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ORIGINAL PAPER

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Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx

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Abstract Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* and *F. culmorum*, can significantly reduce the grain quality of wheat (*Triticum aestivum* L.) due to mycotoxin contamination. The objective of this study was to identify quantitative trait loci (QTLs) for FHB resistance in a winter wheat population developed by crossing the resistant German cultivar Dream with the susceptible British cultivar Lynx. A total of 145 recombinant inbred lines (RILs) were evaluated following spray inoculation with a *F. culmorum* suspension in field trials in 2002 in four environments across Germany. Based on amplified fragment length polymorphism and simple sequence repeat marker data, a 1,734 cM linkage map was established assuming that the majority of the polymorphic parts of the genome were covered. The area under disease progress curve (AUDPC) was calculated based on the visually scored FHB symptoms. The population segregated quantitatively for FHB severity. Composite interval mapping analysis for means across the environments identified four FHB resistance QTLs on chromosomes 6AL, 1B, 2BL and 7BS. Individually the QTLs explained 19%, 12%, 11% and 21% of the phenotypic variance, respectively, and together accounted for 41%. The QTL alleles conferring resistance on 6AL, 2BL and 7BS originated from cv. Dream. The resistance QTL on chromosome 6AL partly overlapped with a QTL for plant height. The FHB resistance QTL on 7BS coincided with a QTL for heading date, but the additive effect on heading date was of minor importance. The resistance QTL on chromosome 1B was associated with the T1BL.1RS wheat-rye translocation of Lynx.

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Introduction

Fusarium head blight (FHB) of wheat is an economically important fungal disease in many wheat-growing areas worldwide. In addition to inflicting damage to the plant, *Fusarium graminearum* and *F. culmorum*, the most common causal pathogens of FHB, are capable of producing mycotoxins, including deoxinivalenol (DON), in infected grains (Parry et al. 1995). In fact, the most serious problem associated with FHB is the accumulation of mycotoxins in wheat and wheat products for human consumption. In 2004, the German Federal Ministry of Consumer Protection, Food and Agriculture released strict threshold values for mycotoxin content in cereals. The maximumly allowed DON contamination of bread wheat products for human consumption is 500 μg kg^{-1}, for bread and bakeries 350 μg kg^{-1} and for baby food 100 μg kg^{-1} (Anonymous 2004).

The cultivation of resistant wheat cultivars is the most promising strategy to reduce the risk of FHB epidemics. The FHB resistant spring wheat germplasm originating from Asia and South America as well as resistant winter wheat cultivars from Europe are widely recognized (Ruckenbauer et al. 2001). Unfortunately, the breeding of resistant cultivars is a difficult task, as reviewed by Rudd et al. (2001). Even the most promising sources have not shown complete resistance or immunity to FHB, and most of the sources of resistance are of exotic origin and not adapted to Central European

agricultural conditions. Climatic conditions greatly affect disease establishment, development and severity, resulting in large genotype-by-environment interactions and variable expression of disease symptoms (Parry et al. 1995; Miedaner et al. 2001).

Molecular mapping and the analysis of quantitative trait loci (QTLs) have assisted in clarifying the inheritance of FHB resistance in wheat. The identification of DNA markers linked to resistance QTLs enables marker-assisted selection.

In spring wheat, Sumai 3 and its relatives have been the main resistance donors for developing mapping populations. The most prominent FHB resistance QTL is located on the short arm of chromosome 3B (*Qfhs.ndsu-3BS*) and was identified by several research groups together with various other chromosomes associated with FHB resistance (Bai et al. 1999; Waldron et al. 1999; Gupta et al. 2000; Anderson et al. 2001; El-Badawy 2001; Buerstmayr et al. 2002, 2003; Zhou et al. 2002). The QTL analysis of resistant spring wheat cultivars from the Chinese gene pool not directly related with Sumai 3 were performed with Wangshuibai (Gonzalez-Hernandez et al. 2002), Ning894037 (Shen et al. 2003b), Huapei57-2 (Bourdoncle and Ohm 2003) and Wuhan-1 (Somers et al. 2003). A major FHB resistance QTL on chromosome 3BS was identified in all of these studies in identical or similar marker intervals as that of *Qfhs.ndsu-3BS*.

Molecular information on sources of resistance in addition to those of the Chinese gene pool is available for the Brazilian spring wheat cultivar Frontana (Steiner et al. 2004), Maringa, a resistant cultivar with Frontana as ancestor of the resistance (Somers et al. 2003), *Triticum macha* (Mentewab et al. 2000), *T. dicoccoides* (Otto et al. 2002; Stack et al. 2002) and *Lophopyrum elongatum* (Shen et al. 2004).

Only a few resistant winter wheat cultivars have been genetically analysed for FHB resistance to date. In a population derived from a cross between Fundulea 201R (resistant) and Patterson (susceptible), Shen et al. (2003a) identified QTLs associated with FHB resistance on chromosomes 1B, 3A, 3D and 5A. Gervais et al. (2003) analysed a Renan (resistant)/Recital (susceptible) population in field trials and identified three stable resistance QTLs—one on chromosome 2BS and two on chromosome 5A. Two of these resistance QTLs coincided with QTLs for plant height and/or flowering time. Several other QTLs with varying stability were identified on chromosomes 2A, 3A, 3B, 5D and 6D. An Arina (resistant)/Forno (susceptible) mapping population was studied by Paillard et al. (2004), who identified resistance QTLs on chromosomes 6DL, 5BL and 4AL. The resistance QTLs on 6DL and 5BL were linked with QTLs for plant height and/or heading date. Additional minor resistance QTLs were detected on chromosomes 2AL, 3AL, 3BL, 3DS and 5AL.

In the study reported here, the German winter wheat cultivar Dream was used as the source of FHB resistance to develop a mapping population of 145 recombinant inbred lines (RILs). The level of resistance to FHB of Dream is comparable to that of the Swiss winter wheat cultivar Arina (Buerstmayr et al. 2004). In order to identify QTLs contributing FHB resistance under field conditions and to develop molecular markers for marker-assisted selection, we genotyped the 145 lines of the Dream/Lynx population with molecular markers and evaluated the lines in field trials for FHB resistance following spray inoculation in four environments.

Materials and methods

Plant materials

A population of 145 $F_{7:9}$ RILs produced by single-seed descent (SSD) was generated by crossing the moderately resistant winter wheat (*Triticum aestivum* L.) cultivar Dream with the highly susceptible cultivar Lynx. Dream, a tall German cultivar with loose heads, was developed by Saatzucht Schweiger (Feldkirchen, Germany) under high natural FHB disease pressure. Dream (Disponent/Kronjuwel//Monopol/3/Orestis) was released in Germany in 1999. Lynx (CWW-4442-64/Rendezvous) is a British dwarf cultivar with dense heads developed by Cambridge Plant Breeders (Cambridge, UK). This population was kindly provided by Saatzucht Schweiger at the F_3 generation.

Field experiments

Field trials were performed in 2002 in four locations: Freising and Hohenheim in the south of Germany, Wetze and Wohlde in the north of Germany. The RILs were grown together with three replicated entries of the parental lines and four replicated entries of the cultivar Drifter. The experimental layout was a lattice design consisting of 36 blocks with ten lines per block and two replications in each environment. Each line was grown in two rows with a plot size of 0.6 m^2 . Plants were inoculated at anthesis with a spore suspension (100 ml m^{-2}) consisting of a mixture of two *Fusarium culmorum* isolates (5×10^5 conidia ml^{-1}) by spraying with an automatic small plot sprayer. The inoculum was produced as described by Miedaner et al. (1996). With the spray inoculation method, the combined effects of disease incidence (type I) and disease spread within the heads (type II) could be evaluated and described as field resistance. In order to take into account variations in flowering time between the lines, we carried out the inoculation procedure two or three times (Freising: 6 and 12 June; Hohenheim: 6, 9 and 12 June; Wetze: 4, 7 and 14 June; Wohlde: 6, 9 and 12 June). FHB severity was scored visually as the percentage of diseased spikelets per plot. Depending on the disease development at the respective location, the scoring of symptoms started 9 days or 10 days following the last inoculation and was

repeated once or twice at intervals of 3–5 days (Freising: 21 and 26 June; Hohenheim: 21, 24 and 27 June; Wetze: 24, 27 June and 1 July; Wohlde: 21, 24 and 27 June). Plant height was measured as the distance from the soil surface to the middle of the head. The heading date was expressed as the number of days from January 1 to heading, and spike compactness was estimated visually on a scale of 1 (loose) to 9 (compact). Both replications were inoculated, and all traits were scored in both replications.

Molecular markers

For the amplified fragment length polymorphism (AFLP) analysis (Vos et al. 1995), DNA was digested using two combinations of restriction enzymes (*Sse*8387I /*Mse*I or *Pst*I /*Mse*I). Restriction digestion, adapter ligation and PCR amplification were performed as described by Hartl et al. (1999) with some modifications. Genomic DNA (250 ng) was digested with the restriction enzymes *Sse*8387I or *Pst*I (2.5 U) and *Mse*I (1 U) for 2 h. For ligation, the *Sse*8387I or *Pst*I adapter (2.5 pmol) and the *Mse*I adapter (25 pmol) were added to the mixture using 1 U T4-DNA ligase and incubated for 8 h. In total, 43 *Sse*8387I /*Mse*I primer combinations with two selective nucleotides and 23 *Pst*I /*Mse*I primer combinations with three selective nucleotides on the 3′ end of either primer were applied for selective amplification. The selective amplification was carried out in 10 µl reaction volumes. The 5′ end of the *Sse*8387I and *Pst*I primers was labelled with fluorescein or cy3. The standard list for AFLP primer nomenclature (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html) was used. The names of AFLP loci were based on the applied primer combination followed by the estimated fragment size in base pairs. For example, S25M15_187 refers to a fragment of 187 bp amplified with *Sse*8387I primer 25 and *Mse*I primer 15. Nulli-tetrasomic lines of Chinese Spring (Sears 1966), kindly provided by F. Zeller, were used to assign AFLP markers to specific wheat chromosomes.

Based on the marker information of Roeder et al. (1998) and Gupta et al. (2002) and on that obtained from the GrainGenes database (http://wheat.pw.usda.gov/ggpages/maps.shtml), about 150 simple sequence repeats (SSRs) were screened between parental lines and, in the case of polymorphism, used for genotyping the RILs. The proprietary SSR GWM1011 was kindly provided by M. Roeder (Institute of Plant Genetics and Crop Plant Research, Gatersleben). SSRs were amplified according to Roeder et al. (1998) with minor modifications. Forward primers were usually labelled with fluorescein. Gels with non-labelled microsatellites were stained with Vistra Green (Amersham, Braunschweig) following electrophoresis.

The IAG95 marker has been developed from restriction fragment length polymorphism (RFLP) for the rye-derived powdery mildew resistance genes *Pm8*/*Pm17* of wheat. This marker enables the amplification of diagnostic DNA fragments from cultivars with the T1AL.1RS and T1BL.1RS wheat-rye translocation (Mohler et al. 2001). The sequence tagged site (STS) marker IAG95 was amplified as described by Mohler et al. (2001).

The DNA fragments were separated using 5% denaturing polyacrylamide gels under standard conditions. For fragment detection, the gels were scanned with a Typhoon 9200 (Amersham, Braunschweig) fluorescence scanner, the digital images analysed with the AFLP QUANTAR software (Keygene, Wageningen, The Netherlands) and visually reviewed.

Statistical analysis

The area under disease progress curve (AUDPC) was calculated for the visually scored FHB symptoms according to Buerstmayr et al. (2000). Lattice analysis of the phenotypic data for single environments was performed with PLABSTAT ver. 2N (Utz 1995). Adjusted means of each environment were used for the analysis of variance across the four environments and for calculating the broad-sense heritability according to Fehr (1987). The SAS programme (SAS Institute 2001) was used for correlation and regression analysis. Spearman correlation coefficients between the recorded traits were estimated based on means across the environments.

Genetic maps for segregating marker data were constructed with MAPMAKER 3.0B (Lander et al. 1987) using the Haldane transformation. For assigning the molecular markers to linkage groups, we employed a minimum logarithm of odds (LOD) of 3.0. The most likely order of markers within a linkage group was established using the ORDER, COMPARE, RIPPLE and TRY commands.

Composite interval mapping (CIM) for means over the environments was carried out using PLABQTL software ver. 1.1 (Utz and Melchinger 1996). Cofactor selection was done automatically with an F-to-enter threshold of ten. Empirical LOD thresholds were estimated after 1,000 permutation tests with an error level of $\alpha = 5\%$. Based on the results of the permutation tests, a LOD threshold of 3.1 for CIM was applied for the following QTL analysis. Fivefold cross-validation was conducted by CIM with a LOD threshold of 3.1 for disease severity averaged over the environments to test sampling effects on QTL estimation (position and effects). With 80% of the genotypes, QTLs were estimated; with the remaining 20%, a validation was performed. The cross-validation was carried out following random permutations of the lines resulting in 1,000 independent validation subsets.

The SMODEL option of PLABQTL was used to test additive × additive epistatic interactions between the detected QTLs.

Results

Field evaluation of FHB severity

The lines of the Dream/Lynx population displayed a quantitative distribution for means of FHB severity across the four environments (Fig. 1). No line was more resistant than Dream, but five lines showed a significantly higher FHB severity relative to the susceptible parent Lynx. Means and ranges of the scored traits of the population Dream/Lynx and of the parental lines are presented in Table 1.

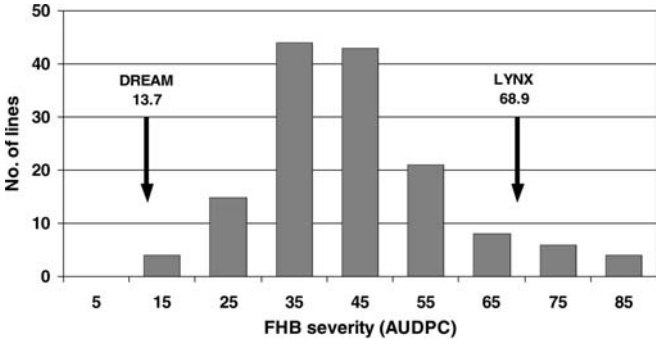

Fig. 1 Frequency distribution of the 145 RILs for FHB severity. Data are based on means across the four environments. *Arrows* indicate values of the parental lines

The analysis of variance for FHB severity revealed a significant variation for environments, genotypes and genotype-by-environment interaction (Table 2). Broad-sense heritability for disease severity was very high ($H = 92\%$; the 95% confidence interval was 90–94%). Broad-sense heritabilities for plant height and heading date were likewise high with $H = 95\%$ and 92%, respectively, while broad-sense heritability for ear compactness was moderate with $H = 75\%$. Correlation coefficients for disease severity between single environments ranged from 0.67 to 0.80 ($P < 0.0001$).

A significant negative correlation ($P < 0.0001$) was found between FHB severity and plant height ($r = -0.33$) and between FHB severity and heading date ($r = -0.43$). Taller lines and lines with a later heading date tended to show less FHB infection. A significant correlation between FHB severity and ear compactness was not observed.

Molecular map

Of the 150 SSR markers tested, 45 were polymorphic between Dream and Lynx, which confers a degree of polymorphism of 30%. The genetic map was established on the basis of 617 AFLPs, 45 SSRs and the STS marker IAG95. None of the 38 unlinked markers showed a significant association with FHB resistance ($P < 0.05$) tested by single marker regression. For the QTL analysis, co-segregating or tightly clustered markers were excluded, resulting in a core map of 38 linkage groups with 283 markers covering 1,734 cM of the wheat genome.

The linkage groups were assigned to chromosomes by 24 AFLP markers, which were localized with

Table 1 Means and ranges of the estimated traits for means across environments in 2002 of the population Dream/Lynx and the parental lines

Trait	Population means	Population range	LSD5%	Dream	Lynx
FHB severity (AUDPC)	45	14–86	11.3	14	69
Plant height (cm)	95	70–113	6.5	99	77
Ear compactness (1–9)	6	3–9	1.6	3	8
Heading date (days after 1 January)	156	153–161	1.3	159	156

Table 2 Analysis of variance of 145 RILs for mean FHB severity AUDPC, plant height, ear compactness and heading date evaluated in four experiments in 2002

Source	*df*	FHB AUDPC		Plant height		Ear compactness		Heading date	
		Variance component	*F*	Variance component	*F*	Variance component	*F*	Variance component	*F*
Environments (E)	3	118.8	262.1*	167.2	1100.7*	0.2	21.1*	5.1	836.2*
Genotypes (G)	144	195.0	12.8*	111.0	21.1*	1.0	4.0*	2.4	11.9*
G × E	432	42.3	4.7*	16.8	4.2*	0.8	2.3*	0.3	1.5*
Error	580	23.7	–	5.3	–	0.6	–	0.6	–

*Significant at $P < 0.0001$

nulli-tetrasomic lines and 31 SSR markers using the reference maps of Roeder et al. (1998), Gupta et al. (2002) and of the GrainGenes database (http://wheat.pw.usda.gov/ggpages/maps.shtml). In total, 13 linkage groups with 100 markers were located on the A genome, 11 linkage groups with 98 markers on the B genome, five groups with 21 markers on the D genome and nine linkage groups with 64 markers remained unassigned. Chromosomes 4B, 1D, 4D and 6D are not represented by any linkage group of the Dream/Lynx map.

A linkage group obtained for chromosome 1B consisted of 39 markers, of which 33 AFLP markers, the STS marker IAG95 and the SSR marker GWM18 clustered tightly at the end of that group, spanning an estimated distance of only 3.1 cM. In this part of the map, only five putative recombination events were found among the 145 lines. The marker IAG95, which is diagnostic for wheat-rye translocations mapped at the end of that group, completely linked with four AFLPs. The banding pattern of IAG95 and protein electrophoresis of gliadins (B. Killermann, personal communication) showed that Lynx carries the 1BL/1RS wheat-rye translocation. The population showed distorted segregation for 1BL/1RS with approximately one-third of the lines carrying the translocated chromosome. According to Roeder at al. (1998), the SSR marker GWM18 is located close to the centromere of chromosome 1B and can not be clearly assigned to the long or to the short arm and has also not been mapped on the translocated rye segment. In our map, GWM18 was linked to IAG95 with an estimated distance of 2.0 cM. Of the 33 AFLP markers representing this 3.1 cM region, two were used for QTL analysis (Fig. 2).

QTL analysis

Based on the CIM approach, four FHB resistance QTLs were identified for the mean FHB severity across the four environments. These QTLs were located on chromosomes 6AL, 1B, 2BL and 7BS, accounting for 19%,

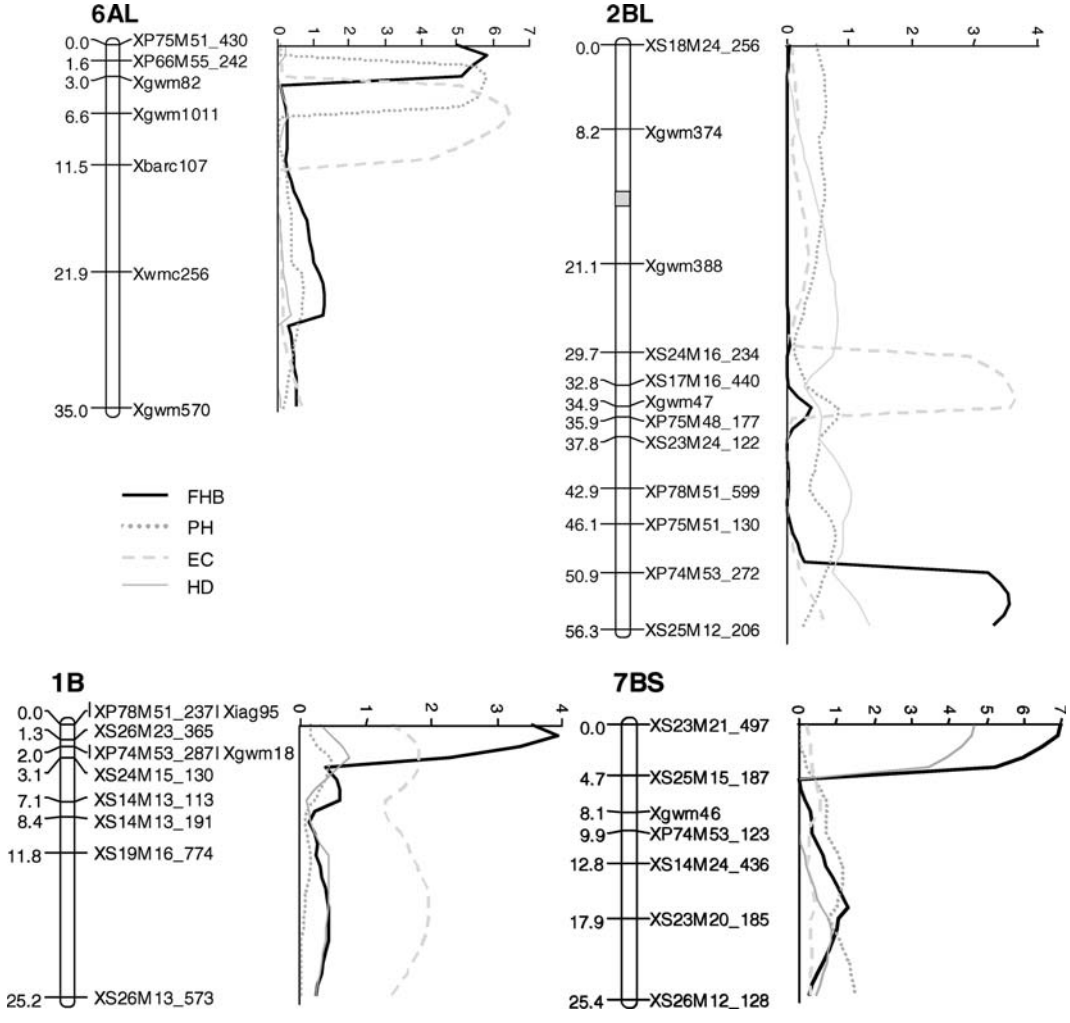

Fig. 2 The CIM analysis of FHB severity (*FHB*), plant height (*PH*), ear compactness (*EC*) and heading date (*HD*) for means across environments corresponding to chromosomes 6AL, 1B, 2BL and 7BS

Table 3 Summary of the QTL analysis for FHB resistance, plant height, ear compactness and heading date of the Dream/Lynx population

Trait	Marker interval	Chromosomal location	Donor[a]	LOD	R^2 %[b]	Additive effect
FHB resistance	XP77M51_430-XP66M55_242	6AL	Dream	5.8	19	5.4
	XP78M51_237-XS26M23_365	1B-1	Lynx	3.9	12	4.2
	XP74M53_272-XS25M12_206	2BL	Dream	3.6	11	4.1
	XS25M15_187-XS23M21_497	7BS	Dream	7.0	21	5.8
Simultaneous fit				18.2	41	
Plant height	XS24M16_485-XP68M52_476	5A	Lynx	3.2	10	3.2
	XP66M55_242-Xgwm82	6AL	Dream	5.8	18	5.8
Simultaneous fit				8.9	23	
Ear compactness	Xgwm1011-Xbarc107	6AL	Dream	6.5	20	0.5
	XS17M16_440-XP75M48_177	2B	Lynx	3.7	11	0.3
	XP68M52_624-XS15M24_238	2DS	Dream	7.1	20	0.5
Simultaneous fit				16.0	37	
Heading date	XS20M20_243-XP75M60_415	1B-2	Dream	4.5	14	0.5
	XS25M15_187-XS23M21_497	7BS	Dream	4.7	14	0.5
	Xgwm302-XP66M55_236	7BL-1	Dream	5.9	17	0.6
	XP78M55_748-XS14M21_286	X1	Dream	4.2	12	0.5
Simultaneous fit				18.7	42	

[a]Donor allele confers fewer diseased heads, taller plants, loose heads and later heading, respectively
[b]R^2 %, Percentage of explained phenotypic variance

12%, 11% and 21% of the phenotypic variance (Table 3). For the QTLs on 6AL, 2BL and 7BS, Dream contributed the allele conferring FHB resistance, while the favourable allele on 1B was inherited by the susceptible parent Lynx. The LOD profiles for the QTLs on 6AL, 1B, 2BL and 7BS indicated that these four major QTLs were located close to or at the end of the respective linkage group (Fig. 2).

Fivefold cross-validation tests clearly confirmed the presence of the QTLs on chromosomes 6AL and 7BS. In 980 of the 1,000 validation subsets with randomly arranged lines, the QTL on 6AL was identified within the expected or adjacent marker interval. The QTL on chromosome 7BS was detected in 998 of the subsets in the identical marker interval compared to the QTL analysis with all lines of the population. The QTL on chromosome 1B was identified in 504 validation subsets in the expected marker interval. The QTL on the long arm of chromosome 2B was detected in just 367 validation subsets in the corresponding marker interval. The mean explained phenotypic variance of all validation subsets of the QTLs on 6AL and 7BS were in a similar range compared to the CIM analysis with all lines of the population. The mean explained phenotypic variance of the QTL on chromosome 1B for the validation subsets was about 25% of that of the CIM analyses with all lines. Fivefold cross-validation showed that the effect of the QTL on 2BL was rather unstable (Table 4).

Two QTLs for plant height, three QTLs for ear compactness and four QTLs for heading date were identified by CIM analysis for means across environments (Table 3). The FHB resistance QTL on 6AL partly overlapped with QTLs for plant height and ear compactness. The resistance QTL on 7BS coincided with a QTL for heading date. Lines carrying the resistance alleles on 6AL, 1B and 7BS showed less severe FHB, with an average AUDPC value of 34, than lines possessing the opposite alleles, which had an average AUDPC of 63. The RILs carrying the resistance allele on 6AL were 6–11 cm taller than those without this resistance allele, and lines carrying the resistance allele on 7BS were 1–2 days later in heading than lines with the alternative allele (Fig. 3).

Because of the segregation distortion of the markers at the QTL regions on chromosomes 6AL, 1B and 7BS, just a few lines possessing only the favourable allele of the 1B chromosomal region were available.

No additive × additive epistatic interactions between the detected resistance QTLs were found by interval analysis with PLABQTL (SMODEL) that significantly increased or decreased the percentage of explained phenotypic variance.

Table 4 Summary of the fivefold cross-validation of the resistance QTLs on 6AL, 1B, 2BL and 7BS identified by CIM

QTL[a]	6AL	1B	2BL	7BS	All QTLs
Mean R^2 % of calibration	13	6	2	15	34
Mean R^2 % of validation	13	3	1	15	28
Frequency	98.2	50.4	36.7	99.8	

[a]The explained phenotypic variance (R^2 %) for the calibration and validation subsets and frequency of QTL detection within the expected marker interval for the resistance QTLs on 6AL, 1B, 2BL and 7BS for FHB means are shown

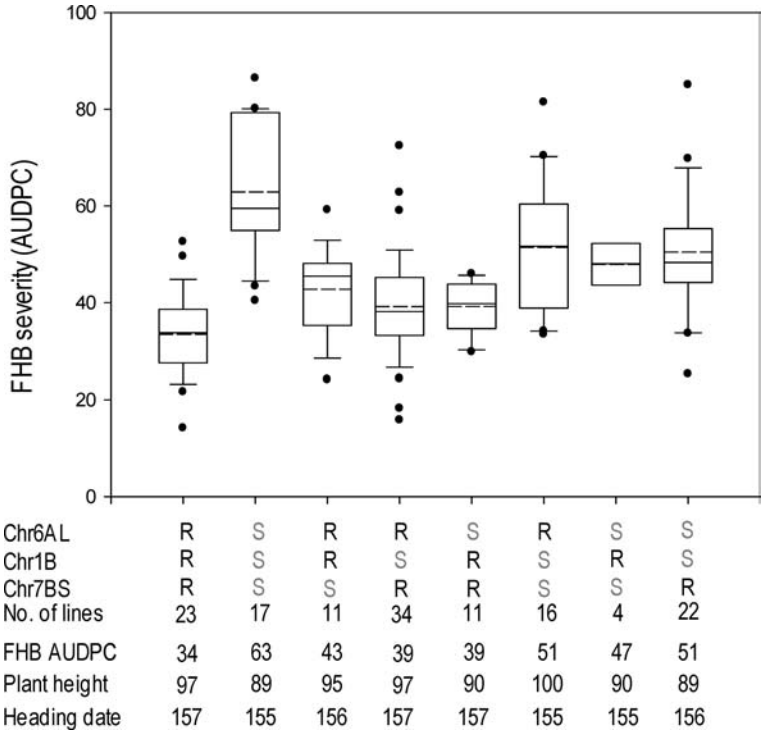

Fig. 3 Boxplot distributions of lines possessing alternative alleles in the QTL regions on 6AL, 1B and 7BS for FHB severity (AUDPC). Data are based on mean values over four experiments. Genotypes were classified by markers linked to the corresponding QTLs; seven lines were not classified because of missing marker values. *R* allele contributing to FHB resistance, *S* alternative allele, *dashed lines* means, *solid lines* median

Discussion

In the present study, the winter wheat population Dream/Lynx was evaluated for FHB severity following spray inoculation in order to identify important resistance components. Schroeder and Christensen (1963) have described two different types of resistance—resistance to initial FHB infection (type I) and resistance to the spread of the pathogen in the heads (type II). Following spray inoculation, the combined effects of types I and II resistance can be investigated.

In the Dream/Lynx population, significant FHB resistance QTLs were detected on chromosomes 6AL, 1B, 2BL and 7BS. Paillard et al. (2004) reported that the long arm of chromosome 6A is associated with FHB resistance and identified a resistance QTL at the distal end of chromosome 6AL in one single environment. Because of the mapped SSR markers, the QTL on chromosome 6AL identified in the Dream/Lynx population is assumed to be located close to the centromere. This resistance QTL partly overlapped with QTLs for plant height and ear compactness. Plant height is also known to be quantitatively inherited, and several dwarfing genes (*Rht*) are commonly present in European cultivars. To our knowledge, no information is available at the present time on dwarfing genes located on the long arm of chromosome 6A. A negative correlation between plant height and FHB resistance following spray inoculation was observed in the present study and in several other studies of winter wheat (Mesterházy 1995; Hilton et al. 1999; Buerstmayr et al. 2000; Gervais et al. 2003; Paillard et al. 2004). The coinciding of FHB resistance QTLs with QTLs for plant height was also reported previously (Gervais et al. 2003; Paillard et al. 2004). This correlation of FHB resistance and plant height following spray inoculation as well as overlapping QTL regions suggests a genetic basis—either linkage between loci or pleiotropy.

With respect to the QTL on chromosome 1B identified in this study, the susceptible parent Lynx contributed the allele conferring resistance. This resistance QTL is most likely located at or closely linked to the T1BL.1RS wheat-rye translocation derived from Lynx. Markers in that region were tightly linked and showed segregation distortion, including the STS marker IAG95 and the SSR marker GWM18. Calculation of an unambiguous order of markers in this QTL region was not feasible because recombination events were very rare. Ittu et al. (2000) reported a significant increase in FHB resistance associated with the *GliR1* allele, which is positioned on the translocated rye chromosome arm 1RS. These investigators suggested that a resistance QTL may not be on the translocated rye arm 1RS, but instead linked with the *GliR1* allele. Shen et al. (2003a) reported a resistance QTL on chromosome 1B based on the position of GWM18 in a similar region as in our study. The favourable allele of this QTL was conferred by the parental line Fundulea 201R, which also carries the T1BL.1RS translocation. These authors assumed that the resistance QTL is outside the translocated 1RS chromosome because of recombination between markers in the QTL interval.

The resistance QTL on the long arm of chromosome 2B identified in the Dream/Lynx population showed a very minor effect when tested with fivefold cross-validation. Nevertheless, the use of CIM enhances

the power for QTL detection in the background of major QTLs. We suggest that the QTL on 2BL is not a ghost but that the effect of this locus on FHB resistance needs to be verified. Zhou et al. (2002) detected a QTL on chromosome 2BL associated with resistance to fungal spread following point inoculation (type II resistance) in the RIL spring wheat population Ning7840/Clark. The small effect of the QTL on 2BL in the present study was possibly due to the spray inoculation method used (combined type I and II resistance). When the mapped SSR markers in the Ning7840/Clark and in the Dream/Lynx population are compared with the reference map of Roeder et al. (1998), it seems possible but unlikely that these QTLs are identical. Zhou et al. (2002) also reported a significant additive × additive epistasis effect of the major 3BS QTL over the QTL on 2BL. Additive × additive epistatic interactions of FHB resistance loci may play a role in wheat (Snijders 1990; Bai et al. 2000). In our study, additive effects of the resistance QTLs on 6AL, 1B, 2BL and 7BS accounted for most but not all of the genetic variance for FHB resistance. The reason why we did not detect significant additive × additive epistasis interactions between the identified resistance QTLs is probably due to the small population size. A precise estimation of QTL interactions requires a population with more lines (Melchinger et al. 1998).

The resistance QTL located on chromosome 7BS was associated with the resistant parent Dream and explained the highest proportion of phenotypic variance of all the QTLs detected. This QTL on 7BS coincided with a QTL for heading date, and a significant negative correlation between FHB severity and date of heading was found. Negative correlations between FHB severity and heading date/flowering time as well as overlapping of resistance QTLs with QTLs for heading date/flowering were also reported by Gervais et al. (2003) and Paillard et al. (2004). Lines carrying favourable alleles at 7BS were on average 1–2 days later in heading. This was a comparatively small effect; there was a total range of about 8 days in heading date between the lines of the population. Gupta et al. (2000) reported that in the Ning7840/Freedom population, the SSR marker GWM46 was significantly associated with FHB resistance in field trials following inoculation with FHB infected maize residues; however, no association between GWM46 and FHB resistance was found in the greenhouse evaluations using single point inoculation. The GWM46 is located on the short arm of chromosome 7B (Roeder et al. 1998) and was mapped in our population close to the identified resistance QTL. El-Badawy (2001) also detected a resistance QTL on chromosome 7BS close to GWM46 following spray inoculation in a population developed by crossing Sagvari/NobeokaBozu//MiniMano/Sumai 3/3/Apollo. This resistance QTL on 7BS is therefore considered to be located in a similar chromosomal region as found in the present investigation.

The four resistance QTLs on chromosomes 6AL, 1B, 2BL and 7BS are located at the very end of the corresponding linkage groups. In the case of the 1B chromosome, this position of the resistance QTL is probably due to the translocation of the rye chromosome. The mapped SSR markers of the linkage group with the QTL on 6AL indicates that this QTL is located close to the centromere, a genomic region where recombination events are rare. Another possible reason for the position of the resistance QTLs at the end of the linkage groups is that our mapping population is based on European cultivars with a similar genetic background. As a result of phenotypic selection of the resistant parent Dream, adjacent regions to the QTLs with negative effects on agronomic performance were excluded, resulting in regions with a poor polymorphism of molecular markers next to the QTL regions.

Although we were able to generate 663 polymorphic markers, only 283 remained in the core map because a great proportion of the markers clustered very tightly. This indicates that Dream and Lynx possess large genomic regions that are highly conserved. This is especially true for the D genome. Due to the incomplete coverage of our genetic map, it is possible that some resistance QTLs could possibly have remained undetected; however, we believe that the most important genomic regions with respect to FHB resistance were recorded and that the application of additional markers will not produce a map that covers the whole genome or reveals additional major FHB resistance loci. This assumption was also supported by the results of the single marker regression analysis. None of the unlinked markers showed a significant association with FHB resistance.

Each of the individual FHB resistance QTLs on 6AL, 1B, 2BL and 7BS in our study explained between 11% and 21% of the total phenotypic variance and together accounted for 41%, which is in a similar range as for other QTL studies on FHB resistance in winter wheat (Gervais et al. 2003; Shen et al. 2003a; Paillard et al. 2004).

Previously published research focussed on the QTL mapping of FHB resistance in spring wheat, mainly using Sumai 3 and its derivatives as the resistance donor (Bai et al. 1999; Waldron et al. 1999; Gupta et al. 2000; Anderson et al. 2001; El-Badawy 2001; Buerstmayr et al. 2002; Zhou et al. 2002) or other resistance sources from the Chinese gene pool (Gonzalez-Hernandez et al. 2002; Bourdoncle and Ohm 2003; Shen et al. 2003b; Somers et al. 2003). In these studies, a major QTL was identified at the distal end of chromosome 3BS together with some other resistance QTLs distributed over the wheat genome. Bimodal frequency distributions for FHB severity suggesting that a few major QTLs control FHB resistance were reported for spring wheat (Bai et al. 1999; Waldron et al. 1999; Buerstmayr et al. 2002). In the present study, FHB severity showed a more quantitative frequency distribution, indicating an oligogenic to polygenic inheritance of FHB resistance. Similar results

were also found by Gervais et al. (2003), Shen et al. (2003a) and Paillard et al. (2004), suggesting that FHB resistance in winter wheat is controlled by several loci in different genomic regions, each with moderate to small individual effects, compared to the major QTL *Qfhs.ndsu-3BS* in spring wheat. This major QTL on 3BS in spring wheat explained up to 60% of the phenotypic variance when assessed by point inoculation (Buerstmayr et al. 2002). To date, there are only a few studies which have combined spring wheat from China and spray inoculation. El-Badawy (2001) detected a resistance QTL on 3BS linked to GWM493 that explained 12% of the phenotypic variance following spray inoculation in a Sagvari/NobeokaBozu//MiniMano/Sumai 3/3/Apollo population. Buerstmayr et al. (2003) reported that the QTL on 3BS originating from CM82036 (Sumai 3/Thornbird) explained 20% of the phenotypic variance following spray inoculation, which is quite similar compared to the QTL effects in our study. Somers et al. (2003) identified a major resistance QTL at the distal end of 3BS which was derived from Wuhan-1 following point inoculation, but not following spray inoculation. These results indicate that the QTL on 3BS is associated mainly with type II resistance. Therefore, we suggest that a higher proportion of the explained phenotypic variance is restricted to the QTL on 3BS based on the analysis of type II resistance using single spikelet inoculation.

Highly resistant germplasm, like Sumai 3 and other spring wheats from China, are not adapted to Central European conditions and possess unfavourable agronomic characteristics. The introgression of resistance QTLs from these sources into breeding materials is feasible but would be time-consuming because several backcross generations would be necessary to restore adapted genotypes. In contrast to Sumai 3, the resistance donor Dream used in this study is well adapted to European climatic conditions. While the QTLs with major effects on FHB resistance of Sumai 3 and its relatives are located on chromosomes 3BS, 6BS and 5A (Anderson et al. 2001; Buerstmary et al. 2002), the identified resistance QTLs conferred by the cultivar Dream were located on different chromosomes (6AL, 2BL and 7BS). These resistance QTLs could be valuable for winter wheat breeding because they enable the pyramiding of resistance genes and may also prevent a potential breakdown of resistance.

Future investigations involve attempts to fine-map the QTL regions on chromosomes 6AL, 2BL and 7BS and to develop diagnostic markers. With the identification of suitable markers linked to these resistance QTLs, our aim is to validate these QTLs in an independent genetic background and to develop near-isogenic lines (NILs) for each QTL.

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