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ORIGINAL PAPER

Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina

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Abstract Fusarium head blight (FHB) of wheat has become a serious threat to wheat crops in numerous countries. In addition to loss of yield and quality, this disease is of primary importance because of the contamination of grain with mycotoxins such as deoxynivalenol (DON). The Swiss winter cultivar Arina possesses significant resistance to FHB. The objective of this study was to map quantitative trait loci (QTL) for resistance to FHB, DON accumulation and associated traits in grain in a double haploid (DH) population from a cross between Arina and the FHB susceptible UK variety Riband. FHB resistance was assessed in five trials across different years and locations. Ten QTL for resistance to FHB or associated traits were detected across the trials, with QTL derived from both parents. Very few of the QTL detected in this study were coincident with those reported by authors of two other studies of FHB resistance in Arina. It is concluded that the FHB resistance of Arina, like that of the other European winter wheat varieties studied to date, is conferred by several genes of moderate effect making it difficult to exploit in marker-assisted selection breeding programmes. The most significant and stable QTL for FHB resistance was on chromosome 4D and co-localised with the *Rht–D1* locus for height. This association appears to be due to linkage of deleterious genes to the *Rht-D1b* (*Rht2*) semi-dwarfing allele rather than differences in height per se. This association may compromise efforts to enhance FHB resistance in breeding programmes using germplasm containing this allele.

Keywords *Fusarium culmorum* · *Triticum aestivum* · Deoxynivalenol · QTL mapping

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Introduction

Fusarium head blight (FHB), of wheat is predominantly caused by *Fusarium graminearum* and *Fusarium culmorum.* FHB is present in most cereal growing regions of the world and, in epidemic years, the disease reduces yield and grain quality via production of mycotoxins such as deoxynivalenol (DON) that are harmful to both humans and

animals. The cultivation of FHB-resistant varieties is thought to be the most viable strategy for controlling disease and reducing mycotoxin contamination (D'Mello et al. 1999; Buerstmayr et al. 2002). Most research attention has focused on the highly resistant Chinese spring wheat variety Sumai 3 and its derivatives. A major QTL for resistance to FHB on the short arm of chromosome 3B (*Qfhs.ndsu-3BS*) has been consistently identified in numerous studies of Sumai 3 and its derivatives (Anderson et al. 2001; Zhou et al. 2002; Buerstmayr et al. 2002, 2003). This QTL has recently been fine-mapped as a Mendelian factor and termed *Fhb1* (Liu et al. 2006; Cuthbert et al. 2006).

Over dependence on a limited number of resistance sources could, however, result in selection pressure on the pathogen leading to the emergence of strains that are pathogenic against Sumai 3-derived resistance (Akinsanmi et al. 2006). Alternative sources may provide effective protection against FHB and could complement Sumai 3-based resistance (Ittu et al. 2000). Recent studies have begun to explore the potential of European winter wheat germplasm as an alternative source of FHB resistance. Several authors have reported moderate levels of FHB resistance in the Swiss winter wheat variety Arina (Miedaner et al. 2001; Wanyoike et al. 2002). Genetic mapping of resistance by Paillard et al. (2004) identified two major QTL on 4A and 6D and three minor QTL on 2AL, 3BL and 5AL in a cross between Arina and Forno. However, more recently, QTL mapping in a cross between Arina and NK93604 failed to detect QTL in similar locations (Semagn et al. 2007).

The current study was undertaken to identify the genomic location of resistance in a doubled haploid population of lines developed from a cross between Arina and the UK semi-dwarf winter wheat variety Riband. In addition to symptom development expressed as area under the disease progress curve (AUDPC), several other resistance parameters, including relative spikelet weight (RSW), fungal DNA (FDNA) and DON content of infected grains were determined to further characterise resistance and study the genetic relationship between parameters. Experiments were conducted over several years and locations both in the field and in polytunnel trials in order to fully account for the influence of environmental variation on the expression of resistance (Mesterhazy et al. 1999).

Materials and methods

Plant materials and assessment of FHB resistance

An Arina × Riband F_1 derived doubled haploid (DH) population of 116 lines was generated using the method of Laurie and Reymonde (1991). The DH lines and parental varieties were phenotyped in five experiments over five different environments. Three polytunnel trials were conducted at the John Innes Centre (JIC) during the summer of 2000, 2001 and 2004 (JIC2000, JIC2001 and JIC2004). Two field-based trials were conducted in 2004 at Tulln, Austria and at Szeged, Hungary (Tulln2004 and Szeged2004). Due to limited seed availability for some lines a reduced number of lines was used in the Tulln2004 and Szeged2004 trials. Inoculum preparation, plant husbandry, trial set-up and disease assessment were as described previously for trials at JIC (Gosman et al. 2005), IFA-Tulln (Buerstmayr et al. 2003) and Szeged (Mesterhazy et al. 2005).

The area under the disease progress curve (AUDPC) was calculated as described by Buerstmayr et al. (2002), to provide an integrated measure of disease severity. The DON and fungal DNA content of grain, along with the relative spikelet weight (RSW) of infected versus control heads were determined in the JIC2000 and JIC2001 trials. Two additional traits, %Fusarium damaged kernels (FDK) and yield loss, were assessed in the Szeged2004 trial.

Fungal DNA and deoxynivalenol (DON) content

Wheat heads were hand harvested and manually threshed and winnowed to ensure retention of small highly infected kernels. For each genotype, DNA was extracted from milled grain and analysed by competitive PCR using *F. culmorum*-specific primers according to the method of Nicholson et al. (1998). The amount of fungal DNA was expressed as a percentage of the total DNA content of the sample as described by Gosman et al. (2005).

The DON content of grain was determined for the JIC2000 and JIC2001 trials. DON was extracted from milled grain samples in 10% methanol and DON content was assessed using the Ridascreen® Fast DON™ (R-Biopharm Rhône Ltd.) enzyme linked immuno-assay (ELISA) according to the manufacturer's instructions and as described previously (Gosman et al. 2005).

Statistical analysis

Analysis of variance (ANOVA) was carried out to assess variability attributable to genotype and PCR replicates for fungal DNA content and to partition the variance attributable to replicates, genotypes and environments for all other data. The data for fungal DNA content were subjected to a logarithmic transformation prior to analysis due to the non-independence of mean and variance. Statistical analysis for genotype differences between doubled haploid lines was carried out by one-way analysis of variance incorporating Dunnett's intervals for treatment means tests (Mead et al.

1994). Over the trials, heritability was estimated from the ANOVA using the formula: $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_{GE}/E) + (\sigma^2_e/rE)]$, 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 line (Nyquist 1991). Analyses were carried out using Minitab release 12 (1994 Minitab Incorporated USA) and Genstat release 8.1 [Copyright 2005, Lawes Agricultural Trust (Rothamsted Experimental Station)].

Map construction

A genetic linkage map was constructed using amplified fragment length polymorphism (AFLP) loci and simple sequence repeat (SSR) markers. AFLP PCR and fragment analysis was similar to that described by Vos et al. (1995) except that for each sample, 250 ng of DNA was simultaneously digested with 2.5 U each of *Sse*8387I and *Mse*I and ligated to adapters. A total of 29 AFLP primer combinations with two selective nucleotides on the 3′ end of either primer were used for selective amplification. PCR products were size separated by electrophoresis through 5% polyacrylamide gel and visualised by the silver staining technique of Bassam et al. (1991). The standard AFLP nomenclature (http://www.wheat.pw.usda.gov/ggpages/keygeneAFLPs.html) was used to identify products. The sources of primer sequences for microsatellites are identified by the marker prefix, e.g. (*Xgwm*) for Röder et al. (1998), (*Xbarc*) for Song et al. (2005), (*Xpsp*) for Bryan et al. (1997) (*XDuPw*) for Eujayl et al. (2002), (*Xwmc*) Gupta et al. (2002) and http://www.wheat.pw.usda.gov/ggpages/SSR/WMC/. PCR amplification for microsatellite analysis was similar to that of Bryan et al. (1997). Fragment analysis and product visualisation using silver staining was also similar to Bryan et al. (1997). The map also included genotype data for the *Rht-D1* dwarfing locus on chromosome 4D. PCR analysis of *Rht-D1a* and *Rht-D1b* alleles was carried out as described by Ellis et al. (2002).

Linkage analysis was performed with the JoinMap program (version 3.0 for the PC) (Van Ooijen and Voorrips 2001) using the Kosambi mapping function (Kosambi 1944). The linkage map was obtained by choosing 0.45 as the maximum recombination fraction and 3.0 as the modified minimum logarithm of the odds ratio (LOD) score value.

QTL analysis

QTL were identified using the MapQTL package (version 4.0 for the PC) (Van Ooijen and Maliepaard 1996). Cofactors with a P-value less than 0.02 were taken into account using automatic co-factor selection prior to multiple-QTL model (MQM) mapping, the number of background markers specified for use as cofactors ranged from one to three. A step size of 5cM around the test interval was used for all analyses. Permutation tests (1,000 permutations) were carried out to identify the appropriate significance thresholds for each trait to declare the presence of a QTL. The putative genomic locations of QTL linked to AFLP loci were inferred by association with microsatellite loci of known genomic location. QTL that explained greater than 10% of the phenotypic variance were arbitrarily classified as major QTL while those that explained less than 10% were classified as minor QTL.

Results

Trait analysis

The frequency distribution for AUDPC in all five trials was continuous and skewed towards greater susceptibility and the population mean was greater than the mid-parent mean (Table 1). The AUDPC distribution was normal for all environments and years except for JIC2004 where a square root transformation was used to normalise the data prior to statistical analysis. AUDPC was higher in JIC2000 than in 2001 and 2004 and the range of AUDPC values (328–3,130) was greatest in the Szeged2004 trial (Table 1).

Genotype and genotype-by-experiment interaction effects were significant for AUDPC ($P < 0.001$) (Table 2). Broad sense heritability for AUDPC calculated over all five trials was 0.70 (Table 2). The correlation for AUDPC between all experiments was highly significant ($P < 0.001$) ranging from $r = 0.42$ to 0.68 across the five trials. The highest correlation was that between JIC2001 and JIC2004 ($r = 0.68$) followed by that between JIC2000 and JIC2001 ($r = 0.66$). There was also significant correlation for AUDPC between the field and polytunnel trials ranging between 0.45 (JIC2000-Szeged2004) and 0.61 (JIC2004-Szeged2004). Interestingly the lowest correlation was that between the Tulln2004 and Szeged2004 field trials ($r = 0.42$).

Table 1 Mean AUDPC scores for Arina and Riband and means and ranges for the Arina/Riband population across the five experiments

Experiment	Mean				Range
	Arina	Riband	Mid-parent	Population	
JIC2000	867	1,528	1,197	1,297	767–1,727
JIC2001	714	1,540	1,127	1,155	584–1,639
JIC2004	249	917	583	597	306–1,381
Szeged2004	559	2,100	1,329	1,452	328–3,130
Tulln2004	368	1,150	759	765	290–1,320

Table 2 Variance components of FHB resistance traits using generalised linear modelling for JIC2000 and JIC2001 and for AUDPC over all five experiments for the Arina/Riband population

Source of variation	2 Experiments (JIC2000–JIC2001)								5 Experiments	
	AUDPC		DON		FDNA		RSW		AUDPC	
	MS	*F* value	MS	*F* value	MS	*F* value	MS	*F* value	MS	*F* value
Genotype (DH line)	975,399	14.08***	7,800	6.78***	0.30	5.87***	1,881.8	10.67***	372,934	28.93***
Genotype x experiment	226,825	3.27***	1,472	1.28	0.12	2.37***	998.2	5.66***	149,251	11.58***
Error	69,264		1,150		0.05		176.4		12,890	
H	0.77		0.75		0.64		0.62		0.70	

MS mean squares, *H* broad sense heritability

*** $P < 0.001$

In the JIC2000 and JIC2001 trials RSW, DON and fungal FDNA content of grain were also assessed. There was continuous variation for all three traits in both trials. Genotype and genotype-by-experiment interaction effects were highly significant ($P < 0.001$) except for DON content ($P = 0.1$) (Table 2). Heritability estimates for these traits were high across years with $H = 0.75$, 0.64, 0.62 for DON, FDNA and RSW, respectively (Table 2). Correlation between the traits and across the two trials was variable. JIC2000FDNA was highly correlated with both RWS and DON content of grain in JIC2001 ($r = –0.76$ and 0.76, respectively). Correlation between JIC2000RSW and other traits was low, ranging from $r = –0.42$ with JIC2001FDNA to $r = 0.48$ with JIC2001DON.

Linkage map

A total of 279 polymorphic AFLP and SSR markers were used to construct a map of the Arina × Riband DH population that consisted of 30 linkage groups with a combined total genetic distance of 1,199 cM. The chromosomal identities of 29 out of 30 linkage groups were determined by inclusion of anchor SSR loci (a minimum of one per chromosome arm) the position of which was determined through reference to published data. There was at least one linkage group representing each of the 21 chromosomes in the wheat genome.

QTL analysis for AUDPC

Significant FHB resistance QTL were detected in all experiments on the basis of AUDPC data (Table 3). With the exception of loci on 7BL and 7DL from Riband, all QTL were contributed by Arina. Using Tulln2004 data, a major QTL for resistance was detected on chromosome 4DS, centred on the gene-based marker for *Rht-D1*, which explained 23.9% of the phenotypic variance (LOD 8.4) for AUDPC (Table 3). QTL of lesser significance were detected at a similar map position with data from JIC2001, JIC2004 and Szeged2004. Additional QTL were detected on 6BL in JIC2001 and JIC2004, on 1BL and 7BL (from Riband) in JIC2000, on 2B and 7DL (from Riband) in JIC2001, on 1BL (at a position >30 cM from that of JIC2000) in Tulln2004 and on 7AL in Szeged2004 (Table 3). The positions of QTL for all measured traits are shown in Fig. 1.

QTL analysis of additional FHB traits

QTL analysis was also performed for the FDNA, RSW and DON traits for JIC2000 and JIC2001. Two QTL for FDNA content of grain, both derived from Riband, were detected in JIC2000 on 3DL and 7BL explaining 16.4 and 16.7% of the phenotypic variance (LOD 2.9 and 3.0 respectively) (Table 3). Two QTL for FDNA were also detected in JIC2001, but originating from Arina and on different chromosomes. The QTL on 4DS appeared closely linked to *Rht-D1* and accounted for 12.9% of the phenotypic variance (LOD 3.8) while the QTL on 6BL accounted for 10.2% of variance (LOD 2.7). A single QTL for RSW was detected in JIC2000 on chromosome 1BL accounting for 15.8% of the phenotypic variance. This QTL was not detected in the JIC2001 trial. Five QTL for RSW were detected in the JIC2001 trial with the most potent being that derived from Arina on chromosome 4DS associated with *Rht-1D*, which accounted for 17.6% of the variance. The other four QTL were on chromosomes 1BL, 2AS, 6BL and 7DL (Riband) (Table 3).

Analysis of variance showed that there was no significant effect of experiment for DON content so QTL analysis for the DON trait was performed using the combined data for JIC2000 and JIC2001. Three QTL were located for DON on 4DS, 6BL and 7DL accounting for 9.1, 11.7 and 7.8% respectively of the variance for this trait (LOD scores of 3.6, 4.6 and 3.1) (Table 3). These three QTL were in similar positions to those detected in JIC2001 for RSW and

Table 3 Summary of QTLs in the Arina/Riband DH population

Trait	Name	Closest marker	Chromosome	Origin	JIC2000		JIC2001		JIC2004		Tulln2004		Szeged2004	
					LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
AUDPC	*Qfhs.jic-1b*	*S16M14.400*	1BL	Arina	1.5	10.1	NS	NS	NS	NS	NS	NS	NS	NS
AUDPC	*Qfhs.jic-1b*	*S12M23.400*	1BL	Arina	NS	NS	NS	NS	NS	NS	3.5	9	NS	NS
AUDPC	*Qfhs.jic-2b*	*S18M23.200*	2B	Arina	NS	NS	5.27	11.1	NS	NS	NS	NS	NS	NS
AUDPC	*Qfhs.jic-4d*	*Rht-D1*	4DS	Arina	NS	NS	6.3	13.9	4.1	12.9	8.4	23.9	3.9	14.9
AUDPC	*Qfhs.jic.6b*	*Xpsp3131*	6BL	Arina	NS	NS	6.7	14.8	3.2	9.9	NS	NS	NS	NS
AUDPC	*Qfhs.jic.7a*	*S14M14.225*	7AL	Arina	NS	NS	NS	NS	NS	NS	NS	NS	4.3	16.9
AUDPC	*Qfhs.jic.7b*	*Xwmc276*	7BL	Riband	2.4	16.5	NS	NS	NS	NS	NS	NS	NS	NS
AUDPC	*Qfhs.jic.7d*	*Xpsp3123*	7DL	Riband	NS	NS	3.8	8	NS	NS	NS	NS	NS	NS
FDNA	–	*Xwmc418b*	3DL	Riband	2.9	16.4	NS	NS	–	–	–	–	–	–
FDNA	–	*Rht-D1*	4DS	Arina	NS	NS	3.8	12.9	–	–	–	–	–	–
FDNA	–	*Xgwm219*	6BL	Arina	NS	NS	2.7	10.2	–	–	–	–	–	–
FDNA	–	*S12M23.190*	7BL	Riband	3.0	16.7	NS	NS	–	–	–	–	–	–
RSW	–	*Xgwm018*	1BL	Arina	2.3	15.8	NS	NS	–	–	–	–	–	–
RSW	–	*S20M19.240*	2AS	Arina	NS	NS	2.9	11	–	–	–	–	–	–
RSW	–	*Rht-D1*	4DS	Arina	NS	NS	7.4	17.6	–	–	–	–	–	–
RSW	–	*Xpsp3131*	6BL	Arina	NS	NS	3.6	8.1	–	–	–	–	–	–
RSW	–	*Xpsp3123*	7DL	Riband	NS	NS	2.8	6	–	–	–	–	–	–
DON [c]	–	*Rht-D1*	4DS	Arina	3.6 [a]	9.1 [a]			–	–	–	–	–	–
DON [c]	–	*Xpsp3131*	6BL	Arina	4.6 [a]	11.7 [a]			–	–	–	–	–	–
DON [c]	–	*Xwmc488a*	7DL	Riband	3.1 [a]	7.8 [a]			–	–	–	–	–	–
FDK	–	*Xwmc457*	4DS	Arina	–	–	–	–	–	–	–	–	3.7	11.4
FDK	–	*S18M24.330*	5AS	Riband	–	–	–	–	–	–	–	–	3.6	11.7
FDK	–	*S14M14.225*	7AL	Arina	–	–	–	–	–	–	–	–	2.2	8.7
Yield loss	–	*S14M14.225*	7AL	Arina	–	–	–	–	–	–	–	–	3.3	12.6
Plant height	–	*S14M15.278*	2B	Riband	–	–	–	–	–	–	6.7	13.8	NS	NS
Plant height	–	*S12M18.250*	2B	Riband	–	–	–	–	–	–	NS	NS	8.2	9.7
Plant height	–	*Rht-D1*	4DS	Arina	–	–	–	–	–	–	16.4	42.4	27.3	51.5
Plant height	–	*XBarc146c*	6A	Riband	–	–	–	–	–	–	2.8	5.2	7.3	8.1
Plant height	–	*S16M20.70*	7BL	Arina	–	–	–	–	–	–	NS	NS	3.1	3.2

Resistance to FHB measured as area under the disease progress curve (AUDPC) in JIC2000, JIC2001 JIC2004, Tulln2004 and Szeged2004 trials; fungal DNA content of grain (FDNA), relative spikelet weight (RSW) and deoxynivalenol (DON) content of grain in JIC2000 and JIC2001 trials; percentage FHB (%FHB), Fusarium damaged kernels (FDK) and yield loss in the Szeged2004 trial and plant height in the Tulln2004 and Szeged2004 trials

R^2 is the percentage phenotypic variance explained

[a] QTL for DON content were derived from the 2000 and 2001 combined data

coincident with those found for AUDPC, with *Rht-D1* the closest linked marker on 4DS and *Xpsp3131* the closest linked marker on 6BL. The map position of the QTL for DON on 7DL overlapped with that for AUDPC and RSW detected in JIC2001 so it is possible that a single QTL within this interval conditions all three traits. While Arina alleles were associated with the QTL for reduced DON on 4DS and 6BL, the Riband allele was associated with reduced DON for the QTL on 7DL.

Two additional traits, yield loss and Fusarium damaged kernels (FDK) were measured in the Szeged2004 trial. A QTL contributed by Arina, detected on chromosome 7AL, accounted for 12.6% of the phenotypic variance (LOD 3.3) for yield loss and also explained 8.7% of the phenotypic variance for FDK. Two additional QTL for FDK were located on chromosomes 4DS (from Arina) and 5AS (from Riband) and accounted for 11.4 and 11.7% of variance respectively (LOD of 3.7 and 3.6) (Table 3).

Plant height

While Arina is a tall variety, Riband carries the *Rht-D1b* allele on 4DS that confers a semi-dwarf habit. This allele accounted for 42–52% of the variance in height at the

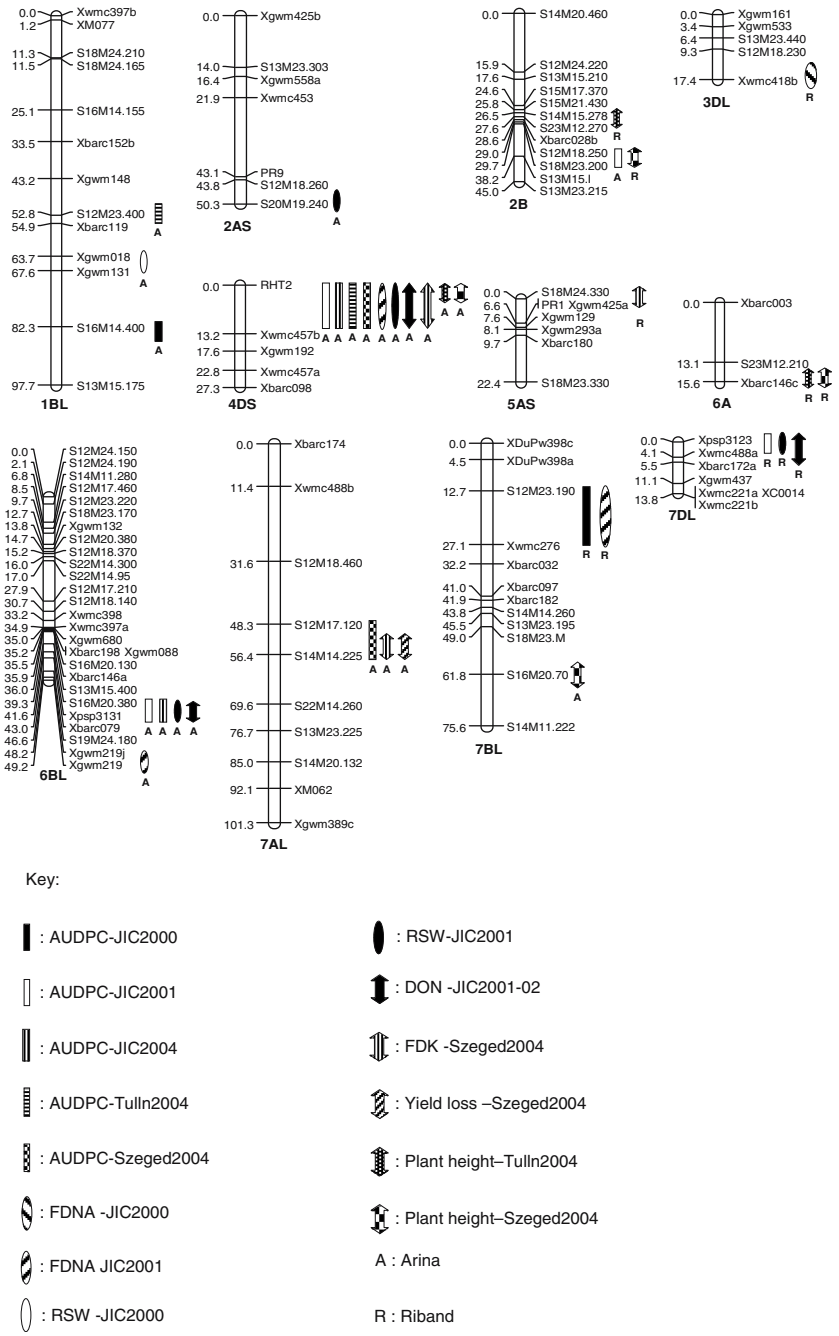

Fig. 1 Linkage maps of chromosome segments constructed from the Arina × Riband double haploid population. Putative QTL positions for FHB-associated traits and plant height are shown on the right of each linkage group and described in the key. Traits are: area under disease progress curve (*AUDPC*), fungal DNA content of grain (*FDNA*), relative spikelet weight of infected versus non-infected spikelets (*RSW*), deoxynivalenol content of grain (*DON*), Fusarium-damaged kernels (*FDK*). Genetic distances are shown in centimorgans to the left of each linkage group

Szeged2004 and Tulln2004 trials (Table 3). Two additional plant height QTL from Riband on 2B and 6A were detected in both trials. The QTL on 2B accounted for 13.8 and 9.7% of the phenotypic variance at the Szeged and Tulln sites, respectively. Although the closest marker differed at the two sites, the distance between them was only 2.5 cM. The QTL on 6A was of lesser effect, accounting for 8.1 and 5.2% of the phenotypic variance in the Szeged2004 and Tulln2004 trials, respectively (Table 3). An additional height QTL of minor effect was detected on 7BL in the Szeged2004 trial.

Discussion

FHB resistance in the Arina/Riband population appears to be a quantitatively inherited trait as indicated by the distributions for AUDPC and other FHB-related traits and both parents appear to be contributing different FHB-resistance QTL. These results are in agreement with the findings from the two previous studies of FHB resistance QTL in Arina (Paillard et al. 2004, Semagn et al. 2007). Paillard and co-workers detected 14 QTL for FHB resistance segregating in their Arina × Forno population with

several QTL originating from the susceptible parent Forno. Many of these were not stable and/or overlapped with those for earliness or plant height. The more recent study of a DH population derived from a cross between Arina and the Norwegian breeding line NK93604 detected four FHB-resistance QTL of moderate effect and two QTL of lesser effect with both parents contributing positive alleles (Semagn et al. 2007).

In the present study at least 10 QTL for resistance to FHB were detected. The major QTL on 4DS was the most consistent, being observed in four trials and for five FHB-related traits (AUDPC, DON, FDNA, RSW and FDK). Although it is possible that the coincidence of QTL for the different FHB-related traits is due to the presence of linked genes separately affecting each trait, we believe that it is more likely that it represents a resistance that reduces fungal colonisation, yield loss, mycotoxin accumulation and disease symptoms.

QTL influencing more than one FHB-associated trait were also detected on several other chromosomes (Table 3, Fig. 1). For example, in JIC2001 QTL for the traits AUDPC, FDNA and DON were detected on chromosomes 6B and 7D. These traits were highly correlated to each other (data not shown) suggesting a common genetic influence, which is reinforced by the QTL analysis. Zhu et al. (1999) reported similar results and found that coincident QTL supported their observed pattern of phenotypic correlation.

A negative relationship between plant height and FHB resistance has been reported from several populations (Buerstmayr et al. 2000; Somers et al. 2003) and the 4DS FHB resistance QTL was coincident with the *Rht-D1* locus that confers the semi-dwarf phenotype in wheat. While Arina has the wild-type allele at this locus, Riband, like most UK winter wheat varieties has the *Rht-D1b* allele, also known as *Rht2*. A moderate overall correlation between FHB-resistance and plant height was observed ($r = 0.35$; $P < 0.001$), but no such correlation remained when lines were classified according to their *Rht-D1* status as *Rht-D1a* or *Rht-D1b* ($P = 0.7$ and 0.65, respectively) (Fig. 2). The mean AUDPC of lines carrying the *Rht-D1b* allele was significantly higher ($P < 0.001$) than that of those carrying the wild-type allele (831 and 667, respectively), irrespective of height. The association between susceptibility to FHB and *Rht-D1b* is supported by the finding that UK winter wheat varieties, the great majority of which carry the *Rht-D1b* semi-dwarfing allele are generally highly susceptible to FHB (Gosman et al. 2007).

QTL for both plant height and FHB resistance were observed on both 2B and 7B. However, the QTL on 2B for FHB resistance was derived from Arina while that for height originated from Riband. In addition, the QTL for height and FHB resistance on 7B did not overlap, being separated by over 30 cM. Furthermore the plant height

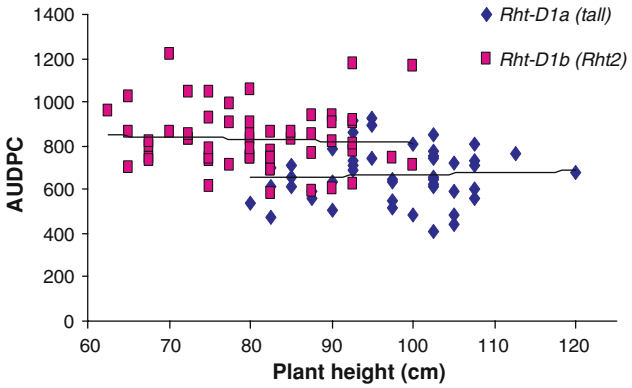

Fig. 2 Relationship between area under disease progress curve (*AUDPC*) and plant height for individual lines in the Arina × Riband double haploid population. The status of each line for the *Rht*-D1 locus is shown: *Rht-D1a* (*tall*) wild-type allele and *RhtD1b* (*Rht2*) semidwarf allele. The regression line of AUDPC against height are shown for each allele

QTL on 6A did not overlap with any FHB resistance QTL. Overall, these findings suggest that the relationship between plant height and FHB susceptibility in this population is not due to plant height per se but, rather to either linked genes conferring FHB susceptibility present in some intervals and/or a pleiotropic physiological effect of the dwarfing allele at *RhtD1* enhancing susceptibility. Further work involving lines carrying different alleles at the *RhtD1* (*Rht2*) and *RhtB1* (*Rht1*) loci is required to differentiate between these possibilities.

A major FHB QTL was detected on chromosome 6B in two experiments (JIC2001 and JIC2004) that appeared to condition multiple parameters of FHB resistance, reducing visual disease (AUDPC), DON and FDNA content of grain and increasing RSW. A QTL for FHB resistance from Arina was also detected on chromosome 6B by Semagn et al. (2007). However, the FHB QTL in the present study was on the long arm, whereas that observed by Semagn and co-workers was determined to be on the short arm of 6B.

A major QTL was also observed on the long arm of chromosome 1B in two trials in the present study. Unlike those on 4DS and 6BL this QTL only reduced AUDPC and increased RWS suggesting that this locus reduces symptoms and yield loss but does not significantly reduce fungal colonisation or mycotoxin accumulation in grain. This QTL is in a similar location to that detected previously in Arina (Semagn et al. 2007). Interestingly, the 1B QTL detected in Arina by Semagn et al. (2007) was also not found to influence mycotoxin accumulation. This finding is similar to that of other reports in which FHB infection and DON accumulation were determined to be controlled, in part, by independent loci (Somers et al. 2003). While disease symptoms have generally been found to correlate with DON accumulation in grain and fungal infection of grain it

is important to differentiate between those QTL that reduce symptoms but not DON so that resistance QTL that influence both traits are preferentially used in breeding programmes.

It is notable that very few QTL were common across the three studies involving Arina. Two QTL of major effect (4A and 6D) were detected in Arina with the Arina/Forno population (Paillard et al. 2004). Another three QTL on the long arm of 5B derived from Forno. Minor QTL were also detected on 1BL, 2AL, 3AL, 3BL, 3DS, and 5AL (Paillard et al. 2004). In the Arina/NK93603 population major QTL on 1BL and 6BS derived from Arina while QTL on 1AL and 7AL derived from NK93604 (Semagn et al. 2007). Only the major QTL on 1B of Arina appears common among the trials being detected in the present study and both previous reports (Paillard et al. 2004; Semagn et al. 2007). In contrast to the report by Semagn et al. (2007) who found this QTL to be stable across environments and of major effect, in both the present study and that of Paillard et al. (2004) this QTL was only observed in some environments. It is possible that differences in disease evaluation methods, the *Fusarium* species, genotype-by-environment interactions and/or differences in the genetic background of the 'susceptible' parent all contribute to this effect. Notably, in the current study, the influence of the *Rht-D1b* allele was observed across different environments in which different *Fusarium* species were used and in which scoring methods also differed. The previous studies involved populations that did not possess this allele. In the present study, the presence of this major QTL may have reduced our ability to detect other QTL of somewhat lesser effect.

Taken together, it appears that the FHB resistance of Arina is due to a number of QTL of moderate to small effect and this, in part may explain the results obtained across the three studies. Previous reports have demonstrated that the stability of resistance appears to depend upon the level of resistance. While highly FHB resistant genotypes remain relatively free of disease under nearly all epidemic conditions, the response of moderately resistant and susceptible germplasm is greatly influenced by epidemic conditions (Mesterhazy 1995; Miedaner et al. 2001). Another factor that may be, in part, responsible for the lack of common FHB resistance QTL is the "susceptible" parent used to generate the populations in each of the Arina studies. QTL analysis can only identify those components that differ between the parents from which the population was derived and it is likely that the three "susceptible" parents differ significantly with respect to the QTL in each of them that influence FHB resistance and DON accumulation. Overall, the results from the three studies indicate that, while Arina may be a useful source of FHB resistance, the genetic basis of this resistance is complex and may not be amenable to breeding using a marker-assisted selection approach.

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