

Mapping of QTL Associated with Fusarium Head Blight in Spring Wheat RL4137

SRINIVASACHARY¹, NICOLAS GOSMAN¹, ANDREW STEED¹, SEBASTIEN FAURE¹,
ROSEMARY BAYLES², PHILIP JENNINGS³ and PAUL NICHOLSON¹

¹John Innes Centre, Norwich Research Park, Colney, Norwich, UK; ²National Institute of Agricultural Botany (NIAB), Huntington Road, Cambridge, UK; ³Central Science Laboratory, Sand Hutton, York, UK

Abstract: Fusarium head blight (FHB) is a destructive disease of wheat worldwide. We aimed to map QTL for FHB resistance in RL4137, a FHB resistant line derived from Frontana using 90 recombinant inbred lines (RIL) from a cross between RL4137 and the moderately FHB resistant variety Timgalen. A total of seven putative FHB resistance QTL (1B, 2B, 3A, 6A, 6B, 7A and 7D) were identified and in all but one instance, the alleles from RL4137 had a positive effect on FHB resistance. The QTL, *Qfhs.jic-2b* and *Qfhs.jic-6b* contributed by the alleles from RL4137 and Timgalen, respectively were detected in multiple trials. Our study also identified three QTL for plant height (2B, 4A and 5B), two QTL for weight of infected spikelets from infected ears (2B and 6A) and one QTL for “awns” (2B). The QTL mapped on 2B for PH, WIS and awns co-localized with *Qfhs.jic-2b*. The FHB QTL on 1B and 6B were not associated with PH QTL and that the minor PH QTL on 4A and 5B, did not co-localise with any other FHB resistance QTL.

Keywords: wheat; FHB traits; plant height; awn measurements; DON tolerance; DArT; genetic map; QTL

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* and *F. culmorum* is one of the major diseases of wheat world wide, results in significant loss in terms of both grain yield and quality (BAI & SHANER 1994; PARRY *et al.* 1995; WALDRON *et al.* 1999). The disease is favoured by warm humid conditions during anthesis and the early stages of kernel development (GILBERT & TEKAUZ 2000). FHB can cause bleached spikes, spikelet sterility, poor seed filling, low-test weight and tombstone seeds, and in epidemic years the yield losses can range from 10 to 40% (WANG 1996). Furthermore, the grain is often contaminated with mycotoxins such as deoxynivalenol (DON) which is a significant health risk for humans and animals (GILBERT & TEKAUZ 2000). FHB also affects the milling and baking quality of wheat (BECHTEL *et al.* 1985).

FHB resistance is a quantitatively inherited trait controlled, in many spring wheat varieties by a few genes of major effect plus several genes of more minor effect (LIU *et al.* 2005). Although fungicides and good agronomic practices can limit damage, the use of genetic resistance is considered to be a key component in the management of FHB (BAI & SHANER 1994). The accepted model of resistance to FHB in wheat is resistance to initial infection (type I) and resistance to spread within the spikelet (type II) (SCHROEDER & CHRISTENSEN 1963), although additional components of FHB resistance have been proposed (BAI & SHANER 1994; YANG 1994; MCMULLEN *et al.* 1997). To date, immunity to FHB has not been identified and there is still a relative paucity of potent resistance available to plant breeders. Resistance from the Chinese spring

wheat Sumai 3 (MESTERHAZY 1983; CHEN *et al.* 1997; BUERSTMAYR *et al.* 2002) and its derivatives has been deployed in breeding programmes for several years, and, as a result, this source tends to predominate worldwide (DEL BLANCO *et al.* 2003). Use of a limited number of host resistance gene sources imposes a strong selective pressure for virulent pathogen strains (GERVAIS *et al.* 2003) and consequently there is a need to identify additional sources of FHB resistance.

Phenotyping for FHB resistance is costly and time-consuming and the expression of FHB resistance is greatly influenced by environmental factors such as temperature, humidity and crop stage at the time of inoculation (BAI & SHANER 1994; PARRY *et al.* 1995; MIEDANER *et al.* 2001; KLAHR *et al.* 2007). In addition, previous authors have reported that morphological characters including plant height (PH), awns and spike compactness (STEINER *et al.* 2004) are linked to, or have a pleotropic effect on FHB resistance in wheat. Such an association with physical characteristics further complicates efforts to understand the physiological basis of FHB resistance.

The Brazilian spring wheat Frontana has the pedigree Fronteira/Mentana and is known to have moderate type I and type II resistance (SINGH *et al.* 1995; BUERSTMAYR *et al.* 1996; VAN GINKEL *et al.* 1996; STEINER *et al.* 2004). In addition to this, *in vitro* experiments suggest that Frontana may be able to degrade and tolerate higher levels of DON (MILLER & ARNISON 1986; WANG & MILLER 1988). Previous mapping studies indicate that resistance in Frontana is conditioned by several QTL of minor effect (STEINER *et al.* 2004; MARDI *et al.* 2006). RL4137 is a Canadian spring wheat derived from Frontana and was found to be highly resistant to FHB in preliminary trials at the John Innes Centre. A mapping study was undertaken to identify and characterise FHB resistance in recombinant inbred lines (RILs) derived from a cross between RL4137 and the moderately resistant variety Timgalen and to study the relationship between FHB resistance traits (disease symptoms and weight of infected spikelets) and some developmental and morphological traits (plant height and measurements of awns).

MATERIALS AND METHODS

Mapping population, markers and genotyping

Ninety F₂ recombinant inbred lines (RILs) from a cross between the FHB resistant variety RL4137

(Frontana/3/McMurachy/Exchange//2*Redman/4/Thatcher*6/Kenya Farmer) and the moderately resistant variety Timgalen (Aguillera/Kenya//Marroqui/3/Supremo/4/Gabo/5/Winglen), were examined in this study along with the parental lines. The population was originally developed at the John Innes Centre and used to study pre-harvest sprouting resistance (BASSOI & FLINTHAM 2005).

Phenotyping

The RILs and the parents, RL4137 and Timgalen, were phenotyped in two polytunnel and field experiments. The polytunnel experiments were conducted at the John Innes Centre (JIC), Norwich, UK in 1999 (J1999) and 2000 (J2000). The field trials were conducted in 2006 at the National Institute of Agricultural Botany (NIAB), Cambridge (N2006) and Central Science Laboratory (CSL), York, UK (C2006). All the lines were tested in J2000, C2006 and N2006 experiments but, due to a shortage of seed, only half of the population was tested in J1999. In both field and polytunnel experiments, the lines were grouped according to flowering time (early, medium and late) and used in the study. Twelve seeds from each recombinant inbred line and parents RL4137 and Timgalen were germinated in Petri-dishes for 48 h at 4°C in total darkness in 10 ml of distilled water. After germination, they were planted in pots in special John Innes cereal mix (3 barrows of loam, 1 barrow of grit, 1 bale of medium peat, 5 ½ lb. Oscomote, 5 lb. Chalk and 1 bushel of tap water.) adjusted to pH 8.0, with one seed per pot and grown in a polytunnel (National Poly tunnels, Preston, UK) allowing protection from the rain, but not from the wind, atmospheric humidity or relative sun warmth. Pots containing individual plants were arranged in a randomised complete block design with three plants per block, prior to inoculation. The field experiments at NIAB (N2006) and CSL (C2006) were conducted in small hand sown plots—each plot comprised of 1 row of approximately 40 cm using a randomized complete block design with two replicates.

The inoculum preparation, plant husbandry, trial set-up and disease assessments were carried out as described in GOSMAN *et al.* (2005). Plants were spray inoculated at mid-anthesis (growth stage 65, ZADOKS *et al.* (1974) with a conidial suspension (1×10^5 conidia ml⁻¹) of a DON-producing isolate

of *F. culmorum*. Inoculum was amended with 0.05% Tween 20 prior to use. In the field, inoculation was carried out in the evening with a knapsack sprayer and plants in the polytunnel experiments were inoculated with a hand-held sprayer. In the polytunnel, inoculated spikes were covered with cellophane crossing bags for 72 h post inoculation to maintain high humidity.

Disease was assessed as the percentage (0 to 100%) of visually infected spikelets (GOSMAN *et al.* 2005). In the polytunnel trials, disease was assessed four times at 7, 14, 21 and 28 days post inoculation (dpi). The lines in the N2006 trial were assessed at 20, 28 and 34 dpi. The area under the disease progress curve (AUDPC) was calculated (BUERSTMAYR *et al.* 2000) and used in subsequent analysis. The disease levels were low at C2006 trial where a single assessment was made. The weight of infected spikelets (WIS) was determined at harvest after counting the number of spikelets per head and weighing heads to provide an additional FHB resistance trait. In addition to this, the effect of DON toxin on the growth and germination of seeds (Petrifox test) was measured as described in GOSMAN *et al.* (2005) and the area under germination curve (DON-AUG) was measured. Due to the high cost associated with the test, only half of the population and the parents RL4137 and Timgalen were examined for DON response. The DON-AUG data was only used in QTL detection and no further statistical analysis was performed on this data. The overall AUDPC was calculated from J1999, J2000 and N2006 and was treated as another experiment (pooled AUDPC) for QTL analysis (the C2006 data were omitted as only a single score was taken).

Morphological traits were assessed in the J2000 experiment. Awns were scored on a 1 to 5 scale (1 = no awns, 5 = long awns). The plant height (PH) to the top of the spike in cm was recorded during mid-anthesis.

Statistical analysis

All the statistical analyses were performed using GenStat for Windows 9th 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. The experimental repeatability (h^2) was estimated from the ANOVA using the formula:

$$h^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_e^2/r)]$$

where:

σ_G^2 – genetic variance

r – number of replicates per genotype (NYQUIST 1991).

Markers, map construction and QTL analysis

A genetic linkage map was constructed using simple sequence repeat (SSR) markers, amplified fragment length polymorphism (AFLP) and diversity arrays technology (DArTTM) markers, a sequence independent, relatively cheap and high throughput dominant marker system (JACCOUD *et al.* 2001, Triticarte Private Limited, Australia, http://www.triticarte.com.au/content/wheat_diversity_analysis.html) which has been used in crop plants. Simple sequence repeat (SSR) loci of known map location (primer prefix *Xpsp* and *Xgwm* from BRYAN *et al.* (1997) and RÖDER *et al.* (1998), respectively) were used. The PCR master mix comprised 20 µl containing 60 ng of DNA, 0.02 µM each of forward and reverse primers, 0.13 mM of dNTPs, 2 U of *Taq* DNA polymerase (Roche). The PCR profile was as follows: 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 61°C for 1 min (ramp 0.5°C/s) and 72°C for 1 min (ramp 0.5°C/s) with a final extension of 72°C for 4 min. AFLPs were amplified as described by Vos *et al.* (1995) using 81 combinations of *Mse*I and *Pst*I primers, and the amplified products were visualised by silver staining. The linkage map was constructed using JoinMap (version 3.0) (VAN OOIJEN & VOORIPS 2001) and the map distances were calculated using the Kosambi mapping function (KOSAMBI 1944). The map comprised a total of 341 loci of which, 90 were AFLPs, 15 SSRs and 236 DArT markers. The SSRs and DArT markers of known map location permitted the assignment of linkage groups (LGs) to chromosomes according to the previously published wheat genetic maps (SOMERS *et al.* 2004; SEMAGN *et al.* 2007; <http://www.triticarte.com.au/pdf/TriticartewhmapalignV1-2.xls>). QTL detection and mapping was carried out using MapQTL[®] 4.0 (VAN OOIJEN 2004). For each trait, one-way ANOVA (Kruskal-Wallis test) was performed to detect the association between markers and traits individually. In a second step, interval mapping (IM) was performed to identify the major QTL. Automatic cofactor selection was used to fit the multiple QTL model (MQM) (backward elimination ($P > 0.02$)). For each trait, a permutation test

(1000 iterations) 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, a threshold LOD value of 3 was used to declare the presence of QTL. The QTL that explained more than 10% of the variance (R^2) in at least one environment/experiment were arbitrarily classified as major QTL and those explaining less than 10% as minor QTL. Linkage Maps were drawn using MapChart (VOORRIJS 2002).

RESULTS

Phenotyping of RILs

The parents, RL4137 and Timgalen, and the progeny were phenotyped for FHB resistance in the polytunnel (J1999 and J2000) and field experiments (N2006 and C2006). The frequency distribution of AUDPC was continuous for all the experiments and distribution was slightly skewed towards the resistant parent RL4137. The frequency distribution of pooled AUDPC is shown in Figure 1. Summary statistics of disease and other FHB related traits recorded in different experiments are shown in Table 1.

The correlations between N2006 and polytunnel experiments for AUDPC were highly significant ($P < 0.001$) and the correlation coefficient ranged from 0.5 (between J2000 and N2006) to 0.7 (J1999 and J2000) (Table 2). The correlation between C2006 and N2006 was also highly significant ($P = 0.005$). WIS had a significant negative relationship with AUDPC except for C2006 ($P = 0.082$) and the correlation coefficient ranged

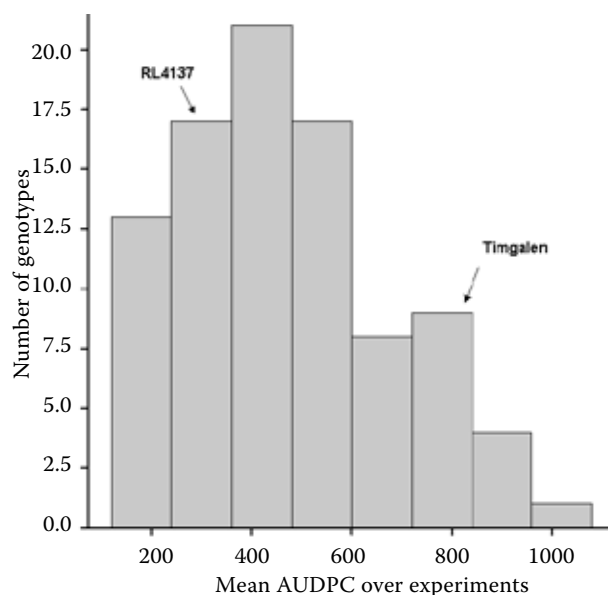


Figure 1. Frequency distribution of pooled AUDPC of Fusarium head blight in RL4137 X Timgalen RIL population

from -0.29 to -0.80 over experiments. Correlation coefficients between PH and AUDPC were in all experiments negative but this was only significant for J2000, N2006 and for the pooled data and the correlation coefficient ranged from -0.12 (C2006) to -0.47 (N2006). Significantly positive was the relation between PH and WIS. The trait “awns” showed non-significant relationship with AUDPC, but significantly negative association with PH ($r = -0.46$). For AUDPC, the genotypic variance was highly significant in all the experiments ($P < 0.001$) (Table 3). The experimental

Table 1. Summary statistics for Fusarium head blight (FHB) disease assessed in J1999, J2000 and N2006 and other FHB related traits assessed in J2000 trial

Experiment and trait recorded	RL4137	Timgalen	Mid-parent value	Population mean	Range
J1999-AUDPC	545.64	1161.08	853.36	835.9	50–1796.7
J2000-AUDPC	357.38	983.85	670.62	477.4	0–1540
N2006-AUDPC	75.31	435.88	255.59	276.7	12–835
C2006 (%)	0.25	1.5	0.75	1	0–7.75
J2000 – spikelet weight (g)	0.08	0.04	0.06	0.59	0.04–1.49
J2000 – awns	1	5	2.5	4.336	1–5
J2000 – plant height (cm)	162.3	97.7	130.0	121.1	73–170

J1999 = JIC1999, J2000 = JIC2000, N2006 = NIAB2006, C2006 = CSL2006

Awns were measured on 1 to 5 scale

Table 2. Correlation coefficients of AUDPC and other FHB related traits in RL4137/Timgalen RIL population

Trait	J1999-AUDPC	J2000-AUDPC	N2006-AUDPC	C2006-AUDPC	Pooled-AUDPC	J2000-PH	J2000-A	J2000-WIS
J1999-AUDPC	1							
J2000-AUDPC	0.70 ($P < 0.001$)	1						
N2006-AUDPC	0.56 ($P < 0.001$)	0.51 ($P < 0.001$)	1					
C2006-AUDPC	0.18 ($P = 0.273$)	0.1 ($P = 0.395$)	0.31 ($P = 0.005$)	1				
Pooled-AUDPC	0.90 ($P < 0.001$)	0.88 ($P < 0.001$)	0.77 ($P < 0.001$)	0.08 ($P = 0.501$)	1			
J2000-PH	-0.22 ($P = 0.173$)	-0.42 ($P < 0.001$)	-0.47 ($P < 0.001$)	-0.12 ($P = 0.274$)	-0.42 ($P < 0.001$)	1		
J2000-A	-0.22 ($P = 0.311$)	0.12 ($P = 0.062$)	-0.27 ($P = 0.271$)	-0.14 ($P = 0.210$)	-0.13 ($P = 0.65$)	-0.46 ($P < 0.001$)	1	
J2000-WIS	-0.68 ($P = 0.001$)	-0.75 ($P < 0.001$)	-0.61 ($P < 0.001$)	-0.29 ($P = 0.082$)	-0.80 ($P < 0.001$)	0.54 ($P < 0.001$)	-0.06 ($P = 0.997$)	1

J = JIC; N = NIAB; C = CSL; PH = plant height; A = awns; WIS = weight of infected spikelet

repeatability for AUDPC (h^2) ranged from 0.68 (N2006) to 0.79 (C2006) (Table 3).

The linkage map

The genetic map comprised of a total of 341 loci mapped onto 44 LGs and the map included 15 SSRs, 90 AFLPs and 236 DArT loci covering a genetic distance of 973 cM. LGs were assigned to chromosomes either based on the consensus wheat DArT maps from the Triticarte website and/or using the marker information from previously published maps. Genetic distance and marker coverage varied between genomes. There was a good coverage of the B genome with 198 markers spanning 571 cM. The A genome map contained 99 markers with the map length of about 282 cM however, the D genome map was less well covered and contained only 40 markers spanning 119 cM. There were two anonymous small LGs with two markers each and no LG corresponding to 5A.

FHB QTL

QTL analysis was performed on AUDPC values for individual experiments and for data pooled across experiments. The pooled average AUDPC from the three experiments was treated as another environment. The analysis identified a total of seven putative QTL for AUDPC on chromosomes 1B, 2B, 3A, 6A, 6B, 7A and 7D (Table 4 and Figure 2). Of these, five QTL were detected above the LOD score 3. Alleles from RL4137 contributed the positive effect for all the QTL except that on 6B. The QTL *Qfhs.jic-2b* (LOD = 2.1 to 5.2; $R^2 = 8.4$ to 21.5) contributed by RL4137 and *Qfhs.jic-6b* (LOD = 2.4 to 3.8; $R^2 = 9.2$ to 14) contributed by the alleles from Timgalen were the major QTL detected in more than one environment indicating that they are relatively stable. QTL for WIS, the yield associated FHB trait, were detected on chromosomes 2B and 6A with RL4137 contributing the positive effect alleles (greater WIS) in each case (Table 4). In the N2006 field trial a major QTL on 3A was detected, contributed by RL4137. QTL analysis for DON tolerance identified two minor QTL (contributed by alleles from RL4137) one

Table 3. Components of variation for AUDPC and other FHB associated traits in RL4137/Timgalen RIL population

Source of variation	FHB-AUDPC			FHB associated traits		
	MS	F	P	MS	F	P
J1999				plant height – J2000		
Blocks	239953	2.66	0.035	1402.96	36.28	< 0.001
Genotypes	430371	4.77	< 0.001	3347.97	86.57	< 0.001
Residual	90194			38.67		
h^2			0.7			0.94
J2000				awns – J2000		
Blocks	819205	10.08	< 0.001	0.2808	1.53	0.218
Genotype	458747	5.65	< 0.001	7.2132	39.25	< 0.001
Residual	81256			0.1838		
h^2			0.71			0.92
N2006				spikelet weight – J2000		
Blocks	143	0.01	0.934	0.00224	4.48	0.012
Genotypes	54885	2.69	0.007	0.00457	9.14	< 0.001
Residual	20396			0.0005		
h^2			0.68			0.58
C2006						
Blocks	0.55628	8.92	0.004			
Genotypes	0.09855	1.58	0.021			
Residual	0.06237					
h^2			0.79			

MS = mean square, F = variance ratio, P = F probability, h^2 = repeatability, J = JIC, N = NIAB

each on 2B and 7A with the closest markers being S24/M16I and wPt-6273, respectively.

QTL for morphological traits

QTL for PH (positive effect of “greater height” allele from RL4137) and “awns” (positive effect of “long awns” allele from Timgalen) were coincident on 2B and associated with the AFLP marker S13/M23G (Tables 4 and 5; Figure 2).

Co-incidence of trait QTL

The QTL for PH (LOD = 15.9; R^2 = 47.9), WIS (LOD = 3.5; R^2 = 33.9) and “awns” (LOD = 3.4; R^2 = 14.6) co-localized with major FHB resistance QTL *Qfhs.jic-2b* (LOD = 2.1 to 5.2; R^2 = 8.4 to 24.2) (Table 4 and Figure 2). RL4137 contributed the positive effect alleles for the FHB (greater

resistance), PH (greater height) and WIS (greater weight) while Timgalen contributed the positive effect alleles for “awns”.

DISCUSSION

To date, almost 50 QTL studies have been published on FHB resistance in hexaploid wheat. Many of these are either on Sumai 3 or other varieties of Chinese origin with a limited number of studies on known resistance sources from elsewhere. The red-grained, awnless, Canadian FHB resistant spring wheat RL4137, showing “extremely” great plant height, was derived from the Brazilian cultivar Frontana. Timgalen is a white-grained Australian spring wheat that has moderate resistance to FHB. It carries a large introgressed segment on 2B from *Triticum timopheevi* (AAGG) (Devos *et al.* 1993). Classical genetic studies suggest that a

Table 4. Summary of QTL identified for FHB resistance in RL4137/Timgalen recombinant inbred population

QTL	Closest marker	Position	Origin	J1999		J2000		N2006		C2006		Pooled data	
				LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²
AUDPC													
<i>Qfhs,jic-1b</i>	wPt-6425	51.3	RL4137					2.6 ^a	9.9			2.4 ^a	9.1
<i>Qfhs,jic-2b</i>	wPt-5292	4.6	RL4137					4.8	21.5				
<i>Qfhs,jic-2b</i>	S24/M16I	6.2	RL4137			5.2	19.8					2.1 ^a	8.4
<i>Qfhs,jic-2b</i>	S21/M13G	29.37	RL4137							4.4	24.2		
<i>Qfhs,jic-3a</i>	S22/M14F	48.1	RL4137					4.6	20.8				
<i>Qfhs,jic-6a</i>	wPt-9132	12.3	RL4137	3.4	25.8								
<i>Qfhs,jic-6b</i>	wPt-3376	16.6	Timgalen							3.6	19.7	2.4 ^a	9.2
<i>Qfhs,jic-6b</i>	wPt-4930	17.3	Timgalen			3.8	14						
<i>Qfhs,jic-7a</i>	wPt-3836	3.1	RL4137			2.1 ^a	7.4						
<i>Qfhs,jic-7d</i>	S14/M20M	5.3	RL4137							3.7	20.2		
WIS													
<i>Qfhs,jic-2b</i>	S13/M23G	5.5	RL4137					3.5	33.9				
<i>Qfhs,jic-6a</i>	wPt-9132	12.3	RL4137					4.2	40.4				
DON-AUG													
<i>Qfhs,jic-2b</i>	S24/M16I	6.2	RL4137	2.1 ^a	23.3								
<i>Qfhs,jic-7A</i>	wPt-6273	3.3	RL4137	2.3 ^a	20.3								

J = JIC, N = NIAB, R² = Percentage phenotypic variance explained, a = LOD below the permutation test threshold for significance, AUDPC = area under disease progress curve, WIS = weight of infected spikelets, DON = deoxynivalenol, AUG = area under growth curve

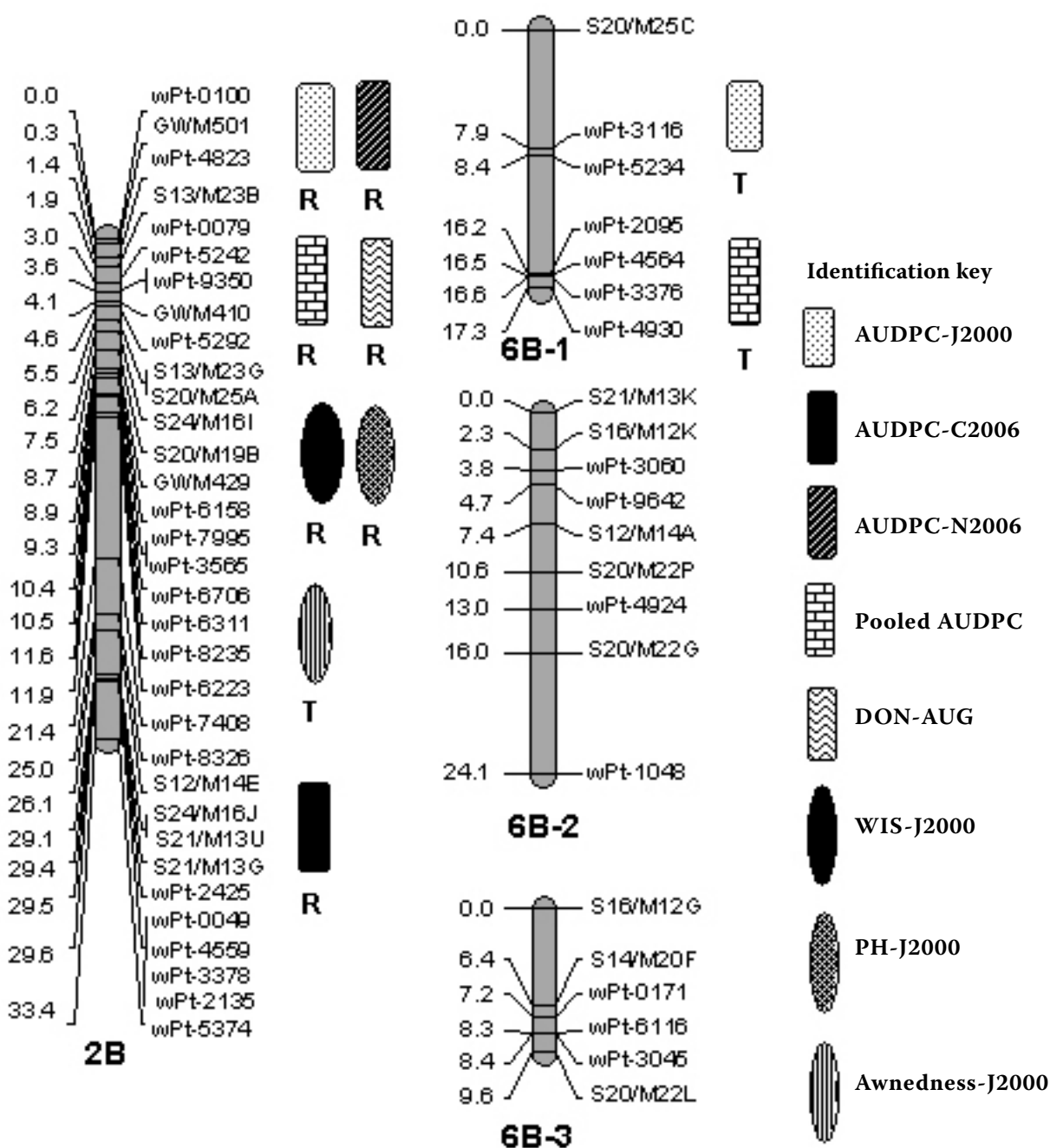


Figure 2. Linkage maps of chromosome segments constructed from the RL4137/Timgalen recombinant inbred population; putative QTL positions for the major consistent FHB resistance along with the co-localized QTL for the DON-tolerance (effect of DON on seed germination and growth, DON-AUG), plant height (PH), weight of infected spikelet (WIS) and awns are shown on the right of each linkage map; J and N refer to JIC and NIAB trial sites, respectively; genetic distances are shown in centimorgans to the left of each linkage map; R and T refer to alleles contributed by RL4137 and Timgalen, respectively

minimum of two or three additive genes control FHB resistance in Frontana (SINGH *et al.* 1995; VANGINKEL *et al.* 1996). In the current study, RILs from a cross between RL4137 and Timgalen were screened for FHB resistance in two polytunnel and two field experiments using a DON-producing

isolate of *F. culmorum*. Generally, distribution of AUDPC was continuous and was marginally skewed towards RL4137. Positive transgression of FHB resistance was observed indicating that both parents contributed unique alleles of positive effect. In the present study, the RL4137 FHB

Table 5. Summary of QTL identified for plant height and awns in RL4137/Timgalen RIL population

Trait	Closest marker	Chr.	Position	Origin	LOD	R^2
Plant height (cm)	S13/M23G	2B	5.5	RL4137	15.9	47.9
Plant height (cm)	wPt-7524	4A	3.4	Timgalen	2.9	5.4
Plant height (cm)	wPt-9454	5B	57.8	RL4137	2.7 ^a	5.9
Awns	S13/M23G	2B	5.5	Timgalen	3.4	14.6

R^2 = percentage phenotypic variance explained, Chr. = chromosome, a = LOD below the permutation test threshold for significance

QTL of greatest effect was on 2B (*Qfhs.jic-2b*), explaining up to 24% of the phenotypic variance. The shift in the QTL peak for C2006 could be due to the introgression of the large chromosomal segment on 2B from *T. timopheevi* into RL4137 (DEVOS *et al.* 1993) and this made it difficult to reliably position FHB QTL on 2B. RL4137 also contributed a putative FHB QTL of minor effect on 1B (*Qfhs.jic-1b*) and less environmentally stable, but more potent QTL on 3A, 6A and 7A. The moderately resistant variety Timgalen contributed a QTL of major effect on 6B (*Qfhs.jic-6b*) and, together, these accounted for a total of about 40% of the total phenotypic variance. It is not uncommon for a moderately resistant or susceptible parent to contribute alleles for resistance. For example, WALDRON *et al.* (1999) reported a QTL for FHB resistance from Stoa, a moderately susceptible parent and SHEN *et al.* (2003), found that Alondra, a FHB susceptible parent contributed an allele for resistance. In addition to visual disease, a second FHB-associated trait was assessed. Two QTL (2B and 6A) contributed by alleles from RL4137 explained most of the variation for WIS (weight of infected spikelets) (> 74%) in the population. The QTL on 2B co-localized with that for the major FHB QTL in this population.

Despite Frontana being acknowledged as a useful source of FHB resistance (VAN GINKEL *et al.* 1996), only two QTL studies have been reported to date (STEINER *et al.* 2004; MARDI *et al.* 2006). STEINER *et al.* (2004) reported stable QTL for field resistance on 3A and 5A and less stable QTL on 2B and 6B in a Frontana/Remus cross. For type II resistance, these researchers found only a single QTL of small effect on 2B. In addition, a QTL of small effect on 1BL was contributed by Remus, the susceptible parent. Subsequently, MARDI *et al.* (2006) confirmed the QTL on 3A and detected an additional QTL on 7AS. They also detected a QTL

on 1BL originating from Seri82, the susceptible parent of their mapping population. The results of the present QTL analysis partially support the findings of these earlier studies, but also highlight some inconsistencies that may reflect differences in the genotype of the susceptible parent and/or the methodologies employed. The 3A QTL for field resistance reported in both previous studies was detected in the N2006 field trial of the present study but, significantly, was not observed in the C2006 field trial where disease pressure was very low or the polytunnel trials where disease pressure was very high. This highlights the importance of appropriate testing for the detection of some FHB QTL that are, perhaps, only effective under particular conditions. We observed FHB QTL on 2B and 7A, in common with the reports of STEINER *et al.* (2004) and MARDI *et al.* (2006), respectively. Both previous studies found Frontana to carry alleles on 1B that were deleterious for FHB resistance compared to the other parent. In contrast, alleles on 1B from Frontana were marginally more beneficial than those of Timgalen. This probably reflects differences in the genotypes of the susceptible parent in each study.

In addition to physiological, or active, components of FHB resistance, morphological traits including PH, and the presence and size of awns have previously been reported to be associated with FHB resistance, either through linkage or pleiotropy. For this reason PH and awnedness were also measured to assess their relationship to FHB resistance in the present materials. Studying the relationship between FHB resistance and other important agronomic traits is crucial for the development of resistant cultivars. Breeding programmes can be seriously hampered if FHB resistance is linked to undesirable traits. For example, tall stature has widely been assumed to be an FHB escape mechanism reducing spike infec-

tion through reduced spore transfer by rain splash and by providing a less favourable micro-climate for spore germination on the spike (MIEDANER 1997; HILTON *et al.* 1999; KLAHR *et al.* 2007). However, tall cultivars are prone to lodging and have a low harvest index. In the current study, plant height (PH) had a significant negative correlation with AUDPC. QTL mapping detected a major PH QTL, which co-localized with a major FHB resistant QTL on 2B with alleles for a positive effect both contributed by RL4137. QTL for these two traits were also detected on 2B by STEINER *et al.* (2004). Some studies have revealed a more complex relationship between FHB resistance and PH (SCHMOLKE *et al.* 2005; DRAEGER *et al.* 2007; SRINIVASACHARY *et al.* 2008a,b). These studies suggested that co-incidence of QTL for FHB resistance and PH has a genetic basis, e.g. linkage or pleiotropy, rather than being the result of escape. The observation that the FHB QTL on 1B and 6B were not associated with PH QTL and that the minor PH QTL on 4A and 5B, did not co-localise with any other FHB resistance QTL. The FHB QTL *Qfhs.jic-2b* remained highly significant even after co-variance analysis was carried out on the AUDPC data adjusted in relation to PH. The existence of FHB resistance that is independent of PH is of importance since it allows for the selection of resistant cultivars of any height.

The precise role of awns in FHB resistance is not clear and the literature is often contradictory. TAMBURIC-ILINCIC *et al.* (2007) found that awned genotypes had a lower FHB index than awnless genotypes while BAN and SUENAGA (2000) reported that fully awned genotypes were more resistant than tip-awned genotypes. SNIJDERS (1990) suggested that awns could be used as a marker to select FHB resistant lines among progenies from crosses in which the resistant parent carries awns. However, MESTERHAZY (1995) found that the presence of awns was associated with higher levels of disease under field conditions but, interestingly, not under conditions of artificial inoculation. In the current study, a major QTL for awns was also found to co-localise in repulsion phase (associated with the long awn allele from Timgalen) with the major FHB resistance QTL on 2B contributed by RL4137. The present result, combined with that of other studies suggests that QTL for both FHB resistance/susceptibility and awns/no awns are located on 2B and that they are sufficiently closely linked to provide a means to enhance FHB resist-

ance in breeding programmes by selecting for either presence or absence of awns as appropriate depending upon the association in the original FHB resistance donor.

The trichothecene mycotoxin deoxynivalenol (DON) has been shown to be required by *E. graminearum* to facilitate spread, from the point of infection, into adjacent spikelets (DESJARDINS *et al.* 1996; BAI *et al.* 2002). Furthermore, LEMMENS *et al.* (2005) found that a major QTL for DON tolerance co-localised with that for type II resistance on chromosome 3BS supporting the view that type II resistance is associated with resistance to DON. Most of the FHB resistance in Frontana appears to be of type I (STEINER *et al.* 2004). However, early studies reported that Frontana possesses resistance to DON (MILLER & ARNISON 1986). Germination in the presence of DON toxin, known as *in vitro* DON tolerance, has previously been reported to be associated with FHB resistance in wheat (LEMMENS *et al.* 1994; GOSMAN *et al.* 2005). Interestingly, in the current study, QTL analysis for DON tolerance identified two minor QTL one each on 2B and 7A both of which co-localized with the FHB resistance QTL contributed by RL4137. These findings correlate with those from previous studies that reported moderate correlation between FHB symptom development, DON accumulation in kernels and *in vitro* DON tolerance (LEMMENS *et al.* 1994, 1997). This probably reflects a relatively minor role for type II resistance in RL4137 as was reported by STEINER *et al.* (2004) in Frontana. Interestingly, STEINER *et al.* (2004) also reported that the most consistent QTL for Type II resistance in Frontana was on 2B.

The current study identified two major consistent QTL one each on 2B and 6A, contributed by alleles from RL4137 and Timgalen, respectively. The QTL for the FHB related traits co-localized with those for PH and awns on 2B. Co-localization of QTL may result from linkage or pleiotropy. However, at the level of resolution afforded by the present mapping population, linkage or pleiotropy could not be distinguished. The development of iso-genic lines carrying individual FHB resistance loci in a common genetic background may provide the opportunity to distinguish linkage from pleiotropy in the relationship between FHB resistance and morphological traits. Unfortunately, the 2B chromosome of Timgalen carries a large DNA segment from *T. timopheevi* (DEVOS *et al.* 1993),

which reduces recombination making it impractical to attempt to undertake precise mapping of the RL4137 2B QTL in a Timgalen background. Additional crosses to an alternative FHB susceptible parent will be required to differentiate between linkage and pleiotropy for these traits.

Acknowledgements. We wish to express our sincere gratitude to the 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 Seeds (UK) Ltd., Elsoms Seeds Ltd., Advanta Seeds (UK) Ltd. and RAGT (UK) Ltd. (collaboration initiated with Monsanto (UK) Ltd., who subsequently transferred their wheat breeding programme to RAGT) for their invaluable contributions. We also acknowledge help rendered by all those involved in field and polytunnel related work.

References

- BAI G.H., SHANER G. (1994): Scab of wheat – prospects for control. *Plant Disease*, **78**: 760–766.
- BAI G.H., DESJARDINS A.E., PLATTNER R.D. (2002): Deoxynivalenol non-producing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia*, **153**: 91–98.
- BAN T., SUENAGA K. (2000): Genetic analysis of resistance to *Fusarium* head blight caused by *Fusarium graminearum* in Chinese wheat cultivar Sumai 3 and the Japanese cultivar Saikai 165. *Euphytica*, **113**: 87–99.
- BASSOI M.C., FLINTHAM J. (2005): Relationship between grain colour and pre-harvest sprouting-resistance in wheat. *Pesquisa Agropecuaria Brasileira*, **40**: 981–988.
- BECHTEL D.B., KALEIKAU L.A., GAINES R.L., SEITZ L.M. (1985): The Effects of *Fusarium graminearum* infection on wheat kernels. *Cereal Chemistry*, **62**: 191–197.
- BRYAN G.J., COLLINS A.J., STEPHENSON P., ORRY A., SMITH J.B., GALE M.D. (1997): Isolation and characterisation of microsatellites from hexaploid bread wheat. *Theoretical and Applied Genetics*, **94**: 557–563.
- BUERSTMAYR H., LEMMENS M., GRAUSGRUBE H., RUCKENBAUER P. (1996): Scab resistance of international wheat germplasm. *Cereal Research Communication*, **24**: 195–202.
- BUERSTMAYR H., STEINER B., LEMMENS M., RUCKENBAUER P. (2000): Resistance to fusarium head blight in winter wheat: Heritability and trait associations. *Crop Science*, **40**: 1012–1018.
- BUERSTMAYR H., LEMMENS M., HARTL L., DOLDI L., STEINER B., STIERSCHNEIDER M., RUCKENBAUER P. (2002): Molecular mapping of QTL for *Fusarium* head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). *Theoretical and Applied Genetics*, **104**: 84–91.
- CHEN P., LIU, D., SUN W. (1997): New countermeasures of breeding wheat for scab resistance. In: DUBIN H.J., GILCHRIST L., REEVES J., MCNAB A. (eds): *Fusarium Head Scab-Global Status and Future Prospects*. CIM-MYT, Mexico.
- DEL BLANCO I.A., FROHBERG R.C., STACK R.W., BERZONSKY W.A., KIANIAN S.F. (2003): Detection of QTL linked to *Fusarium* head blight resistance in Sumai 3-derived North Dakota bread wheat lines. *Theoretical and Applied Genetics*, **106**: 1027–1031.
- DESJARDINS A.E., PROCTOR R.H., BAI G.H., MCCORMICK S.P., SHANER G., BUECHLEY G., HOHN T.M. (1996): Reduced virulence of trichothecene-non-producing mutants of *Gibberella zeae* in wheat field tests. *Molecular Plant-Microbe Interactions*, **9**: 775–781.
- DEVOS K.M., MILLAN T., GALE M.D. (1993): Comparative RFLP maps of the homeologous group 2 chromosomes of wheat, rye and barley. *Theoretical and Applied Genetics*, **85**: 784–792.
- 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. *Theoretical and Applied Genetics*, **115**: 617–625.
- GERVAIS L., DEDRYVER F., MORLAIS J.Y., 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. *Theoretical and Applied Genetics*, **106**: 961–970.
- GILBERT J., TEKAUZ A. (2000): Review: Recent developments in research on *Fusarium* head blight of wheat in Canada. *Canadian Journal of Plant Pathology*, **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. *European Journal of Plant Pathology*, **111**: 57–66.
- HILTON A.J., JENKINSON P., HOLLINS T.W., PARRY D.W. (1999): Relationship between cultivar height and severity of *Fusarium* ear blight in wheat. *Plant Pathology*, **48**: 202–208.
- JACCOUD D., PENG K., FEINSTEIN D., KILIAN A. (2001): Diversity Arrays: a solid-state technology for sequence

- information independent genotyping. *Nucleic Acids Research*, **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 D.D. (1944): The estimation of map values from recombination value. *Annals of Eugenetics*, **12**: 172–175.
- LEMMENS M., REISINGER A., BUERSTMAYER H., RUCKENBAUER P. (1994): Breeding for head blight (*Fusarium* spp.) resistance in wheat: development of a mycotoxin-based selection method of seedlings. *Acta Horticultura*, **355**: 223–232.
- LEMMENS M., JOSEPHS R., SCHUHMACHER R., GRAUSGRUBER H., BUERSTMAYER H., RUCKENBAUER P., NEUHOLD G., FIDESSER M., KRŠKA R. (1997): Head blight (*Fusarium* spp.) on wheat: Investigations on the relationship between disease symptoms and mycotoxin content. *Cereal Research Communication*, **25**: 459–465.
- LEMMENS M., SCHOLZ U., BERTHILLER F., D'LL'ASTA C., KOUTNIK A., SCHUHMACHER R., ADAM G., BUERSTMAYER H., MESTERHAZY A., KRŠKA R., RUCKENBAUER P. (2005): The ability to detoxify the mycotoxin deoxynivalenol co-localises with a major quantitative trait locus for *Fusarium* head blight resistance in wheat. *Molecular Plant-Microbe Interaction*, **18**: 1318–1324.
- LIU S., ABATE Z.A., MCKENDRY A.L. (2005): Inheritance of *Fusarium* head blight resistance in the soft red winter wheat Ernie. *Theoretical and Applied Genetics*, **110**: 454–461.
- MARDI M.L., PAZOUKI L., DELAVAR H., KAZEMI M.B., GHAREYAZIE B., STEINER B., NOLZ R., LEMMENS M., BUERSTMAYER H. (2006): QTL analysis of resistance to *Fusarium* head blight in wheat using a 'Frontana-derived' population. *Plant Breeding*, **125**: 313–317.
- MCMULLEN M., JONES R., GALLENBERG D. (1997): Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease*, **81**: 1340–1348.
- MESTERHAZY A. (1983): Breeding wheat for resistance to *Fusarium graminearum* and *Fusarium culmorum*. *Journal of Plant Breeding*, **91**: 295–311.
- MESTERHAZY A. (1995) Types and components of resistance to *Fusarium* head blight of wheat. *Plant Breeding*, **114**: 377–386.
- MIEDANER T. (1997): Breeding wheat and rye for resistance to *Fusarium* diseases. *Plant Breeding*, **116**: 201–220.
- MIEDANER T., REINBRECHT C., LAUBER U., SCHOLLENBERGER M., GEIGER H.H. (2001): Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale, and wheat. *Plant Breeding*, **120**: 97–105.
- MILLER J.D., ARNISON P.G. (1986): Degradation of deoxynivalenol by suspension-cultures of the *Fusarium* head blight resistant wheat cultivar Frontana. *Canadian Journal of Plant Pathology*, **8**: 147–150.
- NYQUIST W.E. (1991): Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews on Plant Sciences*, **10**: 235–322.
- PARRY D.W., JENKINSON P., MCLEOD L. (1995): *Fusarium* ear blight (Scab) in small-grain cereals - a Review. *Plant Pathology*, **44**: 207–238.
- RODER M.S., KORZUN V., WENDEHAKKE K., PLASCHKE J., TIXIER M.H., LEROY P., GANAL M.W. (1998): A microsatellite map of wheat. *Genetics*, **149**: 2007–2023.
- SCHMOLKE M., ZIMMERMANN G., BUERSTMAYER H., SCHWEIZER G., MIEDANER T., KORZUN V., EBMAYER E., HARTL L. (2005): Molecular mapping of *Fusarium* head blight resistance in the winter wheat population Dream/Lynx. *Theoretical and Applied Genetics*, **111**: 747–756.
- SCHROEDER H.W., CHRISTENSEN J.J. (1963): Factors affecting resistance of Wheat to Scab Caused by *Gibberella zeae*. *Phytopathology*, **53**: 831–838.
- SEMAGN K., SKINNES H., BJORNSTAD A., MAROY A.G., TARKEGNE Y. (2007): Quantitative trait loci controlling *Fusarium* head blight resistance and low deoxynivalenol content in hexaploid wheat population from Arin' and NK93604. *Crop Science*, **47**: 294–303.
- SHEN X.R., ITTU M., OHM H.W. (2003): Quantitative trait loci conditioning resistance to *Fusarium* head blight in wheat line F201R. *Crop Science*, **43**: 850–857.
- SINGH R.P., MA H., RAJARAM S. (1995): Genetic analysis of resistance to scab in spring wheat cultivar Frontana. *Plant Disease*, **79**: 238–240.
- SNIJDERS C.H.A. (1990): *Fusarium* head blight and mycotoxin contamination of wheat, a review. *Netherlands Journal of Plant Pathology*, **96**: 187–198.
- SOMERS D.J., ISAAC P., EDWARDS K. (2004): A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, **109**: 1105–1114.
- SRINIVASACHARY, GOSMAN N., STEED A., SIMMONDS J., LEVERINGTON-WAITE M., WANG Y., SNAPE J., NICHOLSON P. (2008a): Susceptibility to *Fusarium* head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. *Theoretical and Applied Genetics*, **116**: 1145–1153.
- SRINIVASACHARY, GOSMAN N., STEED A., BAYLES R., JENNINGS P., HOLLINS B., NICHOLSON, P. (2008b): Semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* differ in

- their influence on resistance to Fusarium head blight of wheat. Theoretical and Applied Genetics, published online DOI: 10.1007/s00122-008-0930-0.
- STEINER B., LEMMENS M., GRIESSER M., SCHOLZ U., SCHONDELMAIER J., BUERSTMAYER H. (2004): Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theoretical and Applied Genetics, **109**: 215–224.
- TAMBURIC-ILINCIC L., SCHAAFSMA A.W., FALK D.E. (2007): Indirect selection for lower deoxynivalenol (DON) content in grain in a winter wheat population. Canadian Journal of Plant Sciences, **87**: 931–936.
- VAN OOIJEN J.W. (2004): MapQTL™. Version 5.0. Software for the Mapping of Quantitative Trait Loci in Experimental Populations. Kyazma B.V., Wageningen.
- VAN OOIJEN J.W., VOORRIPS R.E. (2001): Joinmap 3.0. Software for the Calculation of Genetic Linkage Maps. Plant Research International, Wageningen.
- VANGINKEL M., VANDERSCHAAR W., YANG Z.P., RAJARAM S. (1996): Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Disease, **80**: 863–867.
- VOORRIPS R.E. (2002): MAPCHART: software for the graphical presentation of linkage maps and QTLs. Heredity, **93**: 77–78.
- VOS P., HOGERS R.M., ZABEAU M. (1995): AFLP – a new technique for DNA fingerprinting. Nucleic Acids Research, **23**: 4407–4414.
- WALDRON B.L., MORENO-SEVILLA B., ANDERSON J.A., STACK R.W., FROHBERG R.C. (1999): RFLP mapping of QTL for fusarium head blight resistance in wheat. Crop Science, **39**: 805–811.
- WANG Y.Z. (1996): Epidemiology and management of wheat scab in China: In: Fusarium Head Scab: Global Status and Future Prospects. CIMMYT, El Batan, 97–105.
- WANG Y.Z., MILLER J.D. (1988): Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. Journal of Phytopathology, **122**: 118–125.
- YANG Z.P. (1994): Breeding for Resistance to Fusarium Head Blight of Wheat in the Mid-to Lower Yangtze River Valley of China. CIMMYT, Mexico.
- ZADOKS J.C., CHANG T.T., KONZAK C.F. (1974): Decimal code for growth stages of cereals. Weed Research, **14**: 415–421.

Received for publication November 11, 2008
Accepted after corrections December 10, 2008

Corresponding author:

Dr. PAUL NICHOLSON, John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK
tel.: + 44 1603 450 616, fax: + 44 1603 450 045, e-mail: paul.nicholson@bbsrc.ac.uk
