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Effects of non-adapted quantitative trait loci (QTL) for Fusarium head blight resistance on European winter wheat and *Fusarium* isolates

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[1] **Von der Ohe**, C., E. Ebmeyer, V. Korzun, and T. Miedaner. 2010a. Agronomic and quality performance of winter wheat backcross populations carrying non-adapted Fusarium head blight resistance QTL. Crop Sci 50:2283–2290.

[2] **Von der Ohe**, C., V. Gauthier, L. Tamburic-Ilincic, A. Brule-Babel, W.G.D. Fernando, R. Clear, T.J. Ward, and T. Miedaner. 2010b. A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur J Plant Pathol 127:407–417.

[3] **Von der Ohe**, C., and T. Miedaner. 2010. Competitive aggressiveness in binary mixtures of *Fusarium graminearum* and *F. culmorum* isolates inoculated on spring wheat with highly effective resistance QTL. J Phytopathol. Doi: 10.1111/j.1439-0434.2010.01778.x.

1 General Introduction

Fusarium head blight in wheat

Winter wheat (*Triticum aestivum* L.) is a main crop in Germany grown on 3 million ha (FAOSTAT 2009). Annual yields of European winter wheat varieties in Germany and in Great Britain average between 7 to 8 t ha^{-1} (FAOSTAT 2009). In addition to improving grain yields the deployment of Fusarium head blight (FHB) resistance is an important objective of most wheat breeding programs. In the Maritimes of Canada FHB causes yield losses of up to 70 % (Bai and Shaner 1994), while losses in Europe were estimated between 10 and 30 % (Bottalico and Perrone 2002; Logrieco et al. 2002). In the U.S. from 1991 to 1997 severe epidemics occurred which caused a total loss of $2.6 billion due to FHB and subsequent mycotoxin contamination of wheat and barley (Windels 2000). Recent epidemic outbreaks of FHB were also seen in South America, Asia, and Europe (Parry et al. 1995; McMullen et al. 1997). FHB infection on wheat results in both reduced grain yield and reduced seed quality and vigor due to blighted spikes producing shrunken, bleached and shriveled kernels (McMullen et al. 1997; Goswami and Kistler 2004).

FHB is caused by the filamentous ascomycetes *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schw.) Petch) and *F. culmorum* (W.G. Smith) Sacc. (teleomorph: unknown). Out of a large number of *Fusarium* species *F. graminearum* and *F. culmorum* are the most common and important species in cereals (Parry et al. 1995; Miedaner et al. 2008). *F. graminearum* is prevalent in continental regions of Asia, North and South America, and Europe, whereas in temperate regions *F. culmorum* is most common (Parry et al. 1995; Miedaner et al. 2008). Epidemic incidences can arise suddenly, but their appearance is dependent on environmental conditions such as high humidity and rainfall occurring during flowering in the presence of susceptible hosts and aggressive isolates of the pathogen.

Wheat plants can be infected in all growth stages by seed- or soil-borne inocula produced on debris during the saprophytic life stage of the pathogen (Sutton 1982; Goswami and Kistler 2004). Heads are infected either by water-splashed conidiospores (asexual) transferred from the stem base or soil surface upward to the leaves, or directly by wind-dispersed ascospores (sexual). Disease symptoms are characterized by spikelets that eventually become bleached out completely or only in segments of the head. When conditions are highly favorable, pink-red mycelium and conidia develop on the spikelets

and infection spreads throughout the entire head with infected kernels finally become shriveled and chalky white in appearance. In addition, infected grains can be contaminated considerably with various mycotoxins (Gareis et al. 1989; Benett and Klich 2003).

F. graminearum and *F. culmorum* produce trichothecene type B toxins (deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol) and zearalenones (D'Mello and Macdonald 1997). Type B toxins are subdivided into two major chemotypes: (1) nivalenol chemotype which produces nivalenol (NIV), and (2) deoxynivalenol chemotype which produces deoxynivalenol (DON) and is often accompanied with acetylated derivatives (ADON: 3-ADON or 15-ADON). Potentially, DON inhibits protein synthesis of eukaryotic cells and possesses neurotoxic and immunosuppressive activity (Snijders 1994; Benett and Klich 2003). Even low concentration of DON in a diet can reduce food consumption of exposed animals, while higher doses induce vomiting (Benett and Klich 2003). Consequently, mycotoxin production of the fungus is a major concern for food safety. In years with epidemics of FHB the mycotoxin deoxynivalenol (DON) was found in more than 90 % of harvest samples (Gareis et al. 1989). Even without any epidemic infection 0.1 mg kg^{-1} of DON were detected in wheat samples (Lepschy 1992). Due to food safety concerns, approximately 100 countries had regulated the maximum levels of mycotoxins in food or feedstuffs by the end of 2003 (van Egmond et al. 2007). In the EU the limits in grains and food products allow a maximum DON content in unprocessed bread wheat of 1.25 mg kg^{-1}, in bread and bakeries of 0.5 mg kg^{-1}, and 0.2 mg kg^{-1} of baby food (Anonymous 2005).

Considering the current situation of crop production systems it is doubted, however, whether these legal limits can be consistently met. *Fusarium* colonizes also on maize stubbles which consequently supply the inoculum for the successive wheat crop grown (Maiorano et al. 2008). A shift in field management practices all over the world to reduced tillage, increased maize cultivation and narrower crop rotation provides the optimal growing condition for the fungus to survive and persist within fields. Controlling FHB with fungicides is still difficult and its success is largely dependent on the environment and the genotype, in particular due to the narrow time frames in which an application of fungicides would be possible. The optimal application time should be the time of flowering, which is a very short period which can be limited by rainfall conditions. Consequently, resistance breeding is the most environmentally friendly, effective and economical way to control FHB (Schroeder and Christensen 1963).

FHB resistance

Suitable sources for FHB resistance have been intensively explored in wheat (Snijders 1994; Mesterhazy 1995; Miedaner 1997). FHB resistance is quantitatively inherited with a considerable genetic variation among breeding materials (Mesterhazy 1995; Miedaner 1997) and is not species or isolate specific (van Eeuwijk et al. 1995; Toth et al. 2008). Highly resistant varieties reduce the mycotoxin levels significantly (Miller et al. 1985). Since high correlations between FHB resistance to *F. culmorum* and *F. graminearum* have been reported (Mesterhazy 1987; Miedaner et al. 1993), resistance breeding can be conducted independently of the *Fusarium* species or isolate.

In Germany, resistance breeding resulted mainly in moderately resistant varieties (Anonymous 2009). In order to improve resistance levels and detect new sources of resistance further efforts were made for identification, validation, and finally fine mapping of FHB resistance quantitative trait loci (QTL) or genes from non-adapted resistance sources in recent years. In a comprehensive meta-analysis Loeffler et al. (2009) compared 176 FHB published resistance QTL and found that most of the chromosomes of hexaploid wheat were associated with FHB resistance. The most important and widely used QTL is *Fhb1* on chromosome 3BS (Liu et al. 2006), which explained 20 to 40 % of the phenotypic variance in the mapping population (Anderson et al. 2001; Buerstmayr et al. 2002, 2003; Zhou et al. 2002). The second important QTL is *Qfhs.ifa-5A*, which is located on chromosome 5A, and was also detected in the spring wheat Sumai3-derived line CM-82036. This QTL explained 23 % of the phenotypic variation (Buerstmayr et al. 2003). Further major resistance QTL with comparably smaller effects are *Fhb2* and *Fhb3* that were detected and fine mapped on chromosomes 6BS and 7AL, respectively (Cuthbert et al. 2007; Qi et al. 2008).

For the QTL *Fhb1* a proven diagnostic marker is available and therefore, *Fhb1* is used widely as it is seen in the US cultivar *Alsen* (Gamotin et al. 2007; Mergoum et al. 2007). However, *Fhb1* is so far not incorporated in commercial varieties in Germany (Holzapfel 2009; E. Ebmeyer, personal communication). The demands on the highly competitive market for high grain yield and specific quality in Europe restricts the use of non-adapted sources of wheat with unknown side effects for breeders. In contrast, rather intensive multi-step selection that leads to accumulation of minor FHB resistance QTL has commonly been used in the European winter wheat pool (Holzapfel et al. 2008). One

positive aspect is that the major resistance QTL can be introgressed into breeding material due to marker-based selection in the shortest possible time (Wilde et al. 2007). A procedure of phenotypic and marker-based introgression was started incorporating three QTL, *Fhb1* and *Qfhs.ifa-5A* mapped in CM-82036, and *Qfhs.ifa-3A* mapped in Frontana into spring wheat material (Fig. 1; Buerstmayr et al. 2002, 2003; Steiner et al. 2004; Miedaner et al. 2006; Wilde et al. 2007).

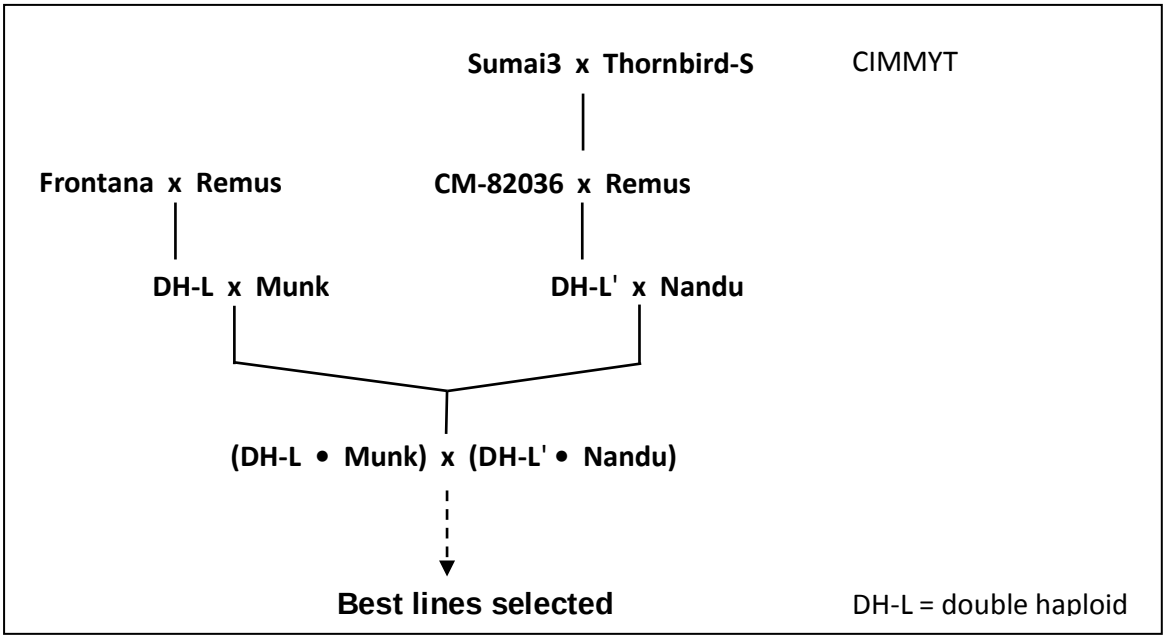

Fig. 1 Introgression of non-adapted resistance QTL *Fhb1* and *Qfhs.ifa-5A* from the line CM-82036, as well as, *Qfhs.ifa-3A* from Frontana into susceptible spring wheat material

Out of the developed population, several lines were detected by marker-assisted selection as described by Miedaner et al. (2006), in addition to phenotypic testing for FHB resistance in the field. The four best lines selected containing either one of the QTL *Fhb1* (aaBB) or *Qfhs.ifa-5A* (AAbb), both (AABB) or neither of them (aabb) were used as starting point for this thesis research.

The use of non-adapted wheat lines bearing resistance QTL in European winter wheat breeding as donor plants is feared to have negative side effects on the agronomic performance. To our knowledge, no attempts have been made to verify these possible side effects of the two important QTL, *Fhb1* and *Qfhs.ifa-5A* on agronomic and quality traits in practical breeding populations.

Genetics of *Fusarium*

F. graminearum and *F. culmorum* are both haploid. For *F. culmorum* no sexual stage is known. *F. graminearum* is homothallic (i.e. sexual reproduction of a single mycelium type is possible) but can be outcrossed under laboratory conditions to allow meiotic analysis (Bowden and Leslie 1999). More recently, outcrossing of *F. graminearum* has been observed in the field by Chen and Zhou (2009). Based on the comparison of sequence homologies of six coding regions of nuclear DNA, O'Donnell et al. (2000) classified the fungus into seven biogeographically structured lineages that were later extended to include eleven biogeographical lineages or species that were ranked as phylogenetically distinct species (O'Donnell et al. 2004). Lineage 7 corresponds to the name *F. graminearum* and is the most dominant clade species in the northern hemisphere, however, it has also been found in Asia (O'Donnell et al. 2000; Gale et al. 2002) Hence, the nine lineages cannot be strictly located to defined geographical regions (Miedaner et al. 2008). In accordance, Bowden et al. (2006) demonstrated fertile crosses between lineage 7 and all other lineages, bringing the validity of the lineage concept into question. In this study, the entire species complex is *sensu lato* represented by *F. graminearum*.

Mycotoxins as aggressiveness factor

The extent of *Fusarium* infection is different for *Fusarium* genotypes (isolates) and depends on environmental factors. The intensity of infection capability of one pathogenic isolate is defined as its aggressiveness (Vanderplank 1968) and is used in pathosystems with quantitative variation, as in the example for FHB (Mesterhazy 1984; Snijders and Perkowski 1990). Evaluation of aggressiveness is generally measured using a mean FHB rating of the disease (Voss et al. 2008). Additionally, mycotoxin production can be used to define differences between *Fusarium* isolates.

Mycotoxins produced by *Fusarium* are mainly the two trichothecenes DON and NIV with the appearance of chlorosis, necrosis and wilting on plants being the main phytotoxic effects (McLean 1996). *Fusarium* mycotoxins are thought to be one factor affecting aggressiveness on wheat heads (Snijders 1990; Desjardins et al. 1996; Eudes et al. 2001; Bai et al. 2002). In addition, several authors have shown that the presence of other fungi can affect DON production (Cooney et al. 2001; Velluti et al. 2001), suggesting

that trichothecene production is important for the competition with other fungi in the environment.

Detectable quantities of different types of trichothecenes can be produced by all isolates of *F. graminearum*. The ability to cause the disease is not influenced by the type of trichothecenes produced (Goswami and Kistler 2005). Ward et al. (2008) detected a shift from 15- to 3-ADON producing isolates in North America. They showed *in vitro* that on average 3-ADON isolates produced higher concentrations of DON than 15-ADON isolates, but the difference in aggressiveness was not significant between chemotypes in the greenhouse. Therefore, this shift could be caused by competition advantage of 3- over 15-ADON producing isolates. Screening of European isolates at the Universitaet Hohenheim detected primarily 15-ADON chemotypes suggesting a prevalence of 15-ADON isolates in Europe. However, studies investigating the differences between 3-ADON and 15-ADON chemotypes in aggressiveness and DON production in the field were not available.

Shifts in aggressiveness in the context of host-pathogen relationship

Little is known about the mechanisms that control aggressiveness. However, changes towards greater aggressiveness in *Fusarium* populations were theoretically described and experimentally proven (O'Donnell et al. 2000; Cumagun and Miedaner 2004), but studies explaining this phenomenon are scarce. Shifts between and within *Fusarium* species have been observed, for example in the Netherlands a shift from DON to NIV producers and from *F. graminearum* to *F. culmorum* were detected by Waalwijk et al. (2003). In China, a population of the *F. graminearum* clade developed a fungicide resistance (Gale et al. 2002) and in Canada a shift from 15-ADON to 3-ADON chemotypes was shown (Ward et al. 2008). All these examples support the hypothesis of adaptation to changing hosts and environments and may involve also the overcoming of improved host resistance. Consequently, this topic has to be considered in respect to the wheat/*Fusarium* pathosystem.

Breakdowns of disease resistance are known from other pathosystems when the resistance was dependent on only few major resistance genes in wheat (McDonald and Linde 2002). Varieties with major resistances genes for cereal rust and powdery mildew were overcome by adaptation of the pathogen population over several lifecycles per year

enhancing selection for better mutants, recombinants or immigrants (Wolfe and McDermott 1994; McIntosh and Brown 1997). In general, the genetic basis of aggressiveness will influence the outcome of fungal adaptation. In contrast to more easily broken, monogenic rust resistance, FHB resistance in wheat is quantitatively inherited and the counterpart of the resistance in the host system is the complex inheritance of aggressiveness in the fungal system (Cumagun et al. 2004; Holzapfel et al. 2008).

One condition for effective selection of highly aggressive isolates within a population is the availability of sufficient genetic variation. For aggressiveness and DON production, large genotypic variation was found in isolate collections within individual field populations, as well as, by the comparison of field populations from different origin (Miedaner and Schilling 1996; Miedaner et al. 2001; Goswami and Kistler 2005; Miedaner et al. 2008). Even on single heads isolates differing in their haplotype were located (Miedaner et al. 2008). However, when analyzing haplotypes in detail most were unique and low differentiation between subpopulations of a local population in North America was detected (Zeller et al. 2004). These contradictory results emphasize the involvement of various counteracting mechanisms influencing the amount of variation.

Different mechanisms such as sexual reproduction, outcrossing and high gene flow lead to genetic variation. Sexual recombination is important for the evolution of pathogen populations with the occurrence of transgressive segregants indicating that sexual recombination can result in increased aggressiveness of *F. graminearum* populations (Cumagun and Miedaner 2004; Miedaner et al. 2008). The impact of outcrossing of *Fusarium* was proven under field conditions, when the outcrossing rate ranged from 5.7 to 20.9 % in three different crosses (Chen and Zhou 2009). Nevertheless, until now it is still not known whether outcrossing and recombination occur regularly or periodically and how this can affect the adaptation ability of *Fusarium* populations.

Environmentally stable host by isolate interaction could lead to selection that eventually causes fungal adaptation to increased host resistance as described by Mesterhazy (1984). However, in the majority of published studies specific host by isolate interactions between *Fusarium* isolates and wheat varieties varying in their FHB resistance were absent across environments (van Eeuwijk 1995; Mesterhazy et al. 1999; Mesterhazy 2003). In the absence of an interaction, adaption to the host is theoretically expected to be quantitative and slower. Nonetheless, the widespread use of a few resistance QTL grown

worldwide poses a yet undefined risk of pathogen adaptation and, therefore, a shift towards increased aggressiveness and mycotoxin production. Finally, this could lead to long-term slower erosion instead of a more rapid breakdown of expected durable quantitative FHB resistance. Cumagun and Miedaner (2004) suggested that genetic potential for gradual unspecific adaptation exists. One main factor presumably enhancing this process is the competition between different *Fusarium* isolates present on the plant. In case only highly aggressive isolates can survive and reproduce one growth period on the resistant host, and this continuously over time, adaptation due to competition can be expected. Whether competition among *Fusarium* isolates is involved in shifts towards higher aggressiveness is so far not sufficiently investigated.

Objectives

The overall goal of this research was to estimate the effect of non-adapted resistance QTL in European elite wheat material on the resistance level and on the development of *F. graminearum* and *F. culmorum* populations. The specific objectives were:

1. (a) to test a marker-based selected winter wheat population subdivided into different QTL classes containing either *Fhb1* and/or *Qfhs.ifa-5A*, or neither of them, for FHB resistance, agronomic and quality traits;

   (b) to analyze side effects of these QTL by comparing the four QTL classes;

2. to compare the aggressiveness and DON production of 3-ADON *vs.* 15-ADON chemotypes in *F. graminearum*;

3. (a) to investigate the competitive aggressiveness in binary mixtures of *F. graminearum* by *F. graminearum* and *F. graminearum* by *F. culmorum* isolates differing in aggressiveness and chemotype; and

   (b) to test whether highly resistant and susceptible lines influence the competition between *Fusarium* isolates.

For references please see chapter 6.

2 Agronomic and quality performance of winter wheat backcross populations carrying non-adapted Fusarium head blight resistance QTL

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Abbreviations: BC, backcross; FHB, Fusarium head blight; QTL, quantitative trait loci; DH, double haploid line; SSR, simple sequence repeat

Abstract

Two $BC_3F_{2:5}$ winter wheat populations carrying Fusarium head blight (FHB) resistance QTL *Fhb1* and *Qfhs.ifa-5A* were tested for resistance, agronomic and baking-quality performance in 10 high yielding environments. The objectives of this study were to determine (i) the resistance effect of the two non-adapted QTL, (ii) their side effects on agronomic and quality performance and (iii) their relative advantage in European breeding programs. The two populations were split into four QTL classes containing either *Fhb1*, *Qfhs.ifa-5A*, both QTL or none of them with about 25 (*Opus* $BC_3F_{2:5}$ population) and 15 (*Anthus* $BC_3F_{2:5}$ population) lines each and check varieties. Resistance trials on microplots and yield trials on large plots had two replicates at five locations in Germany in each of two years. Mean FHB ratings (%) between QTL classes carrying either one or two QTL showed significantly ($P < 0.05$) improved FHB resistance and a significant variation within QTL classes. Small significant negative effects on grain yield were detected in the *Anthus* but not in the *Opus* $BC_3F_{2:5}$ population. However, selection of lines with improved resistance level and similar high yield level like the recurrent parent was feasible. All other differences in agronomic and quality traits were in all cases small although often significant. The introgression of both QTL did not significantly improve the FHB resistance compared to *Qfhs.ifa-5A* only indicating that this QTL suffice for European breeding programs.

3 A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat

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Abstract

Twenty four isolates of *Fusarium graminearum*, half of which were 3-acetyldeoxynivalenol (3-ADON) and half 15-acetyldeoxynivalenol (15-ADON) producers, were tested for their ability to produce deoxynivalenol and to cause Fusarium head blight (FHB), in spring wheat cultivars. The objectives of this study were to determine (i) whether 3-ADON producers differ in aggressiveness, as measured by the FHB index, and DON production from 15-ADON producers under field conditions, and (ii) whether the performance of resistant host cultivars was stable across isolates. Field tests of all isolates were conducted with three replications at two locations in Canada and Germany in 2008, with the three host genotypes differing in FHB resistance level. The resistant host genotype showed resistance regardless of the isolate or location. The differences between mean FHB indices of 3-ADON and 15-ADON chemotypes were not significant. In contrast, average DON production by the 3-ADON isolates (value) was significantly ($P < 0.05$) higher than for the 15-ADON isolates (value) at three of the four locations where moderately resistant lines were tested, and at both locations where susceptible lines were evaluated. These results indicate that 3-ADON isolates could pose a greater risk to food safety. However, as the mean aggressiveness and DON production of 3-ADON and 15-ADON isolates was quite similar on highly resistant lines, breeding and widespread use of highly resistant lines is still the most effective measure of reducing the risks associated with DON in wheat.

Keywords: chemotype, DON, FHB Index

4 Competitive aggressiveness in binary mixtures of *Fusarium graminearum* and *F. culmorum* isolates inoculated on spring wheat with highly effective resistance QTL

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Abstract

Fusarium head blight (FHB) caused by Fusarium graminearum and F. culmorum is a devastating disease with high effects on grain yield and quality. We developed spring wheat lines incorporating the highly effective FHB resistance quantitative trait loci (QTL) Fhb1 and Qfhs.ifa-5A. Whether these QTL lead to competition within Fusarium populations in the field resulting in isolates with higher aggressiveness has not been analyzed. The aims of this study were to determine (i) the aggressiveness potential of F. graminearum and F. culmorum isolates, (ii) competition effects of these isolates in binary mixtures, and (iii) the stability of resistant hosts. Six F. graminearum, two F. culmorum isolates and seven binary mixtures containing these isolates were tested for their aggressiveness and mycotoxin production at two locations in South Germany in 2007 and 2008. Host lines were four spring wheat lines containing the resistance QTL Fhb1 and/or Qfhs.ifa-5A or none and one standard variety. Re-isolates were sampled from plots inoculated with the binary mixtures to identify the percentage of each isolate in the mixture by simple sequence repeat markers. Resistant host lines reacted as expected and had a high stability to all isolates and mixtures. Only less important host x mixture interactions were detected. Aggressiveness among isolates and mixtures were significantly different. Type and amount of mycotoxin and high single isolate aggressiveness were not necessarily advantageous in the mixture. However, both F. culmorum isolates outcompeted F. graminearum isolates. Significant deviations from the inoculated 1:1 proportions occurred in 34 of 49 cases illustrating that competition effects appeared in the mixtures. These differences depended mainly on the year and not on the level of host resistance. We conclude that resistance should not be affected by the Fusarium isolates and mixtures.

Keywords: aggressiveness, competition, Fhb1, Fusarium head blight, mycotoxins, QTL, SSR marker

5 General Discussion

Usefulness of FHB resistance QTL

One main goal of wheat breeding is the continuous development of varieties to improve the yield levels within wheat crops. Common breeding strategies for FHB resistance were mainly multi-step selection and population development by accumulating favorable alleles (Holzapfel et al. 2008). With the implementation of genetic markers used in wheat breeding, trait selection has been accelerated by marker-assisted selection (MAS) (Anderson et al. 2007). MAS became an important tool for directed introgression of *Fusarium* resistance due to existing and sufficiently powerful QTL (Anderson et al. 2007; Wilde et al. 2007, 2008). In theoretical studies MAS has the potential to reach higher selection gains than phenotypic selection (Moreau et al. 2000), due to the ability of MAS to shorten the breeding period (Loeffler et al. 2009). However, it has to be considered that in advanced backcross generations the QTL effect is often smaller than in the mapping population. The following general discussion on the usefulness of certain FHB resistance QTL will proceed first with the introgression of QTL, second with the variation of the QTL effects itself and in different genetic backgrounds, and third with the analysis of possible side effects on different agronomic traits.

In our study, the two *Fusarium* resistance QTL *Fhb1* and *Qfhs.ifa-5A* were introgressed from spring into winter wheat material by crossing a selected spring wheat line AABB (Miedaner et al. 2006) containing both resistance QTL with two winter wheat varieties (Fig. 2; von der Ohe et al. 2010a). The recipient parents were the moderately resistant variety Anthus and the susceptible variety Opus. In all backcross (BC_x)-generations lines were analyzed with three flanking markers for *Fhb1* and two for *Qfhs.ifa-5A* to ensure that the QTL alleles were present. The number of analyzed offspring (*n*) was large, because an additional target was the dissection of the QTL into smaller segments.

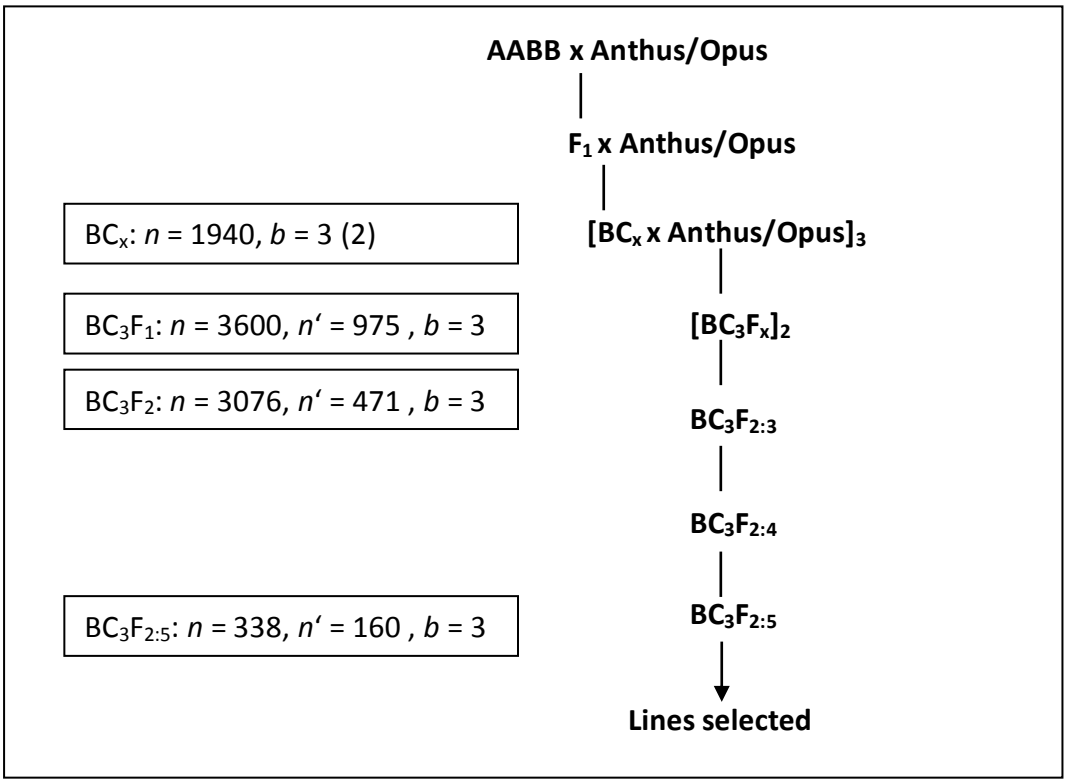

Figure 2 Introgression of non-adapted resistance QTL *Fhb1* and *Qfhs.ifa-5A* from the selected spring wheat donor line AABB into the moderately resistant variety Anthus and the susceptible variety Opus, respectively. Where, *n* is the population size, summed up for both populations, before and *n'* that after preselection for presence of donor alleles at the target markers, *b* is the number of selection markers per locus analyzed in the *n* plants

After the first two selfing steps the populations were selected for the target-QTL to be homozygous. The BC_3F_2 seeds were screened with markers for the following four QTL classes: QTL *Qfhs.ifa-5A* (AAbb), *Fhb1* (aaBB), both (AABB) or neither of them (aabb) in the homozygous state. Selected BC_3F_2 derived-bulks in generation $BC_3F_{2:3}$ were propagated twice resulting in $BC_3F_{2:5}$. In this generation, for each of the four QTL classes 25 and 15 lines were marker-assisted selected in the Anthus and Opus population, respectively. Afterwards, these lines were phenotypically tested in the field (von der Ohe et al. 2010a).

Table 1 Mean FHB rating (%) of selected lines containing either *Fhb1* (aaBB), *Qfhs-ifa.5A* (AAbb), both QTL (AABB) or none of them (aabb). The spring wheat lines were tested across nine different isolate mixtures at two locations (EWE, HOH) in 2007 and 2008 and the winter wheat lines were inoculated with one isolate across five locations (HOH, OLI, SEL, WET, WHO) in 2008 and 2009

Entry	Spring wheat	Winter wheat	
		Opus background	Anthus background
AABB	4.3	26.9	14.2
aaBB	9.5	36.0	16.5
AAbb	9.2	30.7	15.6
aabb	40.0	42.9	19.6

The resistance improvement was analyzed between spring wheat lines without and with FHB QTL *Fhb1* and *Qfhs.ifa-5A* in generation BC_0, and similarly between selected winter wheat lines in generation BC_3 (von der Ohe and Miedaner 2010; von der Ohe et al. 2010a). When comparing single lines containing both QTL (AABB) or neither QTL (aabb), disease symptoms were decreased in highly susceptible backgrounds by 36 % (spring wheat) and 16 % (winter wheat), respectively (Table 1). The different effect of the resistance QTL could be explained (1) by the genetic backgrounds (i.e. genetically different recipient varieties, for example Anthus and Opus varieties), or general genetic difference between spring and winter wheat, (2) by additional minor QTL contained either in the donor or the recipient parent, and (3) by background effects of non-adapted chromosome segments. In accordance with the findings of Salameh (2005), we detect that these QTL function were additive and independent of the differences between the absolute effects in the different backgrounds. This underlines the interest in combining major QTL to increase resistance level.

Explanations for differing QTL effects are the variability of the genetic background and the varying proportion of the donor genome. Theoretical considerations define the expected proportion of the donor genome in generation BC_n as $1/2^{n+1}$. The theoretical expected donor percentage in generation BC_3 was, on average, 6.25 %. In our testing material a $BC_3F_{2:5}$-population was analyzed with 92 SSR markers covering the entire genome. The percentage of the donor genome was, close to what was theoretically expected, on average 6.7 % (von der Ohe et al. 2010a).

Large phenotypic variation of FHB resistance was detected within each QTL class in the $BC_3F_{2:5}$ population of our study, which underlines the importance of phenotypic selection parallel to MAS (von der Ohe et al. 2010a). Wilde et al. (2007) suggested that the best way to use the full range of quantitative variation for resistance is to first apply MAS followed by phenotypic selection. This technique allows the incorporation of minor QTL which have been undetected in QTL mapping studies by phenotypic selection. The phenotypic variation was dependent on the environment, particularly in the amount of disease severity present in different years (von der Ohe and Miedaner 2010; von der Ohe et al. 2010a). Consequently, major QTL effects can be less pronounced in new genetic backgrounds and environments and, therefore, their effects have to be validated in each breeding population.

In contrast to the worldwide importance of *Fhb1* and *Qfhs.ifa-5A*, in Europe, and especially in Germany, these QTL have not been employed in breeding material till now (Holzapfel et al. 2008; E. Ebmeyer, personal communication), because the extent of the QTL effect is uncertain in the European material. The main reason is attributed to the fear of negative side effects (linkage drag) due to the introgression of these QTL because of their origin from non-adapted sources. In the best case, MAS would select the smallest segment of the non-adapted chromosome incorporating the QTL, which is dependent on the position of the linked markers. Today, diagnostic markers for *Fhb1* are available within a 1.2 cM interval (Liu et al. 2006, Buerstmayr et al. 2009) improving MAS and usefulness for breeders.

We initiated our study at the University of Hohenheim in 2002, at this time no fine-mapping analyses on *Fhb1* and *Qfhs.ifa-5A* were available. In our mapping population the marker used flanked a chromosome segment of 17.5 cM on 3BS and 13.6 cM on 5A. The target QTL position is near the teleomer and the total length of chromosome 3BS and 5A is 148 cM and 184 cM, respectively (Somers et al. 2004). However, the exact size of the non-adapted introgressed donor chromosome segment still surrounding the flanking markers is unknown. This implies that a large number of non-adapted donor genes could be introduced into the genetic background of the recipient elite line, therefore, negative side effects may be possible.

Yield and quality decreases due to negative side effects were reported in several introgression studies in the *Fusarium* and other pathosystems (Koen et al. 2002; McCartney et al. 2007; Mergoum et al. 2007; Brevis et al. 2008; Fedak et al. 2008).

However, yield was not tested up to now in the context of the FHB-resistance QTL. In the presented experiment, significantly reduced grain yield of 0.4 Mg ha^{-1} across lines containing two QTL compared to lines without QTL were found in the Anthus background (von der Ohe et al. 2010a), whereas the difference was small and not significant in the Opus background. Lines containing either one of the QTL were significantly improved in resistance without significantly decreased yield levels. Due to marginal negative side effects, we conclude that the segment of the donor genome in generation BC_3 was sufficient reduced in our lines.

In conclusion, the introgression of the QTL *Fhb1* and *Qfhs.ifa-5A* can be recommended, when using a combination of MAS with phenotypic selection. Neither QTL led to major negative side effects in the two $BC_3F_{2:5}$ populations. However, lines carrying *Qfhs.ifa-5A* performed better than *Fhb1* in the tested German winter wheat background (von der Ohe et al. 2010a).

Stability of resistance QTL across isolates

An important advantage of *Fusarium* resistance QTL is their stability across isolates. Toth et al. (2008) tested 15 isolates of *F. graminearum* and six isolates of *F. culmorum* on 20 wheat genotypes differing in their resistance level including Sumai3-derived lines. Buerstmayr et al. (2008) observed with 56 wheat lines ranging from susceptible to resistant when tested across ten environments that the more resistant lines were more stable. Accordingly, in all of our field experiments moderately resistant or susceptible lines varied more in mean FHB rating and DON production due to isolates than the highly resistant wheat lines (Fig. 3; von der Ohe and Miedaner 2010; von der Ohe et al. 2010a; von der Ohe et al. 2010b). We, therefore, confirmed that the FHB resistance QTL *Fhb1* and/or *Qfhs.ifa-5A* improved the resistance stably compared to lines without these QTL.

Aggressiveness was estimated for isolates from two continents. In Canada concerns about outstanding highly aggressive isolates are present due to recurring FHB epidemics (Paulitz et al. 1999; Clear and Patrick 2008). The aggressiveness of single isolates from Europe and Canada showed similar range of variation for both groups when tested in Germany (Fig. 3). These were confirmed by Miedaner and Schilling (1996), who found large genotypic variation already within field populations from different continents.

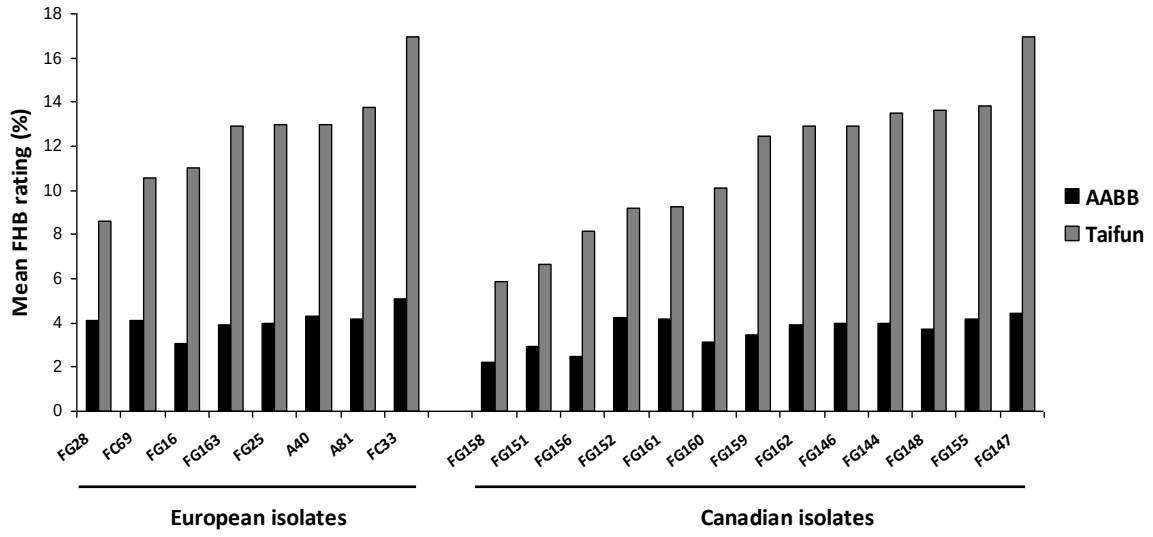

Figure 3 Variation of aggressiveness tested as mean FHB rating of six *F. graminearum* (FG) und two *F. culmorum* (FC) European isolates and 13 Canadian FG isolates on the highly resistant lines AABB and the susceptible cultivar Taifun across two locations (Hohenheim and Eckartsweier) and two years (2007 and 2008)

Additionally, our results emphasize the influence of the host genotype on the level of aggressiveness. The resistant line with both QTL AABB had mean FHB ratings of only 2 - 5 %, as compared to the susceptible hosts where aggressiveness ranged comparably wide. We conclude that in general aggressiveness of isolates from different parts of the world has similar ranges of aggressiveness independent of the epidemic pressure, but mainly dependent on the host resistance.

The absolute level of the resistance, however, is always depending on the environment due to favorable growing conditions such as optimal temperature and relative humidity in specific years. Several studies found, hence, high isolate by environment interactions. For example, Cumagun and Miedaner (2004) reported from a field test with a *F. graminearum* population an isolate by environment interaction accounting for 29 % of the total variance for aggressiveness and 19 % of the variance for DON production. Similar findings for highly significant interactions of two *F. graminearum* crossing populations in the field were described by Voss (2010). Our experiments confirmed these results by detecting significant ($P < 0.01$) isolate by environment interactions for *Fusarium* mixtures and single isolates in four environments (von der Ohe and Miedaner 2010). Wheat lines and isolates are both mainly influenced by the genotype by environment interactions that always refer to the development of FHB symptoms.

In contrast to the interaction with environment, isolate by host line interactions were not confirmed in our experiments, which is in accordance with several other studies (van Eeuwijk et al. 1995; Mesterhazy 2003; Voss 2010). In studies detecting contradictory results, the interactions were mainly due to scaling effects of the evaluated isolates, which varied widely in their aggressiveness (Dusabenyagasani et al. 1997; Mesterhazy 2002). We observed significant ($P < 0.05$) host line by isolate interaction, but in the absence of the susceptible line in the ANOVA the interactions was not significant (von der Ohe and Miedaner 2010).

In general FHB resistance in wheat is non-specific and horizontal for *F. graminearum* and *F. culmorum*, which was found by several authors in accordance to our findings (van Eeuwijk et al. 1995; Mesterhazy et al. 1999; Goswami and Kistler 2005). In fact, this is also true for highly aggressive isolates and highly resistant wheat lines. Consequently, there should not be concern for fast adaptation to single widely used resistance QTL, which is strengthened by the high environmental stability of the resistance QTL and the lack of interactions of isolates with highly resistant hosts.

Aggressiveness changes of *Fusarium* populations

Sources for variation among isolates include several mechanisms like mutation, mating, outcrossing, geneflow and competition effects (McDonald and Linde 2002; Miedaner et al. 2008). The role of mutation as variation cause is important when considering high propagation number of *Fusarium* under epidemic conditions. The average spontaneous mutation frequency per aggressiveness locus in fungi is generally given as 1×10^{-6} (Cumagun 2004). In large populations of *Fusarium* asexual reproduction is more frequent than sexual one but a wheat-maize crop rotation would support sexual reproduction allowing at least one recombination per year leading to a new variation. A recent study of Chen and Zhou (2009) estimated an outcrossing rate of 5.7 to 20.9 % of three *F. graminearum* crosses in rice fields. Nevertheless, it still remains unclear whether outcrossing occurs regularly or rather episodically under field conditions. To sustain genotypic variation, rare sexual recombination would suffice (Leslie and Klein 1996; Zeller et al. 2004).

Genetic variation among *Fusarium* isolates is known from miscellaneous studies detecting different haplotypes and species on one wheat head, as well as, in collections

from different parts of the world (Miedaner et al. 2008). Molecular marker techniques enable to distinguish between different haplotypes arising from natural infection on a single wheat head (Miedaner et al. 2001). Moreover, genotypic diversity detected between haplotypes even exists between countries and continents (Miedaner et al. 2001; Gale 2003; Akinsami et al. 2006a). The genetic variation is reflected by a high phenotypic variation. Morphological variation among *F. graminearum* and *F. culmorum* isolates in culture were described by several authors (Mitter 1929; Oswald 1949; Puhalla 1981). Futhermore, a large variation was detected for aggressiveness measured by symptom development, host colonization, and the type and amount of mycotoxin production between isolate collections from different continents (Mesterhazy 1984; Miedaner and Schilling 1996; Miedaner et al. 2000; Desjardins et al. 2004; Toth et al. 2004; Akinsami et al. 2006b).

In our study, DON production varies similarly according to aggressiveness (von der Ohe and Miedaner 2010). Several authors detected high correlations between aggressiveness and DON production in artificial infections (Cumagun and Miedaner 2004; Goswami and Kistler 2005; Nicholson 2009). The DON content should always be a second selection criteria besides the visible symptoms, for example the shift of chemotypes from 15-ADON to 3-ADON led to higher DON production without a significant detectable increase in mean FHB rating (von der Ohe et al. 2010b). Higher amounts of DON would be problematic if susceptible lines were mainly grown. The association between symptoms and trichothecenes is complex and dependent on the resistance level of the host line but also is controlled by the isolates and their competition. How these mechanisms influence each other is still not clear and therefore, all detected changes in variation of aggressiveness and DON production may indicate adaptation processes of the isolates.

Adaptation of isolates and competition within *Fusarium* populations

Adaptation of isolates to resistant hosts cannot be detected on the single isolate level but on the population level. Within a population exists competition which could enhance the survival of the most aggressive isolate. From other pathosystems it is known that artificial selection for quantitative traits like aggressiveness can result in pathogens that quantitatively adapt to the host. Selection towards higher aggressiveness may be driven by the competition between the isolates in the population.

Methods detecting such competitions on plants are either marker techniques differentiating diseased material with quantitative PCR on isolate level or the re-isolation of inoculated mixtures on wheat heads. The first method has not been practiced because isolate-specific markers are not available for quantification within *F. graminearum*. Only markers distinguishing between isolates without quantification can be found. The second method uses SSR markers and is, therefore, mainly depending on the sample size, which should be large enough to detect small differences in re-isolation proportion. This method was used in our study and resulted in a data set for analyzing the competition of *Fusarium* isolates in binary mixtures, which can be defined as simplified model for highly complex *F. graminearum* populations occurring in the field (von der Ohe and Miedaner 2010). In this model population the competition between two isolates was quantified by changes in mixture proportions.

In the presented experiment, the competition in populations on the field, measured as mixtures aggressiveness and re-isolation rate, were not reflected by differences in single isolate aggressiveness (von der Ohe and Miedaner 2010). Therefore, isolate aggressiveness itself cannot predict the ability of one isolate to compete against another isolate because after analyzing competition in binary mixtures, one isolate could not always prevail against its mixture partners (von der Ohe and Miedaner 2010). These varying results might be explained by intra tissue competition effects. Cell wall degrading enzymes might be different among isolates and may lead to differences in the speed of infestation on the wheat head.

We detected significant ($P < 0.05$) but not directed competition effects (von der Ohe and Miedaner 2010). These findings agreed well with a study of Reid et al. (1999) about competition between *F. verticilioides* and *F. graminearum* on maize. They concluded that the outcome of fungal competition is environmentally unstable and changes from year to year. In addition, the host plant of *Fusarium* is always changing due to crop rotation with *Fusarium* isolates having the ability to change from parasitical to saprophytic life stage. In a field study the genetic diversity were compared within a saprophytic field populations from maize stubble (Naef and Defago 2006) with a pathogenic population from wheat (Miedaner et al. 2001). The genotypic diversity of the saprophytic population was significantly higher than in the parasitic population, and allelic richness and gene diversity were similar in both populations. Large genetic variation would persist if the forces of selection act differentially on the two subpopulations. Balanced selection may

explain the results from experiments on artificial media, in which only a weak association between aggressiveness on wheat, fecundity and growth rate were reported for *F. graminearum* (Akinsanmi et al. 2007). When the selection effects of host, environment and change of saprophytic to parasitic phase counteract each other, then eventually, competition among *Fusarium* isolates will not lead to directed selection but rather to a balanced one. The fluctuating and changing factors seem to counteract the selection of highly aggressive isolates. Rather new genetic variation could be generated due to balanced selection (Miedaner et al. 2008), which concurs well with the assumption of non-adapted *Fusarium* species to the host. However, without monitoring the gene flow between parasitical and saprophytic phase any conclusions can be drawn on pathogen adaptation and on the potential specialization to crop rotation and the deployment of resistant sources.

Conclusions for breeding FHB resistance

Our research demonstrated that the *Fusarium* head blight resistance can be stably improved by the non-adapted major QTL *Fhb1* and *Qfhs.ifa-5A*. Concerns about negative side effects of these QTL decreasing the yield level or downgrading the quality could be negotiated, because the variation among BC progeny was wide enough to detect optimal genotypes. *Qfhs.ifa-5A* is preferable in comparison to *Fhb1* in German populations and climatic conditions. The use of the additive effect of both QTL together is not suggested, since no significant differences were detected between one single QTL or the combination of both QTL. *Qfhs.ifa-5A* is especially interesting in susceptible backgrounds. The introgression is only possible with the help of MAS, because phenotypic introgression of non-adapted QTL would be too time consuming. However, wide ranges of resistance level in each marker class were detected; therefore, phenotypic selection is still advantageous after MAS selection. Conclusively, this study underlines the usefulness of the non-adapted QTL *Fhb1* and *Qfhs.ifa-5A* for European breeding programs.

The variation in aggressiveness and DON production among *Fusarium* isolates from Europe and Canada was demonstrated to have a similar range. However, we detected a higher DON production of 3-ADON than of 15-ADON producing isolates on susceptible host genotypes. Consequently, resistant host genotypes should be grown to reduce mycotoxin contamination.

One goal of my research was to understand the complex interactions of isolates within *Fusarium* spp. We therefore analyzed competition effects in binary mixtures by counting the re-isolation rate. The detected competition between isolates was neither predictable nor directed towards selection of higher aggressive isolates. Similarly, the effects of these non-adapted QTL on the *F. graminearum* and *F. culmorum* isolates and mixtures were analyzed as stable. Consequently, adaptation to resistant hosts is unlikely on the short term.

In a long term perspective we suggest the introgression of the major resistant QTL into practical breeding material since no linkage drag was found for both QTL individually. The breeding for Fusarium resistance will be easier in later stages, because molecular markers are not essential for controlling that the resistance QTL are kept in the material. Phenotypic selection under artificial inoculation is sufficient. Additional positive effects are that the non-adapted QTL region (1) is going to be smaller due to recombination in further generations and (2) can be combined with minor QTL due to additive effects.

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7 Summary

Fusarium head blight (FHB), caused by *Fusarium graminearum* and *F. culmorum*, is a devastating disease responsible for tremendous damage in wheat fields and contamination of grain with mycotoxins deoxynivalenol (DON) and nivalenol (NIV), rendering the harvest unsafe for human and animal consumption. The variability of *Fusarium* populations is high and changes in aggressiveness, chemotypes or species within and among *Fusarium* populations are known. Stable FHB resistance combined with high yield is one main target in wheat breeding programs. Mapping studies detected several quantitative trait loci (QTL) for FHB resistance in non-adapted sources, such as Sumai3 from China. The two most important and commonly used major QTL are located on chromosome 3BS (*Fhb1*) und 5A (*Qfhs.ifa-5A*). However, negative side effects of non-adapted resistance sources introgressed in elite winter wheat material are feared in Europe. Furthermore, the stability of the QTL effect against changing *Fusarium* populations is unknown.

The objectives of this research were to analyze whether (1) the QTL *Fhb1* and *Qfhs.ifa-5A* introgressed from a non-adapted resistance source into two winter wheat varieties have possible side effects on agronomic and quality performance, (2) 3-ADON and 15-ADON chemotypes are significantly different in their aggressiveness and DON production, (3) competition among *Fusarium* isolates in mixtures exists, and if so, how the resistant host will influence this competition.

All experiments incorporated disease resistance trials on microplots measuring severity and incidence on a whole-plot basis and mycotoxin production. The winter wheat trails were tested in two replicates ten environments across Germany. The spring wheat trails were replicated three times at four environments.

For the first experiment, we incorporated the highly effective FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A* in two spring wheat lines. One spring wheat line containing the two QTL *Fhb1* and *Qfhs.ifa-5A* (AABB) was selected and used as donor line to introgress both QTL in the two winter wheat varieties Anthus and Opus. Two $BC_3F_{2:5}$ winter wheat populations with 60 (Anthus) and 100 (Opus) lines were developed. Both populations were split into four QTL classes containing either *Fhb1*, *Qfhs.ifa-5A*, both QTL or neither of them. FHB resistance was significantly ($P < 0.05$) improved between QTL classes carrying either one or two QTL, however, genotypes showed a significant variation within QTL classes in both populations. In the class containing both QTL grain yield was significantly negatively affected by 1.6 % in the Anthus but not in the Opus $BC_3F_{2:5}$ population.

However, for breeders selection of lines with improved resistance level and similar high yield level like the recurrent parent would be feasible. For agronomic and quality traits, differences were in all cases small and negligible but often significant.

The second experiment consisted of 24 isolates of *Fusarium graminearum*, half of which were 3-acetyldeoxynivalenol (3-ADON) and half 15-acetyldeoxynivalenol (15-ADON) chemotypes randomly sampled across Canada. Mean FHB ratings of 3-ADON *vs.* 15-ADON isolates were not significantly different in any spring wheat genotype. In contrast, average DON production of the 3-ADON isolates (10.44 mg kg^{-1}) was significantly ($P < 0.05$) higher than for the 15-ADON isolates (6.95 mg kg^{-1}) on most of the moderately resistant and susceptible lines. On highly resistant lines, mean aggressiveness and DON production of 3-ADON and 15-ADON chemotypes were similar. However, changes towards increased occurrence of 3-ADON isolates might lead to higher DON levels.

The third experiment contained six *F. graminearum*, two *F. culmorum* isolates and seven binary mixtures containing these isolates. Host lines were four spring wheat lines containing either *Fhb1* (aaBB) or *Qfhs.ifa-5A* (AAbb), both (AABB) or neither of them (aabb). Re-isolates were sampled from plots inoculated with the binary mixtures to identify the percentage of each mixing partner by simple sequence repeat markers and to detect competition effects. Resistant host lines showed a high stability to all isolates and mixtures. Host by mixture interactions were detected, but no change in rank order occurred. Isolates differed significantly ($P < 0.05$) in their aggressiveness when tested individually and in mixtures. The type and amount of mycotoxins and high aggressiveness of single isolate does not necessarily give greater competitive ability in the mixture. Significant deviations from the inoculated 1:1 proportions occurred in 34 of 49 cases illustrating that competition effects appeared in most instances and these effects depended mainly on the year and not on the level of host resistance.

In conclusion, both resistance QTL are effective and stable in elite spring and winter wheat backgrounds. For improvement of FHB resistance both QTL are valuable, but *Qfhs.ifa-5A* would suffice for European breeding programs. Due to chemotype shifts, 3-ADON isolates could pose a greater risk to food safety than 15-ADON but breeding and use of highly resistant lines can reduce the risks associated with DON in wheat. Accordingly, resistant spring wheat lines were less affected by the tested *Fusarium* isolates and mixtures and, therefore, confirmed a high stability of these QTL. Directed selection of highly aggressive isolates due to the resistance QTL seems to be unlikely in the short term.

8 Zusammenfassung

In der europäischen Weizenproduktion verursachen Ährenfusariosen hohe Ertrags- und Qualitätsverluste. Die Hauptpathogene im Weizen sind *Fusarium graminearum* und *F. culmorum*. Diese kontaminieren das Erntegut mit den von ihnen produzierten Mykotoxinen Deoxynivalenol (DON) und Nivalenol (NIV). Die genetische Variabilität der Pilze ist groß, so dass immer wieder Veränderungen in ihrer Aggressivität auftreten. Es gibt sowohl Verschiebungen zwischen Arten und zwischen Chemotypen als auch innerhalb von Populationen. Ein großes Ziel der Pflanzenzüchtung ist es daher ertragreichen Sorten mit stabiler Resistenz zu etablieren. Bisher konnten in verschiedenen Kartierungspopulationen quantitative vererbte Genorte (quantitative trait loci, QTL) für die *Fusarium*-Resistenz gefunden werden. Eine Resistenzquelle ist die chinesische Weizensorte Sumai3, welche zwei weit verbreitete major QTL auf den Chromosomen 3BS (*Fhb1*) und 5A (*Qfhs.ifa-5A*) trägt. Bei einer Einkreuzung dieser QTL in Elitematerial werden jedoch negative Nebeneffekte aufgrund der nicht adaptierten Quelle befürchtet. Des Weiteren ist die Stabilität dieser QTL gegenüber sich verändernden *Fusarium*-Populationen nicht bekannt.

Die Ziele dieser Studie waren (1) die Analyse möglicher Nebeneffekte der zwei QTL *Fhb1* und *Qfhs.ifa-5A* auf agronomische Eigenschaften und Qualitätsparameter, da die QTL aus nicht adaptierten Resistenzquellen in zwei Elite-Winterweizensorten eingekreuzt wurden, (2) die Messung der Aggressivität und der DON-Produktion von 3-acetyldeoxynivalenol (3-ADON) im Vergleich zu 15-ADON Chemotypen, und (3) die Modellierung des Wettbewerbs zwischen *Fusarium*-Isolaten in Mischungen in Abhängigkeit von unterschiedlich resistenten Wirtsgenotypen.

Resistenztests in Mikroparzellen wurden in allen Versuchen durchgeführt, um das Auftreten und die Ausbreitung des *Fusarium*-Befalls zu bestimmen und die DON-Produktion zu messen. Während die Winterweizeversuche in zwei Wiederholungen in zehn Umwelten deutschlandweit getestet wurden, fanden die Sommerweizenversuche in drei Wiederholungen an vier Umwelten statt.

Im ersten Versuch wurden die zwei hoch effektiven *Fusarium*-Resistenz-QTL *Fhb1* und *Qfhs.ifa-5A* in zwei Sommerweizen-Sorten eingelagert. Aus den Nachkommen erfolgte eine Selektion von vier Linien, die entweder den QTL *Fhb1* (aaBB) oder *Qfhs.ifa-5A* (AAbb), beide (AABB) oder keinen der beiden (aabb) beinhalten. Die Linie AABB war der Donor, um beide QTL in die zwei Winterweizensorten Anthus und Opus einzukreuzen.

Zwei $BC_3F_{2:5}$-Populationen mit 60 (Anthus) und 100 (Opus) Linien wurden entwickelt. Diese enthielten vier QTL-Klassen, die entweder *Fhb1*, *Qfhs.ifa-5A*, beide QTL oder keinen der beiden beinhalteten. Die *Fusarium*-Resistenz konnte in den QTL-Klassen sowohl mit einem als auch mit zwei QTL signifikant ($P < 0,05$) verbessert werden, jedoch zeigten sich signifikante Unterschiede zwischen den Genotypen innerhalb der QTL-Klassen in beiden Populationen. In der Klasse mit beiden QTL war der Kornertrag in der Anthus-Population um 1,6 % signifikant ($P < 0,05$) reduziert. Für den Einsatz in der praktischen Züchtung könnten aber vor allem aus der Opus-Population Linien mit einer verbesserten Resistenz bei gleichem Ertragsniveau wie der rekurrente Elter selektiert werden. Die Unterschiede in den agronomischen Eigenschaften und in den Qualitätsparametern waren signifikant, in allen Fällen jedoch klein und vernachlässigbar.

Im zweiten Versuch wurden 24 *F. graminearum*-Isolate verwendet, die in ganz Kanada gesammelt wurden und zur Hälfte 3-ADON-Chemotypen und zur anderen Hälfte 15-ADON-Chemotypen waren. Der *Fusarium*-Boniturmittelwert der 3-ADON-Isolate unterschied sich auf allen getesteten Sommerweizengenotypen nicht signifikant von dem der 15-ADON-Isolate. Im Gegensatz dazu war die mittlere DON-Produktion der 3-ADON-Isolate (10,44 mg kg^{-1}) auf den meisten der moderat resistenten und anfälligen Wirtsgenotypen signifikant ($P < 0,05$) höher als die der 15-ADON-Isolate (6,95 mg kg^{-1}). Auf den hochresistenten Wirten waren die mittlere Aggressivität und die DON-Produktion der 3-ADON- und 15-ADON-Chemotypen gleich. Eine Veränderung hin zu einem vermehrten Auftreten der 3-ADON-Isolate hin könnte jedoch zu einem höheren DON-Niveau im Erntegut führen.

Der dritte Versuch bestand aus sechs *F. graminearum*-, zwei *F. culmorum*-Isolaten und sieben binären Mischungen aus den genannten Einzelisolaten. Als Wirtsgenotypen dienten die vier beschriebenen Sommerweizenlinien mit den Resistenz-QTL. Die Mikroparzellen wurden mit den binären Mischungen inokuliert und nach 28 Tagen Re-Isolate gesammelt. Aus den Re-Isolaten wurde der Anteil jedes Mischungspartners mit Hilfe von Mikrosatelliten-Markern identifiziert, um daraus Schlüsse auf den möglichen Wettbewerb zwischen den Mischungspartnern zu ziehen. Es wurden Wirt-Mischungs-Interaktionen ohne Veränderung in der Rangordnung festgestellt. Die Art und Menge der produzierten Mykotoxine und die Höhe der Aggressivität der Einzelisolate war nicht unbedingt mit einer höheren Wettbewerbsfähigkeit in der Mischung verbunden. Signifikante Abweichungen vom inokulierten 1:1 Verhältnis wurden in 34 von 49 Fällen

bestimmt, was zeigt, dass in den meisten Fällen ein Wettbewerb vorhanden war. Diese Effekte hingen meistens vom Jahr und nicht vom Resistenzniveau des Wirtes ab.

Beide Resistenz-QTL waren im Elite-Sommer- und Winterweizenhintergrund effektiv und stabil. Zur Verbesserung der *Fusarium*-Resistenz sind beide QTL sinnvoll einsetzbar. Die Ergebnisse zeigten, dass der Einsatz von *Qfhs.ifa-5A* in europäischen Zuchtprogrammen vorteilhaftesten wäre. Durch die Veränderung innerhalb der Chemotypenzusammensetzung von *Fusarium*-Populationen könnten 3-ADON-Isolate ein höheres Risiko für die Nahrungsmittelsicherheit bedeuten als 15-ADON-Isolate. Die Züchtung und der Anbau von hochresistenten Sorten kann dieses Risiko, das mit erhöhten DON-Werten im Erntegut verbunden wäre, verringern. Die resistenten Sommerweizenlinien waren weniger mit den getesteten *Fusarium*-Isolaten und -Mischungen infiziert als die anfälligen, was die Stabilität der QTL bestätigt. Eine gerichtete Selektion von hoch aggressiven Isolaten durch den Anbau von Sorten mit Resistenz-QTL ist zumindest kurzfristig unwahrscheinlich.

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Employment record

Since Jun 2010:	KWS LOCHOW GMBH, Bergen

Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und nur unter Zuhilfenahme der angegebenen Quellen und Hilfsmitteln angefertigt wurde. Dementsprechend wurden wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass nicht zu einem früheren Zeitpunkt oder gleichzeitig ein Antrag auf Eröffnung eines Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt wurde.

Stuttgart, Juni 2010

Christiane von der Ohe