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ORIGINAL PAPER

Inheritance of resistance to Fusarium head blight in three European winter wheat populations

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Abstract Fusarium head blight (FHB) resistance is of particular importance in wheat breeding programmes due to the detrimental effects of this fungal disease on human and animal health, yield and grain quality. Segregation for FHB resistance in three European winter wheat populations enabled the identification of resistance loci in well-adapted germplasm. Populations obtained from crosses of resistant cultivars Apache, History and Romanus with susceptible semi-dwarfs Biscay, Rubens and Pirat, respectively, were mapped and analysed to identify quantitative trait loci (QTL) for FHB severity, ear emergence time and plant height. The results of the present study together with previous studies in UK winter wheat indicated that the semi-dwarfing allele *Rht-D1b* seems to be the major source for FHB susceptibility in European winter wheat. The high resistance level of the cultivars Romanus and History was conditioned by several minor resistance QTL interacting with the environment and the absence of *Rht-D1b*. In contrast, the semi-dwarf parents contributed resistance alleles of major effects apparently compensating the negative effects of *Rht-D1b* on FHB reaction. The moderately resistant cultivar Apache contributed a major QTL on chromosome 6A in a genome region previously shown to carry resistance loci to FHB. A total of 18 genomic regions were repeatedly associated with FHB resistance. The results indicate that common resistance-associated genes or genomic regions are present in European winter wheats.

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Introduction

Fusarium head blight (FHB), caused by different *Fusarium* species, is an important ear disease in many wheat cropping areas worldwide. The attention given to this disease is largely due to the mycotoxins produced by the fungus. Besides agricultural control practices such as crop rotation and ploughing, the breeding and cultivation of resistant varieties is a very efficient means to reduce the risk of FHB infection (Koch et al. 2006). However, FHB resistance is a complex trait controlled by multiple genes (Snijders 1990). Moreover, Miedaner et al. (2001) showed that resistance evaluations can be confounded by large environmental effects and genotype × environment interactions. Different types of resistance are known, e.g. resistance to initial infection (type I) and resistance to fungal spread within the ear (type II) (Schroeder and Christensen 1963). Morphological resistance to natural FHB infection is mediated, among other causes, through increased plant height (Mesterházy

1995). However, tall genotypes are not desired by breeders. Combined type I and II resistance can be assessed by artificial spray inoculation. Visual FHB rating is a good predictor of DON content of infected wheat (Miedaner et al. 2004).

Marker-assisted breeding could help to overcome the problems associated with phenotypic assessment and enables the combining of resistance genes from different sources (Wilde et al. 2008). Therefore, various wheat populations have been analysed for the presence of quantitative trait locus/loci (QTL). Great progress was made by utilising resistance from the Chinese cultivar Sumai 3 in many wheat breeding programmes worldwide (Bai and Shaner 2004). However, its introduction into high-yielding European wheat is hampered, or is at least very time consuming due to the negative agronomic performance of exotic germplasm. An alternate strategy being pursued is the search for resistance QTL in germplasm adapted to European winter wheat growing areas (Gervais et al. 2003; Shen et al. 2003; Paillard et al. 2004; Klahr et al. 2007). Schmolke et al. (2005) found two major QTL, which explained 19 and 21% of the phenotypic variance and originated from the high-yielding variety Dream. The effects of these QTL (*Qfhs.lfl-6AL*, *Qfhs.lfl-7BS*) were validated by Häberle et al. (2007).

Recent studies showed that the genomic region containing the *Rht-D1b* (*Rht2*) semi-dwarfing allele on chromosome 4DS significantly contributes to FHB susceptibility in European winter wheats (Hilton et al. 1999; Draeger et al. 2007; Srinivasachary et al. 2008; Voss et al. 2008). *Rht-D1b*-containing semi-dwarf wheats are commercially very important not only in Europe, but also in all countries utilising CIMMYT-derived cultivars. Voss et al. (2008) reported the effects of the *Rht-D1* locus on FHB severities in a broad-based study involving three segregating winter wheat populations, which were also the basis for the present study. Draeger et al. (2007) and Srinivasachary et al. (2008) found that *Rht-D1* has the strongest effect on FHB and related traits in UK winter wheats. The latter authors also showed that lines carrying the *Rht-D1b* allele are compromised in type I resistance while being unaffected in type II resistance. Whether the association between *Rht-D1b* and FHB susceptibility is due to close linkage or pleiotropy of *Rht-D1* remains unclear.

The objectives of this study were (1) to identify FHB resistance QTL in three segregating winter wheat populations originating from well-adapted high-yielding European germplasm, (2) to investigate whether the identified resistance QTL are associated with QTL for ear emergence time and plant height and (3) to compare the locations of resistance QTL from different populations to assess the commonality of respective genes across genetic backgrounds.

Materials and methods

Plant materials and field experiments

The development of the mapping populations, design of the field experiments, artificial inoculation and trait assessment were described in detail by Voss et al. (2008). Three winter wheat (*Triticum aestivum* L.) mapping populations, Apache (*Rht-D1a*)/Biscay (*Rht-D1b*), History (*Rht-D1a*)/Rubens (*Rht-D1b*) and Romanus (*Rht-D1a*)/Pirat (*Rht-D1b*), consisting of 190, 103 and 216 recombinant inbred lines (RIL), respectively, were developed by KWS LOCHOW GmbH, the Bavarian State Research Centre for Agriculture and RAGT 2n. Apache and Rubens are French cultivars; Romanus is a Dutch cultivar. The other parents were bred in Germany. All populations segregated for FHB severity and *Rht-D1* alleles. Field trials were carried out at two Northern (Silstedt, Wohlde) and three Southern German (Freising, Hohenheim, Moosburg) locations in 2005 and 2006. Each population was examined in five location × year-combinations (environments) with two replications per environment. Repeated artificial spray inoculation with *F. culmorum* conidia during anthesis provoked FHB infection. FHB severities were averaged over five visual ratings as a percentage of infected spikelets per plot beginning with the onset of symptom development at 3- to 4-day intervals. Plant height was measured in centimetres; ear emergence time (heading date) was counted from January 1. The traits were evaluated plot-wise.

Molecular analyses

DNA isolation and *Rht-D1* analyses were described by Voss et al. (2008). Amplified fragment length polymorphism (AFLP) analyses were conducted using *Pst*I/*Mse*I restriction enzymes and 45 selective primer combinations for each population. The AFLP protocol (Vos et al. 1995) was modified as described by Schmolke et al. (2005). Marker loci were designated as recommended by McIntosh et al. (2003). Furthermore, we named AFLP loci co-migrating in different populations with the starting character of the population. For example, marker locus *XP6451-190.AHR* refers to a fragment of about 190 bp amplified with primer combination P64/M51 and co-migrating in Apache/Biscay, History/Rubens and Romanus/Pirat. Co-migration with AFLPs of nulli–tetrasomic Chinese Spring lines enabled the chromosomal assignments of various AFLPs. Simple sequence repeat (SSR) primer sequences and marker probe information were obtained from the GrainGenes database (GrainGenes 2008) except for GWM767, GWM905 and GWM1027, which were kindly provided by M. Röder (Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany). PCR conditions for

sequence-tagged site markers IAG95, diagnostic for the wheat–rye translocated chromosome 1BL.1RS, and ACT/CAA (*Xwhs2001*), a resistance gene-like sequence, were as described by Mohler et al. (2001) and Schwarz et al. (1999), respectively.

Statistical analysis

For disease reaction comparisons, the arithmetic means of individual ratings that showed genotypic differentiation were used; the resultant trait was called mean FHB rating. All statistical analyses were based on single-plot data. Normal distributions of residuals were confirmed using the PROC UNIVARIATE procedure for mean FHB rating, plant height and heading date by SAS/STAT (SAS Institute Inc. 2004).

Genetic maps were constructed with JoinMap software version 3.0 (Van Ooijen and Voorrips 2001), using the Haldane mapping function. Linkage of loci was claimed at logarithm of odds LOD $\geq$ 3.0 with a maximum recombination fraction of 0.4. The threshold value for removal of loci with respect to jumps in goodness-of-fit was 5.0 without performing a "third round".

Linkage groups and the phenotypic means adjusted from block effects of each environment were the data basis for QTL analyses. MultiQTL software version 2.5 (Korol et al. 2005) with the multiple-environment option was used for QTL analyses. With this option, the phenotypic data for individual environments enter the analytical model. After performing simple-interval mapping (SIM) for each trait, significances of detected QTL were estimated by permutation tests ($N = 1{,}000$). For the entire genome analysis, we included all chromosomes with significant ($P < 0.05$) putative QTL detected by SIM into the multiple-interval mapping (MIM) model to reduce "background" variation by taking into account QTL effects from other chromosomes. QTL obtained with MIM were tested for significance ($P < 0.001$) with a global permutation test ($N = 10{,}000$). Confidence intervals (99.9%) of QTL were estimated by bootstrapping with 10,000 samples. The probability of epistasis among QTL was tested by permutation ($P < 0.05$, $N = 1{,}000$), comparing sub-models with and without epistatic effects. In the same way, the probability of two linked QTL on one chromosome was tested. A major QTL was claimed, if it explained more than 10% of the phenotypic variance (R^2) in at least one environment. Genetic maps with QTL confidence intervals were drawn using MapChart software version 2.1 (Voorrips 2002).

Comparison of QTL locations and construction of an integrated QTL map

Comparability of QTL maps from the populations analysed in this study was achieved by using the same set of microsatellite markers and co-migrating AFLPs. Regarding the populations of this study, confidence intervals of QTL were compared. In cases of clear overlap, QTL were considered as coincident. In some cases, a direct comparison of published QTL maps with our maps was not feasible due to the lack of corresponding markers in QTL regions. Reference maps, mainly the physical Chinese Spring deletion bin SSR map (Sourdille et al. 2004) and the genetic high-density consensus SSR map (Somers et al. 2004), were used for comparison via the comparative map viewer software CMap of GrainGenes (2008). Marker order and linkage distances of those reference maps also comprised the backbone of the integrated QTL map. QTL for FHB severity that were coincident among our mapping populations were plotted in this schematic map. Furthermore, all QTL of published European winter wheat mapping populations, whose most likely position (marker interval, LOD curve) overlapped with QTL confidence intervals in our study, were included. Regarding the published studies, all QTL for FHB-related traits (e.g. type I resistance, type II resistance, DON accumulation, FHB severity, Fusarium damaged kernels) were considered.

Results

Phenotypic trait evaluation

The results of phenotypic trait evaluation were described by Voss et al. (2008) (Table 1). Correlation coefficients of FHB severities ranged between 0.60 and 0.84 (Apache/Biscay), 0.72 and 0.82 (History/Rubens) and 0.50 and 0.83 (Romanus/Pirat) among individual environments ($P < 0.001$). Because of unfavourable weather conditions during inoculation in Moosburg 2006, this environment was excluded from further analysis. The full data set of correlation coefficients between individual environments can be viewed in the electronic supplementary material section (S1).

Molecular mapping

The numbers of mapped markers were 293 for Apache/Biscay, 394 for History/Rubens and 276 for Romanus/Pirat, and the total linkage distance of the maps were 2,048, 2,187 and 1,722 cM, respectively. Generally, only markers that did not co-segregate or cluster (linkage distance < 1 cM) were included. In the population History/Rubens, two linkage groups of 13 cM could not be assigned to particular chromosomes. In each population, all 21 wheat chromosomes were at least partially represented by linkage groups. The genetic maps with the QTL confidence intervals for FHB severities are provided as electronic supplementary

Table 1 Population (Pop.) sizes (*N*), means, ranges and least significant differences (LSD) for FHB severity and plant height

Data represent overall means across environments

Population	*N*	FHB severity (%)			Plant height (cm)		
		Pop. mean	Range	$LSD_{5\%}$	Pop. mean	Range	$LSD_{5\%}$
Apache/Biscay	190	32.2	12.0–56.0	7.5	78.9	69.0–94.3	3.1
History/Rubens	103	33.5	14.7–59.1	7.7	96.3	66.8–116.6	5.0
Romanus/Pirat	216	35.2	14.1–68.9	10.3	90.4	70.6–111.5	4.2

material (S2). Confidence intervals of QTL for ear emergence time and plant height were also included in the maps in the cases of overlap with FHB severity QTL.

Quantitative trait analyses for FHB severity

Analysing the data with the MIM procedure and the multiple-environment model of MultiQTL, we detected 13, 8 and 14 QTL for FHB severity in the populations Apache/Biscay, History/Rubens and Romanus/Pirat, respectively (Table 2, S2). QTL were found on all chromosomes with the exception of chromosomes 3A, 5D and 7D. Seven resistance QTL had coefficients of determination (R^2) > 10% in at least one environment and were therefore regarded as major QTL. They were located on chromosomes 2D, 4DS, 6A (Apache/Biscay), 4DS, 6BL (History/Rubens) and 1BL, 4DS (Romanus/Pirat). The majority of the QTL showed significant ($P < 0.05$) QTL × environment interactions. QTL on chromosomes 4DS (Apache/Biscay), 2A, 2BS, 7BS, 7B (History/Rubens) and 6DL (Romanus/Pirat) showed no QTL × environment interactions. QTL with overlapping confidence intervals in all populations were found on chromosomes 1BL and 4DS (*Rht-D1*). The total phenotypic variance explained for FHB severity ranged between 45.1% (Apache/Biscay, Wohlde 2005) and 79.0% (History/Rubens, Silstedt 2005).

The QTL with the highest additive effect in each population was detected on chromosome arm 4DS (LOD 26.5–142.8) and reduced FHB severity between 16.3 and 31.5%. This QTL explained most of the phenotypic variance in every population and environment, with the exception of Apache/Biscay in Silstedt 2006. The donors of the resistance alleles were the parents carrying the wild-type allele *Rht-D1a* (Apache, History, Romanus). The QTL was located close to the *Rht-D1* gene, with a confidence interval (99.9%) of less than 1 cM (S2). In each population, it coincided with a major QTL for plant height (LOD 76.3–274.2), which reduced height by an average of 5.7 cm (Apache/Biscay), 17.1 cm (History/Rubens) and 16.1 cm (Romanus/Pirat) (data not shown).

In the population Apache/Biscay, a major resistance QTL on chromosome 6A reduced FHB severity by 13.9% compared to lines without the QTL allele. This QTL did not co-locate with QTL for plant height or ear emergence time (Table 2, S2) but interacted significantly ($P < 0.05$) with the QTL at *Rht-D1*. The QTL showed a relative FHB reduction of 27% in the subpopulation containing the *Rht-D1a* wild-type allele, whereas it had no significant effect in the semi-dwarf subpopulation (data not shown). A major QTL originating from the susceptible parent Biscay was found on chromosome 2DS, overlapping with a major QTL for ear emergence time and a minor QTL for plant height.

Besides the QTL in the genomic region of *Rht-D1*, another major QTL was detected on chromosome 6B in the population History/Rubens. The resistance allele was inherited from the susceptible parent Rubens and this QTL coincided with a QTL for ear emergence time.

In the population Romanus/Pirat, a major QTL was detected on chromosome 1BL. It came from the susceptible parent Pirat and reduced FHB severity on average by 18.1%. The QTL peaked at *XP6451-190.AHR*, which also marked the peaks for FHB resistance QTL found in the populations Apache/Biscay and History/Rubens. However, in the latter populations, the QTL had smaller effects. Depending on the population, this QTL overlapped with QTL for ear emergence time and/or plant height.

Comparison of QTL locations

A comparison of FHB QTL locations among the current populations and with previously published results from European winter wheat identified a total of 18 genomic regions repeatedly associated with FHB resistance and distributed over 14 chromosomes (Table 3, Fig. 1). Overlaps occurred for nine FHB QTL in Apache/Biscay, four in History/Rubens and eight in Romanus/Pirat. The most frequent coincidences included five at *Rht-D1* on chromosome 4DS, and three for each of chromosomes 1BL, 1DS, 3B and 6A.

Discussion

In the present study, we found between 8 and 14 QTL for FHB severity in three different European winter wheat populations. Most of these QTL interacted significantly with the environments. A high number of QTL and QTL × environment interactions observed in many studies seems to be characteristic for European winter wheat (Paillard et al. 2004; Draeger et al. 2007; Klahr et al. 2007; Srinivasachary et al. 2008). The resistant parents Apache,

Table 2 Significant ($P < 0.001$) QTL for FHB resistance in the populations Apache/Biscay, History/Rubens and Romanus/Pirat

Chr.	Resistance donor	Closest marker locus	LOD	Overlap[a]	R^2 environment[b] (%)					Effect[c] (%)
					1	2	3	4	5	
Apache/Biscay										
1BL	Biscay	*XP6451-190.AHR*	11.8	Eet, Ht	6.5	4.6	2.7	2.1	–	10.1
1D	Apache	*Xgwm458-1D*	8.3	Ht	4.4	2.4	0.6	2.4	–	7.5
2B	Biscay	*XP6853-119*	14.9	Ht	6.3	6.7	3.3	1.8	–	10.6
2D	Biscay	*Xgwm484-2D*	14.3	Eet, Ht	10.7	5.6	0.6	0.6	–	9.0
3B	Apache	*XP7455-203.AR*	10.2	–	2.1	4.8	2.0	7.5	–	9.8
3D[d]	Biscay	*Xgwm52-3D*	12.0	–	3.7	4.0	4.3	2.9	–	10.1
4AL	Apache	*XP7452-646*	12.0	–	1.6	1.0	7.8	3.4	–	8.6
4DS[d]	Apache	*Rht-D1*	26.5	Ht	15.2	8.9	2.1	19.4	–	16.3
4DL	Apache	*Xgwm265-4D*	8.1	–	1.5	3.3	3.0	2.1	–	7.9
5AL	Apache	*Xwmc410-5A*	7.2	–	0.4	0.3	4.0	3.5	–	4.9
5BL	Apache	*XP7058-189.AHR*	11.6	–	2.4	0.5	7.9	4.4	–	8.9
6A[e]	Apache	*XP7647-189*	18.6	–	3.5	7.2	14.1	5.7	–	13.9
6BL[e]	Apache	*XP6347-204*	8.0	–	1.0	1.0	4.9	2.8	–	7.5
			R^2 total		58.3	45.1	50.8	52.7	–	
History/Rubens										
1A	History	*XP6851-352*	12.2	–	4.1	9.2	5.7	3.1	3.2	12.0
1BL	History	*XP6451-190.AHR*	14.5	Ht	6.6	0.7	2.5	7.9	4.3	10.7
2A[d]	Rubens	*Xgwm425-2A*	10.4	–	1.5	2.9	2.0	4.1	3.4	8.9
2BS[d]	History	*XP6852-318.AHR*	15.0	–	2.1	4.8	8.0	2.4	5.0	11.1
4DS	History	*Rht-D1*	66.6	Ht	30.4	35.5	28.1	30.9	29.6	29.2
6BL	Rubens	*XP7753-178.AHR*	28.0	Eet	19.1	20.7	6.9	18.9	15.8	22.1
7BS[d]	History	*XP6653-115*	8.4	Eet	2.8	0.9	3.2	1.7	4.7	8.4
7B[d]	Rubens	*Xgwm43-7B*	11.2	–	3.0	4.3	3.0	1.9	3.1	9.6
			R^2 total		67.2	79.0	56.6	70.0	68.4	
Romanus/Pirat										
1AS	Pirat	*Xwmc818-1A*	8.1	–	0.2	2.3	0.4	0.2	2.1	4.3
1B	Romanus	*XP7056-308.AR*	9.1	Eet, Ht	3.0	0.4	0.4	1.6	0.7	6.0
1BL	Pirat	*XP6451-190.AHR*	57.9	Eet	7.7	4.6	6.6	13.8	7.5	18.1
1DS	Pirat	*Xbarc149-1D*	13.2	Eet, Ht	2.8	0.9	0.7	1.8	1.8	8.0
2DS	Romanus	*Xcfd56-2D*	9.1	Ht	2.5	1.2	0.8	0.5	0.4	4.0
3DL	Romanus	*XP6452-257*	15.4	–	0.5	2.3	0.6	4.2	4.2	9.1
4AL	Pirat	*XP7553-254.AR*	25.6	Eet	4.7	0.1	3.1	5.0	2.9	10.6
4BL	Pirat	*Xgwm375-4B*	18.2	Eet	2.3	1.5	1.3	4.7	0.2	5.7
4DS	Romanus	*Rht-D1*	142.8	Ht	31.9	30.2	36.4	20.7	22.1	31.5
5AL	Pirat	*Xgwm410-5A*	33.4	Eet	9.2	0.5	5.7	5.9	4.5	14.0
6DL[d]	Romanus	*Xbarc96-6D*	15.6	Eet	0.9	1.2	1.9	3.4	2.0	8.9
7A	Romanus	*XP6655-351*	27.6	Ht	4.8	7.7	5.7	1.5	4.1	13.3
7AL	Romanus	*Xgwm344-7A*	14.3	–	0.5	6.1	1.4	0.2	0.7	6.1
7BL	Romanus	*XP7061-206*	23.5	–	1.6	5.5	2.3	1.5	3.9	10.6
			R^2 total		73.7	67.5	69.1	62.1	56.8	

Chromosomes (Chr.) with major QTL effects ($R^2 > 10\%$ in at least one environment) are given in bold

[a] QTL confidence interval (99.9%) overlaps between QTL for FHB severity and either plant height (Ht) and/or ear emergence time (Eet)

[b] Apache/Biscay, environments 1–4: Moosburg 2005, Wohlde 2005, Silstedt 2006, Wohlde 2006. Moosburg 2006 was excluded from QTL analysis due to unfavourable infection conditions; History/Rubens, environments 1–5: Freising 2005, Silstedt 2005, Freising 2006, Hohenheim 2006, Silstedt 2006; Romanus/Pirat, environments 1–5: Hohenheim 2005, Silstedt 2005, Hohenheim 2006, Silstedt 2006, Wohlde 2006

[c] Relative additive effect: relative FHB reduction of lines with QTL allele compared to lines without QTL allele in %, averaged over 4 and 5 environments, respectively

[d] No significant QTL × environment interaction ($P > 0.05$)

[e] Significant ($P < 0.05$) epistatic QTL × QTL interaction with chr. 4DS (*Rht-D1*)

History and Romanus contributed the majority of the QTL. Despite their susceptible FHB reaction, Biscay, Rubens and Pirat also contributed major FHB resistance alleles.

The QTL explaining by far the largest component of variance for FHB severity was located at the *Rht-D1* locus in each population. In a companion study, Voss et al. (2008) already reported that the semi-dwarfing *Rht-D1b* allele enhanced FHB susceptibility by 22 to 53%. In the present study, we additionally showed that the QTL at *Rht-D1* had the strongest effect on FHB compared to the residual wheat

Table 3 Chromosome (Chr.) locations of QTL regions common to this study and other European winter wheat populations

Chr.	Present study		Comparative study			
	Donor(s) of resistance allele(s)	Closest marker locus	Mapping population[a]	Measured FHB-related trait[b]	Closest marker locus	Reference
1AS	Pirat	*Xwmc818*	G16-92/**Hussar**	AUDPC	*XP68M52-309*	Schmolke et al. (2008)
1B	Romanus	*XP7056-308.AR*	**Arina**/Riband	RSW	*Xgwm18*	Draeger et al. (2007)
1BL	Biscay, History, Pirat	*XP6451-190.AHR*	–	–	–	not reported previously
1DS	Pirat	*Xbarc149*	**Sincron**/F1054 W	AUDPC, RSW	*Gli-D1*	Ittu et al. (2000)
			Cansas/**Ritmo**	AUDPC	*Xwhs2001*	Klahr et al. (2007)
2A	Rubens	*Xgwm425*	**Spark**/Rialto	AUDPC	*Xgwm515*	Srinivasachary et al. (2008)
2B	Biscay	*XP6853-119*	**Renan**/Récital	FHB severity	*Xgwm374*	Gervais et al. (2003)
3B	Apache	*XP7455-203.AR*	**Renan**/Récital	FHB severity	*Xgwm383*	Gervais et al. (2003)
			Arina/Forno	AUDPC	*Xcfa2134*	Paillard et al. (2004)
3D	Biscay	*Xgwm52*	Fundulea 201R/ **Patterson**	FHB severity	*Xgwm341*	Shen et al. (2003a)
4AL	Pirat	*XP7553-254.AR*	**Arina**/Forno	AUDPC	*Xgwm160*	Paillard et al. (2004)
4DS	Apache, History, Romanus	*Rht-D1*	**Arina**/Riband	AUDPC, FDNA, RSW, DON, FDK	*Rht-D1*	Draeger et al. (2007)
			Spark/Rialto	AUDPC	*Rht-D1*	Srinivasachary et al. (2008)
4DL	Apache	*Xgwm265*	**Spark**/Rialto	AUDPC	*Xgwm265*	Srinivasachary et al. (2008)
5AL	Apache	*Xwmc410*	**Arina**/Forno	AUDPC	*Xgwm291*	Paillard et al. (2004)
5AL	Pirat	*Xgwm410*	**Arina**/Forno	AUDPC	*Xgwm291*	Paillard et al. (2004)
6A	Apache	*XP7647-189*	**Dream**/Lynx	AUDPC	*XP66M55-242*	Schmolke et al. (2005), Häberle et al. (2007)
			Spark/Rialto	AUDPC	*XwPt-8833*	Srinivasachary et al. (2008)
6BL	Apache	*XP6347-204*	**Arina**/Riband	FDNA	*Xgwm219*	Draeger et al. (2007)
7A	Romanus	*XP6655-351*	**Spark**/Rialto	AUDPC	*Xpsp3050.2*	Srinivasachary et al. (2008)
7B	Rubens	*Xgwm43*	**Cansas**/Ritmo	AUDPC	*Xgwm46*	Klahr et al. (2007)
7BL	Romanus	*XP7061-206*	Arina/**Riband**	AUDPC	*Xwmc276*	Draeger et al. (2007)

[a] Resistant parent/susceptible parent; the donor of the QTL resistance allele is given in bold

[b] *AUDPC* area under the disease progress curve, *DON* DON content, *FDK* % Fusarium-damaged kernels, *FDNA* % fungal DNA content, *RSW* relative spikelet weight of infected versus control heads

genomes of the three populations. This is in agreement with the results of recent studies from the United Kingdom, where the same observations were made in different winter wheat populations (Draeger et al. 2007; Srinivasachary et al. 2008). Additional results of our working group showed that *Rht-D1* had significant ($P < 0.001$) effects on FHB in six other winter wheat populations, viz. Apache (*Rht-D1a*)/Contra (*Rht-D1b*), History (*Rht-D1a*)/Excellenz (*Rht-D1b*), Solitär (*Rht-D1a*)/Travix (*Rht-D1b*), Dream (*Rht-D1a*)/Lynx (*Rht-D1b*), Cansas (*Rht-D1a*)/Ritmo (*Rht-D1b*) and G16-92 (*Rht-D1a*)/Hussar (*Rht-D1b*) (data not shown). In published QTL studies with the latter three populations (Schmolke et al. 2005, 2008; Klahr et al. 2007), chromosome 4DS was not covered with markers: therefore, no QTL at or near *Rht-D1* were identified. All these studies and the observations made by Gosman et al. (2007) on semi-dwarf UK winter wheats indicate that the genomic region at *Rht-D1b* is a major contributor to FHB susceptibility. The similar position of this QTL in different mapping populations and the fact that the *Rht-D1b* allele used in wheat breeding programmes originated from a common genotype (Norin 10) strongly suggests that the same undesirable genes were inherited by linkage drag in all populations, or that *Rht-D1b* has a negative pleiotropic effect on FHB response.

Rht-D1b and FHB susceptibility—pleiotropy or linkage drag? This question is crucial for European wheat breeders to develop further breeding strategies. Draeger et al. (2007)

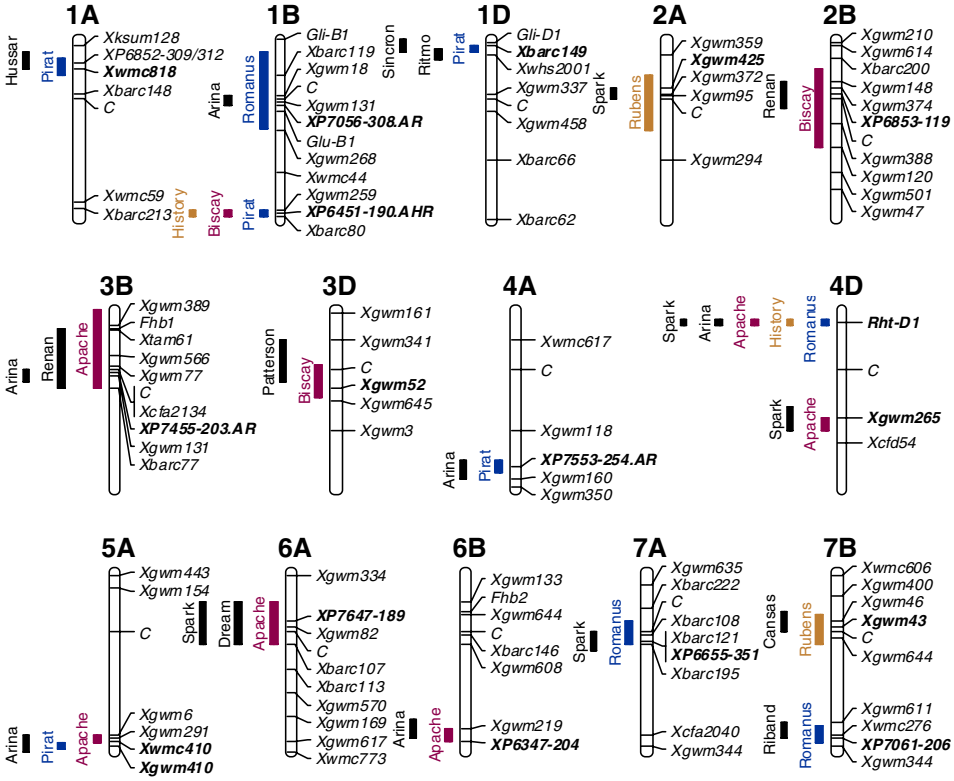

Fig. 1 Integrated QTL map with genomic regions repeatedly associated with FHB resistance in European winter wheat populations. *Bar lengths* for QTL detected in the present study represent 99.9% confidence intervals for FHB severity QTL. *Bar lengths* for QTL from published studies mark the most likely QTL positions (marker interval, LOD curve). *Shorter bars* indicate more precise QTL locations. QTL are designated with the name of the cultivar inheriting the resistance allele. For references of published QTL, see Table 3. Loci closest to the peaks of QTL identified in this study are given in *bold*

suggested linkage of the *Rht-D1b* allele with undesirable genes or pleiotropic effects of *Rht-D1b* on FHB susceptibility rather than plant height per se was responsible for the association. Srinivasachary et al. (2008) assumed pleiotropy rather than linkage drag due to the fact that the orthologous semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* are associated with increased FHB susceptibility. A recent study confirmed that both orthologues enhanced FHB reaction in near-isogenic lines of different origin (Miedaner and Voss 2008). The accumulating evidence supports pleiotropy rather than linkage. *Rht-D1* is an orthologue of the *Arabidopsis Gibberellin Acid Insensitive* gene (*GAI*), encoding a growth-restraining protein with a conserved sequence in the so-called DELLA region (Hedden 2003). A deletion in the DELLA domain causes gibberellic acid insensitive dwarf mutants (e.g. *Rht-D1b*). Cao et al. (2006) found that DELLA proteins in *Arabidopsis* are involved in the regulation of a range of genes, including some involved in response to disease and pathogens, toxin catabolism and multidrug transport. They suggested that DELLA proteins might also mediate disease resistance. Therefore, it is possible that DELLA proteins could be associated with FHB resistance in wheat.

A limited genetic diversity in the Central European gene pool gives reason to suppose that some FHB resistance loci should be common to different wheat cultivars. In our study, FHB resistance QTL with overlapping confidence intervals in three populations were found on chromosomes 1BL and 4DS (S2). An additional comparison with published studies revealed 18 genomic regions repeatedly associated with FHB resistance in European winter wheats. Coincidences in map position were found for a comparatively large number of QTL identified in our study (Table 3, Fig. 1). Although the effects of these QTL varied strongly in different environments and populations, and moreover, different FHB resistance-related traits were measured, several QTL occurred in similar genomic regions. Thus, in spite of the major effect of *Rht-D1*, less potent QTL were identified reliably. QTL originating from resistance sources other than European winter wheats also mapped into those genomic regions (data not shown). This suggests that FHB resistance is inherited in a complex manner, at least partially by similar genes or genomic regions with varying effects. The high resistance level of the two European cultivars History and Romanus—the latter being one of the most resistant genotypes cultivated in Germany—was mediated by various minor QTL and the absence of *Rht-D1b*, as shown for the resistant European cultivars Arina and Spark (Draeger et al. 2007; Srinivasachary et al. 2008). The application of such donors in marker-assisted breeding programmes is not recommended, because too many loci had to be considered for an efficient reduction of FHB susceptibility. However, these donors might be used successfully in classical breeding approaches. Interestingly, the more susceptible parents Biscay, Rubens and Pirat also contributed major QTL for FHB resistance (Table 2). This is in agree-

ment with Draeger et al. (2007) and Srinivasachary et al. (2008), who also found major QTL inherited from the semi-dwarfs Riband and Rialto. This suggests that semi-dwarf cultivars carry effective resistance alleles that counteract the strong negative effect of *Rht-D1b*. Moreover, these QTL are essential to achieve acceptable levels of FHB resistance for cultivar registration.

QTL for FHB resistance were detected at the distal end of chromosome 1BL with the LOD curve peaking at *XP6451-190.AHR* in all three populations. The narrow confidence interval in all populations (<15 cM) provided evidence for a common gene. The effect of this coincident QTL varied between populations (Romanus/Pirat > History/Rubens > Apache/Biscay) and among environments, and overlapped with QTL for plant height and/or ear emergence time (Table 2, S2). FHB resistance loci from the cultivar Arina were found on chromosome 1BL in three QTL studies (Paillard et al. 2004; Semagn et al. 2007; Draeger et al. 2007); however, map comparisons indicated that the Arina QTL found in these studies were more likely proximal to the QTL detected in our study.

A major QTL for FHB resistance originating from Apache was located on chromosome 6A in the Apache/Biscay population. Schmolke et al. (2005) located a resistance QTL in the same chromosomal region in a Dream/Lynx winter wheat population. This QTL (*Qfhs.lfl-6AL*) was validated by Häberle et al. (2007) in a backcross population and by Wilde et al. (2008) in complex crosses of genetically unrelated European winter wheats. Srinivasachary et al. (2008) also mapped a winter wheat FHB resistance QTL in this region. Because of significant epistatic interactions with *Rht-D1*, there was no significant effect of the Apache QTL allele on FHB severity in the semi-dwarf background. However, the effect of *Qfhs.lfl-6AL* in Dream/Lynx is independent of the *Rht-D1* allele (data not shown).

A very important region for FHB resistance on chromosome 3BS contains the major Sumai 3 resistance gene *Fhb1* (Cuthbert et al. 2006). Although this region was included in the confidence interval of an Apache/Biscay QTL on chromosome 3B due to a smaller peak of the LOD curve in that region (data not shown), the main peak is clearly located much closer to the centromere. The possibility of two linked QTL on chromosome 3B was not significant ($P > 0.05$), giving no evidence for a QTL in the *Fhb1* region. This is consistent with other QTL studies in European winter wheat, showing no variation in FHB severity at the *Fhb1* locus. Gervais et al. (2003) and Paillard et al. (2004) also identified the chromosome 3B centromeric region as a source of FHB response in European winter wheats (Table 3, Fig. 1).

A minor QTL was located on chromosome 1B in the population Romanus/Pirat, which segregates for the 1BL.1RS wheat–rye-translocation. Ittu et al. (2000), Shen et al. (2003), Zhang et al. (2004), Schmolke et al. (2005), Mardi et al. (2006), Klahr et al. (2007) and Srinivasachary et al. (2008) reported positive effects of QTL alleles located in or near the 1BL.1RS translocation. In contrast, the parental line Pirat containing the translocation contributed the susceptibility allele in our study, whereas the favourable allele originated from Romanus, which lacks the translocation. Co-dominant SSR markers confirmed that the QTL from Romanus was derived from wheat chromatin, indicating that the QTL detected in our study is different. This suggests that the positive effect of 1BL.1RS on FHB resistance depends on genetic background. Nevertheless, the Romanus QTL detected in the present study coincided with a QTL found by Draeger et al. (2007).

Conclusions

In this study, loci conferring FHB resistance were mapped using three European winter wheat populations. Several resistance loci showed interactions with the environment or coincided with positions of QTL for plant height and/or ear emergence time, confirming the complexity of FHB resistance. The *Rht-D1*-bearing genomic region had the strongest QTL effect in each population. Along with other studies, we showed that the *Rht-D1* genomic region is the major source of variation in FHB reaction in the European gene pool compared to the residual genome. The literature gives strong evidence that FHB susceptibility is caused by pleiotropic effects of *Rht-D1* semi-dwarfing alleles. Nevertheless, semi-dwarf lines with acceptable resistance levels could be selected in all populations due to the variance contributed by QTL with smaller effects. Thus, breeding resistant semi-dwarfs should be feasible. The high resistance levels of the winter wheats History and Romanus were conferred by the absence of the semi-dwarfing allele and the presence of various minor resistance QTL. On the other hand, semi-dwarf cultivars carried major QTL, which were important in compensating the large negative effect of *Rht-D1b* on FHB response. Most of the FHB resistance QTL found in this study mapped to genomic regions repeatedly shown to mediate FHB resistance in European winter wheats. These findings strengthen the value of previous reports and make small QTL effects more reliable.

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References

Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol 42:135–161

Cao D, Cheng H, Wu W, Soo HM, Peng J (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in Arabidopsis. Plant Physiol 142:509–525

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112:1465–1472

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 QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor Appl Genet 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. Theor Appl Genet 106:961–970

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

GrainGenes (2008) GrainGenes: a database for *Triticeae* and *Avena*. Agricultural Research Service, US Department of Agriculture. http://wheat.pw.usda.gov/GG2/index.shtml. Cited 29 March 2008

Häberle J, Schmolke M, Schweizer G, Korzun V, Ebmeyer E, Zimmermann G, Hartl L (2007) Effects of two major Fusarium head blight resistance QTL verified in a winter wheat backcross population. Crop Sci 47:1823–1831

Hedden P (2003) The genes of the green revolution. Trends Genet 19:5–9

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

Ittu M, Saulescu NN, Hagima I, Ittu G, Mustatea P (2000) Association of Fusarium head blight resistance with gliadin loci in a winter wheat cross. Crop Sci 40:62–67

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

Koch H-J, Pringas C, Maerlaender B (2006) Evaluation of environmental and management effects on Fusarium head blight infection and deoxynivalenol concentration in the grain of winter wheat. Eur J Agron 24:357–366

Korol A, Ronin Y, Minkov D, Britvin E, Mester D, Korostishevsky M, Malkin I, Frenkel Z, Orion O, Cohen L, Brailovsky A (2005) MultiQTL version 2.5. Institute of Evolution, Haifa University, Haifa

Mardi M, Pazouki L, Delavar H, Kazemi MB, Ghareyazie B, Steiner B, Nolz R, Lemmens M, Buerstmayr H (2006) QTL analysis of resistance to Fusarium head blight in wheat using a 'Frontana'-derived population. Plant Breed 125:313–317

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. http://wheat.pw.usda.gov/ggpages/wgc/2003. Cited 29 March 2008

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

Miedaner T, Reinbrecht C, Lauber U, Schollenberger M, Geiger HH (2001) Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale, and wheat. Plant Breed 120:97–105

Miedaner T, Heinrich N, Schneider B, Oettler G, Rohde S, Rabenstein F (2004) Estimation of deoxynivalenol (DON) content by symptom rating and exoantigen content for resistance selection in wheat and triticale. Euphytica 139:123–132

Miedaner T, Voss H-H (2008) Effect of dwarfing *Rht* genes on *Fusarium* head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Sci (in press)

Mohler V, Hsam SLK, Zeller FJ, Wenzel G (2001) An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8*/*Pm17* locus of common wheat. Plant Breed 120:448–450

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

SAS Institute Inc. (2004) SAS OnlineDoc® 9.1.2. SAS Institute Inc., Cary

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

Schmolke M, Zimmermann G, Schweizer G, Miedaner T, Korzun V, Ebmeyer E, Hartl L (2008) Molecular mapping of quantitative trait loci for field resistance to Fusarium head blight resistance in an European winter wheat population. Plant Breed (in press)

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:813–838

Schwarz G, Michalek W, Mohler V, Wenzel G, Jahoor A (1999) Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers. Theor Appl Genet 98:521–530

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

Shen X, Ittu M, Ohm HW (2003) Quantitative trait loci conditioning resistance to Fusarium head blight in wheat line F201R. Crop Sci 43:850–857

Snijders CHA (1990) The inheritance of resistance to head blight caused *by Fusarium culmorum* in winter wheat. Euphytica 50:11–18

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

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

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

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a

new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Voss H-H, Holzapfel J, Hartl L, Korzun V, Rabenstein F, Ebmeyer E, Coester H, Kempf H, Miedaner T (2008) Effect of the *Rht-D1* dwarfing locus on *Fusarium* head blight rating in three segregating populations of winter wheat. Plant Breed 127:333–339

Wilde F, Schön CC, Korzun V, Ebmeyer E, Schmolke M, Hartl L, Miedaner T (2008) Marker-based introduction of three quantitative-trait loci conferring resistance to *Fusarium* head blight into an independent elite winter wheat breeding population. Theor Appl Genet 117:29–35

Zhang X, Zhou M, Ren L, Bai G, Ma H, Scholten OE, Guo P, Lu W (2004) Molecular characterization of *Fusarium* head blight resistance from wheat variety Wangshuibai. Euphytica 139:59–64