHB resistance was derived from Spark which carries the wild type allele (*Rht-D1a*) while susceptibility was associated with the *Rht-D1b* allele (*Rht2*) carried by Rialto. An additional, but minor QTL (*Qfhs.jic-4d.2*), contributed by Spark was also detected on 4D in the J2006 trial. The closest loci to the major and minor QTL were at least 24 cM apart.

Another major QTL (*Qfhs.jic-6a.2*,  $R^2 = 7.8$ – $10.5$ ), closely linked to the DArT locus, *wPt-8833*, was detected on 6A in the J2006 trial and across the trials when data was pooled for A2005, N2005 and J2006. A QTL for FHB resistance of major effect was also detected on chromosome 1B in a single environment (N2005) with resistance conferred by the allele from Rialto, the susceptible parent (Table 3; Fig. 1). Two minor FHB QTL on chromosome 3A, separated by approximately 50 cM were observed in more than one environment, while one QTL (*Qfhs.jic-3a.1*) was derived from Rialto, the other (*Qfhs.jic-3a.2*) was derived from Spark (Table 3; Fig. 1). In addition, FHB

**Table 1** Summary statistics for *Fusarium* head blight (FHB) disease assessed in J2006, A2005 and N2005 trials and plant height assessed in the J2006 trial

	Spark	Rialto	Mid-parent value	Population mean	Range
<i>FHB</i>					
A2005–AUDPC	246	557	401	529	128–1,100
N2005–AUDPC	208	339	273	294	37–695
J2006–AUDPC	144.5	792	468	515	61–1,270
<i>Plant height (cm)</i>					
J2006–height	103.3	101.1	102.2	101.7	82–130

A2005 Advanta seeds 2005 trial, N2005 Nickerson seeds 2005 trial, J2006 John Innes Centre 2006 trial, AUDPC Area under the disease progress curve

**Table 2** Analysis of variance and heritability of different traits in individual trials and across environments

Experiment	Variance factor	df	m.s.	F pr.	$R/h^2$
A2005–FHB (AUDPC)	Blocks	1	99,487	0.07	0.48 <sup>a</sup>
	Genotypes	121	56,045	<.001	
	Residual	121	30,288		
N2005–FHB (AUDPC)	Blocks	1	18,136	0.22	0.62 <sup>a</sup>
	Genotypes	121	39,255	<.001	
	Residual	121	11,864		
J2006–FHB (AUDPC)	Blocks	1	442,255	<.001	0.88 <sup>a</sup>
	Genotypes	121	194,638	<.001	
	Residual	121	13,626		
Across sites –FHB (AUDPC)	Blocks within environments	3	136,450	0.002	0.49
	Environments	2	4,057,129	<.001	
	Genotypes	121	119,229	<.001	
	Genotypes <sup>a</sup> environments	237	96,061	<.001	
	Residual	421	26,552		
J2006–height (cm)	Blocks	1	52.71	0.078	0.85 <sup>a</sup>
	Genotypes	121	183.98	<.001	
	Residual	119	16.63		

df, Degrees of freedom; m.s., Variance expressed as mean squares; F pr., F probability; R Repeatability represented by superscript a;  $h^2$  heritability

**Table 3** Summary of QTL for FHB resistance identified in Spark/Rialto DH population in A2005, N2005 and J2006 trials

Name	Closest marker locus	Origin	Chr.	Position	A2005		N2005		J2006		Across A, N, J	
					LOD	$R^2$	LOD	$R^2$	LOD	$R^2$	LOD	$R^2$
<i>FHB resistance</i>												
<i>Qfhs.jic-1b</i>	<i>wPt-0705</i>	Rialto	1B	11.5			5.2	13.4				
<i>Qfhs.jic-2a</i>	<i>Xgwm515</i>	Spark	2A	96.3			2.9	7				
<i>Qfhs.jic-3a.1</i>	<i>Xwmc11(2)</i>	Rialto	3A	0					2.7 <sup>a</sup>	2.7		
<i>Qfhs.jic-3a.1</i>	<i>wPt-7992</i>	Rialto	3A	3.4							2.8	4
<i>Qfhs.jic-3a.2</i>	<i>Xgwm497</i>	Spark	3A	54			3.3	8				
<i>Qfhs.jic-3a.2</i>	<i>Xbarc19</i>	Spark	3A	62	2.8	7.6						
<i>Qfhs.jic-4d.1</i>	<i>Xpsp3103</i>	Spark	4D	24			9.3	25.5				
<i>Qfhs.jic-4d.1</i>	<i>XRht-D1b</i>	Spark	4D	29	6.8	21.4			28.2	52.7	23.5	50.9
<i>Qfhs.jic-4d.2</i>	<i>Xgwm265</i>	Spark	4D	0					3.1	3.1		
<i>Qfhs.jic-5a</i>	<i>Xgwm443</i>	Spark	5A	0					3.5	3.9		
<i>Qfhs.jic-6a.2</i>	<i>wPt-8833</i>	Spark	6A	35.4					8.3	10.5	5.2	7.8
<i>Qfhs.jic-7a</i>	<i>Xpsp3050(2)</i>	Spark	7A	76.7					2.9	3.7		
<i>Qfhs.jic-7a</i>	<i>wPt-7299</i>	Spark	7A	82.5			2.2 <sup>a</sup>	5.2				
Total percentage variance explained							29	64.3	71.4		62.7	

Chr. Chromosome,  $R^2$  Percentage phenotypic variance explained

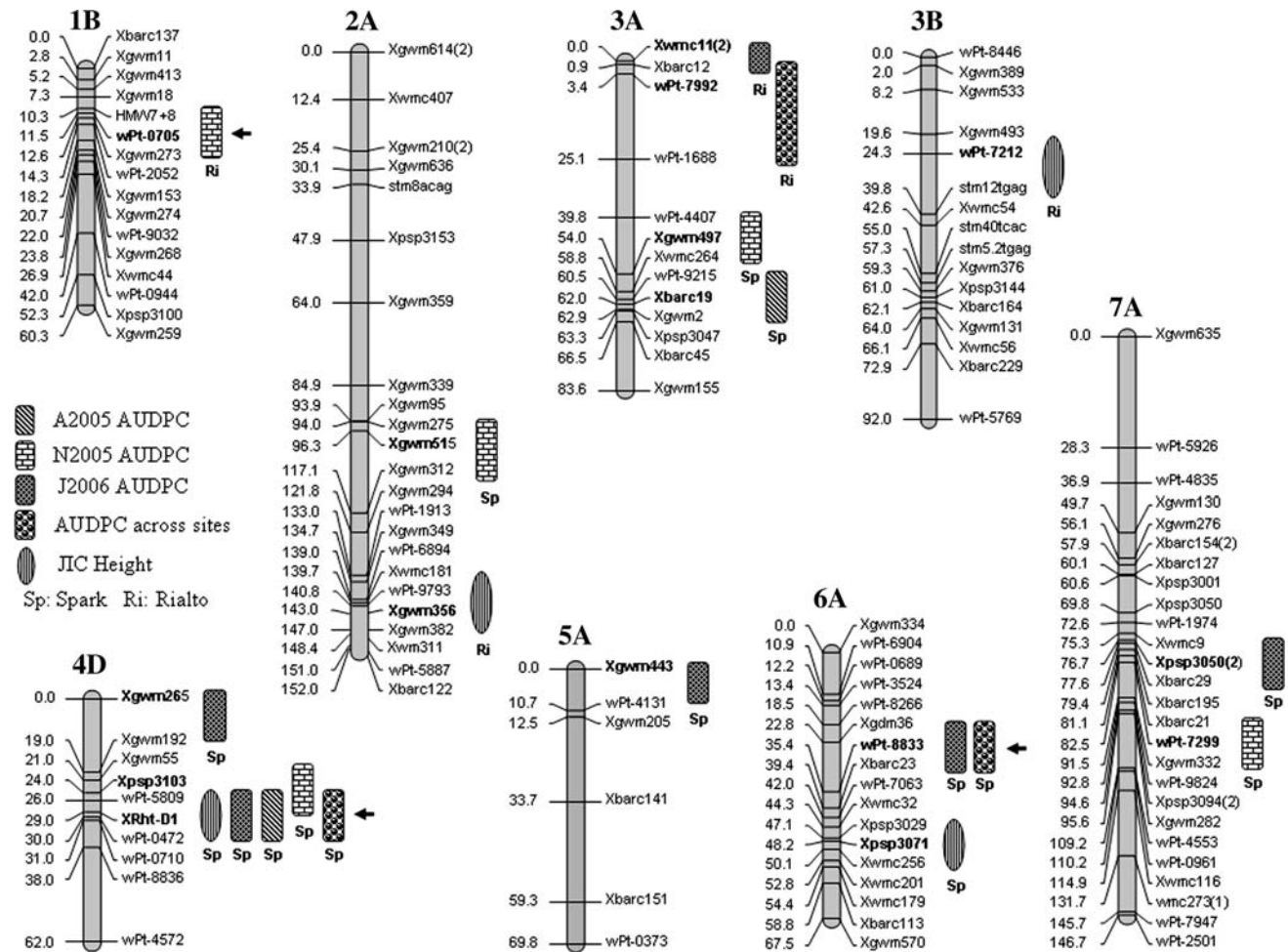
<sup>a</sup> LOD below the permutation test threshold for significance

QTL of minor effect, observed only in individual environments, were observed on chromosomes 2A, 5A and 7A.

#### Plant height QTL

Although Rialto carries the semi-dwarfing allele *Rht-D1b* on chromosome 4D, while Spark has the wild type allele (*Rht-D1a*) at this locus, the height of the parents differed

by only 2 cm in the J2006 field trial. Significant transgressive segregation for PH was observed among the progeny with heights ranging from 82 to 130 cm (Table 1). MQM analysis revealed that both parents contributed QTL for PH and identified one major and three minor PH QTL (Table 4; Fig. 1). The major QTL centred about the *Rht-D1* locus on chromosome 4D with the positive effect contributed by Spark (*Rht-D1a*) (LOD value of 29.2 and



**Fig. 1** Linkage maps of chromosome segments constructed from the Spark  $\times$  Rialto doubled haploid population. Putative QTL positions for FHB resistance and plant height are shown on the *right* of each

linkage group. Genetic distances are shown in centimorgans to the *left* of each linkage group. Major QTL have been indicated with an *arrow* and loci in *bold* are closest to the peak LOD score

$R^2 = 53\%$ ). A minor QTL also contributed by Spark was mapped on 6A but accounted for only 3.1% of variance (Table 4). Rialto contributed two QTL of minor effect, one on each of chromosomes 2A and 3B accounting for 8 and 3.7% of the phenotypic variance, respectively.

#### Coincidence of FHB and PH QTL

The FHB QTL of major effect (*Qfha.jic-4d.1*) was coincident with the major QTL for PH associated with the locus *Rht-D1*. QTL for FHB (*Qfhs.jic-2a*) and PH were also present on chromosome 2A but the contribution to the two traits did not come from the same parent (Spark and Rialto, respectively) and were not coincident on the chromosome. Similarly, although the QTL for FHB (*Qfhs.jic-6a*) and PH on 6A were both derived from Spark, they were not coincident. QTL analysis was carried out on the AUDPC data following adjustment for height by covariance analysis. The *Qfha.jic-4d.1* remained highly significant even after

**Table 4** Summary of QTL for plant height in the Spark/Rialto DH population identified in the J2006 trial

Closest locus	Chromosome	Position	LOD	$R^2$	Origin
<i>Xgwm356</i>	2A	143	6.9	8	Rialto
<i>wPt-7212</i>	3B	24.3	3.3	3.7	Rialto
<i>XRht-D1b</i>	4D	29	29.2	53.1	Spark
<i>Xpsp3071</i>	6A	48.2	2.9	3.1	Spark

$R^2$  Percentage phenotypic variance explained

the effect of height was accounted, although the variance accounted for across the trials (A2005, N2005, J2006) was somewhat reduced ( $R^2 = 41.9\%$ ). Covariance analysis also had no marked effect on the other FHB QTL and all were observed following height covariance analysis.

#### FHB resistance of near-isogenic lines

*Experiment 1.* Following spray inoculation in the glasshouse, disease levels on Maris Huntsman *Rht-D1a* (tall)

were significantly less ( $P < 0.001$ ) than on the *Rht-D1b* (*Rht2*) isogenic line. Mean AUDPC for *Rht-D1a* was 530 while that for *Rht-D1b* was 1,092. In contrast, following point inoculation, the two lines did not differ significantly in the number of damaged spikelets ( $P = 0.68$ ), being 11.6 for *Rht-D1a* and 12.6 for *Rht-D1b*.

**Experiment 2.** In the field trial Huntsman appeared slightly, but not significantly, more resistant than Mercia with AUDPC of 473 and 519, respectively. The *Rht-D1b* isogenic lines of both varieties were significantly more susceptible than their respective parents ( $P = 0.002$ ) with AUDPC for Huntsman *Rht-D1b* and Mercia *Rht-D1b* of 723 and 803, respectively.

**Experiment 3.** Following spray inoculation with *F. graminearum* in the polytunnel, disease levels on Mercia *Rht-D1a* (tall) were significantly less ( $P = 0.002$ ) than on the *Rht-D1b* isogenic line. The mean AUDPC for *Rht-D1a* was 939 while that for *Rht-D1b* was 1153. In contrast, following the point inoculation disease levels in the two lines did not differ significantly ( $P = 0.354$ ). The mean number of damaged spikelets for *Rht-D1a* and *Rht-D1b* were 14 and 15, respectively, following inoculation with *F. graminearum*.

## Discussion

Numerous authors have reported an association between PH and resistance to FHB (Mesterhazy 1995; Somers et al. 2003; Klahr et al. 2007) but the importance of this has been found to vary in magnitude, depending upon the population under study. For example, Somers et al. (2003) observed a very significant negative correlation in progeny from a cross between Wuhan-1 and Maringa; whereas, Klahr et al. (2007) observed a moderate effect in only two of four environments.

It has been suggested that taller lines may escape from the infection because their heads are farther from the soil or that the microclimate about the ear may differ in short and tall varieties (Somers et al. 2003; Klahr et al. 2007). However, several studies indicate that the relationship between FHB resistance and plant height may be more complex. Somers et al. (2003) determined that a QTL on 2DS, influencing the accumulation of DON in grain was coincident with a QTL for PH, although this QTL had no apparent effect on disease symptoms. In a cross between Renan and Recital, QTL for FHB and PH were coincident on 5A whereas the PH QTL on 4A was not associated with differences in resistance to FHB (Gervais et al. 2003). Paillard et al. (2004) also found that, while some PH QTL overlapped with those for FHB (e.g. 5B), the main PH QTL (2 and 5AL) in their population from a cross between Arina and Forno were not coincident with FHB QTL. Similarly,

QTL for FHB and PH on 6A were coincident in a cross between Dream and Lynx, although other FHB and PH QTL were independent in this cross (Schmolke et al. 2005). These authors concluded that the coincidence of FHB and PH QTL following spray inoculation suggested that it has a genetic basis, linkage or pleiotropy, rather than being due to escape. While PH may be correlated with resistance to FHB, the finding that some QTL for PH coincide with those for FHB, while others do not, supports this view. However, it is possible that only QTL with a large effect on height also confer a significant effect on resistance to FHB.

The present study has clearly demonstrated that increased susceptibility to FHB is associated with the *Rht-D1b* semi-dwarfing allele in wheat. The negative effect of the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* (formerly termed *Rht1* and *Rht2*, respectively) on resistance to FHB has been observed or inferred in previous studies. Hilton et al. (1999) observed that, in crosses between varieties carrying these alleles (semi-dwarf) with those carrying the wild type alleles (tall), there was a clear tendency for tall strawed lines to be more resistant than short strawed lines. These authors also spray-inoculated near-isogenic lines differing in *Rht-B1* and *Rht-D1* alleles with a mixture of *Fusarium* species and *Microdochium nivale*. In most cases, lines carrying either *Rht-B1b* or *Rht-D1b* alleles were more susceptible than lines carrying the wild type alleles (Hilton et al. 1999). Relative humidity at ear height was not found to differ between tall and short isogenic lines and it was concluded that the effect on FHB susceptibility was not due to higher humidity at ear height in the shorter genotypes.

The negative effect on FHB resistance associated with the *Rht-D1b* allele carried by Rialto has also been observed in Riband, another UK variety (Draeger et al. 2007). As in the present study, Draeger et al. (2007) showed that the main FHB QTL segregating among progeny from a cross between Arina and Riband co-localised with the *Rht-D1* locus. These authors concluded that the effect is not due to the PH per se but rather due to pleiotropy or linkage. It was also noted that the great majority of UK wheat varieties carry the *Rht-D1b* allele and that they are generally highly susceptible to FHB (Gosman et al. 2007). In combination with the present work these studies indicate that *Rht-D1b* (*Rht2*) is associated with reduced resistance to FHB. The effect of *Rht-B1b* (*Rht1*) on FHB resistance is less clear. Hilton et al. (1999) found no evidence for differences in the effect of the *Rht-B1b* and *Rht-D1b* alleles. However, in a separate study of a population derived from a cross between Frontana and Remus, a FHB QTL identified on 4B coincident with a PH QTL thought to correspond to *Rht-B1b* accounted for only 7.4% of phenotypic variance (Steiner et al. 2004). Further study is required to determine whether *Rht-B1b* and *Rht-D1b* alleles differ in their influence on susceptibility to FHB. It is perhaps significant that

the semi-dwarfing alleles of *Rht-B1* and *Rht-D1* carried on separate chromosomes (4B and 4D, respectively) are associated with increased susceptibility to FHB, suggesting that the effect may be due to pleiotropy rather than linkage to deleterious genes.

The results from different types of inoculation procedure used in the present study clearly demonstrate that the negative effect of the *Rht-D1b* allele on FHB resistance acts on resistance to initial infection (type I resistance) (Schroeder and Christensen 1963) with little, if any effect on spread within the head (type II resistance). It is possible that no effect on type II resistance was observed because both Mercia and Maris Huntsman are highly susceptible to FHB making it difficult to detect any further reduction in resistance. Other studies also found that the relationship between resistance to FHB and PH was evident in field trials measuring type I resistance (incidence) or a combination of type I and type II resistance (severity) but not where point inoculation was used or spread within the head was measured (Somers et al. 2003; Steiner et al. 2004). It remains to be determined how the *Rht-B1b* and *Rht-D1b* alleles might alter susceptibility to infection (type I resistance) and this is the subject of ongoing research.

In addition to the *Qfhs.jic-4d.1* coincident QTL for FHB resistance and PH, two other chromosomes carried QTL for both FHB resistance and PH. On 2A; however, the allele from Spark contributed the FHB resistance while that for PH was contributed by Rialto. Additionally, although, QTL for FHB and PH were located on 6A, they did not overlap (Fig. 1).

In the present study, nine putative FHB QTL were observed to segregate within the Spark × Rialto population. Of these, the alleles from Spark and Rialto contributed resistance for seven and two QTL, respectively. The contribution to FHB resistance of alleles from both the parents is not uncommon and previous studies of FHB resistance in winter wheat have indicated that resistance is controlled by several loci on different chromosomes, each with only a weak or moderate effect (Paillard et al. 2004; Schmolke et al. 2005; Draeger et al. 2007). An exception appears to be *Qfhs.jic4d.1* associated with the *Rht-D1* locus in winter wheat which explained up to 53% of phenotypic variance in the Spark × Rialto population used here and up to 24% in the Arina × Riband population (Draeger et al. 2007).

In addition to *Qfhs.jic-4d.1*, two relatively stable QTL of moderate effect (*Qfhs.jic-6a.2* and *Qfhs.jic-3a.2*) were also observed to segregate in the Spark × Rialto population, both originating from Spark. *Qfhs.jic-6a.2* appears to be in a similar location to a QTL identified in the FHB resistant winter wheat variety Dream (Schmolke et al. 2005). These authors reported that the FHB QTL from Dream partially overlapped with a PH QTL and, interestingly, Spark also possessed a PH QTL near *Qfhs.jic-6a.2* but distinct from

this locus. The *Qfhs.jic-3a.2* from Spark is located in a similar position on chromosome 3A to FHB QTL reported from the spring wheat variety Frontana (Steiner et al. 2004) and Fundulea 201R, a winter wheat line (Shen et al. 2003). Further work is required to establish the relationship between these three FHB QTL.

Rialto carries the 1BL/1RS wheat-rye translocation (Snape et al. 2007). A QTL of major effect associated with chromosome 1B from Rialto was observed at a single location (N2005). Several studies have found that the 1BL/1RS translocation is associated with type II resistance to FHB but it appears that expression of the FHB QTL associated with the 1BL/1RS wheat-rye translocation is influenced by the genetic background in which it is present (Buerstmayr et al. 2002; Shen et al. 2003; Schmolke et al. 2005).

Spark has previously been shown to be one of the most resistant UK winter wheat varieties (Gosman et al. 2007). The present work has demonstrated that most of this effect may be due to the presence of wild type (tall) allele at the *Rht-D1* locus in this variety in contrast to the semi-dwarfing allele (*Rht-D1b*) that is present on most UK varieties. The *Rht-D1b* allele appears to confer enhanced susceptibility to FHB by reducing the level of type I resistance. This allele however, has little or no effect on type II resistance. Additional FHB QTL of lesser effect are also present in Spark (3A and 6A) but additional work is required to establish their value in breeding programmes.

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