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ORIGINAL PAPER

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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

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Abstract *Fusarium* head blight (FHB) is a devastating disease in wheat that reduces grain yield, grain quality and contaminates the harvest with deoxynivalenol (DON). As potent resistance sources Sumai 3 and its descendants from China and Frontana from Brazil had been analysed by quantitative trait loci (QTL) mapping. We introgressed and stacked two donor QTL from CM82036 (Sumai 3/Thornbird) located on chromosomes 3B and 5A and one donor QTL from Frontana on chromosome 3A in elite European spring wheat and estimated the effects of the three individual donor QTL and their four combinations on DON, *Fusarium* exoantigen content, and FHB rating adjusted to heading date. One class with the susceptible QTL alleles served as control. Each of the eight QTL classes was represented by 12–15 F_3-derived lines tested in F_5 generation as bulked progeny possessing the respective marker alleles homozygously. Traits were evaluated in a field experiment across four locations with spray inoculation of *Fusarium culmorum*. All three individual donor-QTL alleles significantly reduced DON content and FHB severity compared to the marker class with no donor QTL. The only exception was the donor-QTL allele 3A that had a low, but non-significant effect on FHB severity. The highest effect had the stacked donor-QTL alleles 3B and 5A for both traits. They jointly reduced DON content by 78% and FHB rating by 55% compared to the susceptible QTL class. Analysis of *Fusarium* exoantigen content illustrates that lower disease severity is associated with less mycelium content in the grain. In conclusion, QTL from non-adapted sources could be verified in a genetic background of German elite spring wheat. Within the QTL classes significant ($P < 0.05$) genotypic differences were found among the individual genotypes. An additional phenotypic selection would, therefore, be advantageous after performing a marker-based selection.

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Introduction

Fusarium head blight (FHB) is a devastating disease in most wheat-growing countries resulting in yield and quality loss, and contamination by mycotoxins. In Central Europe, the disease is caused by a complex of *Fusarium graminearum, F. culmorum* and some species of minor importance. The most prominent mycotoxin is deoxynivalenol (DON) and its derivatives (Mueller et al. 1997). Schroeder and Christensen (1963) identified two components of FHB resistance: resistance to initial infection (type I) and to fungal spread within the spike (type II). The latter is tested by injecting spores directly into individual spikelets and observing the symptoms at different intervals. Spray inoculation affects both type I and type II resistance. Type I resistance can be tested only indirectly when the results after spray and single-spikelet inoculation are different (Miedaner et al. 2003a). Selection progress for FHB resistance in wheat is hampered by its quantitative inheritance (Snijders 1990), the high importance of genotype × environment interaction (Miedaner et al. 2001), and the necessity to test at flowering stage. These features make FHB resistance a valuable candidate trait for marker-assisted selection (MAS).

In all spring materials analysed so far, several FHB resistance loci have been found. In the Chinese source Sumai 3, a major quantitative trait loci (QTL) on chromosome 3BS explained up to 50% of the phenotypic variation (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001). Additionally, QTL on chromosomes 6B (Waldron et al. 1999; Anderson et al. 2001), 2A, and 2B (Zhou et al. 2002) with minor influence were reported. In CM82036 (Sumai 3/Thornbird) the QTL allele 3BS (*Qfhs.ndsu*-3BS) was verified and additionally a QTL on chromosome 5A was detected (*Qfhs.ifa-5A*, Buerstmayr et al. 2003). The locus *Qfhs.ndsu*-3BS seems to be primarily associated with type II resistance (Buerstmayr et al. 2002) and has already been successfully validated in several wheat backgrounds and environments (Shen et al. 2003; Zhou et al. 2003). The QTL allele 5A, in contrast, contributed more to resistance to initial infection (type I) than to fungal spread (Buerstmayr et al. 2002, 2003) and has not been verified in another genetic background yet. The same is true for one QTL on chromosome 3A from the old Brazilian spring wheat cultivar Frontana (Steiner et al. 2004).

Mapping of the QTL regulating DON content in the harvested grain has been scarce. It is, therefore, largely undetermined whether the reported effect of the mapped spring wheat QTL on disease severity is also true for DON content. In the population Wuhan1/Maringa, Somers et al. (2003) detected two QTL on chromosomes 2DS and 5AS controlling DON accumulation independently of FHB resistance, whereas the favourable allele on chromosome 3BS reduced disease spread and DON content simultaneously. A close relationship between resistance and DON content has been reported in different materials (Mesterházy et al. 1999; Miedaner et al. 2003b, 2004). The latter study additionally showed a covariation between these traits and the exoantigen content of the grain measured by a *Fusarium*-specific ELISA (enzyme-linked immunosorbent assay).

Introgressing resistance loci with high additive effects from non-adapted spring wheat in the European gene pool might be a successful breeding strategy. Main disadvantages of non-adapted sources are their low grain yield, susceptibility to powdery mildew and leaf rust, low lodging resistance and generally their non-adaptedness to high-input farming as practised in Central and Western Europe. Their introgression needs, therefore, several cycles of backcrossing and selection, a time consuming, laborious and costly procedure. Marker-assisted backcrossing will shorten this procedure considerably if the high effects of the QTL from exotic background are transferable into elite breeding material. It might be favourable to stack QTL from different chromosomes and responsible for different FHB resistance components to gain the maximum effect. Pyramiding monogenically inherited resistance genes by DNA markers has been successfully used previously (e.g. Sanchez et al. 2000). Much less is known on stacking QTL that are responsible for oligogenic traits. QTL normally have a much lower effect than major loci, are more environmentally sensitive, and prone to effects of the genetic background. In this study we aimed to estimate the effect of stacking two QTL for type I resistance (chromosomes 3A, 5A) and one QTL for fungal spread within the head (type II resistance, chromosome 3BS) from non-adapted CM82036 and Frontana in elite German spring wheat material. Additionally, we wanted to test whether less symptoms and DON go along with less fungal mycelium in the grain by a *Fusarium*-specific ELISA. Genotypes containing the three donor-QTL alleles individually and in all combinations in homozygous conditions were produced by marker-based selection and compared to genotypes with the respective susceptibility QTL alleles.

Materials and methods

Identification of QTL in the original mapping populations

In two previous studies, mapping populations of 364 and 210 doubled haploid (DH) lines were developed from a cross between CM82036 (resistant) and Remus (susceptible) and a cross between Frontana (resistant) and Remus, respectively (Buerstmayr et al. 2003; Steiner et al. 2004). DH lines were evaluated for FHB severity by spray inoculation in each of three environments. The SSR marker WMS 533 on chromosome 3B and WMS 156 on chromosome 5A showed the highest, significantly with FHB rating associated effects in the CM82036 population that were mainly additive. The simultaneous fit of both marker loci amounted to 47% of explained phenotypic variance. In the Frontana population, nine markers were found to be significantly associated with FHB severity. The major QTL mapped to chromosome 3A near the marker WMS 720 and was consistent across three years. In individual years, this donor QTL accounted for 12–13% of the variation. For simplicity, we refer to the chromosomal segment defined by the marker intervals as QTL in the paper throughout. The QTL alleles from the non-adapted donor lines associated with FHB resistance are referred to as donor QTL or donor-QTL alleles, numbered according to their chromosomal localization, e.g. donor-QTL allele 3B.

Development of lines with specific QTL combinations

Basis of the experiment was the double cross DH-L[CM82036/Remus]/Nandu//DH-L[Frontana/Remus]/Munk, where DH-L refer to double-haploid lines from the respective mapping populations selected for FHB resistance and presence of the respective donor-QTL alleles. Our aim was to combine the QTL from the two genetically different resistance sources CM82036 and Frontana. CM82036 is a resistant line from the cross Sumai3/Thornbird (Buerstmayr et al. 2002, 2003); Frontana is an old Brazilian spring wheat (Steiner et al.

2004). Nandu and Munk are both high yielding, but medium to highly FHB susceptible German spring wheat varieties. Single crosses were performed in an off-season greenhouse programme in early 2000. Seeds from the F_1 crosses were used to produce a double cross in the greenhouse in late 2000. This double cross resulted in equal frequencies of all donor-QTL alleles of 0.25. In 2001, 1,200 randomly chosen double cross (DC)-F_1 kernels were planted in plastic trays. At the three-leaf stage, about 2 cm of leaf tissue was harvested separately from each seedling and frozen at −80°C for DNA isolation. Using SSR markers, all 1,200 plants were screened for the two donor-QTL alleles of CM82036. Sixty plants having the donor-QTL alleles 3B and 5A individually or jointly were selected. Plants were transferred to a climate chamber for 8 weeks of vernalization at 4°C with 16 h photoperiod, afterwards transplanted into the field nursery in 2001 and selfed. Each plant was harvested separately at maturity to result in seed of DC-F_1-derived F_2 lines. According to the nomenclature of Fehr (1987), F_n-derived lines in F_{n+x} generation will be called $F_{n:n+x}$ lines. Thirty-five individual plants of each of the marker-selected 60 $F_{1:2}$ lines were again planted in plastic trays in 2002 and leaf tissue was cut for SSR analyses. After screening these 35×60 plants for the two QTL alleles of CM82036, those plants containing the donor-QTL alleles 3B or 5A or their combination homozygously were propagated individually in the field nursery to result in 60 $F_{2:3}$ lines. From each of the 60 lines, 30 plants were genotyped again. Plants possessing the searched combination with the Frontana-derived QTL allele 3A homozygously were selected and multiplied in the nursery plantwise resulting in $F_{3:4}$ lines. Due to segregation, each selected plant had none, one, two, or three of the donor-QTL alleles in homozygous condition. According to Mendelian theory for unlinked loci, the progeny should contain eight QTL classes that occur in equal frequencies of 0.125. From each of the 8 QTL classes, 15 $F_{3:4}$ lines were advanced with each of 20 plants in the nursery in 1 row. At harvest, seed from each row was bulked to result in F_3-derived lines tested as bulked progeny in generation F_5 ($=F_{3:5}$ bulks) for FHB resistance.

Marker analyses

All plant DNA was isolated from young leaves by using the mini-prep CTAB method adapted from Saghai Maroof et al. (1984). Microsatellite designation, composition and primer sequences were reported by Röder et al. (1998) or not yet published. In general, a PCR protocol according to Röder et al. (1998) with 35 cycles instead 45 cycles of 1 min 94°C denaturation, 1 min 50°C (or 55 or 60°C depending on the primer) annealing and 1 min of 72°C extension followed by a final extension step of 5 min 72°C was used. For detection of PCR product on an ABI3700 or ABI3100 DNA sequencer running Genescan and Genotyper software, one of the primers from each pair was synthesized with the 5′end-nucleodite labelled with fam, hex or ned.

At the first selection, the markers WMS 389 and WMS 304 were used for detecting the donor-QTL alleles 3BS and 5A derived from CM82046, respectively. At the second selection, these markers and additionally WMS 533 and BARC 133 for the donor-QTL allele 3B and WMS 156 for the donor-QTL allele 5A were used. At the third selection, plants homozygous for the above-mentioned two donor QTL and their combinations were additionally analysed by WMS 720 to detect plants with the Frontana-derived donor-QTL allele 3A in homozygous condition.

FHB field tests

A total of 110 $F_{3:5}$ bulks were tested, ten bulks of the originally marker-selected progeny were lost caused by insufficient amount of selfed seed. Each marker class was represented by 12–15 bulks (see Fig. 1 for exact numbers). In 2004, testing was performed at each of four locations: Hohenheim (HOH) near Stuttgart (geographic location latitude 48.8°, longitude 9.2°; 400 m above sea level, 8.5°C mean annual temperature, 685 mm mean annual precipitation), Seligenstadt (SEL) near Würzburg (geographic location latitude 50.0°, longitude 8.9°; 281 m above sea level, 9.1°C mean annual temperature, 622 mm mean annual precipitation), Wohlde (WOH) near Celle (geographic location latitude 52.8°, longitude 9.98°; 80 m above sea level, 8.8°C mean annual temperature, 753 mm mean annual precipitation), and Wetze (WET) near Einbeck (geographic location latitude 51.7°, longitude 9.0°; 130 m above sea level, 8.6°C mean annual temperature, 645 mm mean annual precipitation). Trials were arranged as randomized complete block designs with three replications. The respective parents were tested within the same experiment in duplicate to enhance accuracy. All genotypes were planted in two-row microplots (0.42×1.0 m^2) with 40 kernels per row. To avoid infection by other pathogens, all plots were sprayed once with Opus Top™ (BASF, Ludwigshafen, Germany) shortly before heading.

Inoculation and field traits

Inoculum of two highly aggressive, DON-producing isolates of *F. culmorum* (FC 33, FC 46) was produced on wheat grain medium as described in Miedaner et al. (1996). During flowering the spore suspension with a density of 5×10^5 spores ml^{-1} was applied at a rate of approximately 100 ml m^{-2} onto the heads with a machine-driven small-plot field sprayer (Hege 75, Waldenburg, Germany) in the evening. To consider the variation in flowering date between entries, all genotypes including the parents were inoculated four times. Inoculation could not be done by inoculating each genotype

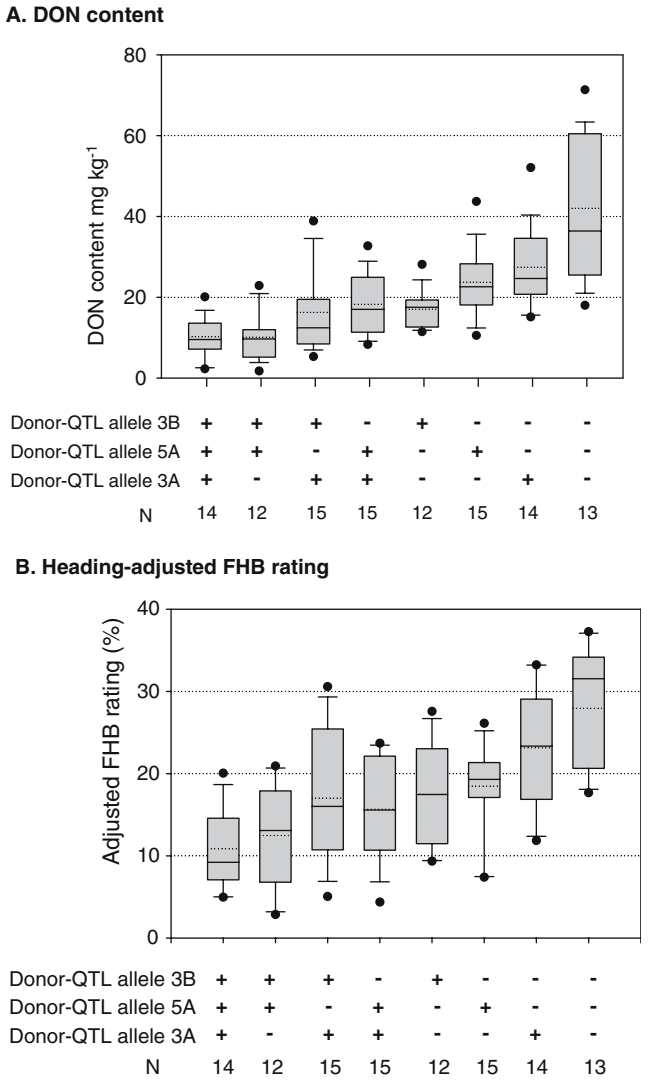

Fig. 1 Boxplot distributions of $F_{3:5}$ bulks possessing alternative alleles (+, − donor QTL allele present or absent, respectively) at the FHB-associated QTL regions on chromosomes 3B, 3A, and 5A for DON content (**a**) and heading-adjusted FHB rating (**b**) after inoculation with *Fusarium culmorum*. Data are based on the respective number (*N*) of bulks across three (**a**) and four locations (**b**), respectively. *Boxes* indicate the median (*solid line*), mean (*dashed line*), 25 and 75 percentiles, respectively, *lines* the 10 and 90 percentiles, respectively, and *dots* refer to outlying data points

exactly at mid-anthesis because the bulks were still segregating. Instead, we inoculated the whole experiment at the same dates such that each genotype was inoculated at least once at mid-flowering. Accordingly, FHB rating was assessed three times beginning with the onset of symptom development, i.e. 14–22 days after inoculation depending on the environment in 3–4 days intervals. For each plot, the percentage of infected spikelets was rated (0–100), i.e. the product of percentage of infected heads per plot and percentage of infected spikelets per head. This measure integrates both resistance components, type I and II. For data analysis, the arithmetic mean of all three ratings was used. Additionally, heading date was recorded on a time scale starting at January 1st, and plant height as a mean height per plot.

DON and exoantigen analysis

The entire plots of all $F_{3:5}$ bulks from two replications at three locations (HOH, WOH, WET) were harvested at full ripening by hand and dried to minimal water content at a temperature of about 30°C. Parents were harvested in duplicate. The fourth location could not be analysed due to lodging. Samples were threshed, sieved by hand to remove fragments of the rachis and glumes, and carefully cleaned in a machine with adjustable forced air. Samples of approximately 100–200 g were ground to a particle size of about 1 mm with a laboratory mill and stored at −20°C until analysis. The competitive immunotest for DON and acetyl-DON contents was applied according to the manufacturer's description (RIDA-SCREEN™FAST DON, R-Biopharm GmbH, Darmstadt, Germany) and reported recently (Miedaner et al. 2004). The detection limit is 0.222 mg kg^{-1}. The measurement was made with a microtitre-plate spectrophotometer (Spectra Basic, TECAN Deutschland GmbH, Crailsheim, Germany) at 405 nm. The extinction values were calculated for DON content by analyses of five standard solutions (0, 0.222, 0.666, 2, 6 ppm) per microtitre plate and multiple regression of the sample contents with these standards by a software package distributed by the manufacturer. From the same grain samples, the *Fusarium*-exoantigen content was determined in the lab of F. Rabenstein, Aschersleben, Germany. The application of this newly developed PTA-ELISA (plate-trapped antigen enzyme-linked immunosorbent assay) has been described recently in detail (Miedaner et al. 2004).

Statistical analyses

Residual analyses showed that a logarithmic transformation was necessary to achieve normality of the data for all traits. The delta method was employed to back transform means of genotypes and standard errors to the original scale (SAS Institute, Cary, NC, USA). Despite multiple inoculation dates, a significant ($P < 0.05$) correlation between FHB rating and heading date existed that varied according to the environment. Thus, a covariance analysis was conducted with heading date set as co-variable. FHB rating was adjusted for the heading date of each genotype by multiple regression analysis and pooled analysis was conducted across locations. This was called heading-adjusted FHB rating throughout the paper. No significant ($P > 0.05$) correlation between FHB rating and heading date existed after the procedure. Between DON or *Fusarium* exoantigen content and heading date only negligible correlations ($r < 0.2$) were found. Consequently, no adjustment was performed. Mean FHB rating was

Table 1 Means for deoxynivalenol (DON) content, heading date, *Fusarium* head blight (FHB) rating, *Fusarium* exoantigen content, and plant height heading-adjusted FHB rating, of each of four parents, and the marker-selected progeny after inoculation with *Fusarium culmorum* at three (DON, *Fusarium* exoantigen content) and four locations, respectively, in 2004

	DON content (mg kg^{-1})	Heading date (days after Jan 1)	FHB rating (%)	Heading-adjusted FHB rating (%)	*Fusarium* exoantigen content (OD[a])	Plant height (cm)
Parents						
CM82036	15.1	164.4	3.5	2.0	0.018	90.2
Frontana	15.0	165.4	15.5	14.6	0.067	117.4
Nandu	34.5	169.0	35.9	35.7	0.128	94.6
Munk	76.4	168.1	42.2	41.5	0.337	88.0
Parental mean	35.3	166.7	24.3	24.4	0.138	97.5
$F_{3:5}$ bulks ($N = 110$)						
Mean	25.5	166.4	21.8	21.3	0.119	95.6
Genotypic range	1.8–71.4	147.3–173.1	3.7–46.2	6.4–37.2	0.01–0.41	69.1–118.1
$LSD_{5\%}$	27.1	1.6	9.7	6.7	0.1	5.6

[a]OD = optical density at 405 nm

estimated using the REML method (residual maximum likelihood method) within the MIXED procedure on the basis of a complete block design (SAS 2001) resulting in standardized estimates of variance components. To investigate the individual and combined effects of the donor QTL, data from the $F_{3:5}$ bulks were sorted into the eight possible QTL classes with either none, one, two, or all three of the donor QTL. The number of individual $F_{3:5}$ bulks ranged from 12 to 15 (exact numbers see Fig. 1). This analysis should remove the confounding effects of segregating QTL associated with FHB resistance but not monitored by our markers ("genetic background effects"). Analysis of variance of data was conducted to examine the sources of variation associated with DON content and FHB rating when data were analysed for the eight possible QTL classes. The statistical model described the effects of location, replication nested within locations, QTL class, genotype ($= F_{3:5}$ bulks) nested within QTL classes, QTL × location and genotype × location interaction, and error. For paired *t*-test comparisons between QTL classes, means averaged across all locations were used. Rejection or acceptance of the null hypothesis was based on probability values of $P < 0.05$. Average standard errors of differences between genotypes were calculated (Littell et al. 1996). Parents were analysed separately in a randomized complete block design and were set as fixed factors, all other factors were considered as random.

Results

Both resistant parents, CM82036 and Frontana, had the lowest DON content and did not differ from each other (Table 1). Nandu had only half the mean DON content of Munk that was highly contaminated. *Fusarium*-exoantigen content and both FHB ratings ran largely in parallel to the DON content; CM82036, however, had lower values than Frontana. Both non-adapted sources were earlier in heading, and Frontana was rather tall. The mean of the 110 $F_{3:5}$ bulks showed only minor deviations from parental mean. Significant ($P = 0.05$) genotypic variance for all traits was found. No significant correlations existed between plant height and all other traits. Accordingly, the marker classes did not differ substantially for plant height (data not shown for brevity). In the individual locations, DON contents were lowest at HOH, WET showed contents twice as high. At all four locations comparable mean ratings were achieved.

Deoxynivalenol content was significantly reduced by all donor-QTL alleles individually over the class lacking donor alleles (Table 2). The highest single effect had the donor-QTL alleles 3B and 5A, both differing significantly from the effect of the donor-QTL allele 3A. The classes with two stacked donor QTL had on average a lower DON content than those with one donor QTL only (12.7 vs. 21.3 mg kg^{-1}). The donor-QTL allele 3A had only a low, non-significant effect on DON content

Table 2 Means and effects of eight QTL classes for deoxynivalenol (DON) content and heading-adjusted FHB rating after inoculation of 12–15 $F_{3:5}$ bulks per class by *Fusarium culmorum* across three (DON content) and four locations (heading-adjusted FHB rating) in 2004

QTL class	DON content		Heading-adjusted FHB rating	
	Mean (mg kg^{-1})	Effect[a] (mg kg^{-1})	Mean (%)	Effect[a] (%)
3B + 5A + 3A	8.5 a[b]	30.3	14.2 a	17.3
3B + 5A	8.1 a	30.7	16.2 ab	15.3
3B + 3A	13.5 b	25.3	20.6 b	10.9
3A + 5A	16.5 bc	22.3	19.1 ab	12.4
3B	16.0 bc	22.8	21.1 bc	10.4
5A	22.2 cd	16.6	21.4 bc	10.1
3A	25.7 d	13.1	26.6 cd	4.9
None	38.8 e	–	31.5 d	–

[a]Difference to the class with no donor-QTL alleles
[b]Different letters indicate significant differences by multiple comparison of means by $P < 0.05$

when combined with the other donor-QTL alleles. A reduction of DON content by 78% was observed in the two classes with the most beneficial donor-QTL alleles compared to the class lacking donor-QTL alleles. Frontana as a variety had a lower DON content than the mean of $F_{3:5}$ bulks carrying the donor-QTL allele 3A from this source (15.0 vs. 25.7 mg kg^{-1}). The opposite was true for the comparison of CM82036 and the class having jointly the donor-QTL alleles 3B and 5A (15.1 vs. 8.1 mg kg^{-1}).

Genotypes within QTL classes reacted quite similar for heading-adjusted FHB rating than for DON content (Table 2). Both traits were significantly ($P < 0.01$) correlated across all 110 progeny ($r = 0.78$). Genotypes with the donor-QTL alleles 3B and 5A individually were, on average, significantly less affected by FHB than those without any donor-QTL alleles. The donor-QTL allele 3A mediated 5% points less FHB infection but the difference was not significant. Accordingly, this QTL allele brought only low, non-significant additional effects in either combination. The donor-QTL alleles 3B and 5A jointly improved FHB resistance on average by 15% points (= 55%) compared to the class lacking donor QTL. Again, the class with the two most beneficial donor-QTL alleles did not significantly differ from the class with all three donor-QTL together. The high-resistance level of CM82036 could not be reached by any of the QTL class means. Accordingly, the variety Frontana itself had a higher resistance level than those genotypes carrying the donor QTL allele 3A from this source (14.6 vs. 26.6%). The effects of the combined donor-QTL alleles for both DON content and adjusted FHB rating were predominantly additive although the observed effects were smaller than expected when the individual effects are summed up.

Genotypic ranges within the QTL classes (Fig. 1) were rather small for DON content in those classes where donor QTL alleles were present, but considerably higher for heading-adjusted FHB rating in most classes. This was confirmed by the analysis of variance. Differences between QTL classes and genotypes within QTL classes accounted for the greatest portion of variance for both traits (Table 3). Genotype × location interaction had also a significant impact on both traits.

Fusarium head blight rating and *Fusarium* exoantigen content correlated with a coefficient of $r = 0.74$ ($P = 0.01$) across all 110 genotypes (Fig. 2). The distribution among the marker classes is roughly as expected: the more donor QTL available, the lower is the mycelium content in the grain. Similarly, *Fusarium* exoantigen and DON content correlated with $r = 0.84$ ($P = 0.01$).

Discussion

Quantitative trait loci mapping is a powerful tool to dissect quantitative traits into single Mendelian factors that can be handled more efficiently in practical breeding programmes. Concerning the low precision of QTL localization, the large upward bias for estimated QTL effects as well as the low chances to detect QTL in small-sized populations (Utz et al. 2000), a validation of QTL before their use for MAS in breeding programmes is urgently necessary. The feasibility of using MAS in practical breeding programmes is further determined by the reproducibility of the marker-QTL association across generations, populations, and environments (Dudley 1993). We used for the validation in this broad sense an empirical test by crossing up to three donor QTL from superior descendents of two mapping populations into German elite spring wheat material and estimating their effects in multi-location field trials. Because the three donor QTL were from two different resistance sources, we used a double cross to combine them in elite background. We could validate two major QTL from a Sumai 3 descendant and one QTL from a Frontana descendant. Both the spring wheat background and the environments differed with the original mapping populations.

The effects of the QTL classes on all traits were rather similar although the original mapping was performed only for disease severity. The same donor QTL that were responsible for lower disease severity reduced DON and *Fusarium* exoantigen content in the grain. Thus, lower FHB rating also results in lower fungal mycelium content illustrating the presence of specific resistance mechanisms. In practical breeding this allows to select for FHB resistance alone when using the appropriate parents.

Table 3 Estimates of variance components and exact probability values of the sources of variation of the 8 QTL classes for DON content and heading-adjusted FHB rating after inoculation of 12–15 $F_{3:5}$ bulks per class by *Fusarium culmorum* across three (DON content) and four locations (heading-adjusted FHB rating) in 2004 (data were transformed)

Source of variation	DON content		Heading-adjusted FHB rating	
	Estimate	*P* value	Estimate	*P* value
Location (L)	0.498	0.164	0.1886	0.116
Replication within L	0.018	0.131	0.0027	0.075
QTL class	0.305	0.041	0.1611	0.0539
QTL class × L	0.0009	0.422	0.0073	0.1084
Genotype (G) within QTL class	0.255	< 0.0001	0.2817	< 0.0001
G × L within QTL class	0.061	0.0001	0.1219	< 0.0001
Error	0.180		0.1150	

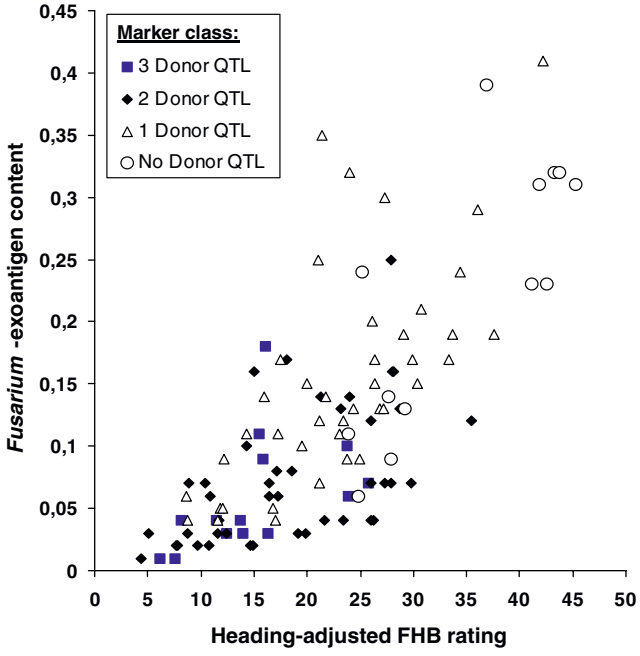

Fig. 2 Association between heading-adjusted FHB rating and *Fusarium*-exoantigen content of 110 genotypes grouped according to their number of donor QTL after inoculation with *Fusarium culmorum* at three locations

Also in non-related winter wheat materials high correlations between both traits were found (Mesterházy et al. 1999; Miedaner et al. 2003a, b, 2004). This does not imply that there might not be other QTL that affect DON content solely as previously reported in barley (Smith et al. 2004) and wheat (Somers et al. 2003). In our study, the QTL allele 3A from Frontana had a higher reduction effect on DON content than on FHB rating.

All eight classes were represented by at least 12 $F_{3:5}$ bulks homozygous for the respective donor QTL. Using means for comparison should rule out background effects caused by genome segments that are associated with FHB resistance, but not detected by the markers used. As expected from the original mapping populations, the effects of donor QTL were mainly additive. The realized effects of stacking donor QTL were smaller than expected from the addition of the individual beneficial QTL alleles. This is, however, a common observation and was already published by Eshed and Zamir (1996) in tomato. They explained these "less than additive effects" by epistasis. Indeed, the donor-QTL allele 3A reduced DON content by 34%, but did not significantly contribute to lower DON content when combined with the other donor-QTL alleles.

No QTL class had a FHB resistance as low as the best parent CM82036 although the best bulks possessed both QTL from this source. This is an indirect hint that CM82036 contains additional QTL with a considerable phenotypic effect on FHB resistance that are not covered by the donor-QTL alleles 3B and 5A. Accordingly, the effect of the donor-QTL allele 3A did not reach the resistance of its origin, Frontana. In the original mapping population, this QTL had the largest effect (Steiner et al. 2004). Compared to the two CM82036-derived QTL (Buerstmayr et al. 2003), however, its additive effect was much smaller, thus confirming the data of our study.

Genotypic variation within QTL classes, i.e. between all individual bulks carrying the same donor QTL alleles or their combinations homozygously, was observed for both traits (Fig. 1). Indeed, this was one of the most important source of variation in the analysis of variance (Table 3). The 14 bulks possessing all three donor-QTL alleles, for example, ranged from 2.3 to 20.1 mg DON kg^{-1} and from 5 to 20% for heading-adjusted FHB rating. Accordingly, the class with three susceptibility alleles yielded some rather resistant individuals with lower DON content. Recombination between marker and QTL surely contributed to these results. Moreover, both findings indicate that there are additional loci segregating for DON content and FHB resistance in the population that were not covered by the three mapped donor QTL. Phenotypic selection following the marker-based selection would be, therefore, clearly advantageous to reach the maximum gain from selection. The best three genotypes of this study had a mean DON content of 2.2 mg kg^{-1} and a mean FHB rating of 3.9% and originated from the QTL classes with the stacked donor alleles 3B, 5A, and 3A or 3B and 5A. This is a rather high selection differential compared to the population mean of 18.8 mg kg^{-1} for DON content and 24.1% for FHB rating and indicates the beneficial effect of stacking QTL to improve FHB resistance and reduce DON in the grain. Marker-assisted introgression of the two non-adapted donor-QTL alleles 3B and 5A into European wheat germplasm should be a promising strategy to reduce the vulnerability of adapted materials to FHB epidemics, especially when it is accompanied by marker-assisted background selection. An additional phenotypic selection during backcrossing should further reduce DON content in the grain and increase FHB resistance.

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