Theor Appl Genet (2009) 119:325–332
DOI 10.1007/s00122-009-1041-2

ORIGINAL PAPER

A major QTL for resistance against Fusarium head blight in European winter wheat

Jennifer Häberle · Josef Holzapfel · Günther Schweizer · Lorenz Hartl

Received: 12 December 2008 / Accepted: 9 April 2009 / Published online: 6 May 2009

Abstract We report on the verification of a resistance quantitative trait locus (QTL) on chromosome 1BL (now designated *Qfhs.lfl-1BL*) which had been previously identified in the winter wheat cultivar Cansas. For a more precise estimation of the QTL effect and its influence on plant height and heading date lines with a more homogeneous genetic background were created and evaluated in four environments after spray inoculation with *Fusarium culmorum*. *Qfhs.lfl-1BL* reduced FHB severity by 42% relative to lines without the resistance allele. This QTL did not influence plant height, but significantly delayed heading date by one day. All of the most resistant genotypes of the verification population carried this major QTL displaying its importance for disease resistance. This resistance QTL has not only been found in the cultivar Cansas, but also in the three European winter wheat cultivars Biscay, History and Pirat. A subsequent meta-analysis confirmed the presence of a single QTL on the long arm of chromosome 1B originating from the four mentioned cultivars. Altogether, the results of the present study indicate that *Qfhs.lfl-1BL* is an important component of FHB resistance in European winter wheat and support the view that this QTL would be effective and valuable in backcross breeding programmes.

Communicated by B. Keller.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1041-2) contains supplementary material, which is available to authorized users.

J. Häberle (✉) · G. Schweizer · L. Hartl
Bavarian State Research Center for Agriculture,
Institute for Crop Science and Plant Breeding,
Am Gereuth 8, 85354 Freising, Germany
e-mail: Jennifer.haeberle@lfl.bayern.de

Present Address:
J. Holzapfel
Saatzucht Josef Breun GdbR,
Amselweg 1, 91074 Herzogenaurach, Germany

Introduction

Fusarium head blight (FHB) is a serious problem in many wheat-growing areas worldwide. The most serious problem associated with this disease is the contamination of grains with mycotoxins including deoxynivalenol (DON). Therefore, a regulation has been decided at the level of the European Union with threshold values for *Fusarium* mycotoxins (Commission regulation No 1881/2006; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006: 364: 0005:0024:EN:PDF). The maximum level for deoxynivalenol in unprocessed cereals other than durum wheat, oats and maize is 1250 µg/kg, and for cereals intended for direct human consumption and cereal flour it is 750 µg/kg. The breeding and cultivation of resistant wheat cultivars is the most promising strategy to reduce the risk of FHB. However, FHB resistance breeding is a difficult and time consuming task due to its quantitative inheritance (Snijders 1990) and the high genotype × environment interactions (Miedaner et al. 2001). Therefore, marker-assisted selection could be a promising tool to facilitate the selection of resistant cultivars and to enhance breeding efficiency.

Resistance quantitative trait loci (QTL) against FHB have been detected in several European winter wheat populations (Gervais et al. 2003; Shen et al. 2003; Paillard et al. 2004; Schmolke et al. 2005, 2008; Draeger et al. 2007; Klahr et al. 2007; Semagn et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008). Except for a QTL identified near or at the *Rht-D1* locus (Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008), effects of QTL found in adapted germplasm are generally smaller

compared to those found in Sumai 3 and its derivatives (Anderson et al. 2001; Buerstmayr et al. 2002; Cuthbert et al. 2006, 2007). Therefore, Sumai 3 has been used in many breeding programmes worldwide (Bai and Shaner 2004). However, when using exotic FHB resistance donors in European breeding programmes it is expected that several backcross generations are required to overcome the inferior genetic background and even more when linkage occurs to loci adversely affecting agronomically important traits.

Nevertheless, a verification of QTL effects either in a homogeneous genetic background (Häberle et al. 2007) or in an independent genetic background (Wilde et al. 2008) is generally recommended before using them in marker-assisted breeding programmes. Another approach to validate QTL is to compare positions of published QTL from different studies to subsequently identify genomic regions repeatedly associated with FHB resistance (Holzapfel et al. 2008). Recently, a summary of 52 QTL mapping studies, nine articles on marker-assisted selection and seven on marker-assisted germplasm evaluation was published by Buerstmayr et al. (2009). If QTL, found in independent experiments and located in the same genomic region of a chromosome, are in fact identical in position can be elucidated by means of statistical tools like meta-analysis (Goffinet and Gerber 2000).

Recently, Klahr et al. (2007) identified FHB resistance QTL in a Cansas (resistant)/Ritmo (susceptible) mapping population explaining between 10 and 20% of the phenotypic variation. The major QTL was mapped to the long arm of chromosome 5B (*Qfhs.whs-5B*). Other QTL with the resistance alleles originating from Cansas were detected on chromosomes 1BS, 3DL and 7BS. The objective of the present study was to construct a detailed genetic map of the QTL region on chromosome 5B. The enrichment of the genetic map with molecular markers in this particular region led to the relocalisation of the QTL to the long arm of chromosome 1B. Furthermore, the presence and effect of this major QTL required verification in lines with a more homogeneous genetic background, evaluated in field trials for FHB resistance after spray inoculation. The chromosomal location of the QTL reported here was compared with chromosomal locations of other published QTL on chromosome 1BL and, subsequently, a meta-analysis was performed for this particular genomic region.

Materials and methods

Plant material

The phenotypic effect of the major FHB resistance QTL (now designated *Qfhs.lfl-1BL*), identified in the Cansas/Ritmo mapping population, was to be verified in a homogeneous genetic background. A mapping population of recombinant inbred lines (RIL) was derived from Cansas (resistant) × Ritmo (susceptible) and advanced to $F_{4:7}$ by single seed decent (Klahr et al. 2007). Four RILs of this mapping population, which were still heterozygous at *Qfhs.lfl-1BL* were grown in the field and analysed with molecular markers (see below) to select single plants which were either fixed for the resistant or the susceptible allele at *Qfhs.lfl-1BL*. In total, 90 single plants deriving from the four $F_{4:7}$ lines were selected and harvested separately in 2006. Seeds of these single plants were used for the Fusarium head blight experiments in 2007 and in parallel grown in the field without inoculation for seed propagation to obtain seeds for the field trials in 2008. Cansas carries the T1BL.1RS wheat-rye translocation and Ritmo the *Rht-D1b* semi-dwarfing allele, however, the lines selected for the verification of *Qfhs.lfl-1BL* carried neither of them. The presence or absence of other FHB QTL in these lines was not known.

FHB field trials and inoculation

Field trials were performed in four environments (years × locations). In 2007, the population was grown in Freising and Leutewitz, and in 2008 in Leopoldshöhe and Leutewitz. Freising is in Southern Germany, and Leopoldshöhe and Leutewitz are situated in Northern and Eastern Germany, respectively. The field experiments were arranged as lattice design with two replications per environment. Together with the selected genotypes, the parents Cansas and Ritmo, as well as the FHB resistant check cultivar Solitär were tested several times within each experiment. Each genotype was grown in two rows with a plot size of 0.6 m^2.

The inoculation of plants was performed during flowering time using a highly aggressive *Fusarium culmorum* isolate (FC33), which was produced as described by Miedaner et al. (1996). The spore suspension had a concentration of 3×10^5 spores/ml and was sprayed onto the plants (100 ml/m^2). Due to differences in flowering date, all genotypes were spray-inoculated two to three times within a period of 10 days to inoculate all plants at least once at mid-anthesis. FHB severity was scored visually as percentage of infected spikelets per plot and started with the first visible symptoms 9–10 days after the last inoculation and was repeated two to three times at intervals of 3–5 days. For further calculations, the arithmetic means of individual ratings were considered. By the use of spray inoculation to cause FHB in field trials, the combined effects of type I and type II resistance can be evaluated. In addition, heading date in days from 1st January was determined, as well as the mean plant height in cm per plot. In 2007, heading date was not

determined in Leutewitz. All other traits were scored in all environments.

DNA isolation and marker analysis

The plant DNA was isolated from young green leaves using the CTAB method (Saghai-Maroof et al. 1984).

To enrich the QTL region of the major QTL on chromosome 5BL (Klahr et al. 2007) with AFLP markers, a screening was performed based on genotypic pools according to Giovannoni et al. (1991). Bulks of DNA from six to ten genotypes of the original Cansas/Ritmo mapping population homozygous for opposing alleles for the desired chromosomal region were assorted and screened with around 100 AFLP primer combinations using the standard list for the AFLP primer nomenclature (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). AFLP marker loci were designated as recommended by McIntosh et al. (2003) and consisted of the applied primer combination followed by the estimated fragment size in base pairs. Subsequently, AFLP markers showing polymorphisms between the genotypic pools were tested on the entire mapping population, which consisted of 94 recombinant inbred lines (Klahr et al. 2007). One of the AFLP markers was previously assigned to the long arm of chromosome 1B in the linkage maps of three winter wheat populations (Holzapfel et al. 2008). Thus, SSR markers GWM140, GWM259 and WMC728—specific for chromosome 1BL—were applied to the mapping population. The data indicated that the major QTL *Qfhs.whs-5B* (Klahr et al. 2007) had to be relocated to chromosome 1BL and, therefore, renamed as *Qfhs.lfl-1BL* (see "Results").

The AFLP markers P6450-108 and P6451-190, as well as the SSR markers WMC728 and GWM140 were used for the selection of the genotypes for the verification of *Qfhs.lfl-1BL* in a homogeneous genetic background.

The procedure for the AFLP markers was performed as described by Hartl et al. (1999) with some modifications (Schmolke et al. 2005) and the amplification of the SSR markers was done according to Roeder et al. (1998). SSR primer sequences were obtained from the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). All used forward primers were labelled with fluorescein. The separation of the DNA fragments on 5% denaturing polyacrylamide gels was followed by the fragment detection with a fluorescence scanner (Typhoon 9200, Amersham, Braunschweig).

Statistical analysis

The existing genetic map of the Cansas/Ritmo mapping population (Klahr et al. 2007) was extended by additional AFLP and SSR markers using Joinmap version 3.0 (van Ooijen and Voorrips 2001). The subsequent QTL analysis for the marker-enriched linkage group on chromosome 1BL was recalculated with MultiQTL version 2.5 (Korol et al. 2005) using the multiple environment option. The confidence interval (95%) of the QTL was estimated via a bootstrap test with 1000 samples. The meta-analysis for chromosome 1BL was performed with BioMERCATOR version 2.1 (Arcade et al. 2004). First, an integrated map was created applying the loci position data for chromosome 1BL of the Cansas/Ritmo, Apache/Biscay, History/Rubens and Romanus/Pirat populations (Holzapfel et al. 2008). Meta-analysis computing was based on the position of each input QTL, and on the variance of this position, assessed through confidence interval values. Models with one, two, three, four or more QTL were tested and the one with the lowest AIC value was considered the best fit. Genetic maps with QTL confidence intervals were drawn using the software Mapchart version 2.2 (Voorrips 2002).

The phenotypic data of the field trials was analysed with Plabstat version 2P (Utz 2001) to calculate the adjusted single values and means of individual environments using the LATTICE option. Broad-sense heritabilities were calculated according to Fehr (1987). Analysis of variance was performed with the adjusted single values of individual environments using the SAS programme version 9.1 (SAS Institute Inc. 2004). The data were only approximately normally distributed, therefore, both a parametric and a non-parametric approach was applied to estimate if *Qfhs.lfl-1BL* had a significant effect on FHB severity. As a parametric method, the PROC GLM procedure was carried out to estimate sources of variance associated with FHB severity. The effects in the statistical model were environment, replication within environment, family (considering the different pedigree of the selected lines which were based on four $F_{4:7}$ lines still segregating for *Qfhs.lfl-1BL*), *Qfhs.lfl-1BL* and interactions. Environment, family and their interactions were defined as random effects. Subsequently, a Scheffé test was conducted for comparisons between the two marker classes possessing the resistance vs. the susceptible allele at *Qfhs.lfl-1BL* with a probability value of $P < 0.05$ using means averaged across all four environments. As a non-parametric method, the Kruskal–Wallis test within the NPAR1WAY procedure was applied for the comparison of the above mentioned marker classes ($P < 0.05$). This test was calculated for both the data of the entire 90 genotypes and the data of the genotypes divided by the four $F_{4:7}$ lines on which the genotypes for the QTL verification were based as indicated above. The parametric as well as the non-parametric analysis resulted in the same significances showing that *Qfhs.lfl-1BL* had a significant effect on FHB severity. Therefore, only the results of the parametric analysis, including all variables mentioned above in one model, are presented in the results. The relative effect of the

resistance QTL refers to the reduction of FHB severity in the presence of the resistance allele at *Qfhs.lfl-1BL* in relation to the susceptible class with no resistance allele (=100% FHB severity).

Results

Relocalisation of the major QTL and meta-analysis of chromosome 1BL

Based on genotypic pools, the region of the major QTL *Qfhs.whs-5B* (Klahr et al. 2007) could be enriched with two additional AFLP marker loci (*XP6450-108* and *XP6451-190*; Fig. 1). However, the marker locus *XP6451-190* was previously assigned to the long arm of chromosome 1B in the linkage maps of three winter wheat populations (Holzapfel et al. 2008). The integration of the SSR marker loci *Xgwm259-1B*, *Xwmc728-1B* and *Xgwm140-1B* into this linkage group (Fig. 1) confirmed the relocalisation of *Qfhs.whs-5B* to chromosome 1BL. Therefore, this QTL was renamed *Qfhs.lfl-1BL*. The proportion of explained phenotypic variation and the effect of the QTL after marker enrichment and relocalisation differed only marginally in comparison to the former QTL analysis (Klahr et al. 2007). The confidence interval of *Qfhs.lfl-1BL* overlapped with QTL which had been previously identified in the European winter wheat populations Apache/Biscay, History/Rubens and Romanus/Pirat (Holzapfel et al. 2008). The AFLP marker locus *XP6451-190* was common to all four populations and located in all cases within the QTL confidence intervals (Fig. 1). This AFLP marker is characterized by a consistent linkage phase in all four populations: genotypes with the resistance allele of *Qfhs.lfl-1BL* lacked the marker fragment. The estimated allele sizes of the SSR marker loci *Xgwm140-1B* and *Xwmc728-1B* from the parental lines of the four mentioned mapping populations are shown in Table 2. The genetic maps of chromosome 1BL of the four mapping populations had these two loci in common and they lay in three out of the four populations within the QTL confidence interval (Fig. 1). Meta-analysis confirmed the presence of a single QTL on the long arm of chromosome 1B originating from the cultivars Biscay, Cansas, History and Pirat (Fig. 2). The QTL was positioned at 28 cM of the integrated map with a confidence interval ranging from 26 to 29 cM. Thus, the most likely QTL position was narrowed down to a 3 cM interval. In the original mapping studies, the confidence intervals of this QTL ranged from 5 cM in the Apache/Biscay population to 13 cM in the Cansas/Ritmo population (Fig. 1). After meta-analysis, the AFLP marker locus *XP6451-190* mapped also within the QTL confidence interval again indicating that this marker is tightly linked to the QTL in all four mapping populations (Fig. 2).

Phenotypic effect of *Qfhs.lfl-1BL* in a homogeneous genetic background

90 selected progenies of four $F_{4:7}$ lines of the original Cansas/Ritmo mapping population segregating for the molecular markers at *Qfhs.lfl-1BL* were spray inoculated with a highly aggressive *Fusarium culmorum* isolate. Development of FHB symptoms after spray inoculation was sufficient in all environments for an accurate rating of disease severity. The 90 selected genotypes for QTL verification ranged from 7 to 37% for FHB severity, from 79 to 108 cm for plant height and from 150 to 157 days from 1st January for heading date based on data averaged across all four environments. The parental lines Cansas and Ritmo differed between FHB severity (11 vs. 39%) and plant height (93 vs. 84 cm), but not between heading date. A table of means values for the individual environments is

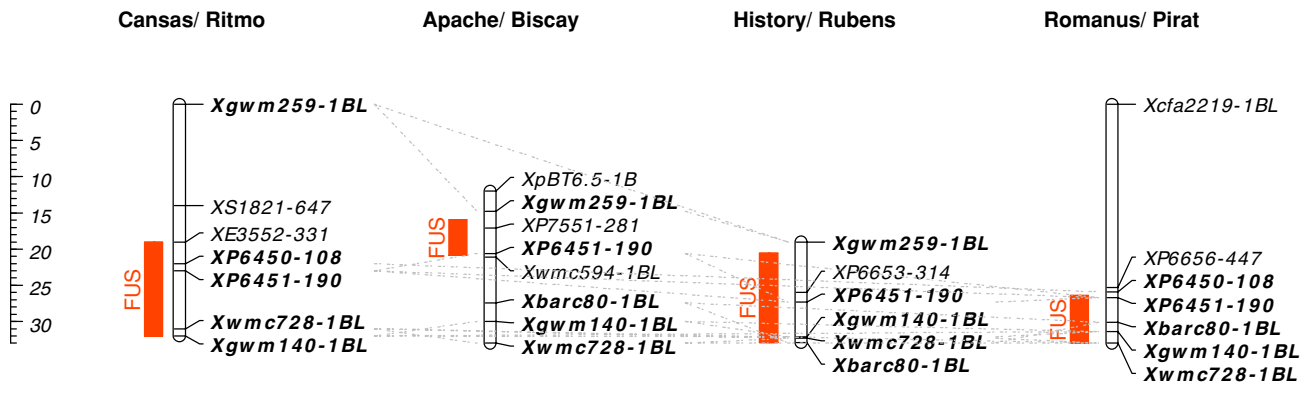


Fig. 1 QTL map of chromosome 1BL in the Cansas/Ritmo population compared to positions of QTL identified in the European winter wheat populations Apache/Biscay, History/Rubens and Romanus/Pirat (Holzapfel et al. 2008). *Bars* represent the 95% confidence intervals of resistance QTL against FHB. Common marker loci of different maps are given in bold and connected with *dotted lines*. The ruler shows the marker distances in cM

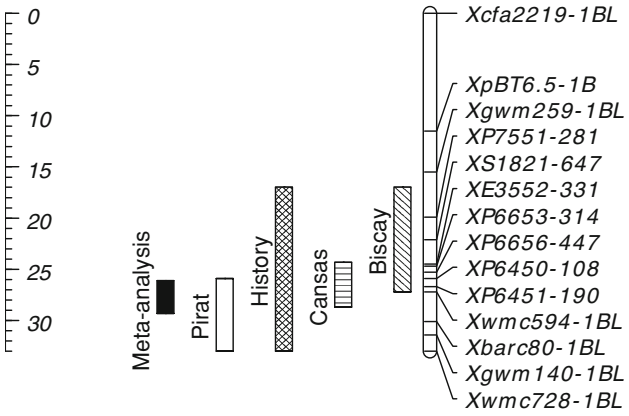


Fig. 2 Integrated QTL map of chromosome 1BL applying the loci position of the four European winter wheat populations Cansas/Ritmo, Apache/Biscay, History/Rubens and Romanus/Pirat (Holzapfel et al. 2008). *Bars* represent 95% confidence intervals of resistance QTL against FHB with the cultivars Biscay, Cansas, History and Pirat as QTL donors. The most likely position of *Qfhs.lfl-1BL* after the meta-analysis is indicated by the *black bar*. The ruler shows the marker distances in cM

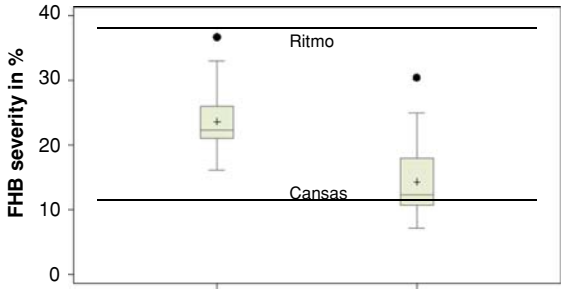
Table 1 Analysis of variance of the sources of variation for FHB severity in the 90 selected progenies of $F_{4:7}$ lines still segregating for *Qfhs.lfl-1BL* after spray inoculation with *Fusarium culmorum*

	Disease severity			
	df	M.S.	*F* value	*P* value
Environment	3	7500.2	16.26	0.0006
Replication within environment	4	166.2	3.88	0.0040
Qfhs.lfl-1BL	1	4489.9	104.98	<0.0001
Family	3	111.3	0.65	0.5986
Environment × family	9	435.5	10.18	<0.0001
Qfhs.lfl-1BL × family	3	946.4	22.13	<0.0001
Environment × *Qfhs.lfl-1BL*	3	370.5	8.66	<0.0001
Error	679	42.8		

Data are based on adjusted single values of the four environments in 2007 and 2008

given in S1. Broad-sense heritability for FHB severity was high ($H^2 = 80\%$), as well as broad-sense heritabilities for plant height ($H^2 = 96\%$) and heading date ($H^2 = 91\%$) indicating a good reproducibility of the phenotypic data.

A significant effect of the major QTL *Qfhs.lfl-1BL* on FHB severity was confirmed in the 90 selected genotypes (Table 1). Furthermore, analysis of variance also revealed significant variations for environment, replication within environment and the interactions environment × family, *Qfhs.lfl-1BL* × family and environment × *Qfhs.lfl-1BL*. No significant variation was found for the variable family. Alternatively, the statistical analysis calculated separately for the families to which the genotypes for the QTL verification belong confirmed that *Qfhs.lfl-1BL* leads to a reduction of FHB severity; however, this effect was only



Qfhs.lfl-1BL	S	R
FHB severity in %	24[a]	14[b]
Relative effect in %	-	42
Plant height in cm	96	99
Heading date in days from January 1st	153[a]	154[b]
Number of lines	29	61

Fig. 3 Boxplot distributions of the 90 selected progenies of $F_{4:7}$ lines still segregating for *Qfhs.lfl-1BL* after spray inoculation with *Fusarium culmorum*—differentiated by the two marker classes possessing the susceptible (S) or resistance (R) allele at *Qfhs.lfl-1BL*. Data are based on means averaged across all four environments. *Boxes* represent the 25 and 75 percentiles, respectively, *vertical lines* the 5 and 95 percentiles, respectively. The horizontal line within the *boxes* refers to the median and + to the mean. Outliers are indicated by *dots*. The *solid horizontal lines* display FHB severities of the parental lines. Significant differences after a Scheffé test are indicated by *different letters* ($P < 0.05$)

significant ($P < 0.05$) in two out of four families (data not shown). The 90 genotypes could be differentiated by the two marker classes possessing the resistant or susceptible allele at *Qfhs.lfl-1BL*. The disease severity of these lines was distributed quantitatively with a clear shift to a lower FHB severity in the presence of the Cansas resistance allele at *Qfhs.lfl-1BL* (Fig. 3). The phenotypic variation within the marker classes was relatively high. In comparison to the susceptible marker class, *Qfhs.lfl-1BL* reduced the relative disease severity by 42%. *Qfhs.lfl-1BL* had no effect on plant height, however, a significant delay in heading date of one day was associated with this QTL.

Discussion

It is well known that FHB resistance in European winter wheat is inherited by a higher number of loci with minor to moderate effects (Gervais et al. 2003; Paillard et al. 2004; Schmolke et al. 2005, 2008; Draeger et al. 2007; Semagn et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008) compared to resistance found in Sumai 3 and its derivatives (Anderson et al. 2001; Buerstmayr et al. 2002; Cuthbert et al. 2006, 2007). Nevertheless, FHB resistance QTL identified in adapted European material are of great interest for wheat breeding programmes due to the most probable lack of linkage drag which occurs when using exotic FHB resistance donors.

Table 2 Estimated allele sizes of the two SSR marker loci *Xgwm140-1B* and *Xwmc728-1B* from the parental lines of the four mapping populations used in meta-analysis

	Allele sizes in base pairs							
	Cansas	Ritmo	Apache	Biscay	History	Rubens	Romanus	Pirat
Xgwm140-1B	246	232	246	234	260	190	232	282
Xwmc728-1B	282	276	260	282	282	276	274	290

Klahr et al. (2007) identified a major QTL for FHB resistance on chromosome 5B (*Qfhs.whs-5B*) in a Cansas (resistant)/Ritmo (susceptible) winter wheat population. However, this QTL has now been reassigned to the long arm of chromosome 1B (*Qfhs.lfl-1BL*) (Fig. 1). The remaining genetic map was not affected by this relocalisation. The short and long arms of chromosome 1B still formed separate linkage groups when re-mapping the population with the additional AFLP and SSR markers. This might be due to the fact that the groups of chromosome 1BL (Fig. 1) represent the very distal part of this chromosome. Furthermore, the reassignment of *Qfhs.lfl-1BL* did not influence the presence of the other QTL identified in the previous study by Klahr et al. (2007). QTL which had been identified on chromosome 1BL in the European winter wheat cultivars Biscay, History and Pirat led to a relative reduction of FHB severity between 10% and 18% (Holzapfel et al. 2008). The narrow confidence interval in all four populations (<15 cM) suggested a common resistance gene in this genomic region. Meta-analysis confirmed the presence of a single QTL (Fig. 2). In meta-analysis, the length of a confidence interval of a QTL is consistently reduced when there are very few actual QTL locations (Goffinet and Gerber 2000). Meta-analysis combining up to hundreds of QTL from different populations has been successfully used in maize (Chardon et al. 2004), cotton (Rong et al. 2007) or rice (Norton et al. 2008) resulting in an increased precision in QTL position estimation. In the review of Buerstmayr et al. (2009) the QTL region on the very distal part of chromosome 1BL was not considered because the results from the study of Holzapfel et al. (2008) were not yet published at that time. Furthermore, the major QTL of Cansas was still located on chromosome 5BL where it partly overlapped with a QTL inherited by Forno (Paillard et al., 2004). Recently, a QTL meta-analysis was performed in wheat to identify independent meta-QTL involved in the control of FHB resistance (Löffler et al. 2009). They detected 19 meta-QTL on 12 chromosomes including one meta-QTL on chromosome 1BL comprising the three initial QTL of populations of Holzapfel et al. (2008). *Qfhs.lfl-1BL* from Cansas was not included in this meta-analysis, but our results show that this QTL forms one meta-QTL together with those identified in Biscay, History and Pirat (Fig. 2). Other QTL on the long arm of chromosome 1B originating from the cultivar Arina were found in three studies (Paillard et al. 2004; Draeger et al. 2007; Semagn et al. 2007), however, these QTL represented independent meta-QTL (Löffler et al. 2009).Depending on the mapping population *Qfhs.lfl-1BL* was inherited from the resistant (Cansas, History) or the susceptible (Biscay, Pirat) parent. This is in agreement with previous studies indicating that particularly semi-dwarf cultivars like Biscay and Pirat carry effective resistance alleles to compensate the negative effect of *Rht-D1b* (Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008). The varying coefficients of determination and effects of this QTL in different mapping populations are not unexpected. The effect of a QTL depends strongly on the genetic background of both crossing parents, the experimental design, the isolates used for inoculation and the environments in which the field trials are conducted including infection conditions. Even if a resistance QTL cannot be detected in some mapping populations it does not constrain its value in general, because it might be that both parental lines possess the same resistance alleles at a particular locus and therefore, a QTL can not be detected. This is indicated by the study of Holzapfel et al. (2008) who showed that FHB resistance in European winter wheat is inherited in a complex manner by partially similar genes with varying effects.

Genomic regions associated with resistance should be well characterized before utilizing them in classical or marker-assisted crossing programmes. We wanted to estimate the effect of *Qfhs.lfl-1BL* on FHB resistance and its influence on plant height and heading date in a more homogeneous genetic background. Therefore, 90 progenies of $F_{4:7}$ lines still segregating for molecular markers at *Qfhs.lfl-1BL* were evaluated after spray inoculation with a highly aggressive *Fusarium culmorum* isolate. To consider QTL × environment interactions field trials were performed in four environments (years × locations). So far, only few studies have been performed dealing with the verification of QTL detected in adapted European germplasm. *Qfhs.lfl-6AL* and *Qfhs.lfl-7BS* have been validated in a backcross population (Häberle et al. 2007) as well as in an independent genetic background together with an additional QTL on chromosome 2BL originating from G16-92 (Schmolke et al. 2008; Wilde et al. 2008).

Especially in small-sized populations, QTL effects might be overestimated (Utz et al. 2000), however, the results of our study in the same, but more homogeneous genetic background agreed well with previous results of the original mapping population (Klahr et al. 2007). There,

Qfhs.lfl-1BL (previously *Qfhs.whs-5B*) was the QTL with the highest effect on FHB severity and an explained phenotypic variation of 20%. In the homogeneous genetic background, *Qfhs.lfl-1BL* had also a major effect leading to a relative reduction of FHB severity by 42% (Fig. 3). This effect is comparable to the effect of the two Sumai 3 resistance QTL on chromosomes 3B (*Fhb1*) and 5A (*Qfhs.ifa-5A*) in the genetic background of elite European spring wheat with a relative effect on FHB severity of 33 and 32%, respectively (Miedaner et al. 2006). They jointly led to a relative reduction of FHB severity by 49%. However, when comparing these effects, it must be considered that *Qfhs.lfl-1BL* was verified in the same genetic background as the original mapping population which can lead to an overestimation of QTL effects. Thus, it might be that effects are smaller when transferring the QTL into another genetic background (Utz et al. 2000; Wilde et al. 2008). Analysis of variance revealed significant *Qfhs.lfl-1BL* × family and *Qfhs.lfl-1BL* × environment interactions (Table 1). It has been observed in many studies with European winter wheat that the presence and effect of a QTL is strongly influenced by the genetic background as well as by the environment (Draeger et al. 2007; Klahr et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008).

The relatively high phenotypic variation within the marker classes (Fig. 3) might be caused by other known or unknown QTL segregating in the genetic background. Recombination events between marker and QTL seem rather unlikely, because markers flanking both sides of the QTL peak were used for the selection of the 90 genotypes for QTL verification. When performing a statistical analysis for the individual families, the effect of *Qfhs.lfl-1BL* was only significant in two out of the four families (data not shown). This might be due to the limited number of lines within the individual families (ranging between 13 and 33) and the skewed segregation ratio between the resistant and the susceptible marker class as it has already been observed in the overall analysis combining the four families (Fig. 3). An alternative approach would have been to analyse only one family consisting of more lines, however, we wanted to make a more general conclusion about the effect of this QTL. Nevertheless, the results of the overall analysis clearly confirmed the effect and importance of *Qfhs.lfl-1BL* on FHB resistance in European winter wheat. The results of the original mapping study were also consistent with those of the verification study concerning the influence of *Qfhs.lfl-1BL* on heading date and plant height. A significant effect of *Qfhs.lfl-1BL* on heading date was detected in both studies, whereas plant height was not affected by this QTL (Fig. 3; Klahr et al. 2007). In the Apache/Biscay population the QTL on chromosome 1BL overlapped with QTL for plant height and heading date, in the History/Rubens population with a QTL for plant height and in the Romanus/Pirat population with a QTL for heading date (Holzapfel et al. 2008).

Altogether, *Qfhs.lfl-1BL* is an important component of FHB resistance in European winter wheat which has been identified so far in the cultivars Biscay, Cansas, History and Pirat. In addition, the importance of this QTL for a good FHB resistance level is highlighted by the fact that the most resistant lines with an absolute FHB severity below 16% carried the resistance allele at *Qfhs.lfl-1BL* (Fig. 3). With the study presented here, a good confidence is created that this QTL would be effective and valuable in backcross breeding programmes for FHB resistance. Together with the three QTL on chromosomes 2BL, 6AL and 7BS which had been verified previously (Häberle et al. 2007; Wilde et al. 2008), there are now four effective and validated QTL from adapted European resistance sources available which could be combined. The utilisation of these QTL to achieve higher levels of FHB resistance would be a complementary approach to the use of exotic resistance sources like Sumai 3, which also have some disadvantageous properties (e.g. bad agronomic performance or a high susceptibility against other diseases) and are not adapted to the Central European climate.

Acknowledgments We would like to thank P. Greim, E. Madge-Pimentel, S. Schmidt and the staff of the departments of Biotechnology and Wheat Breeding of the Bavarian State Research Centre for excellent technical assistance. We also thank W. von Borries-Eckendorf GmbH & Co KG (Leopoldshöhe, Germany) and Deutsche Saatveredelung AG (Leutewitz, Germany) for performing the field trials, and especially A. Jacobi and B. Schlieter for evaluating the population in the field trials in Leopoldshöhe and Leutewitz. We thank Volker Mohler for critically reading the manuscript. This project was part of the GABI-Canada (CGAT) cooperative project: reducing *Fusarium* toxins in wheat through genomics (Subproject C no. 0313711C), which was funded by the German Ministry of Education and Research.

References

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for *Fusarium* head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. Bioinformatics 20:2324–2326

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

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for *Fusarium* head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84–91

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. Plant Breed 128:1–26

Chardon F, Virlon B, Moreau L (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci

meta-analysis and synteny conservation with the rice genome. Genetics 168:2169–2185

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

Cuthbert PA, Somers DJ, Brulé-Babel A (2007) Mapping of *Fhb2* on chromosome 6BS: a gene controlling *Fusarium* head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:429–437

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

Fehr WR (1987) Principles of cultivar development. Theory and technique, vol 1. Macmillan, New York

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

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res 19:6553–6558

Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. Genetics 155:463–473

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

Hartl L, Mohler V, Zeller FJ, Hsam SLK, Schweizer G (1999) Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat. Genome 42:322–329

Holzapfel J, Voss H-H, Miedaner T, Korzun V, Häberle J, Schweizer G, Mohler V, Zimmermann G, Hartl L (2008) Inheritance of resistance to *Fusarium* head blight in three European winter wheat populations. Theor Appl Genet 117:1119–1128

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

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

Löffler M, Schön CC, Miedaner T (2009) Revealing the genetic architecture of FHB resistance in hexaploid wheat (Triticum aestivum L.) by QTL meta-analysis. Mol Breed. doi:10.1007/s11032-008-9250-y

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 09 December 2008)

Miedaner T, Gang G, Geiger HH (1996) Quantitative-genetic basis of aggressiveness of 42 isolates of *Fusarium culmorum* for winter rye head blight. Plant Dis 80:500–504

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, Wilde F, Steiner B, Buerstmayr H, Korzun V, Ebmeyer E (2006) Stacking quantitative trait loci (QTL) for *Fusarium* head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. Theor Appl Genet 112:562–569

Norton GJ, Aitkenhead MJ, Khowaja FS, Whalley WR, Price AH (2008) A bioinformatic and transcriptomic approach to identifying positional candidate genes without fine mapping: an example using rice root-growth QTLs. Genomics 92:344–352

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

Roeder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rong JK, Feltus FA, Waghmare VN, Pierce GJ, Chee PW et al (2007) Meta-analysis of polyploidy cotton QTL shows unequal contributions of subgenomes to a complex network of genes and gene clusters implicated in lint fiber development. Genetics 176:2577–2588

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacerlength polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8019

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 127:459–464

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, Zhou M, Lu W, Ohm H (2003) Detection of *Fusarium* head blight resistance QTL in a wheat population using bulked segregant analysis. Theor Appl Genet 106:1041–1047

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

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

Utz HF (2001) PLABSTAT: a computer programm for statistical analysis of plant breeding experiments. Version 2.P. Institute of Plant Breeding, Seed Science and Population Genetics. University of Hohenheim, Germany

Utz HF, Melchinger AE, Schön CC (2000) Bias and sampling error of the estimation of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849

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

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