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H. Buerstmayr · M. Lemmens · L. Hartl · L. Doldi
B. Steiner · M. Stierschneider · P. Ruckenbauer

Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance)

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Abstract Fusarium head blight (FHB, scab) is a fungal disease of wheat and other small cereals that is found in both temperate and semi-tropical regions. FHB causes severe yield and quality losses, but the most-serious concern is the possible mycotoxin contamination of cereal food and feed. Breeding for FHB resistance by conventional selection is feasible, but tedious and expensive. This study was conducted to identify and map DNA markers associated with FHB resistance genes in wheat. A population of 364 F_1-derived doubled-haploid (DH) lines from the cross 'CM-82036' (resistant)/'Remus' (susceptible) was evaluated for Type II resistance (spread within the spike) during 2 years under field conditions. Marker analysis was performed on 239 randomly chosen DH lines. Different marker types were applied, with an emphasis on AFLP and SSR markers. Analysis of variance, as well as simple and composite interval mapping, were applied. Three genomic regions were found significantly associated with FHB resistance. The most-prominent effect was detected on the short arm of chromosome 3B, explaining up to 60% of the phenotypic variance for Type II FHB resistance. A further QTL was located on chromosome 5A and a third one on 1B. The QTL regions on 3B and 5A were tagged with flanking SSR markers, the 1B QTL was found associated with the high-molecular-weight glutenin locus. These results indicate that FHB resistance is under control of a few major QTLs operating together with unknown numbers of minor genes. Marker-assisted selection for these major QTLs involved in FHB resistance appears feasible and should accelerate the development of resistant and agronomically improved wheat cultivars.

Keywords *Triticum aestivum* · QTL · Fusarium head blight · Scab · Resistance

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H. Buerstmayr (✉) · M. Lemmens · L. Doldi · B. Steiner
M. Stierschneider · P. Ruckenbauer
IFA-Tulln, Institute for Agrobiotechnology,
Department of Biotechnology in Plant Produktion,
Konrad Lorenz Strasse 20, A-3430 Tulln, Austria
e-mail: buerst@ifa-tulln.ac.at
Fax: +43-2272-66280-77

L. Hartl
Bayerische Landesanstalt für Bodenkultur und Pflanzenbau,
Vöttingerstrasse 38, D-85354 Freising, Germany

Introduction

Fungi of the group *Fusarium spp.* have long been recognized as pathogens of many plant species. Wheat and other small grain cereals may be attacked by *Fusarium spp*. on different plant organs, but the infestation of the ear appears to be most critical, leading to Fusarium head blight (FHB), also known as scab. The risk of a FHB epidemic is high when the natural inoculum is abundant (e.g. conidia or ascospores on crop debris on the soil surface) during warm and humid weather at flowering. Fusarium head blight of wheat is a common disease in many wheat-growing regions worldwide with increasing importance in recent years. Changes in crop management practices (minimum or reduced tillage), changes in rainfall patterns and a low resistance level among current cultivars are considered to be the principal causes for severe FHB epidemics in parts of the USA and Canada since 1993 (Dill-Macky and Jones 1997; McMullen et al. 1997). Despite the range of species implicated in the disease, *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium avenaceum* appear to predominate worldwide (Parry et al. 1995). The disease may cause severe losses in grain yield and grain quality. Furthermore, the most-serious threat of FHB is the contamination of the harvested grain with mycotoxins. Chemical and agronomic control measures are either not available or not feasible. The cultivation of genetically resistant cultivars is the most cost-effective method to control the disease. Genetic variation for resistance to FHB is well documented in wheat and its relatives (Mesterhazy 1983, 1995; Snijders 1990; Saur 1991; Wilcoxson et al. 1992; Lemmens et al. 1993; Buerstmayr et al. 1996a, b). The classical model proposed by Schroeder and Christensen (1963), who suggest two components of resistance (Type I and Type II), has been widely accepted by most

authors. Type I resistance operates against initial infection and Type II against spread of the pathogen within the host. Further types or components of FHB resistance have been described (Mesterhazy 1995). In recent years, increased efforts have been devoted to FHB resistance breeding, with successes reported from Hungary (Mesterhazy 1995, 1997), China (Chen et al. 1997), USA (Rudd 1997; Stack et al. 1997), Canada (Gilbert and Tekauz 2000) and Austria (Buerstmayr et al. 2000). However, breeding for FHB resistance is difficult for various reasons: (1) the most-resistant germplasm is of exotic origin and has poor agronomic traits, (2) the inheritance is oligogenic to polygenic, and (3) screening for FHB resistance is environmentally biased, tedious and expensive. The application of molecular markers could complement classical plant breeding. A number of successful reports on the development and application of molecular markers in wheat resistance improvement have been published; for instance for resistance to powdery mildew, leaf rust, hessian fly, cereal cyst nematode and frost (see reviews by Langridge and Chalmers 1998; Gupta et al. 1999).

Only few results are available until now on molecular mapping of FHB resistance, although several such projects are underway worldwide (Bai et al. 1999; Waldron et al. 1999; Ban 2000a; Gilbert and Tekauz 2000; Anderson et al. 2001). In 1995 this work was started with the objective to identify and localize individual genes (QTLs) responsible for the expression of resistance against FHB and quantify the magnitude of their effects in a highly resistant spring wheat genotype. The ultimate goal of the marker analysis is to develop tools that are useful for marker-assisted selection (MAS) in practical breeding programs.

Materials and methods

Plant materials

A population of recombinant F_1-derived doubled-haploid (DH) lines was used for this research. The parental lines were 'CM-82036–1TP-10Y-OST-10Y-OM-OFC' (abbreviated to 'CM-82036' in the following text) and 'Remus'. In total 364 DH lines were tested for FHB resistance and 239 lines were genotyped with markers. 'CM-82036' originates from the cross 'Sumai#3'/'Thornbird-S' and was developed in a shuttle breeding program between CIMMYT Mexico and South-America (J Dubin, personal communication). It has been evaluated during several seasons for resistance against FHB and proved to express a very high level, similar to that of 'Sumai#3' (Buerstmayr et al. 1996a), but showed a better agronomic adaptation. 'Remus' ('Sappo'/'Mex'//'Famos') is a spring wheat cultivar developed at the Bavarian State Institute for Agronomy in Freising, Germany. It possesses well-adapted agronomic characters for cultivation in central Europe, but it is highly susceptible to Fusarium ear infection.

In 1994/1995 crosses were made between the parental lines. In 1995/96 doubled-haploids were developed from greenhouse-grown F_1 plants using the wheat by maize pollination procedure. Maize pollination, embryo rescue and embryo regeneration were carried out as described by Bitsch et al. (1998). In vitro regenerated haploid plantlets were transplanted to soil, cultivated in the greenhouse covered by a transparent plastic shelter to ensure high humidity at 15/10°C (day/night). After 1 week the plants were removed from the shelter and cultivated on an open bench in the greenhouse. Plants at the three to five tiller stage were removed from the soil, roots washed out with tap water and cut to 2-cm length. To achieve chromosome doubling the crowns of the haploids were submerged in colchicine solution (0.1% colchicine, 2% DMSO, 10 drops L^{-1} of Tween-20) for five hours at room temperature and then washed in tap water for 30 min. The treated plants were potted in soil and maintained up to maturity. Seed harvested from individual DH plants was multiplied in the greenhouse and in field nurseries.

Field experiments for evaluation of Fusarium head blight resistance

The DH population and the parental lines were tested during a 2-year period (1999 and 2000) at the experimental field of IFA-Tulln, 30 km west of Vienna, at 180 m above sea level. The soil type is a meadow-czernosem; the preceding crop was corn (*Zea mays* L.). The average temperature and annual precipitation were 10.4°C and 611 mm (1999), and 11.6°C and 476 mm (2000). To control seed-borne diseases the seed was treated with 'Rovral-TS' (Rhone-Poulenc, Lyon, France) seed dressing at a rate of 1.5 g kg^{-1} of seed. The experimental layout was a randomized complete block design with four replications. Sowing time was the first half of March in both years. The replications were purposely sown several days apart, resulting in a 1 to 3-days difference in anthesis between replications. Plots consisted of double rows with 17-cm row spacing and 1-m length. Sowing density was 6 g of seeds per plot. At the beginning of April, fertilizer was applied at a rate of 90 kg ha^{-1} N, 14 kg ha^{-1} P, 23 kg ha^{-1} K and 12 kg ha^{-1} Mg. Weed control was done by spraying 15 g ha^{-1} of 'Express' (Du Pont, Cernay, France) and 0.5 l ha^{-1} of 'Starane 250' (Dow Elanco, King's Lynn, UK) in 300 l ha^{-1} of water at the late tillering stage. Replications 1 and 3 were inoculated with *F. graminearum* and replications 2 and 4 with *F. culmorum*.

Inoculum production, inoculation procedure and disease evaluation

Two single-spore Fusarium isolates were used: one *F. graminearum* and one *F. culmorum*. Macroconidia of the *F. culmorum* isolate 'IPO 39–01' were prepared as described in Buerstmayr et al. (2000). Macroconidia of the *F. graminearum* isolate 'IFA 65' were produced in liquid mungbean medium. Dry mungbean [*Vigna radiata* (L.) Wilczek.] seeds (20 g l^{-1}) were boiled in distilled water for 20 min. The liquid phase was transferred to glass bottles and autoclaved. After inoculation continuous aeration with sterile air caused macroconidia development within 1 week. The inoculum was stored at 4°C until use. Conidia concentrations were determined using a Bürker-Türk counting chamber and adjusted to the desired concentration with distilled water. The final spore concentration used for inoculations was 5×10^4 ml^{-1} in all cases except for *F. graminearum* in 2000 where it was 3.125×10^3 ml^{-1}.

At anthesis ten heads per plot were inoculated. A needle was dipped in spore suspension and then punched through a spikelet slightly above the center of the head. Inoculated heads were marked with self-adhesive paper labels. On days 10, 14, 18 and 22 after inoculation, FHB symptoms were recorded. Two FHB related traits were assessed. (1) The number of spikelets with visible FHB symptoms from the infection point downwards over all ten heads was recorded. (2) Susceptible genotypes frequently show that the part of the head above the point of infection wilts and bleaches in addition to, or even without, developing typical fungal symptoms. The proportion of inoculated heads with such 'wilting' symptoms was recorded.

Molecular markers

RFLP analysis

Leaf tissue of the parents and the DH lines was collected for DNA extraction. DNA preparation, restriction digestion, electrophoresis, Southern blotting, hybridization and membrane washing were all performed as described by Hoisington et al. (1994). RFLP clones

were obtained from the collection maintained at CIMMYT (Mexico) with permission of B.S. Gill (KSU clones) and M.E. Sorrells (WG, BCD and CDO clones). RFLP clones were first screened for DNA polymorphism by probing on small 6×10-cm membranes blotted with DNA of the parental genotypes, digested with one of the following enzymes: *Hind*III, *Bam*HI, *Eco*RI, *Eco*RV, *Xba*I. Polymorphic probes were then applied to the DH population.

AFLP analysis

Restriction digestion (with *Sse*8387I and *Mse*I), adapter ligation and PCR amplification were carried out as described by Hartl et al. (1999). In total 29 AFLP primer combinations with two selective nucleotides on the 3´ end of either primer were applied for selective amplification. The 5´ end of the selective *Sse*8387I-primer was labeled with either fluorescin or IRD800. Electrophoresis and gel analysis for fluorescin-labeled AFLPs was done on a Fluorimager 595 (Molecular Dynamics) as described by Hartl et al. (1999). IRD800-labeled AFLPs were analysed on a LI-COR 4200 DNA analyzer. To the PCR reaction, 6 µl of formamide tracking dye (Amersham # 93–79448) was added. Amplicons were denatured at 94°C for 3 min prior to gel electrophoresis. The 25-cm plates of the LI-COR analyzer were used to cast 7% Long Ranger (FMC) sequencing gels. 0.8 µl of denatured samples were loaded onto the 0.25-mm thin gels equipped with 64-well sharktooth combs. The gels were run in 1×TBE at a constant 40 W and an upper threshold of 1,500 V. The heater was set to 48°C. The gels were loaded twice. One run lasts for 180 min to 210 min, depending on the primer combination. The motor speed of the laser scanning microscope was set to step '3' for signal detection. After the collection of the gel image on a personal computer, the picture was printed and scored visually.

Microsatellite (SSR) analysis

One hundred and seventy five SSRs, based on the map of Roeder et al. (1998), were screened for polymorphism between the parental lines. Two or more polymorphic SSRs per wheat chromosome were then applied to the population. PCR amplification was performed similar to Roeder et al. (1998) with the exception that the reaction volume was reduced to 10 µl, and PCR was performed in a 'Primus' 384 well thermocycler (MWG Biotech). Fragment analysis was carried out on a LI-COR 4200 DNA analyzer operating at the same conditions as for AFLP. Each gel was loaded four to six times.

Protein markers

Two high-molecular-weight glutenins (GluB1 and GluD1) and one gliadin polymorphism (GliB1) were detected by using PAGE as described by Gröger et al. (1997).

Statistical analysis

Phenotypic data

Plot means were calculated and used for the statistical analysis of the field data. The FHB resistance data were analyzed by ANOVA as a randomized complete block design over 2 years with PLABSTAT (Utz 1995). Broad-sense heritabilities and their 90% confidence intervals for line means were estimated according to Knapp et al. (1985).

Marker data

Linkage maps were constructed using MAPMAKER 3.0b for MS-DOS (Lander et al. 1987) assuming the Haldane (1919) mapping function. A logarithm of odds (LOD) threshold of 3 was set for grouping. The most-likely marker orders were determined using the MAPMAKER 'ripple' command.

QTL analysis

QTL analysis was done by one- and multi-factor ANOVAs using the SAS GLM procedure (SAS Institute 1989) for line mean values. Furthermore, simple interval mapping (SIM) and composite interval mapping (CIM) were carried out using the PLABQTL software (Utz and Melchinger 1996). A QTL was declared significant with a LOD >3.5. For CIM, cofactor selection was done as suggested by Jansen and Stam (1994). After a first SIM analysis, markers were chosen as cofactors for CIM at or next to the QTL positions and the QTL analysis was repeated and further refined until a stable LOD profile was reached. The dependence of QTL estimation on sampling effects was estimated by a five-fold cross-validation with the 'cross-validate' option of PLABQTL. With 4/5 of the individuals QTLs (positions and effects) were estimated and with the other 1/5 of the genotypes a validation was performed.

Results

Fusarium head blight resistance

The DH lines showed continuous variation with two peaks for the number of visually infected spikelets below the infection point (FHB-22) and the percentage of inoculated spikelets with wilting symptoms (W-22) on day 22 after inoculation. The two parental lines were situated on either end of the distribution (Fig. 1). The resistant

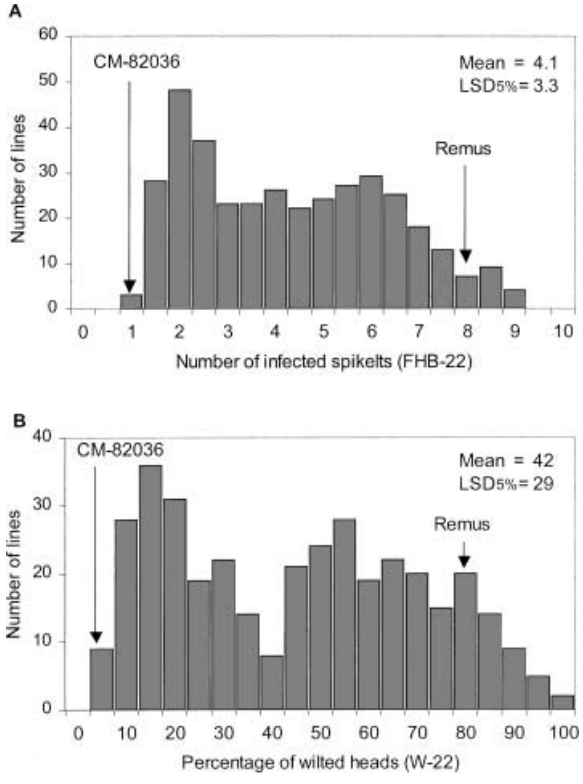

Fig. 1A, B Frequency distribution of 364 DH-lines for mean values of two FHB related traits observed on day 22 after inoculation. **A** Mean number of visually infected spikelets per head below the inoculation point (FHB-22), and **B** proportion of heads with wilting symptoms (W-22). Values of the parental lines are indicated by *arrows*. The overall population mean and the least significant difference for comparison of line means (α=0.05) using the genotype by year interaction mean square as an error term are also given

Table 1 Analysis of variance for the number of FHB infected spikelets (FHB-22) and the percentage of wilted heads (W-22) on day 22 after inoculation across 2 years

Item	*df*	FHB-22			W-22		
		MS	*F*-value	*p*-value	MS	*F*-value	*p*-value
Rep. (year)	6	200.1	65.25	<0.0001	19,656	58.51	<0.0001
Year	1	6,761.4	592.00	<0.0001	354,391	398.80	<0.0001
Genotype	365	33.6	2.94	<0.0001	5,285	5.89	<0.0001
G*Y	365	11.4	3.73	<0.0001	897	2.67	<0.0001
Error	2,150	3.1			336		

Table 2 QTLs for the number of infected spikelets below the inoculation point (FHB-22) and percentage of wilted heads (W-22) on day 22 after inoculation, chromosome location, logarithm of odds (LOD) and the percentage of explained phenotypic variance (VE) in 1999, 2000, and across the 2 years. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM)

Map interval	Chromosome	1999				2000				1999/2000			
		FHB-22		W-22		FHB-22		W-22		FHB-22		W-22	
		LOD	VE	LOD	VE	LOD	VE	LOD	VE	LOD	VE	LOD	VE
SIM													
Xgwm533–Xgwm493	3B	18.6	30.1	28.9	42.7	40.7	54.4	45.15	58.1	41.3	54.9	45.8	58.6
Xgwm293–Xgwm156	5A	–	–	–	–	5.1	9.4	4.4	8.1	4.9	9.1	–	–
Simultaneous fit		18.6	30.1	28.9	42.7	46.4	59.1	49.9	61.8	46.7	59.4	45.8	58.6
CIM													
Xgwm533–Xgwm493	3B	17.2	28.2	28.9	42.5	42.5	55.9	46.6	58.8	43.5	56.8	47.6	60.1
Xgwm293–Xgwm304	5A	–	–	–	–	6.1	11.1	5.1	9.3	5.9	10.9	4.1	7.5
XgluB1	1B	3.9	7.2	4.4	8.1	–	–	–	–	5.3	9.7	4.9	9.0
Simultaneous fit		24.8	38.0	35.9	50.0	46.4	59.1	49.9	61.8	53.9	64.6	56.2	66.2

parent ‘CM-82036’ exhibited a very low level of FHB symptoms. The disease was restricted to the mechanically inoculated spikelet and did not spread across the head. Wilting was rarely observed on inoculated heads of ‘CM-82036’. On the susceptible parent ‘Remus’ on average eight spikelets below the infection point were diseased and 80% of the inoculated heads showed wilting symptoms on day 22 after inoculation (Fig. 1).

Initial ANOVA calculations with all possible factors and interactions included in the model revealed a non-significant effect of genotype by isolate, and genotype by isolate by year interaction, for all FHB related-traits (data not shown). Therefore, the factor isolates was merged with replications in a new model.

ANOVAs and broad-sense heritabilities were calculated for FHB severity and for wilting symptoms observed at each of the four observation dates and for mean values across the four observations. Heritabilities were highest for the assessments on day 22 after inoculation for both visual FHB symptoms (FHB-22) and wilting symptoms (W-22), with heritability estimates and their 90% confidence intervals of H=66.0 (58.3 to 72.3) and H=83.0 (79.1 to 86.2) for FHB-22 and W-22 respectively. The ANOVAs for FHB-22 and W-22 are shown in Table 1. For both FHB related-traits the effects were significant for genotypes as well as genotype by year interaction. Genotype mean-squares were therefore tested against genotype by year interaction mean-squares. Line means for FHB-22 and W-22 were highly correlated (r=0.96; p <0.0001).

Molecular markers

The marker analysis was carried out with 239 randomly chosen DH lines. The 378 polymorphic markers were comprised as follows: one morphological marker (presence or absence of awns), three storage proteins, 27 RFLPs, 79 SSRs and 268 AFLPs; 348 markers could be mapped and they formed 43 linkage groups, covering a total genetic distance of 1,860 cM. For 29 linkage groups their chromosome identity could be determined based on the microsatellite map of wheat published by Roeder et al. (1998); and for all wheat chromosomes apart from 4D at least partial maps were obtained. Fourteen linkage groups did not include a microsatellite marker and their chromosome identity was therefore not determined.

Quantitative trait mapping

Analysis of variance revealed that two genomic regions (located on chromosomes 3B and 5A) were significantly (p<0.0001) associated with FHB-22 and/or W-22 (either in 1999 or 2000, or their combined mean, data not shown). Interval mapping with PLABQTL using SIM also detected prominent QTL effects on chromosomes 3B and 5A with LOD >3.5 (see Table 2). Composite interval mapping with PLABQTL including four cofactors revealed one additional significant QTL effect on chromosome 1B

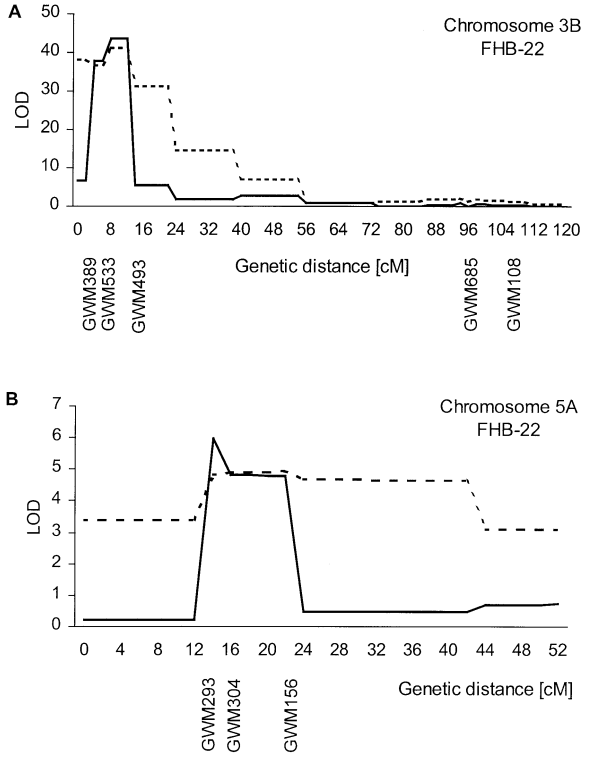

Fig. 2 Interval analysis of QTLs for the number of infected spikelets on day 22 after inoculation (FHB-22) on linkage groups corresponding to chromosomes 3B (**A**) and 5A (**B**). LOD curves were calculated by CIM (*solid line*) and SIM (*dotted line*). Only selected microsatellite markers are presented

Table 3 Five-fold cross validation of QTLs for the mean number of infected spikelets on day 22 after inoculation across 2 years (FHB-22). Data were split in five subsets of 192 lines used for QTL detection (calibration) and the remaining 47 lines were used for QTL validation. For description see Table 2

Cross validation split	Calibration			Validation VE
	QTL detected	LOD	VE	
1	3B 5A	37.8	58.1	59.6
2	3B 5A 1B	37.9	57.7	82.8
3	3B 5A 1B	46.4	65.5	56.2
4	3B 5A	40.8	61.4	45.4
5	3B 5A 1B	44.6	64.8	54.4

associated with the *XgluB1* (high-molecular-weight glutenin) locus (Table 2). The largest and most-consistent effects were associated with the QTL on the short arm of chromosome 3B, and the most-likely position is in the *Xgwm533–Xgwm493* interval. The most-likely QTL position on 5A is in the *Xgwm293–Xgwm304* interval (Table 2 and Fig. 2). The LOD profiles obtained with CIM showed sharper peaks than the LOD profiles obtained with SIM (Fig. 2). For all three detected QTLs the allele conferring FHB resistance originated from the resistant parent 'CM-82036'. In all tested models with combinations of QTLs, the additive effects were significant. In all cases, apart from the CIM analysis for FHB-22 and W-22 in 1999, additive by additive epistatic QTL interactions were found to be non-significant (data not shown).

Five-fold cross validation revealed that the QTLs mapped on chromosomes 3B and 5A were consistently detected. As an example the cross validation result for FHB-22 mean values is given in Table 3. The putative QTL on chromosome 1B was detected in only three out of five runs, whereas the 3B and 5A QTLs were always significant. The magnitude of the explained phenotypic variance was comparable in both the calibration set and the validation set (Table 3), indicating that the QTL effects were estimated correctly.

The association of the two most-prominent QTLs (on 3B and 5A) with the phenotype is shown in Table 4. Lines with the 'resistant' allele (originating from 'CM-82036') at both QTL regions had a mean value for FHB-22 of only one-third compared to lines with the allele from susceptible 'Remus'. Even lines which had the QTL allele conferring resistance at the 3BS region, and the allele from susceptible 'Remus' at the 5A region, had on average less than half the disease severity than the susceptible lines, thus supporting the prominent contribution of this chromosome region to the Type II FHB resistance.

Discussion

FHB resistance assessment

Precise phenotypic evaluation is a prerequisite for QTL mapping. The FHB resistance of wheat is a quantitative trait, confounded by environmental effects like temperature, humidity, plant development stage and abundance of

Table 4 Effect of alternative alleles at two QTL regions with the mean number of FHB infected spikelets below the inoculation point (FHB-22) and the percentage of wilted spikelets (W-22) on day 22 after inoculation

QTL-allele[a]		Number of lines	FHB-22			W-22		
3B	5A		Median	Mean	SE	Median	Mean	SE
CM-82036	CM-82036	60	1.8	2.1	0.8	14	17	11
CM-82036	Remus	53	2.3	2.6	1.1	21	23	13
Remus	CM-82036	46	5.0	5.1	1.2	57	58	13
Remus	Remus	65	6.2	6.1	1.4	67	68	16

[a] Only lines with non-recombined *Xgwm533–Xgwm493* (3B QTL) and *Xgwm293–Xgwm304* (5A QTL) intervals were included in this calculation

inoculum (Parry et al. 1995). Under natural conditions FHB occurs unpredictably and the disease is not evenly spread across the field. Therefore, artificial inoculation is indispensable for a reliable FHB resistance evaluation of wheat genotypes. We applied a single floret inoculation method to determine specifically the spread of the disease within a spike, also designated as Type II resistance by Schroeder and Christensen (1963). Resistance to fungal spread is considered to be a major component of the FHB resistance complex (Bai et al. 2000). Inoculating a single spikelet in a spike reduces the differences in disease incidence among test lines and simplifies the complicated disease system (Bai et al. 1999). Despite the significant genotype by year interaction for FHB related traits, heritabilities were high, indicating that we achieved a reliable measurement of disease spread and wilting 22 days after inoculation. This is in agreement with Bai et al. (1999) who also found maximum genetic differences for Type II resistance among wheat lines 3 weeks after the inoculations. The two FHB resistance-related traits (the number of visually infected spikelets and the percentage of wilted heads) were highly correlated, indicating that resistance to fungal spread and resistance to wilting are under very similar genetic control. One *F. culmorum* and one *F. graminearum* isolate was applied. The non-significance of the genotype by isolate interaction once more underlines the non-specific or horizontal nature of FHB resistance in wheat (Van Eeuwijk et al. 1995). Doubled-haploid populations offer ideal material for studying quantitative traits (Snape et al. 1984; Snape 1988). The wheat by maize method (Laurie and Bennet 1988; Bitsch et al. 1998) proved to be highly efficient to establish a sufficient number of stable homozygous recombinant lines in a relatively short time. The DH lines could easily be maintained and multiplied by selfing; ideal for a proper replication of experiments. We found a quantitative distribution with two major peaks among the DH lines for the two FHB-related traits. A similar distribution for FHB severity after single floret inoculation was reported by Waldron et. al (1999) who speculated that inheritance may be controlled by one or a few major genes and several to many genes with smaller effects. The two parental lines were on either extremes of the distribution, and no significant transgressions were found. The resistant parent ‘CM-82036’ appears to have contributed nearly all the ‘resistant’ alleles compared to ‘Remus’.

QTL mapping

Our marker analysis was mainly based on AFLP and SSR markers. AFLP markers had the advantage to be very economical in terms of the resources input per data point. A map based on AFLP alone, however, has limited value only. With the integration of previously mapped markers, especially SSRs (Roeder et al. 1998), the relation of most linkage groups to the wheat chromosomes could be established. Although we genotyped our population with 378 markers the map obtained was probably incomplete. Nevertheless, we were able to identify two genomic regions with a major effect on FHB resistance by SIM and a third one by CIM. By applying CIM the most-likely QTL positions could be narrowed down compared to SIM, although the peaks of the LOD curves were in the same position. LOD profiles obtained by CIM suggest that in all three genomic regions a single gene or a cluster of tightly linked genes is responsible for the effect on resistance. The importance of the three putative QTLs is ranked in the order: 3B>5A>1B. The most prominent QTL effect was found on the short arm of chromosome 3B, explaining up to 60% of the phenotypic variation for resistance to fungal spread and resistance to wilting. The 3BS QTL was consistently detected in all the experiments and cross validations we performed. Our results confirm the findings of Waldron et al. (1999) and Anderson et al. (2001) who reported an important QTL for Type II resistance in exactly the same region on chromosome 3BS, using two populations segregating for ‘Sumai#3’-derived resistance. Bai et al. (1999) reported a major QTL for fungal spread in a population segregating for ‘Ning 7840’ (also a ‘Sumai#3’ derivative) resistance. Their mapping was based on AFLP markers and the QTL position was initially suspected to be on chromosome 7B. Integration of SSR markers, however, showed that the QTL is indeed on 3BS (Zhou et al. 2000).

The second most-important QTL we detected was on 5 A. A significant effect of chromosome 5 A on FHB resistance was also found in an earlier study, using monosomic analysis (Buerstmayr et al. 1999). Ban (2000b) reported that one resistance gene in ‘Sumai#3’ was linked to the B1 gene, coding for suppression of awns, located on chromosome 5A. Despite the significant QTL effect on chromosome 5 A, this QTL was not associated with the presence or absence of awns in our study. The third putative QTL in our study was detected only by CIM on chromosome 1B, and its effect was lower and less consistent than the effects found for the QTL regions on the chromosomes 3B and 5A. A small but significant effect of chromosome 1B on visual FHB symptoms was also found in the above-mentioned monosomic study (Buerstmayr et al. 1999). Because the results of Waldron et al. (1999) and our own earlier results (Buerstmayr et al. 1999) indicated a significant QTL effect on chromosome 6B of ‘Sumai#3’, we included several markers mapping to that chromosome. We obtained a linkage group corresponding to 6B comprising six SSRs, six RFLPs and 15 AFLPs. Using QTL analysis we did not find a significant association of any 6B marker with FHB resistance (data not shown). The line ‘CM-82036’ most-likely does not carry the putative QTL allele from ‘Sumai#3’ in that region.

Breeding application

Up to now the significant and consistent QTL effect on chromosome 3BS was reported in at least four indepen-

dent populations segregating for 'Sumai#3'-derived FHB resistance (Bai et al. 1999; Anderson et al. 2000; and the present study) and can therefore be considered verified. Lines with the 'resistant' QTL allele in that region showed an average reduction of FHB severity between 50% and 70% compared to the lines carrying the 'susceptible' allele. The 3BS QTL region is well-covered by SSR markers (Roeder et al. 1998). SSR markers are technically relatively easy to perform and are inherited co-dominantly. They are considered a very suitable marker system for practical breeding applications, and marker-assisted selection for the 3BS resistance QTL should therefore be easy and straightforward. The QTL effect on 5A was also consistent in our study, but only limited evidence is found in the literature to confirm this QTL. Also the 5A QTL region is tagged by SSR markers and is therefore amenable to marker-assisted selection. The QTL effect associated with the *XgluB1* locus definitely needs further evaluation and verification before we can recommend its use in marker-assisted selection. All in all, Type II FHB resistance in 'CM-82036' appears to be under the control of only a few QTLs with major and additive effects, together with an unknown number of minor genes.

As highly FHB-resistant sources (like 'Sumai#3' and its derivatives) have too many undesirable agronomic features (low yield, low quality, susceptibility to other diseases), breeders were reluctant to use such sources in their breeding programs. We see the most-promising application of the presented markers linked to FHB resistance QTLs in marker-assisted back-cross breeding. With these techniques and procedures, the development of FHB-resistant and agronomically improved lines will be feasible in a much shorter time than by conventional breeding.

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