Aus der
Landessaatzuchtanstalt
der Universität Hohenheim
Arbeitsgebiet Roggen
Prof. Dr. T. Miedaner

Inheritance of quantitative resistance and aggressiveness in the wheat/*Fusarium* pathosystem with emphasis on *Rht* dwarfing genes

Dissertation
zur Erlangung des Grades eines Doktors
der Agrarwissenschaften
vorgelegt
der Fakultät Agrarwissenschaften

von
Master of Science
Hans-Henning Voß
aus Bad Gandersheim

Stuttgart - Hohenheim
2010

Die vorliegende Arbeit wurde am 30.06.2010 von der Fakultät Agrarwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften (Dr. sc. agr.)“ angenommen.

Tag der mündlichen Prüfung: 09.07.2010

1. Prodekan: Prof. Dr. Andreas Fangmaier

Berichterstatter, 1. Prüfer: Prof. Dr. Thomas Miedaner

Mitberichterstatterin, 2. Prüferin: Prof. Dr. Chris-Carolin Schön

3. Prüfer: Prof. Dr. Ralf T. Vögele

Contents

[1] Voss, H.-H., J. Holzapfel, L. Hartl, V. Korzun, F. Rabenstein, E. Ebmeyer, H. Coester, H. Kempf, and T. Miedaner. 2008. Effect of the *Rht-D1* dwarfing locus on *Fusarium* head blight rating in three segregating populations of winter wheat. Plant Breeding 127:333–339.

[2] Miedaner, T. and H.-H. Voss. 2008. Effect of dwarfing *Rht* genes on *Fusarium* head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Science 48:2115–2122.

[3] Holzapfel, J., H.-H. Voss, T. Miedaner, V. Korzun, J. Haeberle, G. Schweizer, V. Mohler, G. Zimmermann, and L. Hartl. 2008. Inheritance of resistance loci to *Fusarium* head blight in three European winter wheat populations. Theoretical and Applied Genetics 117:1119–1128.

[4] Voss, H.-H., R.L. Bowden, J.F. Leslie, and T. Miedaner. 2010. Variation and transgression of aggressiveness among two *Gibberella zeae* crosses developed from highly aggressive parental isolates. Phytopathology 100:904-912

1. General Introduction

In 1809 the genus *Fusarium* was first described by the German naturalist Johann Heinrich Friedrich Link and comprises a broad spectrum of highly pathogenic species, producing important diseases on roots, stems, leaves, cereal heads and corn cobs of plants at almost any time in their life cycle. *Fusarium* head blight (FHB), also known as scab, or *Fusarium* ear blight is one of the most devastating fungal diseases affecting several small-grain cereals such as wheat, barley, rye, oats and rice worldwide. Throughout the last century repeatedly occurring FHB epidemics have been documented in all main regions of wheat production such as Central and East Europe, Russia, China, Australia, Argentina and especially the US and Canada (Windels 2000). FHB infection on wheat reduces grain yield, seed quality and vigor due to blighted spikes producing shrunken, bleached and shriveled kernels (tombstones) with depressed seed weights (McMullen et al. 1997, Goswami and Kistler 2004).

Among the large number of *Fusarium* species that can cause FHB, relatively few are considered to be of overall significance (Parry et al. 1995). Homothallic *Fusarium graminearum* (teleomorph *Gibberella zeae* (Schwein.) Petch) is the most frequently encountered and most destructive pathogen that causes FHB in cereals as well as *Gibberella* ear rot in maize worldwide (Miedaner et al. 2008). Depending on environmental conditions different species are predominant in different of the world´s wheat-growing areas. Whilst *Fusarium graminearum* generally is associated with warmer and humid conditions mainly of North America, Central Europe and China, anamorph *Fusarium culmorum* (W.G. Smith) Sacc. (teleomorph not known) and *Fusarium avenaceum* (teleomorph *Gibberella avenaceae*) play an important role in cooler, maritime regions of Northern Europe (Leonard and Bushnell 2003, Xu et al. 2005, Miedaner et al. 2008). *Fusarium poae* (teleomorph not known) is associated more with relatively dry warm conditions and is reported to prevail in some European and North and South-American countries (Nicholson 2009).

Owing to yield losses that may reach 50 - 60%, FHB has become a major threat to the worlds´ food supply and is considered by the International Maize and Wheat Improvement Centre (CIMMYT) as one of the most limiting factors of worldwide wheat production (Dubin et al. 1997, Nicholson 2009). In recent years FHB has emerged as a disease of fundamental economic importance, leading to direct economic losses of close to $ 3.5 billion in the 1990s only in the United States and Canada. In addition to yield losses, indirect economic losses due to contamination of grain with mycotoxins, such as trichothecenes, zearalenon and fumonisins, lower market grade or lead to rejection of whole charges.

Trichothecenes are secondary metabolites that are potent inhibitors of protein synthesis in eukaryotic cells, causing feed refusal, vomiting, diarrhea, dermatitis, hemorrhages and weight loss (Ward et al. 2008). Thus, trichothecene mycotoxins pose a serious health hazard to humans and especially nonruminant animals when exposure levels are too high. *F. graminearum* and *F. culmorum* produce deoxynivalenol (DON) and nivalenol (NIV) that are the most prevalent type-B trichothecene mycotoxins associated with FHB in wheat. DON, which is also known as vomitoxin, often is accompanied with acetylated derivatives (3-ADON and 15 ADON) that are less toxic (Nicholson et al. 2009).

By the end of 2003, about 100 countries had specific regulations for maximum levels of mycotoxins in food or feedstuffs (van Egmond et al. 2007). In the EU, since 2005 legally enforceable limits in grain 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 in baby food of 0.2 mg kg^{-1} (Anonymous 2005).

Initial infection e.g. in *F. graminearum* is primarily by ascospores from infected wheat or maize stubbles while conidia produced on flowering spikes may cause secondary infections (Miedaner et al. 2008). Continuous improvement of agricultural productivity such as intensive use of stubble retention practices and non-inversion tillage, vastly increasing maize cultivation and narrow crop rotations facilitate pathogen survival on crop residues. These are considered the principal inoculum source especially for *F. graminearum* infection of the successive wheat crop (Maiorano et al. 2008). The preference of agronomically advantageous but mostly less FHB resistant semi-dwarf wheat varieties further exacerbates disease severity and yield loss. To reduce the impact of FHB epidemics and subsequent DON accumulation within grain, crop management and agrochemical measures are only partly effective because of necessary cost minimization in crop production, insufficient fungicide efficiency and narrow time frames for fungicide application during flowering representing the period of highest susceptibility to *Fusarium* infection of the wheat spike. Therefore, breeding and cultivation of highly disease-resistant varieties plays a key role in effective *Fusarium* control.

Morphological resistance to natural FHB infection is seemingly mediated, among others, through increased plant height due to a longer distance from the infected crop debris to the leaves and spikes (Mesterházy 1995), but tall genotypes are not desired by breeders and growers. Instead, the advantages of using dwarfing genes were soon recognized when largely increased use of inorganic nitrogenous fertilizers, pesticides and irrigation enabled higher grain yields (Gale and Youssefian 1985, Hedden 2006). More and heavier grain per spike caused the tall wheat plants to become prone to lodging in high winds and rain which required

breeding for shorter and stronger plant stature. Owing to its short stiff straw the variety Norin 10, that was registered and released in Japan in 1935, was introduced to the USA in 1946 (Pestsova et al. 2008). In the 1950s the reduced height (*Rht*) genes *Rht-B1b* and *Rht-D1b* derived from Norin 10 were utilized in wheat breeding programmes in the USA in order to improve lodging resistance in winter wheats (Ellis et al. 2002, Borojevic and Borojevic 2005). Through the efforts of Norman E. Borlaug, who led the CIMMYT wheat breeding programme in Mexico, the exploitation of *Rht-B1b* (syn. *Rht1*) and *Rht-D1b* (syn. *Rht2*) rapidly spread throughout the wheat-growing world. The newly developed semi-dwarf wheat varieties gave a quantum jump in productivity when accompanied by intensive agronomic practices and were the basis of the 'Green Revolution' (Swaminathan 2006). The first US variety based on Norin 10 *Rht* genes was the variety Gaines that was released in 1961. Already by 1985 over half the world wheat crop contained dwarfing genes and today approximately 90% of the world´s semi-dwarf wheat varieties carry *Rht-B1b* or *Rht-D1b* (Gale and Youssefian 1985, Worland et al. 1998a, Pestsova et al. 2008). In fact, the merits of globally increased yield performance by *Rht* genes were recognized in 1978 by the award of the Nobel peace prize to Norman Borlaug. The *Rht-D1b* allele was also widely used in the high yielding environments of North-Western Europe. In Great Britain the first semi-dwarf variety was released in 1974, and today the great majority of UK varieties contain *Rht-D1b* (Gale and Youssefian 1985, Gosman et al. 2007).
In Germany, today around 50% of all registered winter wheat varieties carry *Rht-D1b*, whereas only few (6% in 2004) carry *Rht-B1b* (Knopf et al. 2008, E. Ebmeyer *pers. commun.*). As consequence of a dwarfed phenotype with reductions in height of around 16–23%, *Rht-B1b* and *Rht-D1b* most importantly lead to higher overall grain yields of about 8–24% depending on genetic background and environment (Gale and Youssefian 1985, Worland and Petrovic 1988, Flintham et al. 1997a/b, Worland et al. 2001). The yield advantages of these semi-dwarfs result from increased partitioning of assimilates to the developing ear generating increased spikelet fertility and accordingly higher grain numbers per spike but reduced grain size. Due to increased grain yield in combination with slightly reduced total plant biomass the harvest index of *Rht*-varieties e.g. in British varieties rose from 35% in the 1920s to values up to 55% today (Evans 1998, Hedden 2006).

Originating from the wild-type alleles *Rht-B1a* located on chromosome 4B and *Rht-D1a* on chromosome 4D via single gain-of-function base-pair mutations, *Rht-B1b* and *Rht-D1b* encode transcription factors which belong to the DELLA proteins, a subset of the GRAS family of transcriptional regulators (Bolle et al. 2004). DELLA proteins act as repressors of

plant growth, whereas Gibberellins (GAs) promote growth by overcoming DELLA-mediated growth restraint (Achard and Genschik 2009). The point mutations of *Rht-B1b* and *Rht-D1b* lead to the introduction of a stop codon into a conserved region known as the DELLA domain, which is predicted to be in the N-terminus of the protein. Peng et al. (1999) proposed that translation might restart after the introduced stop codon, resulting in shortened proteins which are resistant to GA-induced degradation. Accumulation of the mutant DELLA protein causes continuous growth inhibition and, accordingly, leads to agronomically advantageous dwarfed plant height and improved straw strength by inhibition of stem cell elongation (Dalrymple 1986, Flintham et al. 1997a, Peng et al. 1999). In addition to wheat, DELLA gene orthologues have been described among several species including *Arabidopsis thaliana* (*GAI, RGA, RGL1, RGL2, RGL3*), maize (*dwarf8*), grape (*VvGAI1*), rice (*SLR1/OsGAI*), barley (*SLN1*) and rape seed (*BrRGA1*) indicating that the function of GA-signalling repression is highly conserved in monocots and dicots (Peng et al. 1999, Boss and Thomas 2002, Sun and Gubler 2004, Muangprom et al. 2005).

Another commercially highly important source of GA-insensitivity is *Rht-B1d* (syn. *Rht1S*) which represents an allelic variant to *Rht-B1b* and originated from another old Japanese variety Saitama 27 (Table 1, Worland and Petrovic 1988). Since being incorporated into Italian wheats in 1947, *Rht-B1d* has spread into many Mediterranean countries. By now, 80% of the Italian, 26% of the Bulgarian and a large proportion of the varieties of former Yugoslavia are carrying *Rht-B1d* based on selective advantages under high temperatures due to the weakness of its GA-insensitivity compared to *Rht-B1b* and *Rht-D1b* (Worland and Petrovic 1988, Ganeva et al. 2005). *Rht-B1d* exhibits only half the potency of *Rht-B1b* and reduces height by around 11% combined with an increase in spikelet fertility and grain number. However, a reduction in grain size compensates an advantage in grain yield.
Alternative GA-insensitive allelic variants at the *Rht-B1* and the *Rht-D1* locus are *Rht-B1c* (syn. *Rht3*) derived from the British variety Tom Thumb and *Rht-D1c* originating from the Chinese variety Ai-bian 1 (Worland and Petrovic 1988). Due to an increased magnitude of the GA-insensitivity both alleles confer an extreme dwarfed phenotype with height reductions up to 46%. Nevertheless, both alleles are much less exploited in actual commercial breeding programmes because of enhanced disadvantages such as reduced grain size and quality,

Table 1. Current nomenclature of the most important height reducing (*Rht*) genes and their homoeologous alleles in wheat according to Börner et al. (1996), Worland et al. (1998a), McIntosh et al. (2008), and Holzapfel et al. (2008)

Rht gene	Allele	Chromosome	Old nomenclature / association	Source	GA-sensitivity	Intensity of use in current wheat breeding	Dwarfism
Rht-A1	*a*	4A	*rht*	wild-type		(monomorph)	-
Rht-B1	*a*	4BS	*rht*	wild-type	-	-	-
	b		*Rht1*	Norin 10	-	very high	++ [c]
	c		*Rht3*	Tom Thumb	-	low	++++
	d		*Rht1 Saitama*	Saitama 27	-	high	+
	e		*Rht Krasnodari1*	Krasnodari 1	-	medium	+++
	f		*Rht T. aethiopicum*	W6824D, W6807C (*T. aethiopicum*) [a]	-	?	-
	g		-	fast-neutron mutant of *Rht-B1b*	+	-	-
Rht-D1	*a*	4DS	*rht*	wild-type	-	-	-
	b		*Rht2*	Norin 10	-	very high	+++
	c		*Rht10*	Ai-bian 1	-	low	++++
	d		*Rht Ai-bian 1a*	spontaneous mutation of Ai-bian 1	-	?	++
Rht8	*a*	2DS	*Rht8* WMS261-165bp	Ciano 67, Brevor, Saitama 27	+	high	- [b]
	b		*Rht8* WMS261-174bp	Cappelle-Desprez, Mara, Norin 10	+	high	+
	c		*Rht8* WMS261-192bp	Akakomugi, Bezostaya	+	very high	++
	d		*Rht8* WMS261-201bp	Pliska, Courtot	+	low	?
	e		*Rht8* WMS261-210bp	Chino, Klein Esterello, Klein 157	+	low	?
	f		*Rht8* WMS261-215bp	Klein 49	+	low	?
	g		*Rht8* WMS261-196bp	Mirleben	+	-	?
	h		*Rht8* WMS261-206bp	Weihenstephan M1	+	-	?

[a] tetraploid

[b] WMS261-165bp is promoting height by 3-4cm compared to WMS-174bp (Worland et al. 1998)

+, ++, +++, ++++ = dwarfism severity from low to very severe

chromosomal and environmental instability that are not sufficiently compensated by higher grain numbers so that overall grain yield is mostly reduced (Hedden 2006). As the potency of the GA-insensitive alleles is reflected in the degree of growth retardation, lines being homozygous for the least potent *Rht-B1d* show identical height reduction to those being heterozygous for *Rht-B1b*, and those being homozygous for *Rht-B1b* are indistinguishable from those heterozygous for *Rht-B1c* (Worland 1986).

The distribution of the most widely used GA-insensitive semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* is restricted to geographical areas that are not subjected to heat stress during the time of meiosis as this has been demonstrated to reduce spikelet fertility (Worland and Law 1985, Ellis et al. 2005). The most important GA-responsive *Rht* gene is *Rht8* that is closely linked to the photoperiodic insensitive gene *Ppd-D1a* in a *Rht8/Ppd-D1a* linkage group (Worland et al. 1998a, Ganeva et al. 2005). The Italian wheat breeder Nazareno Strampelli introduced *Rht8/Ppd-D1a* derived from the old Japanese semi-dwarf landrace Akakomugi into European wheats (Table 1, Worland et al. 1998a). In 1913, Strampelli made the first crosses in order to combine short straw, early maturity and high yield potential of Akakomugi with the adaptability of local varieties (Borojevic and Borojevic 2005). The identification of a tightly linked microsatellite marker, WMS261, located 0.6cM distal to *Rht8* on the short arm of chromosome 2D facilitated its recognition (Korzun et al. 1998). The 192bp-allele at this locus named *Rht8c* was generally used as diagnostic for *Rht8*. Recently Ellis et al. (2007) reported that Norin 10 also carries a 192bp allele at the *Xgwm261* locus resulting in a second haplotype that has no association with the height reducing allele *Rht8c*. The authors suggested that, hence, WMS261-192bp is only indicative of *Rht8c* in wheat varieties that have inherited this allele from Akakomugi or a Strampelli wheat ancestor.

In contrast to *Rht8c*, the closely linked *Ppd-D1a* allele has proven extremely important in promoting height reduction by shortening the plant's life cycle due to a 2.089bp deletion upstream of the coding region leading to mis-expression of the 2D pseudo-response regulator gene (Worland et al 1998a/b, McIntosh et al. 2008). Owing to its mode of action the use of *Rht8c/Ppd-D1a* prevails in wheats of South and South-Eastern Europe further to Southern Ukraine and Russia and in the spring wheats introduced by CIMMYT. Therefore, the improved adaptability to these areas suffering from desiccating summer conditions excludes utilization in areas such as much of Northern Europe and America where maximal yields are associated with extended life cycles (Worland et al. 2001, Ganeva et al. 2005). However, Worland et al. already in 1998 accentuated the need to breed for earlier flowering wheats

carrying *Ppd-D1a* with the upcoming effects of global warming in Northern Europe to ensure high yield by summer drought stress avoidance.

Interactions between *Rht8c* and *Ppd-D1a* were detected for increased grain numbers per spikelet, enhanced spikelet fertility, improved grain fill before the onset of summer desiccation and consequently increased yield under these conditions (Worland et al., 1998a/b). Accordingly, e.g. in Bulgaria 84% of the tested modern wheat varieties are carrying *Rht8c/Ppd-D1a*, whereas in Germany and the United Kingdom so far all locally bred varieties are lacking *Rht8c/Ppd-D1a* (Worland et al. 1998a, Ganeva et al. 2005, Knopf et al. 2008). Studies carried out in the UK, Germany and in former Yugoslavia on single chromosome recombinant lines, suggested that solely presence of *Rht8c* reduces plant height by around 10% (5-10cm) without significant adverse effects on plant yield (Worland et al. 1998a). This indicates *Rht8c* possibly being a viable alternative major height reducing allele other than the GA-insensitive or the photoperiod-insensitive ones.

Today, on total 21 different GA-insensitive or -sensitive *Rht* genes are described with additional allelic variants for the most prevalently exploited loci *Rht-B1* (alleles *a-g*), *Rht-D1* (alleles *a-d*) and *Rht8* (alleles *a-h*) as shown in Table 1 (McIntosh et al. 2008). Only a few of the known *Rht* alleles are used agronomically, as typical features such as higher grain numbers and increased harvest index do not always compensate for reduced grain size and shoot biomass (Evans 1998, Hedden 2006).

Lines carrying *Rht* genes for a long time were hypothesized to be more susceptible to soilborne fungal pathogens under natural infection in agricultural practice because of the short stature leading to short distances from the infected crop debris on the soil surface to the leaves and finally the spikes (Mesterházy 1995). Although phenotypic effects of plant height are not the only source of FHB resistance in wheat as demonstrated by registered varieties of similar plant height significantly varying in their FHB resistance, a general negative association between FHB resistance and plant height was reported from several wheat populations (Mesterházy 1995, Buerstmayr et al. 2000, Somers et al. 2003, Anonymous 2009). Both traits show complex inheritance controlled by multiple major and minor genes and, moreover, resistance evaluation can be confounded by large environmental effects and genotype × environment interactions. Nevertheless, in all conducted quantitative trait loci (QTL) mapping studies several QTL for FHB resistance coincided with QTL for straw length (Buerstmayr et al. 2009). Interestingly, until the beginning of this study possible segregation for *Rht* alleles,

especially for *Rht-D1b* as the most important *Rht* allele in Northern Europe, was not monitored in any study.

For example Hilton et al. (1999) analysed two populations segregating for *Rht-B1b* and *Rht-D1b*, but without differentiation for their *Rht* status and hence did not obtain a sound conclusion on the *Rht* effects. Additionally the populations were analysed only at one location excluding a differentiation between the genotypic and the genotypic × location interaction effects that are of great importance in the pathosystem (Miedaner et al. 2001a). In 2007, a major QTL for FHB resistance that co-localised with the *Rht-D1* locus in a QTL mapping study was observed by Draeger et al. in an Arina × Riband population. This QTL explained 13 to 24% of the total phenotypic variance, but was not stable across all environments and was verified only on a limited number of lines. Whether the association of *Rht-D1b* is due to close linkage of the wild-type allele *Rht-D1a* to a QTL conferring FHB resistance or pleiotropy of *Rht-D1b* remains unclear from this study and has to be further examined.

Simón et al. (2004) analysed the influence of different *Rht* genes, including *Rht-D1b*, on resistance to *Septoria tritici* leaf blotch in wheat near isogenic lines in the Mercia and Cappelle-Desprez background by spray inoculation. The authors found strong association of reduced plant height and increased disease severity only in very short wheats carrying *Rht-B1c* and *Rht12* (derived from the variety Karkagi 522), respectively. The Norin 10 alleles *Rht-B1b*, *Rht-D1b* as well as the Saitama 27 allele *Rht-B1d* had no impact on *S. tritici* leaf blotch in the used genetic backgrounds. This indicates that, depending on the pathosystem, common *Rht* alleles do not necessarily lead to increased susceptibility compared to the tall wild-types.

For *Fusarium* resistance evaluation, to separate the epidemiological effects of plant height *per se* and the effects of *Rht* genes, artificial spray inoculation onto the crop canopy is essential and was conducted in the present experiments. Generally, two different types of resistance are known: resistance to initial infection (type I) and resistance to fungal spread within the spike (type II) (Schroeder and Christensen 1963). As the combination of initial disease incidence and spread within the spike is reflected by the percentage of infected spikes per plot multiplicated by the mean percentage of infected spikelets per infected spike, combined type I and II resistance can be assessed by visually rating the percentage of infected spikelets of all spikelets per trial plot. Measuring only type II resistance can be achieved via inoculation of a spore suspension into single spikelets and rating the fungal spread after a few days.

Taken together, until the beginning of the present studies in 2004 a verification of the effects of *Rht-D1b* on FHB resistance was not available on a broad basis of several segregating European winter wheat populations involving appropriate population sizes and being tested in a reliable number of environments. Additionally a direct comparison of the effects of different *Rht* alleles on type I and II FHB resistance has not yet been conducted in a comparable genetic background of e.g. isogenic lines.

At present in agricultural practice of Germany only moderately resistant wheat varieties are available (Anonymous 2009). Until recently the level of FHB resistance within European breeding programmes was usually obtained through the combination of favourable not yet characterized genes derived from adapted European elite germplasm after multi-step phenotypic selection. In this way in the last decades much progress has been achieved in the development of moderately FHB resistant varieties mainly by use of conventional breeding methods. In the recent years high effort was put into the identification and fine mapping of QTL or genes from genetically distant sources possessing high FHB resistance.

Currently the most effective and best validated FHB resistance QTL are *Qfhs.ndsu-3BS*, recently designated as *Fhb1*, that mediates primarily type II resistance and *Qfhs.ifa-5A* being associated primarily with type I resistance. Both QTL were derived from the highly resistant Chinese spring wheat variety Sumai 3 and related lines and reduce FHB disease severity up to 33% and DON content up to 59% compared to the marker class with no donor QTL (Bai and Shaner 2004, Liu et al. 2006, Anderson 2007, Buerstmayr et al. 2009). Further major resistance QTL being recently detected and fine mapped are *Fhb2* and *Fhb3* on chromosomes 6BS and 7AL, respectively (Cuthbert et al. 2007, Qi et al. 2008). Even though the marker-based introgression of resistance QTL seemingly offers a substantial increase in FHB resistance within the shortest time possible, this expectation appears only true in case of sufficiently powerful donor QTL (Kosová et al. 2009). Nevertheless, the utilization of exotic donor QTL in high-yielding European germplasm is always hampered or at least time and cost consuming due to the negative agronomic performance of the exotic genetic backgrounds causing pronounced linkage drag.

Another approach being pursued is the search for effective FHB resistance QTL in adapted Central European elite germplasm (Gervais et al. 2003, Paillard et al. 2004). For example Schmolke et al. (2005) observed two major QTL on chromosomes 6AL and 7BS. However, the effects of these QTL were yet not large enough to justify the expenses of a marker-assisted introgression programme replacing continuous phenotypic selection.

Besides the improvement of host resistance, likewise the pathogens´ capability to adapt to and possibly overcome improved host resistance has to be considered in the wheat/*Fusarium* pathosystem. Breakdowns of disease resistance depending on one or only few major resistance genes in wheat have been documented most thoroughly with regard to cereal rusts (McIntosh and Brown 1997) and powdery mildew (Wolfe and McDermott 1994). In these cases major resistance genes were overcome by evolution of the pathogen population caused by selection for mutants, recombinants or immigrants that were better adapted to the resistant wheat varieties. Generally, the genetic basis of aggressiveness and the ability of mutation, recombination and gene flow influence the success of adaptation of fungal populations to hosts with improved resistance. On contrast to the wheat/rust pathosystems, aggressiveness of *G. zeae* as well as FHB resistance in wheat are quantitatively inherited traits (Cumagun et al. 2004a, Holzapfel et al. 2008). Isolate aggressiveness, in this context, represents the quantity of disease induced by a pathogenic isolate on a susceptible host (van Eeuwijk et al. 1995). On the basis of a QTL mapping population, pathogenicity contrarily segregated in a qualitative manner. The according pathogenicity locus in *G. zeae* was designated as *PATH1* and mapped adjacent to loci that affect pigmentation (*PIG1*), perithecial production (*PER1*) and toxin amount (*TOX1*) (Cumagun et al. 2004a).

One prerequisite for effective selection within a population is sufficient genetic variation. For aggressiveness large genetic variation was found in isolate collections, crossing populations and especially within-field populations among *G. zeae* sampled from different continents within a country or state and even within individual fields (Bai and Shaner 1996, Miedaner and Schilling 1996, Goswami and Kistler 2005, Miedaner et al. 2008). Hence, Zeller et al. (2004) observed that most of the genetic variation in the North American meta-population is already shared by most of the local populations revealing generally low differentiation among subpopulations and relatively few identified unique haplotypes. Although geographic and genetic distance are correlated to some extent, the observed differences between subpopulations are relatively small and genetic isolation might therefore rather reflect the time required for the alleles to diffuse over large geographical distances.

Another important factor in the evolution of pathogen populations is sexual recombination. Within progeny of moderately aggressive parental isolates Cumagun and Miedaner (2004) observed transgressive segregation for isolate aggressiveness and DON production. Although until now it could not be determined whether outcrossing and recombination in the field occur regularly or episodically, the occurrence of transgressive segregants indicates that solely sexual recombination could result in increased aggressiveness of *F. graminearum* field

populations. Thus, determining the segregation variance from crosses of already highly aggressive parents would enable detailed investigation on the fungal capability to continuously increase its level of aggressiveness through sexual recombination.

Furthermore, environmentally stable host × isolate interaction might be a selective force and, thus, could trigger a breakdown of increased host resistance as described by Mesterházy (1984). However, the majority of published literature describes an absence of isolate-specific interaction to wheat varieties varying in their FHB resistance (van Eeuwijk et al. 1995, Goswami and Kistler 2005).

Nevertheless, aggressiveness and its role in adaptation of the *Fusarium* pathogen is still insufficiently investigated. Although quantitative adaptation to the host is theoretically expected to be slower, the widespread use of single highly effective resistance genes or QTL grown on large acreages pose a yet undefined risk of pathogen evolution towards increased aggressiveness and mycotoxin production, possibly leading to long term erosion of expectedly durable quantitative FHB resistance.

In the last decade numerous examples have demonstrated the capability of adaptation between and within *Fusarium spp.* to changing hosts and environments. For example, the predominant species shifted from *F. culmorum* to *F. graminearum* and the more pathogenic NIV-producers are on the rise in maize-dominated crop rotations of Northern Europe (Carter et al. 2002, Waalwijk et al. 2003). Additionally, upcoming benzimidazole fungicide resistance of *F. graminearum* in China, or the shift from 15-ADON to 3-ADON producers featuring higher fitness, fecundity and growth rates than isolates from the 15-ADON population give unambiguous hints that evolutionary selection may drive a rapid shift of a pathogen population towards higher fitness, toxigenicity and aggressiveness (Gale et al. 2002, Ward et al. 2008).

Regarding the population structure and interaction pattern, the ability and time required by e.g. *F. graminearum* to overcome increased FHB resistance in wheat will depend on whether survival and reproduction on a resistant host are important parts of the fungus´s life cycle that imply selective forces thriving evolutionary adaptation. However, both theory and experimental evidence remain scarce and further investigation is needed to gather empirical knowledge on the modalities of *Fusarium spp.* response to selection pressures imposed by increased quantitative FHB resistance in wheat.

Hence, the overall goal of this project was to broaden and deepen empirical knowledge on the inheritance of FHB resistance, with special focus on the effects of *Rht* dwarfing genes on FHB resistance, and isolate aggressiveness in the wheat/*Fusarium* pathosystem. Therefore the specific objectives regarding host, pathogen and their interaction in multi-environmental field trials using artificial spray and/or single floret inoculation of *F. graminearum* or *F. culmorum* spores were to investigate

1. the effects of the *Rht-D1b* semi-dwarfing allele on FHB resistance in three genetically unrelated populations of adapted European elite winter wheat varieties segregating for this allele.
2. the segregation variance for FHB resistance in three crossing populations of adapted European winter wheat parents with the aim to locate effective FHB resistance QTL.
3. the effects of commonly used *Rht*-dwarfing genes on either type of FHB resistance (type I and II) utilizing two sets of near-isogenic lines (NILs) in the genetic background of the winter wheat varieties Mercia and Maris Huntsman.
4. the segregation variance for isolate aggressiveness within progeny of two crosses of highly aggressive parental *F. graminearum* isolates (FG07 × FG153, FG3211 × FG96).
5. whether environmentally stable transgressive recombinants with further increased aggressiveness can be recovered from these crosses of already highly aggressive parents and, thus, whether there is a detectable quantitative upper limit of aggressiveness in *F. graminearum* populations.
6. wheat variety × fungal isolate interactions and to determine whether increased aggressiveness of eleven highly aggressive *F. graminearum* and *F. culmorum* isolates exceeds the resistance capabilities of a presently registered and available set of seven wheat varieties that represent moderate to high FHB resistance.

For references please see chapter 7.

Effect of the *Rht-D1* dwarfing locus on Fusarium head blight rating in three segregating populations of winter wheat

H.-H. Voss[1], J. Holzapfel[2], L. Hartl[2], V. Korzun[3], F. Rabenstein[4], E. Ebmeyer[3], H. Coester[5], H. Kempf[6], and T. Miedaner[1,7]

[1] State Plant Breeding Institute, Universität Hohenheim, 70593 Stuttgart
[2] Institute for Crop Science and Plant Breeding, Bavarian State Research Center for Agricultural Research, 85354 Freising
[3] KWS LOCHOW GMBH, 29296 Bergen
[4] Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, 06484 Quedlinburg
[5] RAGT 2n, 38855 Silstedt
[6] Saatzucht Schweiger, 85368 Moosburg
[7] Corresponding author, E-mail: miedaner@uni-hohenheim.de

Voss et al. (2008) Plant Breeding 127:333-339
DOI:10.1111/j.1439-0523.2008.01518.x
The original publication is available at
http://onlinelibrary.wiley.com/doi/10.1111/pbr.2008.127.issue-4/issuetoc

Abstract

Fusarium head blight (FHB) is one of the major fungal diseases in wheat throughout the world. To control FHB severity, breeding genetically resistant varieties is thought to be the most promising strategy. In wheat breeding programmes, short cultivars predominantly carrying the Norin 10 derived semi-dwarfing allele *Rht-D1b* (*Rht2*) are preferred worldwide because of higher achievable grain yields and lower risk of lodging. This study was conducted to determine the influence of different alleles at the *Rht-D1* locus on FHB reaction. Three winter wheat populations were produced by crossing rather susceptible varieties 'Biscay', 'Pirat' and 'Rubens' carrying mutant-type allele *Rht-D1b* with the more resistant varieties 'Apache', 'Romanus' and 'History' containing the *Rht-D1a* wild-type allele (*rht2*). The 190, 216 and 103 progeny of the F4-derived populations were assayed for the presence of *Rht-D1a* or *Rht-D1b*, plant height, and mean FHB rating after spray inoculation at flowering time with a highly aggressive isolate of *Fusarium culmorum*. Comparably, high mean FHB severities ranging from 28% to 49% for all population × environment combinations were achieved, with significant genotypic variation for FHB rating and plant height within all populations. Both traits were negatively correlated with *r* ranging from -0.48 to -0.61 in the complete populations. However, within the subpopulations homozygous for one or other height allele these correlations decreased considerably. The *Rht-D1b* semi-dwarfing allele resulted in 7–18% shorter plants, depending on the population, but a considerably increased FHB reaction of 22–53%. Nevertheless, significant genotypic variance for FHB resistance remained in all tested *Rht-D1b* subpopulations indicating that selection for moderately FHB resistant genotypes within agronomically beneficial *Rht-D1b* genotypes is still feasible.

Effect of dwarfing *Rht* genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars

T. Miedaner* and H.-H. Voss

Universitaet Hohenheim, State Plant Breeding Institute, D-70593 Stuttgart, Germany.
Both authors contributed equally to the paper.
*Corresponding author (miedaner@uni-hohenheim.de)

Miedaner and Voss (2008) Crop Science 48:2115-2122
DOI: 10.2135/cropsci2008.02.0107
The original publication is available at
https://www.crops.org/publications/cs/abstracts/48/6/2115

Abstract

Reduced height *(Rht)* genes are used in wheat *(Triticum aestivum L.)* breeding throughout the world. Fusarium head blight (FHB) is one of the most destructive wheat diseases caused by *Fusarium graminearum* (Schwabe) and *F. culmorum* (W.G. Sm.) Sacc. Objectives of this study were to analyze the effects of (i) specific *Rht* dwarfing genes on FHB reaction using two sets of near-isogenic lines (NILs) and (ii) genetic background and environment on FHB reaction. We inoculated NILs carrying *Rht-B1b, Rht-B1d, Rht-D1b, Rht8c,* and *Rht-D1b+Rht8c* in the background of the British winter wheat cultivar Mercia possessing medium height and moderate resistance, and NILs carrying *Rht-B1b, Rht-B1c, Rht-D1b* and *Rht-B1b+Rht-D1b* in the background of the rather tall, generally more resistant British cultivar Maris Huntsman, as well as three German check cultivars ('Toras', 'Certo', 'Travix') carrying the *Rht-D1b* allele. Entries were tested in eight (Mercia) and four (Maris Huntsman) environments, respectively, by inoculation with *F. culmorum*. In the Mercia data set, *Rht-B1d* and *Rht-D1b* significantly increased mean FHB rating by 35 and 52%, respectively. *Rht-B1b* and *Rht8c* increased FHB rating only by 19%, being not significantly different to the wild-type line (*rht*). *Rht8c* affected heading date due to its linkage with the photoperiod insensitive *Ppd1* allele. In the Maris Huntsman data set, FHB rating was increased by 22 to 83%, but only the very short *Rht-B1c* and *Rht-B1b+Rht-D1b* lines showed significance. Although the mutant *Rht* alleles increased FHB susceptibility, the checks show that these negative effects can be largely counteracted by a more resistant genetic background.

Inheritance of resistance to Fusarium head blight in three European winter wheat populations

Josef Holzapfel[1], Hans-Henning Voss[2], Thomas Miedaner[2], Viktor Korzun[3], Jennifer Häberle[1], Günther Schweizer[1], Volker Mohler[1], Gerhard Zimmermann[1], and Lorenz Hartl[1]

[1] Bavarian State Research Centre for Agriculture, Institute for Crop Science and Plant Breeding, Am Gereuth 8, 85354 Freising-Weihenstephan, Germany
[2] Universität Hohenheim (720), State Plant Breeding Institute, Fruwirthstraße 21, 70593 Stuttgart, Germany
[3] KWS LOCHOW GmbH, Bollersener Weg 5, 29296 Bergen, Germany

Holzapfel et al. (2008) Theor. Appl. Genet. 117:1119-1128
DOI 10.1007/s00122-008-0850-z
The original publication is available at
http://www.springerlink.com/content/m722331u3l63786m/

Abstract

Fusarium head blight (FHB) resistance is of particular importance in wheat breeding programmes due to the detrimental effects of this fungal disease on human and animal health, yield and grain quality. Segregation for FHB resistance in three European winter wheat populations enabled the identification of resistance loci in well-adapted germplasm. Populations obtained from crosses of resistant cultivars Apache, History and Romanus with susceptible semi-dwarfs Biscay, Rubens and Pirat, respectively, were mapped and analysed to identify quantitative trait loci (QTL) for FHB severity, ear emergence time and plant height. The results of the present study together with previous studies in UK winter wheat indicated that the semi-dwarfing allele *Rht-D1b* seems to be the major source for FHB susceptibility in European winter wheat. The high resistance level of the cultivars Romanus and History was conditioned by several minor resistance QTL interacting with the environment and the absence of *Rht-D1b*. In contrast, the semi-dwarf parents contributed resistance alleles of major effects apparently compensating the negative effects of *Rht-D1b* on FHB reaction. The moderately resistant cultivar Apache contributed a major QTL on chromosome 6A in a genome region previously shown to carry resistance loci to FHB. A total of 18 genomic regions were repeatedly associated with FHB resistance. The results indicate that common resistance-associated genes or genomic regions are present in European winter wheats.

Variation and Transgression of Aggressiveness Among Two *Gibberella zeae* Crosses Developed from Highly Aggressive Parental Isolates

Hans-Henning Voss[1], Robert L. Bowden[2], John F. Leslie[3], and Thomas Miedaner[1,4]

[1] Universität Hohenheim, State Plant Breeding Institute, 70593 Stuttgart, Germany
[2] Plant Science and Entomology Research Unit, United States Department of Agriculture-Agricultural Research Service, 4008 Throckmorton Plant Science Center, Manhattan, USA
[3] Department of Plant Pathology, 4024 Throckmorton Plant Science Center, Kansas State University, Manhattan, USA
[4] Corresponding author: T. Miedaner; E-mail address: miedaner@uni-hohenheim.de

Voss et al. (2010) Phytopathology 100:904-912
DOI:10.1094/PHYTO-100-9-0904
The original publication is available at
http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-100-9-0904

Abstract

Gibberella zeae (anamorph: *Fusarium graminearum*) is the most common cause of Fusarium head blight (FHB) of wheat (*Triticum aestivum*) worldwide. Aggressiveness is the most important fungal trait affecting disease severity and stability of host resistance. Objectives were to analyze in two field experiments (i) segregation for aggressiveness among 120 progenies from each of two crosses of highly aggressive parents and (ii) stability of FHB resistance of seven moderately to highly resistant winter wheat cultivars against isolates varying for aggressiveness. Aggressiveness was measured as FHB severity per plot, *Fusarium* exoantigen absorbance, and deoxynivalenol content. In the first experiment, mean FHB ratings were 20 to 49% across environments and progeny. Significant genotypic variation was detected in both crosses ($P < 0.01$). Isolate-environment interaction explained approximately half of the total variance. Two transgressive segregants were found in cross B across environments. Traits were significantly ($P < 0.05$) intercorrelated. In the second experiment, despite significant ($P < 0.05$) genotypic variance for cultivar and isolate, no significant ($P > 0.05$) interaction was observed for any trait. In conclusion, progeny of highly aggressive parents might exhibit increased aggressiveness due to recombination and may, therefore, adapt nonspecifically to increased quantitative host resistance.

6. General Discussion

6.1 Variation of resistance among wheat

To reduce the impact of *Fusarium* epidemics on yield and grain quality, and to comply with nationally established maximum levels of mycotoxin contamination, in the last decades much emphasis was placed on breeding for improved FHB resistance in wheat worldwide. To reliably identify potent quantitative trait loci (QTL) for FHB resistance suitable for subsequent marker-assisted selection (MAS), in the present study three populations from the adapted European elite winter wheat gene pool each were evaluated across five environments: Apache × Biscay (N=190), History × Rubens (N=103) and Romanus × Pirat (N=216). The progeny of all three populations showed a normal distribution for FHB severity following spray inoculation which confirms the quantitative character of FHB resistance with only small to medium effects of single QTL that are widely spread across the wheat genome (Löffler et al. 2009).

Subsequent QTL analysis from a companion study of Holzapfel et al. (2008) revealed FHB resistance QTL with overlapping confidence intervals in all three populations on chromosomes 1BL and 4DS. The latter, being the QTL with the highest additive effect, was coincident with the *Rht-D1* dwarfing locus and accounted for up to 36.4% of the phenotypic variance. In total, 13 (Apache × Biscay), 14 (Romanus × Pirat) and 8 (History × Rubens) QTL for FHB resistance were detected that were spread across all chromosomes except for chromosomes 3A, 5D and 7D. Mostly these QTL interacted significantly with the environments as observed in several QTL mapping studies comprising European winter wheat (Paillard et al. 2004, Draeger et al. 2007, Srinivasachary et al. 2008).

Moreover, Holzapfel et al. (2008) compared these QTL with FHB resistance QTL derived from published European winter wheat mapping populations. The authors found coincidences in map positions for a comparably large number of QTL identified in the present analysis resulting in 18 genomic regions with varying effects that were repeatedly associated with FHB resistance. This indicates that common resistance-associated genes or genomic regions are present in European winter wheats. Congruently, Buerstmayr et al. (2009) summarized the relevant findings of 51 QTL mapping studies on FHB resistance and reported FHB resistance QTL on all wheat chromosomes except for 7D. Powerful major QTL originated only from Asian (e.g. Sumai 3, Wangshuibai, Nyu Bai, Nobeokabouzu) and a Brazilian variety (Frontana), whereas in European elite winter wheat germplasm solely minor QTL were

detected that are currently not amenable to marker-assisted selection. On total, the authors counted 22 genomic regions that have been repeatedly detected in more than one mapping population.

Applying a novel QTL meta-analysis approach, including 176 initial QTL for FHB type I and type II resistance, Löffler et al. (2009) found six, ten and three independently inherited Meta-QTL (MQTL) located on the A, B, and D genome, respectively, each comprising 2-13 initial QTL. Taken together, coincident genomic regions or MQTL, respectively, indicate that these (M)QTL are stable and therefore potentially useful for marker-assisted selection. Three of the 19 MQTL described by Löffler et al. (2009) already comprised 38% of all QTL included in the meta-analysis and were located on chromosomes 3B, 5A and 6B. These were already suspected to be carriers of major QTL derived from Asian origins prior to analysis (Cuthbert et al. 2007). On chromosome 1B eleven initial QTL aggregated to three separate MQTL located within a range of about 70 cM. Interestingly, one of these three MQTL comprised only the QTL consistently detected by Holzapfel et al. (2008) in the European resistance donors Apache, Romanus and History, whereas the other two included QTL from European (Arina, Dream), Brasilian (Frontana) and Asian (Wangshuibai) material.

The detection of few major and dozens of minor QTL proves the quantitative and complex inheritance of FHB resistance. Many of these resistance QTL coincide with QTL for plant height. In all mapping populations segregating at the *Rht-D1* locus, corresponding mapping analysis revealed a stable QTL at this locus with the largest effect on FHB resistance throughout (Draeger et al. 2007, Holzapfel et al. 2008, Srinivasachary et al. 2009).

6.1.1 Specific role of the *Rht* genes

In general, taller genotypes show less FHB severity, whereas short varieties, especially those carrying the dwarfing allele *Rht-D1b*, are suspect to higher susceptibility to FHB (Miedaner 1997, Hilton et al. 1999). Basically it has been postulated that in case of natural infection conidia spread more easily to the spikes of shorter varieties because of the shortened distance between leaf layers and/or from the spike to crop debris on the soil surface as natural infection source (Mesterházy 1995). Therefore, to minimize any epidemiological influence of plant height *per se*, in the present experiments repeated spray inoculation and spatial separation in the field were applied for short and tall progeny of the History × Rubens and Romanus × Pirat populations, both possessing a wide variation in plant height. However, still moderate negative overall correlation coefficients (r = -0.48 to -0.61) between plant height and FHB severity could be detected. Interestingly, when classified to the *Rht-D1* status, within

subpopulations carrying either *Rht-D1a* or *Rht-D1b* only reduced correlation coefficients with r = -0.33 to -0.44 remained. Although disease escape due to plant height *per se* can be widely excluded, the presence of *Rht-D1b* resulted in considerably higher mean FHB ratings of 22–53% compared to mean FHB ratings of the *Rht-D1a* subpopulations as shown in all three populations.

To further exclude the influence of plant height *per se* due to possible disease escape, from each population a maximum number of genotypes carrying either *Rht-D1a* or *Rht-D1b* were selected within a minimum range of plant height (Apache × Biscay: 3cm, Romanus × Pirat: 7cm, and History × Rubens: 8cm). Additionally, these genotypes were analysed for their DON and exoantigen (ExAg) content that represents the amount of fungal biomass within the host spike tissue (Cumagun et al. 2004b). Hence, on a similar level of plant height the increase in FHB severity due to presence of *Rht-D1b* compared to *Rht-D1a* remained in all three populations, although on a reduced scale of 7–27% being significant only for the Romanus × Pirat population (Table 2). Interestingly, the DON content increased to a much larger extent in all three populations compared to FHB severity, whereas the increases in the ExAg content most strongly varied between the populations.

Table 2. Best linear unbiased predictors (BLUPs) for plant height (PH), FHB rating, deoxynivalenol (DON) content, and exoantigen (ExAg) content of progenies selected for similar plant height and differing in the *Rht-D1* allele from three segregating populations inoculated with *F. culmorum* in 3 locations in 2006

Population	Trait	Subpopulation		Δ*Rht-D1b*/ *Rht-D1a*
		Rht-D1a	*Rht-D1b*	
		N=17	N=18	
Apache × Biscay	PH (cm)	77.6	77.8	+ 0.2% n.s
	FHB (%)	29.9	32.1	+ 7.4% n.s
	DON (mg kg^{-1})	15.8	19.8	+ 24.8% n.s
	ExAg (OD_{405})[a]	0.31	0.35	+ 11.9% n.s
		N=15	N=16	
Romanus × Pirat	PH (cm)	90.5	87.9	- 2.9% *
	FHB (%)	26.5	33.8	+ 27.4% *
	DON (mg kg^{-1})	30.9	45.4	+ 46.8% *
	ExAg (OD_{405})	0.25	0.39	+ 54.7% **
		N=10	N=11	
History × Rubens	PH (cm)	86.8	86.5	- 0.4% n.s
	FHB (%)	34.0	38.1	+ 11.9% n.s
	DON (mg kg^{-1})	37.2	48.4	+ 30.2% n.s
	ExAg (OD_{405})	0.98	1.07	+ 8.84% n.s

*, ** Significantly different at $P < 0.05$, 0.01, respectively

[a] Optical density measured at 405 nm

In accordance with our results similar observations on *Rht-D1b* were made in different European winter wheat populations as Arina × Riband, Spark × Rialto, Apache × Contra, Solitär × Travix and History × Excellenz (Draeger et al. 2007, Srinivasachary et al. 2008, Holzapfel et al. 2008). Throughout, major QTL at or near the *Rht-D1* locus were identified indicating that the QTL allele *Rht-D1b* is a major contributor to FHB susceptibility or, on the contrary, is lacking a major FHB resistance QTL. Accordingly, the wild-type allele *Rht-D1a* showed the highest additive effect in each of the present populations with a mean reduction in FHB severity of 16.3, 29.2 and 31.5%, but similarly led to taller plants of 5.7, 17.1 and 16.1cm, respectively.

Confirming our findings, on chromosome 4D the Meta-QTL with the narrowest confidence interval comprised only QTL seemingly tightly linked to the *Rht-D1* gene and originated only from studies including parents differing in their *Rht-D1* status (Löffler et al. 2009). Close linkage of FHB susceptibility genes that consistently cosegregate with *Rht-D1b* for many generations might be a possible explanation for increased FHB severity as likewise hypothesized by Draeger et al. (2007) and Srinivasachary et al. (2008). Alternatively, *Rht-D1b* conferring insensitivity to gibberellic acid (GA) may have negative pleiotropic effects on FHB response rather than linkage.

This question cannot be fully answered from our and/or literature results. Nevertheless, further evidence supporting pleiotropy was given by comparative evaluation of different *Rht* dwarfing alleles of worldwide agricultural importance and their specific impact on FHB resistance (Miedaner and Voss 2008). Of the 21 currently described *Rht* genes (McIntosh et al. 2008), the most prevalent *Rht-B1b* and *Rht-D1b* derived from Norin 10, *Rht8c* from Akakomugi and *Rht-B1d* from Saitama 27 are possessed by more than half of the worlds´ wheat varieties (Gale and Youssefian 1985, Ellis et al. 2005, Ganeva et al. 2005, Mathews et al. 2006). In accordance to Worland et al. (1998a) and Ganeva et al. (2005), *Rht-B1b*, *Rht-B1d*, *Rht-D1b* and *Rht8c* reduced plant height by 15 to 21% implemented in the genetic background of Mercia, a UK variety possessing medium height and moderate FHB resistance. Interestingly, these *Rht* alleles significantly differed in their FHB severity response following spray inoculation of *F. culmorum*. Whereas *Rht-D1b* and *Rht-B1d* vastly increased FHB susceptibility, *Rht-B1b* as well as *Rht8c* revealed a considerable, but not significant increase across eight environments (Miedaner and Voss 2008). Among the near-isogenic lines of the rather tall and slightly more resistant variety Maris Huntsman, the effects of the *Rht* genes on FHB rating were less pronounced. Only the extremely dwarfed lines containing *Rht-B1c* derived from the variety Tom Thumb and the combination of *Rht-B1b*+*Rht-D1b* showed

significantly increased FHB susceptibility in addition to the strongest reduction in plant height of 53 and 46%, respectively.

Using the same isogenic lines of Mercia and Maris Huntsman carrying *Rht-B1b* and *Rht-D1b*, Srinivasachary et al. (2008) obtained comparable results. *Rht-D1b* significantly increased FHB susceptibility, whereas *Rht-B1b* led to an intermediate increase in FHB severity with no significant difference compared to the tall (*rht*) line in their field trial on one location.

In the present study, the *Rht* effects proved to be robust over years in the Mercia data set and the check varieties. Across both data sets the underlying *Rht* genes, although derived from genetically different sources, consistently enhanced FHB severity from at least 19 up to 83%. Nevertheless, in accordance to Hilton et al. (1999) who analysed the effects of *Rht-B1b* and *Rht-D1b* in near-isogenic lines of Maris Widgeon and Maris Huntsman, the FHB resistance response strongly depends on the genetic background and environmental factors. This becomes obvious by the German check varieties Toras and Certo, which were much less susceptible to FHB compared to the respective Mercia and Maris Huntsman *Rht-D1b* lines. The large differences in FHB response indicate that these varieties might incorporate further QTL for FHB resistance resulting in Toras being among the most FHB-resistant varieties in Germany (Anonymous, 2009). Likewise, Holzapfel et al. (2008) detected resistance QTL with major effects in *Rht-D1b* carrying varieties Biscay, Pirat, Rubens and Travix partly compensating the negative effect of *Rht-D1b* on FHB resistance.

In two populations of the crosses between the Chinese varieties Sumai 3 × Nobeokabouzu-komugi and Sumai 3 × Gamenya, Handa et al. (2008) detected a significant FHB resistance QTL on 2DS near the *Rht8* semi-dwarfing gene locus. Interestingly, both wild-type alleles from the resistant variety Nobeokabouzu-komugi and the highly susceptible variety Gamenya reduced FHB severity and DON accumulation compared to the respective semi-dwarfing allele *Rht8c* present in Sumai 3 (V. Korzun *pers. commun.*). The authors postulated that this QTL is a gene complex consisting of *Rht8a* being associated with type I resistance and a multidrug resistance-associated protein(s) controlling type II resistance by detoxification of DON. This hypothesis of an absent resistance allele in case of *Rht8c* presence agrees with our findings of a moderate negative effect on FHB resistance comparable to *Rht-B1b*, or a negative pleiotropic effect of *Rht8c* on FHB reaction that is greatly diminished compared to *Rht-D1b*.

However, although the exact length of the introgressed segments bearing the respective *Rht* genes are not known (Korzun *pers. commun.*), it is highly unlikely that three Japanese donor

varieties all carry QTL closely linked to the respective *Rht* genes that are differentially promoting FHB susceptibility without any documented segregation over decades. Therefore, the effects of these loci are most likely caused by differential pleiotropic *Rht*-allele effects rather than linkage in multiple cases.

6.1.2 Evaluation of type II resistance in *Rht*-isogenic lines

In the present field trials using spray inoculation the overall rating of the percentage of FHB diseased spikelets per plot inevitably combines two types of resistance: the resistance to initial infection (type I) and resistance to fungal spread within the spike (type II). Owing to the difficulty in measuring *Rht* gene effects solely on type I resistance in the field, evaluation of type II resistance in a point inoculation experiment including near-isogenic lines allows to draw conclusions on *Rht* gene effects on type I resistance when compared to the former spray inoculation results.

In such a way, Srinivasachary and colleagues (2008) recently revealed that *Rht-D1b* confers enhanced FHB severity towards initial infection (type I), but has no effect on fungal spread within the spike (type II). This may be further substantiated by the fact that the meta-QTL on chromosome 4D comprised solely QTL that were detected using spray inoculation (Löffler et al. 2009). Likewise, Srinivasachary et al. (2009) found that *Rht-B1b* significantly increased type I FHB severity, but on the contrary decreased type II FHB severity compared to *Rht-B1a*. However, the authors assessed the resistance of tall (*rht*), *Rht-B1b* and *Rht-D1b* near-isogenic lines of the varieties Mercia and Maris Huntsman on the basis of a comparably small data set of 28 and 30 plants per line, respectively, in an artificial environment of a polytunnel using a less aggressive isolate.

To obtain results on the effects of individual *Rht* genes exclusively on fungal spread (type II FHB severity) on a more comprehensive data base, we evaluated the same near-isogenic Mercia and Maris Huntsman lines containing the tall (*rht*), *Rht-B1b*, *Rht-D1b* and additional *Rht* alleles in a field trial in Hohenheim in 2006 and 2007. Following point inoculation of 25 single spikes in 1m two-row plots of each line, with one replication in 2006 plus each of two replications in 2007, for Mercia and Maris Huntsman lines disease was rated twice as percentage of diseased spikelets per spike at 19 and 24 days post inoculation (Table 3). As a result, *Rht-B1b* significantly ($P < 0.05$) increased fungal spread in both sets of isogenic lines. *Rht-D1b* led to significantly ($P < 0.05$) increased fungal spread compared to the tall (*rht*) line only in the Maris Huntsman data set, but showed no difference in the Mercia background across both years. Within the Mercia background, *Rht8c* most vigorously decreased fungal

spread in absolute terms by 4.3% in comparison to the tall (*rht*) line. This further supports the results from the spray inoculation trials that *Rht8c* decreases combined type I + II FHB resistance to a lesser extent than *Rht-B1b* or *Rht-D1b*, respectively, when compared to the tall (*rht*) line.

Rht-B1d reduced fungal spread, although significant ($P < 0.05$), only to a small extent of 1.9% less infected spikelets per spike compared to the respective tall (*rht*) Mercia line. Unexpectedly from the strongest increase in FHB severity resulting from the spray inoculation experiment, *Rht-B1c* and the combination of *Rht-B1b*+*Rht-D1b* showed no significant effect on type II FHB resistance.

Table 3. Average percentage of *Fusarium* head blight (FHB) infected spikelets per spike of five near-isogenic *Rht*-lines each of the varieties Mercia and Maris Huntsman and three check varieties following point inoculation with a conidial suspension (1×10^6 spores/ml) of *F. culmorum* isolate FC46 for testing type II resistance in a field experiment in Hohenheim

Line	Infected spikelets per spike (%)			
	Mercia			Maris Huntsman
	Both years	2006	2007	2007
rht	25.9 d [a]	29.7 b	23.5 c	15.1 a
Rht-B1b	28.1 e	28.5 b	27.4 d	17.1 b
Rht-B1c				13.6 a
Rht-B1d	24.0 c	28.6 b	21.2 b	
Rht-D1b	25.0 cd	23.7 a	25.2 c	17.8 b
Rht-B1b + Rht-D1b				13.9 a
Rht8c	21.6 b	22.5 a	20.7 b	
Checks with *Rht-D1b*:				
Toras	12.1 A	11.6 A	11.0 A	11.0 A
Certo	20.3 B	24.8 B	16.7 B	15.8 B
Travix	22.7 C	26.5 B	19.5 C	19.1 C

[a] Numbers followed by different letters are significantly different ($P < 0.05$) according to Tukey-test. Checks were calculated as separate data set, significant differences between checks are presented in capital letters.

In conclusion, following point inoculation, all near-isogenic *Rht*-lines revealed only minor absolute differences for type II FHB severity in both genetic backgrounds compared to the spray inoculation experiment measuring type I + II FHB severity (Miedaner and Voss 2008). Although partially significant, the percentage of infected spikelets per spike varied only by 6.5% in the Mercia and 4.2% in the Maris Huntsman background, respectively. In accordance to the results from the spray inoculation trials (Miedaner and Voss 2008), likewise the fungal spread within the spike was highly dependent on the genetic background and line × year interaction. This was particularly illustrated by the disease scores of the *Rht-D1b* carrying

check varieties Toras, Certo and Travix, that significantly ($P < 0.05$) varied within a much larger range of about 10% and showed significant differences between years within the Mercia data set (Table 3). Accordingly, the results from spray and single floret inoculation carried out in the present work demonstrate that the negative effects of *Rht-B1b*, *Rht-B1c*, *Rht-B1d*, *Rht-D1b* and *Rht8c* on FHB resistance mainly affect resistance to initial infection (type I) with little, if any consistent effect on fungal spread within the spike (type II). As a consequence, point inoculation trials carried out in an artificial environment involving only one to two genotypes as conducted by Srinivasachary et al. (2008, 2009) seem not to be sufficient to conclude on effects of individual *Rht* genes in the field.

6.2 Variation of aggressiveness and DON production among *G. zeae* crossing populations

The incremental approach to increase disease resistance, especially when relying on only few major resistance QTL, may pose a yet undefined risk of changes in the genetic structure of fungal populations towards a severe increase in aggressiveness within the pathogen population. For the quantitative inheritance of resistance and aggressiveness in the wheat/*Fusarium* pathosystem little is known about pathogen evolution in response to increasing quantitative host resistance. In the last decade several examples have been documented demonstrating the high adaptability of the fungus to consistently changing environmental conditions (Gale et al. 2002, Carter et al. 2002, Waalwijk et al. 2003, Ward et al. 2008). Therefore, it is possible that *Fusarium* populations likewise adapt to increased quantitative resistance in modern agroecosystems. However, if the quantitative nature of this pathosystem evolution is slower compared to that against major gene resistance, it is more difficult to detect and could better be characterized as a process of erosion rather than a breakdown as described by McDonald and Linde (2002). The authors specified a risk assessment framework on the basis of five factors that have to be considered to evaluate the risk of pathogen evolution: (I) mutation, (II) population size, (III) gene and genotype flow, (IV) reproduction and mating system, and (V) directional selection.

Consistent development of phylogenetically distinct lineages indicates a generally low but constant average mutation frequency per locus in *F. graminearum* at 1×10^{-6} (Fincham et al. 1979, O´Donnell et al. 2000, Miedaner et al. 2008). Nevertheless, mutational adaptation to changing environments should be considered in large populations.

Several studies mainly spanning North America and Asia revealed large population sizes with high genetic diversity sampled across different countries and even within individual fields with little or no substructuring within populations for *F. graminearum* as the most prevalent *Fusarium* species (Miedaner et al. 2001b, Zeller et al. 2004, Schmale et al. 2006, Qu et al. 2008, Burlakoti et al. 2008). In addition, for *G. zeae* high gene flow was observed due to long distance dispersal of primarily sexual ascospores (Markell and Francl, 2003, Maldonaldo-Ramirez et al. 2005, Schmale et al. 2005/2006, Burlakoti et al. 2008). Moderately low clonal genotype flow of asexual propagules, representing a linked package of advantageous alleles that has already been selected over years, pose a high but more regional risk to cause epidemics (Schmale et al. 2006).

In the recent work, main emphasis was put on sexual recombination as the most obvious mechanism to achieve and maintain high genetic diversity and the possible influence of efficient directional selection due to increased FHB resistance levels within the host. Pathogens with mixed (sexual and asexual) reproduction systems, as present in *G. zeae*, are suspected to pose the highest evolutionary risk for adaptation (McDonald and Linde 2002). As used to create the analysed *F. graminearum* populations, sexual recombination can be observed under laboratory conditions (Bowden and Leslie, 1999). In the field, common outcrossing can only be inferred from high genetic diversity and low levels of linkage disequilibrium that are typical for random mating populations (Miedaner et al. 2008).

Accordingly, a negative correlation (r = -0.59, $P < 0.01$) between genetic similarity and geographical distance on the one hand may indicate co-existence of genetically divergent populations of *F. graminearum* on a continental scale (Zeller et al. 2004, Schmale et al. 2006, Gale et al. 2007). On the other hand regional subdivision in population structure might simply reflect the time required for long-distance gene or genotype flow between populations separated by large geographic distances rather than consistent regional differentiation.

Very recently, Chen and Zhou (2009) determined a low outcrossing rate of 5.7–20.9% in three *F. graminearum* crosses under field conditions, but the database was insufficient to prove general validity and it remains unclear whether outcrossing occurs regularly or episodically in the field. However, only rare sexual recombination is needed to sustain high genotypic diversity and the appearance of a randomly mating population (Leslie and Klein 1996, Zeller et al. 2004).

The mixed mating system enables the fungus to recombine alleles for aggressiveness as rapidly as breeders can put together QTL for resistance. Due to clonal reproduction these new combinations that provoke increased aggressiveness can be maintained over years and are

subject to environmental selection. Hence, if increases in pathogen aggressiveness are possible or likely, respectively, efficient directional selection may be one of the main forces that trigger durable changes in population composition towards isolates possessing superior aggressiveness. Similarly, the genes facilitating mycotoxin production, especially DON, might be under a non-specific selection as high DON content is attributed to higher aggressiveness (Cumagun and Miedaner 2004).

Within the progeny of a cross of two moderately aggressive *F. graminearum* isolates, Cumagun and Miedaner (2004) described continuous distributions for the traits aggressiveness and DON production. As a result, moderately aggressive parents gave rise to a comparably high number of recombinants transgressive towards higher aggressiveness and DON levels following only one sexual reproduction phase. Accordingly, within progenies of crosses of parental isolates already representing a high level of aggressiveness, one could expect a narrowed distribution possessing a sharp range of recombinant aggressiveness close to the parental mean without significant transgressions. In strong contrast, in the present study including two such crosses wide significant normal distributed variation and, within progeny of cross B, even some transgressions towards increased aggressiveness, DON-, and fungal mycelium production were observed (Voss et al. 2010).

In 2007, the weather conditions in Hohenheim and Eckartsweier were much more appropriate to infection than in 2006, whereon the tested isolates generally reacted with heightened aggressiveness resulting in increased FHB severity and ExAg content. We found highly significant ($P < 0.01$) isolate × environment (environment = year × location) interaction that accounted for most of the variation observed for FHB rating, fungal biomass and deoxynivalenol content (Voss et al. 2010). Broken down further, the isolate × year interaction proved of predominant relevance, while isolate × location interaction was not significant ($P > 0.1$). Whereas less aggressive isolates as FG24 gained a pronounced increase in aggressiveness in 2007 compared to 2006 (+30% FHB severity on average across Cross A and B), isolates that have already been highly aggressive in 2006 increased in aggressiveness only to a lesser extent (+17.0% average in Cross A, +18.3% in Cross B). Hence, the large differences in aggressiveness in 2006 almost vanished in 2007 demonstrating a rather strong episodic selection due to changed weather conditions during the infection period.

Although not significant, the isolate-specific fungal mycelium production increased up to 3-fold in both pathogen populations from 2006 to 2007. Interestingly, the DON content was inconsistent for both populations. Previous studies on *Fusarium* populations have found

strong correlations between the amount of fungal mycelium represented by the ExAg absorbance and DON production, suggesting the feasibility of using the ExAg absorbance as an indirect measure for DON presence in the grain (Liu et al. 1997, Schnerr et al. 2002, Cumagun et al. 2004b, Burlakoti et al. 2007). Some authors concluded that the amount of produced DON relative to the amount of fungal mycelium in the host tissue remains a constant ratio. In contrast, we observed only weak correlations between ExAg absorbance and DON content (r = 0.43 among Cross A; r = 0.34 among Cross B). In agreement with findings of Miedaner et al. (2004), the highly variable DON/*Fusarium* ExAg ratio possessed significant (P = 0.01) genotypic variation in both progeny sets and, furthermore, no correlation between this ratio and FHB severity could be observed. This implies that DON is rather required at a threshold level to inhibit host resistance reactions and to enable fungal spread, than being a basic aggressiveness factor *per se*. Hence, increasing the amount of DON beyond this threshold seems not to suffice to further increase FHB severity.

Genetic analysis of the role of trichothecenes in pathogenicity or aggressiveness of *F. graminearum* on wheat and maize that were assessed using *tri5*-mutant lines are supporting this point of view. *Tri5*-deficient knockout mutants are lacking DON and any DON-precursors, but still are able to cause initial infection without the ability to spread within the wheat spikes (Bai et al. 2002). In the field, DON-nonproducing strains appeared to be only less aggressive on wheat instead of non-aggressive compared to the DON-producing progenitor strains (Bai et al. 2002, Desjardins et al. 1996/2006, Maier et al. 2006). This confirms that DON is not required for pathogenicity but acts as one aggressiveness factor enhancing host colonization.

Whether increased DON production by highly aggressive isolates is cause or effect of accelerated fungal invasion of host tissue cannot definitively be determined from our or literature results. Hence, more aggressive isolates might rather speed up invasion because of multiple interacting factors that generally confirm the quantitative character of aggressiveness with DON, among other mycotoxins, being just one part of the whole. Conclusive evidence has been obtained from several pathosystems that furthermore composition and activity of cell wall-degrading enzymes play a decisive role in the infection process (Kikot et al. 2009). Phalip et al. (2005) identified a powerful enzymatic arsenal secreted by *F. graminearum* that comprises 24 different enzyme classes necessary for plant cell wall penetration, maceration and digestion. Among these, e.g. fungal cutinases, pectinases, xylanases, lipases, exo-cellulases, endo-cellulases and β-glucosidases are considered as important aggressiveness factors in *Fusarium spp.* (Phalip et al. 2005, Kikot et al. 2009).

Summarized, the analyses of two segregating populations across four environments confirmed the ability of *F. graminearum* to significantly increase isolate-specific aggressiveness and DON production by sexual recombination, but the degree of aggressiveness is highly dependent on location and especially year effects.

6.3 Interaction of wheat resistance and pathogen aggressiveness

The interaction of host plant resistance and pathogen aggressiveness in the wheat/*Fusarium* pathosystem is complex because of quantitative inheritance with a continuous distribution among the progeny of host and pathogen in addition to wide genetic variation for both traits (Snijders 1990, Carter et al. 2002, Cumagun and Miedaner 2004, Cumagun et al. 2004a, Schmolke et al. 2005). As described likewise for FHB resistance of wheat varieties, we observed the aggressiveness of *Fusarium* isolates to be subject to environmental variability that influences disease initiation and development.

Generally, there is no recognized interaction between wheat genotypes and *Fusarium* isolates (Snijders and van Eeuwijk 1991, van Eeuwijk et al. 1995, Bai and Shaner 1996). Nevertheless, in some studies specific interactions were noted that were, however, not consistent across environments (e.g. Dusabenyagasani et al. 1997, Mesterházy 2002). In the according ANOVA, a resulting significant host × isolate interaction was rather caused by scaling effects than by changes in rank order of the evaluated isolates varying widely in aggressiveness.

In the present study seven medium to highly resistant wheat varieties were inoculated with eleven highly aggressive isolates to examine possible variety × isolate interactions, thus to avoid possible effects due to preassigned large differences in isolate aggressiveness (Voss et al. 2010). We observed no significant variety × isolate interaction and the variety rankings also were consistent across isolates indicating that the host variety plays no major role in the genetic composition of a pathogen population. Likewise, Snijders and van Euwijk (1991) found no evidence for isolate-specific resistance in a study containing 17 winter wheat genotypes evaluated with four *F. culmorum* isolates over 3 years. Hence, from our results in accordance to findings of several authors, the FHB resistance in wheat can be described as non-specific and horizontal at least for the most prevalent species as e.g. *F. graminearum* and *F. culmorum* (van Eeuwijk et al. 1995, Mesterhazy et al. 1999). This is even true for highly aggressive isolates and rather resistant wheat genotypes. For both, wheat lines and *F.*

graminearum isolates genotype × environment interactions played the major role in the individual development of FHB severity.

Nevertheless, we found the level of FHB resistance to be correlated with the stability of resistance, meaning varieties being moderately resistant to FHB varied more with respect to disease severity and DON accumulation than did the highly resistant varieties. This was confirmed by Buerstmayr et al. (2009) who similarly evaluated 56 susceptible to resistant wheat genotypes in five locations in two years. Referring to the pathogen, constant levels of FHB severity across varying environmental conditions for infection and disease development largely depend on the level of isolate aggressiveness with less aggressive isolates showing higher isolate × environment interaction.

The most important question remains whether the incremental approach to increase disease resistance in actual breeding programmes worldwide consistently triggers evolutionary selection within *Fusarium* species towards higher aggressiveness and accumulation of aggressiveness alleles by sexual recombination. Furthermore, a crucial aspect to evaluate is whether any improved quantitative FHB resistance in wheat can potentially be lowered by newly evolved isolates.

Taken together the five evolutionary forces that affect the risk of pathogen evolution as described by McDonald and Linde (2002), we can assume a merely constant mutation rate within highly diverse and large *F. graminearum* populations worldwide (O´Donnell et al. 2000, Miedaner et al. 2008). Long-distance transport is rather limited to the sexual ascospores (gene flow), but substantiates extensive interpopulation genetic exchange (Zeller et al. 2003/2004, Schmale et al. 2005/2006, Burlakoti et al. 2008). The mating system of *F. graminearum* can be described as mixed, with common sexual outcrossing at a frequency of up to 21% (Chen and Zhou 2009), although the data for proven outcrossing frequencies or selfing in the field are insufficient yet. However, owing to the presence of transgressive segregation within *F. graminearum* crosses of parental isolates already possessing high levels of aggressiveness, we present evidence that corroborate the major role of sexual recombination as driving force in the pathogen evolution.

In conclusion, following the framework of McDonald and Linde (2002) the evolutionary risk of *F. graminearum* to adapt to and finally overcome improved quantitative FHB resistance in wheat can be characterized as high. Although the present proof of missing variety × isolate interaction indicates that local appearance of highly resistant varieties may not be able to trigger a short-term adaptation of the pathogen, comprehensive use of wheat germplasm with

significantly increased FHB resistance might cause a consistent directed selection on the pathogen especially when it is based only on a few major QTL, like *Fhb1* or *Qfhs.ifa-5A*.

To generate predictions whether the *Fusarium* pathogens will adapt to their hosts and environments in terms of increased aggressiveness, it is essential to be able to link the concept of isolate aggressiveness to other concepts in evolutionary epidemiology such as isolate fitness (Galvani 2003). Aggressiveness describes the combination of speed and degree of disease severity caused by a pathogenic isolate resulting from the expression of several quantitative components, i.e. infection efficiency, fungal biomass and spore production and germination rate of spores, that do not inevitably quantify a change in isolate fitness. Since isolate fitness is determined by additional parameters such as spore viability and saprophytic survival that are not considered as aggressiveness-related traits, isolate aggressiveness cannot be considered strictly equivalent to isolate fitness.

Hence, the ability of a *Fusarium* population to overcome improved FHB resistance in wheat varieties by increased isolate aggressiveness depends on two factors: (1) the intensity of natural selection provoked by increased FHB host resistance being the evolutionary force and (2) the aggressiveness components expected to evolve that are closely related to isolate fitness.

Accordingly, if more aggressive isolates predominantly possess superior fitness and natural selection towards higher aggressiveness occurs based on disadvantageous environmental conditions for disease development, then it is likely that pathogen aggressiveness will increase and host resistance would erode gradually with time. From our results weather conditions unfavorable for infection as were present in 2006 or considerably increased host resistance, respectively, could meet the criteria of these disadvantageous environmental conditions. If highly aggressive isolates are lacking superior fitness for all environments and poorly or moderately aggressive isolates are favored under conditions conducive for disease development, then aggressiveness within the fungal population should not increase continuously due to inconsistent selection towards heightened aggressiveness.

Assuming a high amount of fungal biomass in the host reflects competitive fitness, then a high correlation between FHB severity and ExAg content indicates superior fitness of highly aggressive isolates producing more mycelium compared to less aggressive isolates. In the present study the correlation coefficients of FHB severity and fungal biomass content decreased considerably from 2006 to 2007 in both populations (Cross A: $r = 0.77$ in 2006 to $r = 0.47$ in 2007; Cross B: $r = 0.78$ to $r = 0.47$) but remained significant ($P < 0.01$), indicating a

variable but present advantage of high isolate specific aggressiveness in terms of fungal biomass production across changing environmental conditions. We do not know, however, whether higher fungal biomass in the host supports a higher fecundity of the isolate, an important parameter for fitness.

From literature only few datasets exist on positive or negative (trade-off) correlations between increased aggressiveness and fitness of fungal pathogens. In 1988, Leonard et al. observed that *C. carbonum* revealed low aggressiveness on maize but a great survival ability, whereas *C. heterostrophus* expressed high aggressiveness but a low survival ability. Both findings suggest trade-offs between aggressiveness and fitness. In contrast, Montarry et al. (2007) found no evidence for a trade-off between aggressiveness and overwinter survival of *P. infestans* on potato tubers.

In order to understand the complex interactions of each individual aggressiveness component with competitiveness and finally fitness within a *Fusarium spp.* population in the field, further investigation is required.

6.4 Consequences for resistance breeding in wheat

During the last decades the improvement of FHB resistance in high yielding wheat varieties has been a major effort in wheat breeding programmes worldwide. As in Germany the level of FHB resistance represents a knockout criterion for variety registration the aim remains to combine an acceptable moderate level of FHB resistance with lodging-resistance and high yield performance.

At present, the semi-dwarfing allele *Rht-D1b* is necessary to utilize and assure high yield potential by increased spikelet fertility, higher grain number per ear, increased harvest index and superior lodging resistance and, hence, is preferred within the breeding strategies of many wheat breeders for northern and central Europe (Gale and Youssefian 1985, Li et al. 2006, Addisu et al. 2009, E. Ebmeyer, *pers. commun.*). To cope with future demands for improved and consistent FHB resistance combined with maximum yield performance two strategies seem reasonable: (1) substitution of *Rht-D1b* by other dwarfing alleles with similar agronomic effects less compromising FHB resistance and (2) the accumulation of (new) FHB resistance loci in susceptible but high-yielding *Rht-D1b*-varieties to counterbalance the negative effect of *Rht-D1b* on FHB resistance.

From the currently described 21 *Rht* genes including additional allelic variants for the most prevalently exploited loci *Rht-B1*, *Rht-D1b* and *Rht8* (McIntosh et al. 2008) only a few are used agronomically within commercial European wheat varieties, as typical features such as higher grain numbers and increased harvest index do not always compensate for reduced grain size and shoot biomass (Evans 1998, Hedden 2006). As shown in both varietal backgrounds of Mercia and Maris Huntsman the use of *Rht-B1b* might be the most self-evident alternative for *Rht-D1b* in northwestern Europe because of a reduced negative effect on FHB resistance (Miedaner and Voss 2008). Nevertheless, currently only 6% of the German officially registered varieties contain *Rht-B1b* (Knopf et al. 2008), although e.g. *Rht-B1b*-carrying variety Hermann has been one of the most grown varieties in Germany for the last five years that already combines maximum yield and good FHB resistance (Anonymous 2009).

Considering future climate change scenarios for central Europe, predicted climate warming may have substantial consequences for the structure and dynamics of agroecosystems leading to rapid northern shifts in distribution of adapted plant and pathogen species as well as adapted wheat germplasm (Aerts et al. 2006). Additionally, the likelihood of crop failure is expected to rise sharply because the risk of extreme regional weather conditions such as summer drought stress or increasing variability of rainfall will become more accentuated (Ferrara 2009). Nevertheless, Harrison and Butterfield in 1996 predicted an increasing winter wheat yield rate of 0.2 t ha^{-1} $decade^{-1}$ up to the 2020s and 0.36 t ha^{-1} $decade^{-1}$ beyond. All together this illustrates the persisting need for plant stability ensured by effective and stress insensitive *Rht* genes.

In the varietal background of Mercia, semi-dwarfing *Rht8c* linked with photoperiodic insensitive *Ppd-D1a* offered similar positive performance referring to reduction both in plant height and FHB resistance compared to *Rht-B1b*. Due to the shortening of the plant´s life cycle, integrating the *Rht8c/Ppd-D1a* complex offers the best opportunity for reducing plant height, accelerating time of flowering, increasing spikelet fertility and grain fill before the onset of desiccating dry summer conditions predominant in south and central Europe as well as in Russia (Worland et al. 1998a/b, 2001, Borojevic and Borojevic 2005). The *Rht-B1d* allele is believed to correlate with less temperature sensitivity compared to *Rht-B1b* and *Rht-D1b* (Worland 1986, Worland and Petrovic, 1988). Accordingly, this allele prevails in Italian, Bulgarian and Yugoslavian varieties. Confirming literature, in the present study *Rht-B1d* led to a reduction in plant height similar to *Rht-B1b*. However, a strong increase in FHB susceptibility caused by *Rht-B1d* comparable to *Rht-D1b* may question a future use in Northern Europe.

Hence, the replacement of *Rht-D1b* by *Rht8c/Ppd-D1a* that affects FHB resistance to a lesser extent than the GA-insensitive genes might be an option for future demands on the long run, as indicated by Ellis et al. (2005). For a more short term approach lines solely carrying *Rht8c* or *Ppd-D1a* are already existent for commercial breeding by breaking the *Rht8c/Ppd-D1a* linkage group (Worland et al. 1998a). Depending on the varietal background of the two alleles, *Ppd-D1a* reduces plant height of around 10cm by shortening the plants life cycle due to its photoperiod insensitivity whereas *Rht8c* reduces plant height by 5-10cm without adverse interactive environmental effects on yield (Worland et al. 1998a/b, Korzun et al. 1998, Worland et al. 2001). In conclusion, on the short term the sole use of *Rht8c* might be an alternative for high-yielding, humid environments of Northern Europe to achieve shortened plant stature combined with less negative effects on FHB resistance whereas earlier heading provided by photoperiod-insensitive *Ppd-D1a,* at present, can be counterproductive for achieving maximal grain yield.

Resulting from the optimum balance between increased harvest index and reduced total shoot weight, the maximum grain yields are obtained at intermediate plant heights between 70 and 100cm (Flintham et al. 1997a/b). According to the background varietal height, intrinsically taller varieties require more potent *Rht* alleles to achieve optimum height.

Therefore, to achieve the appropriate height reduction for the target varietal background and environment, for breeders it is useful to utilize a wide range of dwarfing genes at their disposal (Ellis et al. 2005). Since various *Rht* dwarfing alleles as well as combinations of several semi-dwarfing alleles result in too severe plant shortage in homozygous condition, such as *Rht-B1c* or *Rht8c+Rht-D1b*, hybrid wheat breeding might offer advantages for both grain yield and exploitation of additional *Rht* dwarfing alleles that have yet no commercial use. Additional *Rht*-allele specific characteristics would offer further advantages for future hybrid wheat breeding. One example is the inhibition of pre-harvest alpha-amylase production conferred by *Rht-B1c* that is likewise expressed in heterozygous condition (Flintham et al. 1997b). Nevertheless, because in homozygous status *Rht-B1c* as well as the combination of *Rht-B1b+Rht-D1b* resulted in the strongest increase of FHB susceptibility in the Maris Huntsman data set, its effects on FHB resistance in heterozygous status require further investigation. At least the severe plant shortage combined with significantly less increase of FHB susceptibility of *Rht-D1b+Rht-8c* compared to solely *Rht-D1b* in the Mercia isogenic lines suggest yet unknown positive epistatic effects of *Rht*-allele combinations. Taken together, a wider range of *Rht* alleles could be used in homozygous or heterozygous condition

to optimally adapt a wheat line or hybrid to a target environment referring to yield performance, disease and lodging resistance as well as heat and drought tolerance.
However, utilization of *Rht-D1b* often is a prerequisite in actual northern European wheat breeding programmes (H. Coester and E. Ebmeyer *pers. comm.*). In the present study significant transgressions towards higher resistance occurred only in the Apache × Biscay progeny. Nevertheless, significant genotypic variation for FHB resistance and plant height within crosses of susceptible short-strawed varieties (Biscay, Pirat, Rubens) all carrying *Rht-D1b,* with more resistant long-strawed varieties (Apache, Romanus, History) indicate feasibility of selection for short-strawed varieties conferring improved FHB resistance (Voss et al. 2008). In the present populations this was expressed by many recombinants being significantly less FHB susceptible, but offering plant heights similar to the short-strawed parent. Likewise, lines with FHB ratings similar to the resistant parent being significantly shorter could also be found.

To fulfill the criteria for variety registration, selection for *Rht-D1b*-varieties conferring improved FHB resistance to counterbalance the negative effect of *Rht-D1b* results in either increased selection intensity or requires enlarged population sizes when solely using phenotypic selection. Accordingly, fast and low cost incorporation and accumulation of powerful FHB resistance QTL into susceptible but high-yielding *Rht-D1b* germplasm via marker assisted selection (MAS) presently is a major aim and, hence, the second option to achieve increased FHB resistance combined with short plant stature.
MAS is increasingly becoming a common tool in commercial wheat breeding programmes (Wilde et al. 2007/2008). Qualitative mapping of *Fhb1, Fhb2, Fhb3* and further significant QTL on chromosomes 1B, 2B, 2D, 5A, 6A or 7B, respectively, for FHB resistance in wheat already provides tightly linked markers that can reduce linkage drag associated with the incorporation of exotic FHB resistance QTL sources (Cuthbert et al. 2007, Wilde et al. 2007/2008, Holzapfel et al. 2008, Löffler et al. 2008). At present, the most useful QTL are those on chromosomes 3BS (*Fhb1*), 5AS (*Qfhs.ifa-5A*) and 6BS (*Fhb2*) derived from Sumai 3 involving mapping populations.
For the purposes of MAS, diagnostic markers are currently available only for *Fhb1* (Liu et al. 2008). Therefore, the community of wheat breeders strongly focuses on the incorporation of *Fhb1* in their aim to effectively improve *Fusarium* resistance with minimum expenses. In this way *Fhb1* has been successfully introduced into many breeding populations worldwide in recent years, including primarily the USA, Canada, Australia and Germany (Buerstmayr et al.

2009, H. Coester *pers. commun.*). Owing to the glucosylation process fungal DON seemingly is detoxified into less phytotoxic DON-3-glucoside by this QTL (Lemmens et al. 2005). The conjugated form is referred to as masked mycotoxin because DON-3-glucoside escapes routine detection methods but can release its toxic precursor after hydrolysis in mammals or after subsequent food processing (Berthiller et al. 2005, 2009a). In order to investigate the concentrations of DON-3-glucoside Berthiller et al. (2009b) analysed a set of 77 naturally *Fusarium*-contaminated wheat and maize samples from Austria, Germany and Slovakia. The detected DON-3-glucoside concentrations in all cereal samples corresponded to about 5 up to 46 mol% of their DON concentrations which on total varied from 42 to 4130 µg kg^{-1}. The fact that *Fhb1* further shifts this proportion towards DON-3-glucoside indicates the future importance to consider both DON and DON-3-glucoside with regard to food and feed safety. In general, knowledge on the physiological mode of action has to be further deepened when breeding extensively relies on single major resistance genes such as *Fhb1*.

Moreover, in case of reliance on single major resistance genes in agroecosystems many examples of ineffective qualitative resistance offer abundant evidence that selection for pathogen virulence in the field is highly efficient (McDonald and Linde 2002). From literature it has been repeatedly demonstrated that artificial selection for quantitative traits as aggressiveness can likewise result in pathogens´ quantitative adaptation to host varieties (Pariaud et al. 2009). As one of the first studies in this area, Leonard already 1969 observed an increase in the mean infection efficiency of two populations of *P. graminis* f.sp. *avenae* by approximately 10-15% after seven asexual generations only on the quantitatively resistant host from which they were isolated. Confirming Leonard´s results of an adaption to the variety of origin, Chin and Wolfe (1984) as well as Villaréal and Lannou (2000) found significantly increased multiplication rates and infection efficiency of powdery mildew (*B. graminis*) isolates on quantitatively resistant barley and wheat varieties solely on the host variety from which they were collected. Only few experiments have investigated the selective effect of susceptible and quantitatively resistant host varieties on pathogen evolution with yet controversial results depending on the pathosystem. Zhan et al. (2002) found a strong impact of host genotypes on the dynamics of pathogen populations in *Mycosphaerella graminicola* strains. Their experiments revealed that the pathogen populations evolved more slowly on a more resistant host variety referring to genotypic diversity and changes in genetic structure and that reproductive fitness and aggressiveness of a fungal strain were not correlated. Within the same pathosystem Ahmed et al. (1996) similarly demonstrated that pathogen populations isolated from susceptible wheat varieties produced higher levels of disease, whereas Lehmann

and Shaner (1997) found isolates of *Puccinia recondita* f.sp. *tritici* reproductively more fit on partially resistant wheat varieties.

Consequently, cultivation of wheat varieties with increased FHB resistance mainly based on a specific mode of action of single major resistance genes (e.g. *Fhb1*) on large acreages in all main countries of wheat production should induce no directional short-term but a considerable long-term selective force. This poses a definite evolutionary risk of *Fusarium spp.* to significantly increase isolate-specific aggressiveness to an open maximum by sexual recombination as confirmed in the present study (Voss et al. 2010).

Therefore, the emphasis of future research activities is to discover and/or develop more diagnostic markers to aid effective and efficient pyramiding of the most promising FHB resistance QTL originating from genetically unrelated resistance sources. Application of array-based high throughput markers, such as DArT (diversity array technology) markers or SNP (single nucleotide polymorphism) detection methods will complement existing PCR-based markers in the next years (Buerstmayr et al. 2009). Recently, experimental marker-assisted pyramidisation of different FHB resistance loci has successfully been conducted (Miedaner et al. 2006, Wilde et al. 2008, Shi et al. 2008) and seems promising when combined with phenotypic selection as the most effective tool to accumulate additional minor resistance QTL to achieve a reliable and durable level of FHB resistance. A broad base of major and minor resistance genes should weaken the directional selective force of improved quantitative FHB resistance on *Fusarium spp.* populations and may prevent a slow erosion of quantitative FHB resistance due to consistently increasing aggressiveness on the long run.

7. References

Achard, P. and P. Genschik. 2009. Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. J. of Exp. Bot. 60:1085–1092.

Addisu, M., J.W. Snape, J.R. Simmonds, and M.J. Gooding. 2009. Effects of reduced height (*Rht*) and photoperiod insensitivity (*Ppd*) alleles on yield of wheat in contrasting production systems. Euphytica *in press.*

Aerts, R., J.H.C. Cornelissen, and E. Dorrepaal. 2006. Plant performance in a warmer world: general responses of plants from cold, northern biomes and the importance of winter and spring events. Plant Ecology 182:65–77.

Ahmed, H.U., C.C. Mundt, M.E. Hoffer, and S.M. Coakley. 1996. Selective influence of wheat cultivars on pathogenicity of *Mycosphaerella graminicola* (anamorph *Septoria tritici*). Phytopathology 86:454–458.

Anderson, J.A. 2007. Marker-assisted selection for *Fusarium* head blight resistance in wheat. Int. J. Food Microbiol. 119:51–53.

Anonymous. 2005. Commission Regulation (EC) No 856/2005 of 6th June 2005 amending regulation (EC) no 466/2001 as regards *Fusarium* toxins.

Anonymous. 2009. Descriptive list of recommended varieties (BSL). (In German.) Landbuch-Verlag, Hannover, Germany.

Bai, G.H. and G. Shaner. 1996. Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis. 80:975–979.

Bai, G.H., A. Desjardins, and R. Plattner. 2001. Deoxynivalenol nonproducing *Fusarium graminearum* causes initial infection but does not cause disease spread in wheat spikes. Mycopathologia 153:91–98.

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

Berthiller F., C. Dall'Asta, R. Schuhmacher, M. Lemmens, G. Adam, R. Krska. 2005. Masked mycotoxins: Determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 53:3421–3425.

Berthiller, F., R. Schuhmacher, G. Adam, R. Krska. 2009a. Formation, determination and significance of masked and other conjugated mycotoxins. Anal. Bioanal. Chem. 395:1243–1252.

Berthiller F., C. Dall'Asta, R. Corradini, R. Marchelli, M. Sulyok, R. Krska, G. Adam, R. Schuhmacher. 2009b. Occurrence of deoxynivalenol and its 3-β-D-glucoside in wheat and maize. Food Addit. Contam. 26:507–511.

Boerner, A., J. Plaschke, V. Korzun, and A.J. Worland. 1996. The relationship between the dwarfing genes of wheat and rye. Euphytica 89:69–75.

Bolle, C. 2004. The role of GRAS proteins in plant signal transduction and development. Planta 218:683–692.

Borojevic, K. and K. Borojevic, 2005. The transfer and history of ‘‘Reduced height genes’’ (*Rht*) in wheat from Japan to Europe. J. Hered. 96:455–459.

Boss, P.K. and M.R. Thomas. 2002. Association of dwarfism and floral induction with a grape 'green revolution' mutation. Nature 416:847–850.

Bowden, R.L. and J.F. Leslie. 1999. Sexual recombination in *Gibberella zeae*. Phytopathology 89:182–188.

Buerstmayr, H., B. Steiner, M. Lemmens, and P. Ruckenbauer. 2000. Resistance to *Fusarium* head blight in winter wheat: heritability and trait associations. Crop Science 40:1012–1018.

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

Burlakoti, R.R., R. Estrada, V.V. Rivera, A. Boddeda, G.A. Secor, and T.B. Adhikari. 2007. Real-time PCR quantification and mycotoxin production of *Fusarium graminearum* in wheat inoculated with isolates collected from potato, sugar beet, and wheat. Phytopathology 97:835–841.

Burlakoti, R.R., S. Ali, G.A. Secor, S.M. Neate, M.P. McMullen, and T.B. Adhikari. 2008. Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato, and sugar beet in the upper Midwest of the United States. Phytopathology 98:969–976.

Carter, J.P., H.N. Rezanoor, D. Holden, A.E. Desjardins, R.D. Plattner, and P. Nicholson. 2002. Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. Eur. J. Plant Pathol. 108:573–583.

Chen, Y. and M.-G. Zhou. 2009. Sexual recombination of carbendazim resistance in *Fusarium graminearum* under field conditions. Pest Manag. Sci. 65:398–403.

Chin, K.M. and M.S. Wolfe. 1984. Selection of *Erysiphe graminis* in pure and mixed stands of barley. Plant Pathology 33:535–46.

Cumagun, C.J.R. and Miedaner T. 2004. Segregation for aggressiveness and deoxynivalenol production of a population of *Gibberella zeae* (*Fusarium graminearum*). Eur. J. Plant Pathol. 110:789–799.

Cumagun, C.J.R., R.L. Bowden, J.E. Jurgenson, J.F. Leslie, and T. Miedaner. 2004a. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. Phytopathology 94:520–526.

Cumagun, C.J.R., F. Rabenstein, and T. Miedaner. 2004b. Genetic variation and covariation for aggressiveness, deoxynivalenol production and fungal colonization among progeny of *Gibberella zeae* in wheat. Plant Pathology 53:446–453.

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

Dalrymple, D.G. 1986. Development and spread of high-yielding wheat varieties in developing countries. Agency for International Development, Washington, DC.

Desjardins, A.E., R.H. Proctor, and G.H. Bai. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant Microbe Interact. 9:775–781.

Desjardins, A.E. 2006. *Fusarium* Mycotoxins: Chemistry, Genetics and Biology. APS Press, St. Paul, Minnesota, USA.

Draeger, R., N. Gosman, A. Steed, E. Chandler, M. Thomsett, Srinivasachary, J. Schondelmaier, H. Buerstmayr, M. Lemmens, M. Schmolke, M. Mesterhazy, and P. Nicholson. 2007. Identification of QTLs for resistance to *Fusarium* head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor. Appl. Genet. 115:617–625.

Dubin, H.J., L. Gilchrist, J. Reeves, and A. McNab. 1997. *Fusarium* head scab: Global Status and prospects. CIMMYT, Mexico DF, Mexico.

Dusabenyagasani, M., R.C. Hamelin, J. Collin, and D. Dostaler. 1997. Effect of cultivar and strain interactions in the screening of resistance against wheat scab caused by *Fusarium graminearum*. Phytoprotection 78:53–60.

Ellis, M.H., W. Spielmeyer, K.R. Gale, G.J. Rebetzke, and R.A. Richards. 2002. ‘‘Perfect’’ markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theor. Appl. Genet. 105:1038–1042.

Ellis, M.H., G.J. Rebetzke, F. Azanza, R.A. Richards, and W. Spielmeyer. 2005. Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat. Theor. Appl. Genet. 111:423–430.

Ellis, M.H., D.G. Bonnett, and G.J. Rebetzke. 2007. A 192bp allele at the Xgwm261 locus is not always associated with the *Rht8* dwarfing gene in wheat (*Triticum aestivum* L.). Euphytica 157:209–214.

Evans, L.T. 1998. Feeding the ten billion: plants and population growth. Cambridge University Press, UK, 133–150.

Fernando, W.G.D., J.D. Miller, W.L. Seaman, K. Seifert, and T.C. Paulitz. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Can. J. Bot. 78:497–505.

Ferrara, R.M., P. Trevisiol, M. Acutis, G. Rana, G.M. Richter, and N. Baggaley. 2009. Topographic impacts on wheat yields under climate change: two contrasted case studies in Europe. Theor. Appl. Climatol. 99:53–65.

Fincham, J.R.S., P.R. Day, and A. Radford. 1979. Fungal Genetics. 4th ed. University of California Press, Berkeley, US.

Flintham, J.E., A. Boerner, A.J. Worland, and M.D. Gale. 1997a. Optimizing wheat grain yield: effects of *Rht* (gibberelin-insensitive) dwarfing genes. J. Agric. Sci. 128:11–25.

Flintham, J.E., W.J. Angus, and M.D. Gale. 1997b. Heterosis, overdominance for grain yield, and alpha-amylase activity in F1 hybrids between near-isogenic *Rht* dwarf and tall wheats. J. Agric. Sci. 129:371–378.

Gale, L.R., L.F. Chen, C.A. Hernick, K. Takamura, and H.C. Kistler. 2002. Population analysis of *Fusarium graminearum* from wheat fields in eastern China. Phytopathology 92:1315–1322.

Gale, L.R., T.J. Ward, V. Balmas, and H.C. Kistler. 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the upper midwestern United States. Phytopathology 97:1434–1439.

Gale, M.D. and S. Youssefian. 1985. Dwarfing genes in wheat. In: G. E. Russell (ed.), Progress in Plant Breeding, 1–35. Butterworths and Co., London, UK.

Galvani, A.P. 2003. Epidemiology meets evolutionary ecology. Trends in Ecol. and Evol. 18:132–139.

Ganeva, G., V. Korzun, S. Landjeva, N. Tsenov, and M. Atanasova. 2005. Identification, distribution and effects on agronomic traits of the semi-dwarfing *Rht* alleles in Bulgarian common wheat cultivars. Euphytica 145:305–315.

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

Gosman, N., R. Bayles, P. Jennings, J. Kirby, and P. Nicholson. 2007. Evaluation and characterization of resistance to *Fusarium* head blight caused by *Fusarium culmorum* in UK winter wheat cultivars. Plant Pathol. 56:264–276.

Goswami, R.S. and H.C. Kistler. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515–525.

Goswami, R.S. and H.C. Kistler. 2005. Pathogenicity and *in planta* mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology 95:1397–1404.

Handa, H., N. Namiki, D. Xu, and T. Ban. 2008. Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Mol. Breeding 22:71–84.

Harrison, P.A. and R.E. Butterfield. 1996. Effects of climate change on Europe-wide winter wheat and sunflower productivity. Climate Research 7:225–241.

Hedden, P. 2006. Green Revolution Genes. Plant Physiology Online, Fourth Edition, Essay 20.2. http://4e.plantphys.net/article.php?ch=&id=355. Verified 13th Oct. 2009.

Hilton, A.J., P. Jenkinson, T.W. Hollins, and D.W. Parry. 1999. Relationship between cultivar height and severity of *Fusarium* ear blight in wheat. Plant Pathol. 48:202–208.

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

Kikot, G.E., R.A. Hours, and T.M. Alconada. 2009. Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. J. Basic Microbiol. 49:231–241.

Knopf, C., H. Becker, E. Ebmeyer, and V. Korzun. 2008. Occurrence of three dwarfing Rht genes in German winter wheat varieties. Cer. Res. Commun. 36:553–560.

Korzun, V., M.S. Röder, M.W. Ganal, A.J. Worland, and C.N. Law. 1998. Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticium aestivum* L.). Theor. Appl. Genet. 96:1104–1109.

Kosová, K., J. Chrpová, and V. Šíp. 2009. Cereal resistance to *Fusarium* head blight and possibilities of its improvement through breeding. Czech J. Genet. Plant Breeding 45:87–105.

Lehman, J.S. and G. Shaner. 1997. Selection of populations of *Puccinia recondita* f.sp. *tritici* for shortened latent period on a partially resistant wheat cultivar. Phytopathology 87:170–176.

Lemmens, M., U. Scholz, F. Berthiller, C. Dall'Asta, A. Koutnik, R. Schuhmacher, G. Adam, H. Buerstmayr, Á. Mesterházy, R. Krska, and P. Ruckenbauer. 2005. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for *Fusarium* head blight resistance in wheat. Mol. Plant Microbe Interact. 18:1318–1324.

Leonard, K.J. 1969. Selection in heterogeneous populations of *Puccinia graminis* f.sp. *avenae*. Phytopathology 67:1273–1279.

Leonard, K.J., R.P. Thakur, and S. Leath. 1988. Incidence of *Bipolaris* and *Exserohilum* species in corn leaves in North Carolina. Plant Disease 72:1034–1038.

Leonard, K.J. and W.R. Bushnell. 2003. *Fusarium* head blight of wheat and barley. St Paul, MN, US, APS Press.

Leslie, J.F. and K.K. Klein. 1996. Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics 144:557–567.

Li, X.-P., S.-Q. Lan, Y.-P. Liu, M.D. Gale, and A.J. Worland. 2006. Effects of different *Rht-B1b, Rht-D1b* and *Rht-B1c* dwarfing genes on agronomic characteristics in wheat. Cereal Res. Commun. 34:919–924.

Liu, W.Z., W. Langseth, H. Skinnes, O.N. Elen, and L. Sundheim. 1997. Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. Eur. J. Plant Pathol. 103:589–595.

Liu, S., X. Zhang, M.O. Pumphrey, R.W. Stack, B.S. Gill, and J.A. Anderson. 2006. Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to *Fusarium* head blight in wheat. Funct. Integr. Genomics 6:83–89.

Liu, S., M.O. Pumphrey, B.S. Gill, H.N. Trick, J.X. Zhang, J. Dolezel, B. Chalhoub, J.A. Anderson. 2008. Toward positional cloning of *Fhb1*, a major QTL for *Fusarium* head blight resistance in wheat. Cereal Res. Commun. 36:195–202.

Löffler, M., C.C. Schön, and T. Miedaner. 2009. Revealing the genetic architecture of FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-analysis. Mol. Breed. 23:473–488.

Maier, F.J., T. Miedaner, B. Hadeler, A. Felk, S. Salomon, M. Lemmens, H. Kassner, and W. Schäfer. 2006. Involvement of trichothecenes in fusarioses of wheat, barley and maize evaluated by gene disruption of the trichodiene synthase (*Tri5*) gene in three field isolates of different chemotype and virulence. Mol. Plant Pathol. 7:449–461.

Maiorano, A., M. Blandino, A. Reyneri, F. Vanara. 2008. Effects of maize residues on the *Fusarium spp.* infection and deoxynivalenol (DON) contamination of wheat grain. Crop Protection 27:182–188.

Maldonaldo-Ramirez, S.L., D.G. Schmale III, E.J. Shields, and G.C. Bergstrom. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role oof long distance transport in regional epidemics of *Fusarium* head blight. Agric. Forest Meteorol. 132:20–27.

Markell, S.G. and L.J. Francl. 2003. *Fusarium* head blight inoculum: Species prevalence and *Gibberella zeae* spore type. Plant Dis 87:814–820.

Mathews, K.L., S.C. Chapman, R. Trethowan, R.P. Singh, J. Crossa, W. Pfeiffer, M. van Ginkel, and I. DeLacy. 2006. Global adaption of spring bread and durum wheat lines near-isogenic for major reduced height genes. Crop. Sci. 46:603–613.

McDonald, B.A. and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349–379.

McIntosh, R.A. and G.N. Brown. 1997. Anticipatory breeding for resistance to rust diseases in wheat. Annu. Rev. Phytopathol. 35:311–26.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, D.J. Somers, R. Appels, and K.M. Devos. 2008. Catalogue of Gene Symbols for Wheat. Proceedings 11th International Wheat Genetics Symposium, Brisbane, Qld., Australia, 24–28.

McMullen, M., R. Jones, and D. Gallenberg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease 81:1340–1348.

Mesterházy, Á. 1984. A laboratory method to predict pathogenicity of *Fusarium graminearum* in field and resistance of wheat to scab. Acta Phytopathol. Acad. Sci. Hung. 19:205–218.

Mesterházy, Á. 1995. Types and components of resistance to *Fusarium* head blight of wheat. Plant Breeding 114:377–386.

Mesterházy, Á., T. Bartok, C.G. Mirocha, and R. Komoroczy. 1999. Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breeding 118:97–110.

Mesterházy, Á. 2002. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to *Fusarium* head blight. Eur. J. Plant Pathol. 108:675–684.

Miedaner, T. and A.G. Schilling. 1996. Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *Fusarium culmorum* tested on young plants of winter rye. Eur. J. of Plant Path. 102:823–830.

Miedaner, T. 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding 116:201–220.

Miedaner. T., C. Reinbrecht, U. Lauber, M. Schollenberger, and H.H. Geiger. 2001a. Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale, and wheat. Plant Breeding 120:97–105.

Miedaner, T., A.G. Schilling, and H.H. Geiger. 2001b. Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. J. Phytopathology 149:641–648.

Miedaner, T., A.G. Schilling, H.H. Geiger. 2004. Competition effects among isolates of *Fusarium culmorum* differing in aggressiveness and mycotoxin production on heads of winter rye. Eur. J. Plant Pathol. 110:63–70.

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

Miedaner, T. and H.-H. Voss. 2008. Effect of dwarfing *Rht* genes on *Fusarium* head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Science 48:2115–2122.

Miedaner, T., C.J.R. Cumagun, and S. Chakraborty. 2008. Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum.* J. Phytopathology 156:129–139.

Montarry, J., R. Corbiere, and D. Andrivon. 2007. Is there a trade-off between aggressiveness and overwinter survival in Phytophthora infestans? Functional Ecology 21:603–610.

Muangprom, A., S.G. Thomas, T. Sun, T. C. Osborn. 2005. A novel dwarfing mutation in a Green Revolution Gene from *Brassica rapa*. Plant Physiology 137:931–938.

Nicholson, P. 2009. *Fusarium* and *Fusarium*–cereal interactions. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester.

O'Donnell, K., H.C. Kistler, B.K. Tacke, and H.H. Casper. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Nat. Acad. Sci. 97:7905–7910.

Paillard, S., T. Schnurbusch, R. Tiwari, M. Messmer, M. Winzeler, B. Keller, and G. Schachermayr. 2004. QTL analysis of resistance to *Fusarium* head blight in Swiss winter wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:323–332.

Pariaud, B., V. Ravigné, F. Halkett, H. Goyeau, J. Carlier, and C. Lannou. 2009. Aggressiveness and its role in the adaptation of plant pathogens. Plant Pathology 58:409–424.

Parry D.W., P. Jenkinson, and L. McLeod. 1995. *Fusarium* ear blight (scab) in small grains cereals: a review. Plant Pathology 44: 207–238.

Peng, J., D.E. Richards, N.M. Hartley, G.P. Murphy, K.M. Devos, J.E. Flintham, J. Beales, L.J. Fish, A.J. Worland, F. Pelica, D. Sudhakar, P. Christou, J.W. Snape, M.D. Gale, and N.P. Harberg. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261.

Pestsova, E.G., V. Korzun, and A. Börner. 2008. Validation and utilisation of *Rht* dwarfing gene specific markers. Cer. Res. Commun. 36:235–246.

Phalip, V., F. Delande, C. Carapito, F. Goubet, D. Hatsch, E. Leize-Wagner, P. Dupree, A. Van Dorsselaer, and J-M. Jetsch. 2005. Diversity of the exoproteome of *Fusarium graminearum* grown on plant cell wall. Curr. Genet. 48:366–379.

Qi, L.L., M.O. Pumphrey, B. Friebe, P.D. Chen, and B.S. Gill. 2008. Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to *Fusarium* head blight disease of wheat. Theor. Appl. Genet. 117:1155–1166.

Qu, B., H.P. Li, J.B. Zhang, Y.B. Xu, T. Huang, A.B. Wu, C.S. Zhao, J. Carter, P. Nicholson, and Y.C. Liao. 2008. Geographic distribution and genetic diversity of *Fusarium graminearum* and *F. asiaticum* on wheat spikes throughout China. Plant Pathology 57, 15–24.

Schmale, III D.G., J.F. Leslie, K.A. Zeller, A.A. Saleh, E.J. Shields, and G.C. Bergstrom. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021–1026.

Schmale, III D.G., D.A. Shah, G.C. Bergstrom. 2005. Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. Phytopathology 95:472–479.

Schmolke, M., G. Zimmermann, H. Buerstmayr, G. Schweizer, T. Miedaner, V. Korzun, E. Ebmeyer, and L. Hartl. 2005. Molecular mapping of *Fusarium* head blight resistance in the winter wheat population Dream/Lynx. Theor. Appl. Genet. 111:747–756.

Schnerr, H., R.F. Vogel, and L. Niessen. 2002. Correlation between DNA of trichothecene-producing *Fusarium* species and deoxynivalenol concentrations in wheat-samples. Lett. Appl. Microbiol. 35:121–125.

Schroeder, H.W. and J.J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:813–838

Shi, J.R., D.H. Xu, H.Y. Yang, Q.X. Lu, and T. Ban. 2008. DNA marker analysis for pyramided of *Fusarium* head blight (FHB) resistance QTLs from different germplasm. Genetica 133:77–84**.**

Simón, M.R., A.J. Worland, and P.C. Struik. 2004. Influence of plant height and heading date on the expression of the resistance to *Septoria tritici* blotch in near isogenic lines of wheat. Crop Sci. 44:2078–2085.

Snijders, C.H.A. 1990. Genetic variation for resistance to *Fusarium* head blight in bread wheat. Euphytica 50:171–179.

Snijders, C.H.A. and F.A. van Eeuwijk. 1991. Genotype × strain interactions for resistance to *Fusarium* head blight caused by *Fusarium culmorum* in winter wheat. Theor. Appl. Genet. 81:239–244.

Somers, D.J., G. Fedak, and M. Savard. 2003. Molecular mapping of novel genes controlling *Fusarium* head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555–564.

Srinivasachary, N. Gosman, A. Steed, J. Simmonds, M. Leverington-Waite, Y. Wang, J. Snape, and P. Nicholson. 2008. Susceptibility to *Fusarium* head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. Theor. Appl. Genet. 116:1145–1153

Srinivasachary, N., A. Gosman, T. Steed, W. Hollins, R. Bayles, P. Jennings, and P. Nicholson. 2009. Semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to *Fusarium* head blight. Theor. Appl. Genet. 118:695–702.

Sun, T. and F. Gubler. 2004. Molecular mechanism of Gibberellin signaling in plants. Annu. Rev. Plant Biol. 55:197–223.

Swaminathan, M.S. 2006. An Evergreen Revolution. Crop Science 46:2293–2303.

Van Eeuwijk, F.A., A. Mesterházy, C.I. Kling, P. Ruckenbauer, L. Saur, H. Buerstmayr, M. Lemmens, L.C.P. Keizer, N. Maurin, and C.H.A. Snijders. 1995. Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum, F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theor. Appl. Genet. 90:221–228.

Van Egmond, H.P., C.R. Schothorst, and M.A. Jonker. 2007. Regulations relating to mycotoxins in food - Perspectives in a global and European context. Anal. Bioanal. Chem. 389:147–157.

Villaréal, L.M.M.A., and C. Lannou. 2000. Selection for increased spore efficacy by host genetic background in a wheat powdery mildew population. Phytopathology 90:1300–1306.

Voss, H.-H., J. Holzapfel, L. Hartl, V. Korzun, F. Rabenstein, E. Ebmeyer, H. Coester, H. Kempf, and T. Miedaner. 2008. Effect of the *Rht-D1* dwarfing locus on *Fusarium* head blight rating in three segregating populations of winter wheat. Plant Breeding 127:333–339.

Voss, H.-H., R.L. Bowden, J.F. Leslie, and T. Miedaner. 2010. Segregation of heightened aggressiveness to wheat in two *Fusarium graminearum* crosses. Phytopathology *in review*.

Waalwijk, C., P. Kastelein, I. de Vries, Z. Kerenyi, T. van der Lee, T. Hesselink, J. Kohl, and G. Kema. 2003. Major changes in *Fusarium spp.* in wheat in the Netherlands. Eur. J. Plant Pathol. 109:743–754.

Ward, T.J., M.C. Randall, A.P. Rooney, K. O'Donnell, D. Gaba, S. Patrick, D.E. Starkey, J. Gilbert, D.M. Geiser, and T.W. Nowicki. 2008. An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet. and Biol. 45:473–484.

Wilde, F., V. Korzun, E. Ebmeyer, H.H. Geiger, and T. Miedaner. 2007. Comparison of phenotypic and marker-based selection for *Fusarium* head blight resistance and DON content in spring wheat. Molecular Breeding 19:357–370.

Wilde, F., C.C. Schön, V. Korzun, E. Ebmeyer, M. Schmolke, L. Hartl, and T. Miedaner. 2008. Marker-based introduction of three quantitative-trait loci conferring resistance to *Fusarium* head blight into an independent elite winter wheat breeding population. Theor. Appl. Genet. 117:29–35.

Windels, C.E. 2000. Economic and social impacts of *Fusarium* head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology 90: 17–21.

Wolfe, M.S. and J.M. McDermott. 1994. Population genetics of plant pathogen interactions: The example of the *Erysiphe graminis-Hordeum vulgare* pathosystem. Annu. Rev. Phytopathol. 32:89–113.

Worland, A.J. and C.N. Law. 1985. An effect of temperature on the fertility of wheats containing the dwarfing genes *Rht1, Rht2* and *Rht3*. Annual Report, Plant Breeding Institute, Cambridge, UK, 69–71.

Worland, A.J. 1986. Gibberellic acid insensitive dwarfing genes in Southern European wheats. Euphytica 35:857–866.

Worland, A.J. and S. Petrovic. 1988. The gibberellic acid insensitive dwarfing gene from the wheat variety Saitama 27. Euphytica 38:55–63.

Worland, A.J., V. Korzun, M.S. Röder, M.W. Ganal, and C.N. Law. 1998a. Genetic analysis of the dwarfing gene *Rht8* in wheat: Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor. Appl. Genet. 96:1110–1120.

Worland, A.J., A. Börner, V. Korzun, W.M. Li, S. Petrovíc, and E.J. Sayers. 1998b. The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica 100:385–394.

Worland, A.J., E.J. Sayers, and V. Korzun. 2001. Allelic variation at the dwarfing gene *Rht8* locus and its significance in international breeding programmes. Euphytica 119:155–159.

Xu, X.M., D.W. Parry, P. Nicholson, M.A. Thomsett, D. Simpson, S.G. Edwards, B.M. Cooke, F.M. Doohan, J.M. Brennan, A. Moretti, G. Tocco, G. Mulè, L. Hornok, G. Giczey, and J. Tatnell. 2005. Predominance and association of pathogenic fungi causing *Fusarium* ear blight in wheat in four European countries. Eur. J. Plant Pathol. 112:143–154.

Zeller, K.A., R.L. Bowden, and J.F. Leslie. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology 93:874–880.

Zeller, K.A., R.L. Bowden, and J.F. Leslie. 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. Molecular Ecology 13:563–571.

Zhan, J., C.C. Mundt, M.E. Hoffer, and B.A. McDonald. 2002. Local adaptation and effect of host genotype on the rate of pathogen evolution: An experimental test in a plant pathosystem. J. Evol. Biol. 15:634–647.

8. Summary

Fusarium head blight (FHB), or scab, is one of the most devastating fungal diseases affecting small-grain cereals and maize, causing severe yield losses and contamination of grain with mycotoxins such as deoxynivalenol (DON) worldwide. *Fusarium graminearum* (teleomorph *Gibberella zeae*) and *Fusarium culmorum* are the most prevalent *Fusarium* species in wheat production in Central and Northern Europe. Breeding for increased resistance to FHB in wheat is considered the most effective strategy for large scale disease management and mycotoxin reduction as agronomic practices and fungicide application are often insufficient to effectively counteract or prevent FHB epidemics. Height reducing *Rht* genes are extensively used in wheat breeding programmes worldwide in order to improve lodging resistance and yield potential, with *Rht-D1b* being the most important *Rht* allele in Northern Europe. However, their individual effects on FHB resistance are yet unclear. Due to the incremental approach to increase host resistance the question arises whether the *Fusarium* pathogen has the capability to adapt by increased aggressiveness, defined as the quantity of disease induced by a pathogenic isolate on a susceptible host, and/or increased mycotoxin production.

Therefore, the objectives of the present study were to investigate the effects on FHB resistance of *Rht-D1b* and additional *Rht* alleles, the segregation variance for FHB resistance and identification of FHB resistance QTL (quantitative trait loci) in subsequent mapping analyses in three crossing populations segregating for the semi-dwarfing *Rht-D1b* allele and two sets of isogenic wheat lines. Regarding the pathogen, the study aims to determine the segregation variance in two *F. graminearum* crosses of highly aggressive parental isolates and to examine the stability of host FHB resistance, pathogen aggressiveness and the complex host-pathogen-environment interactions in a factorial field trial. All experiments were conducted on the basis of multienvironmental field trials including artificial inoculation of spores. FHB severity was repeatedly visually rated as the percentage of infected spikelets per plot. The DON and fungal mycelium content, measured as exoantigen (ExAg) absorbance, were analysed with two enzyme-linked immunosorbent assays (ELISA).

The presence of *Rht-D1b* resulted in 7-18% reduction in plant height, but considerably increased FHB severity by 22-53% within the 190, 216 and 103 progenies from the European elite winter wheat crosses Apache × Biscay, Romanus × Pirat and History × Rubens, respectively. In a subset of progenies selected for similar plant height, *Rht-D1b* still resulted in increased FHB severity of 7-27%. In the same subset the DON content was increased by 25-46% and fungal mycelium content rose by 9-55%, although only significant for the

Romanus × Pirat population. When divided into subpopulations carrying either *Rht-D1a* or *Rht-D1b*, significant genetic variation for the trait FHB resistance remained in both subpopulations of all three progeny.

In the following QTL mapping analyses the QTL with the strongest additive effects was located at the *Rht-D1* locus on chromosome arm 4DS and accordingly coincided with a major QTL for plant height in all three wheat populations. When combined with a second major QTL located on chromosome 1BL, both QTL explained a total phenotypic variance ranging from 45 to 79% in the different population × environment combinations. On total, a high number of 8 to 14 minor QTL for FHB reaction that were found in the three populations emphasised the quantitative inheritance of FHB resistance in European winter wheat. The detected QTL mostly showed significant QTL-by-environment interactions and often coincided with QTL for plant height. By means of isogenic lines in the genetic background of the variety Mercia, *Rht-D1b* and *Rht-B1d* significantly increased mean FHB severity by 52 and 35%, respectively, compared to the wild-type (*rht*). *Rht-B1b* and *Rht8c* led to non-significant increases in mean FHB severity of 19%. Among the Maris Huntsman data set, the *Rht* alleles increased mean FHB severity by 22 up to 83%, but only the very short lines carrying *Rht-B1c* or *Rht-B1b*+*Rht-D1b* showed significance. Following single floret inoculation the *Rht* effects on FHB resistance to fungal spread within the spike (type II) were less pronounced. Only *Rht-B1b* consistently increased fungal spread up to 13% whereas *Rht8c* led to a significant decrease of 17% in comparison to the wild-type.

The analyses of 120 progenies of the crosses from each of the highly aggressive parental *F. graminearum* isolates FG07 × FG153 and FG3211 × FG96 revealed significant genetic variation for aggressiveness, DON and fungal mycelium production following sexual recombination. This variation resulted in stable transgressive segregants towards increased aggressiveness in one of the two progeny. The factorial field trial, including eleven *F. graminearum* and *F. culmorum* isolates varying in aggressiveness and seven European elite winter wheat varieties, varying in their FHB resistance level, displayed no significant wheat variety × isolate interaction. Nevertheless, isolates possessing increased aggressiveness significantly increased FHB severity and DON production at a progressive rate on varieties with reduced FHB resistance.

In conclusion, the analysed *Rht* alleles led to differently pronounced negative effects on FHB resistance that strongly depended on the genetic background. However, significant genetic variation for FHB resistance exists for selection and, thus, to largely counteract these effects by accumulating major and minor FHB resistance QTL. Significant genetic variation for

aggressiveness among *F. graminearum* and the capability to increase its level of aggressiveness beyond yet known levels simply by sexual recombination may lead to long term erosion of FHB resistance when single exotic major resistance genes such as *Fhb1*, *Fhb2* or *Fhb3* are deployed on a large scale. The rate at which increased aggressiveness develops will depend on the selection intensity and whether it is of constant, episodic or balanced nature. Consequently, the selection pressure imposed on the pathogen should be minimized by creating and maintaining a broad genetic base of FHB resistance that relies on more than one genetically unrelated resistance source by combining phenotypic and marker-assisted selection to achieve a sustainably improved FHB resistance in wheat breeding.

9. Zusammenfassung

Ährenfusariosen zählen aufgrund hoher Ertrags- und Qualitätsverluste sowie der Kontamination des Erntegutes mit Mykotoxinen, vor allem Deoxynivalenol (DON), zu den bedeutendsten Pilzkrankheiten in Getreide und Mais weltweit. *Fusarium graminearum* (teleomorph *Gibberella zeae*) und *Fusarium culmorum* sind die am häufigsten vorkommenden *Fusarium*-Arten in Zentral- und Mittel-Europa. Zur umfassenden Krankheitskontrolle und Reduktion der Mykotoxinbelastung stellt die Resistenzzüchtung in Weizen die effektivste Methode dar, da agronomische Verfahren und Fungizid-Applikation häufig nicht ausreichend wirksam sind um *Fusarium*-Epidemien entgegenzuwirken bzw. zu verhindern. Zur Erhöhung der Halmstabilität und des Ertragspotentials werden weltweit in Weizenzuchtprogrammen Verzwergungsgene, die sogenannten *Rht*-Gene verwendet, wobei das Verzwergungsallel *Rht-D1b* das bedeutendste *Rht*-Allel im nordeuropäischen Raum ist. Jedoch sind ihre individuellen Auswirkungen auf die *Fusarium*-Resistenz bisher unbekannt.

Aufgrund zunehmender Bestrebungen die Wirts-Resistenz gegenüber Ährenfusariosen zu erhöhen, gewinnt die Frage an Bedeutung, ob das *Fusarium*-Pathogen die Fähigkeit zu Anpassungsreaktionen in Form von erhöhter Aggressivität und/oder erhöhter Mykotoxinproduktion besitzt. Die Aggressivität ist definiert als Quantität der Krankheitsausprägung hervorgerufen durch ein pathogenes Isolat auf einem anfälligen Wirt. Zielsetzung dieser Arbeit war es deshalb die Auswirkungen von *Rht-D1b* und weiterer *Rht*-Allele auf die Wirtsresistenz sowie die Aufspaltungsvarianz für *Fusarium*-Resistenz anhand von drei für *Rht-D1b*-spaltenden Populationen und zwei Gruppen isogener Linien zu untersuchen und in einer anschließenden Kartierungsstudie Resistenz-Loci zu identifizieren. Auf Seiten des Pathogens wurde die Aufspaltungsvarianz für Aggressivität in zwei Nachkommenschaften hochaggressiver Eltern-Isolate bestimmt, sowie die Stabilität der Wirts-Resistenz, Pathogen-Aggressivität und der komplexen Wirt-Pathogen-Umwelt-Interaktionen in einem faktoriellen Inokulations-Versuch untersucht. Die Feldversuche wurden über mehrere Umwelten (Jahr × Ort-Kombinationen) angelegt. Der *Fusarium*-Befall wurde mehrfach visuell als prozentualer Anteil befallener Ährchen der gesamten Ährchen einer Parzelle nach künstlicher Inokulation bonitiert. Der Pilzmyzelgehalt, gemessen als *Fusarium*-Exoantigen (ExAg)-Absorption, sowie der DON-Gehalt wurden mittels ELISA (enzyme-linked immunosorbent assay) analysiert.

Innerhalb der 190, 216 bzw. 103 Nachkommen der Kreuzungen Apache × Biscay, Romanus × Pirat und History × Rubens reduzierte die Anwesenheit von *Rht-D1b* die Wuchshöhe um 7-

18% bei gleichzeitig deutlicher Erhöhung des *Fusarium*-Befalls von 22-53%. Bei der Betrachtung von selektierten Nachkommen mit vergleichbarer Wuchshöhe, führte *Rht-D1b* zu einer verbleibenden Erhöhung der *Fusarium*-Anfälligkeit von 7-27%, einer Zunahme des DON-Gehaltes von 25-46% sowie des Pilzmyzel-Gehaltes von 9-55%. Die Effekte waren jedoch nur in der Population Romanus × Pirat signifikant. Getrennt in *Rht-D1a*- bzw. *Rht-D1b*-tragende Subpopulationen war die genetische Varianz für *Fusarium*-Resistenz innerhalb beider Subpopulationen aller Nachkommenschaften signifikant. In der nachfolgenden QTL-Kartierung wurde der stärkste QTL (quantitative trait locus) für *Fusarium*-Resistenz in allen drei Populationen am *Rht-D1*-Lokus auf Chromosomen-Arm 4DS lokalisiert, entsprechend gekoppelt mit einem Major-QTL für Wuchshöhe. In Kombination mit einem zweiten Major-QTL auf Chromosom 1BL erklärten beide *Fusarium*-Resistenz-Loci 45-79% der phänotypischen Varianz in den jeweiligen Population × Umwelt-Kombinationen. Insgesamt bestätigt die größere Anzahl von 8 bis 14 detektierten Resistenz-Loci mit kleineren Effekten in den verschiedenen Populationen den quantitativen Charakter der *Fusarium*-Resistenz in Europäischem Winterweizen. Die detektierten Resistenz-Loci zeigten überwiegend signifikante Interaktionen mit den entsprechenden Umwelten und fielen oft mit Loci für Wuchshöhe zusammen. Auf Basis von isogenen Linien im genetischen Hintergrund der Weizensorte Mercia führten *Rht-D1b* und *Rht-B1b* zu einer signifikanten Erhöhung des *Fusarium*-Befalls von 52 bzw. 35% im Vergleich zum Wildtyp (*rht*). *Rht-B1b* und *Rht-8c* erhöhten den *Fusarium*-Befall um 19%, jedoch nicht signifikant. Im genetischen Hintergrund der Sorte Maris Huntsman konnte durch die *Rht*-Allele eine Erhöhung des *Fusarium*-Befalls von 22-83% beobachtet werden, jedoch waren nur die kürzesten Linien mit *Rht-B1c* bzw. *Rht-B1b+Rht-D1b* signifikant. Nach der Einzelähreninfektion waren die Effekte der *Rht*-Allele auf die Resistenz gegenüber der Ausbreitung des Pathogens in der Ähre (type II) deutlich geringer ausgeprägt. Einzig *Rht-B1b* führte zu einer verstärkten Ausbreitung des Pilzes in der Ähre um bis zu 13% im Vergleich zum Wildtyp (*rht*), während die Pilzausbreitung durch *Rht8c* signifikant um 17% verringert wurde.

Die jeweils 120 Nachkommen der Kreuzungen zweier hochaggressiver *F. graminearum*-Isolate (FG07 × FG153 und FG3211 × FG96) wiesen signifikante genetische Varianz für die Merkmale Aggressivität, DON-Gehalt und Pilzmyzel-Produktion auf. Die Aufspaltungsvarianz nach sexueller Rekombination resultierte in umweltstabilen transgressiven Nachkommen mit erhöhter Isolataggressivität in einer der beiden Populationen. Anhand der faktoriellen Inokulation von elf europäischen Elite Winterweizensorten mit variierenden Resistenzniveaus mit sieben unterschiedlich aggressiven *F. graminearum* und *F. culmorum*-

Isolaten zeigten sich keine signifikanten Weizensorte × Isolat-Interaktionen. Dennoch erhöhten die hochaggressiven Isolate den *Fusarium*-Befall und den DON-Gehalt in den Sorten mit geringerer *Fusarium*-Resistenz überproportional.

Zusammenfassend zeigten die untersuchten *Rht*-Allele unterschiedlich stark ausgeprägte negative Auswirkungen auf die *Fusarium*-Resistenz in starker Abhängigkeit vom genetischen Hintergrund. Ausreichende genetische Varianz für die *Fusarium*-Resistenz erlaubt eine zielgerichtete Selektion um die negativen Effekte der *Rht*-Allele durch Akkumulation von Resistenz-Loci mit größeren und kleineren Effekten weitestgehend zu kompensieren. Demgegenüber zeigen die hohe genetische Varianz für das Merkmal Aggressivität in Nachkommenschaften hochaggressiver Eltern-Isolate und die Fähigkeit des Pathogens das Aggressivitätsniveau mittels sexueller Rekombination graduell weiter zu erhöhen, dass langfristig eine Erosion der *Fusarium*-Resistenz stattfinden könnte, wenn nur wenige exotische Major-Resistenz-Gene wie *Fhb1*, *Fhb2* oder *Fhb3* großflächige Verwendung finden. Die Geschwindigkeit, mit der sich die Pathogenaggressivität steigern könnte, wird dabei entscheidend von der Selektionsintensität, ausgelöst durch verbesserte, oligogen basierte Wirtsresistenz, und der Art der Selektion (konstant, episodisch, oder umweltabhängig) abhängen. Folglich sollte zukünftig der auf das Pathogen ausgeübte Selektionsdruck minimiert werden, indem durch die Kombination von phänotypischer und markergestützter Selektion eine breite genetische Basis für die *Fusarium*-Resistenz in Weizen geschaffen und erhalten bleibt, welche auf mehreren Resistenzquellen beruht um eine dauerhaft verbesserte *Fusarium*-Resistenz zu erreichen.

10. Acknowledgments

I would like to express my highest gratitude and appreciation to…

- Prof. Dr. Thomas Miedaner for his advice, suggestions and support during my Ph.D. thesis. Sincere thanks for introducing me into the State Plant Breeding Institute and all our comprehensive and profound discussions far beyond the topics of plant breeding.
- Dr. Josef Holzapfel and our collaborators from the Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding for our project cooperation among friends.
- Prof. Dr. Chris-Carolin Schön and Prof. Dr. Ralf T. Vögele for serving as members of the examination committee.
- Dr. Erhard Ebmeyer, Dr. Viktor Korzun, Dr. Hubert Kempf, Hilmar Cöster, Dr. Frank Rabenstein, Dr. John F. Leslie, Dr. Robert L. Bowden, Uta Liesenberg, Meike Scholz, and all project partners for providing the wheat as well as the fungal populations and the comprehensive cooperation and technical assistance during the project.
- Bettina Müller, Prof. Dr. H. Friedrich Utz, Prof. Dr. H.P. Piepho, and Jens Möhring for their helpful insights and assistance regarding biostatistical analysis.
- Christiane von der Ohe for being the best possible office mate, proof-reader and unintended secretary during my thesis.
- Dr. Katinka Wilde and Corinna Wilde for bringing me back to Hohenheim after my internship and all expertise in questions of life-science.
- Dr. K. Christin Falke and Prof. Matthias Frisch for the inspiring discussions on breeding plans far beyond closing-time.
- Dr. Hans-Peter Maurer for his extensive assistance and dialog in all agricultural matters and accommodation during the final stage of my thesis.
- To all members of the rye working group - Bärbel, Gunda, Heike, Sigrid, Silke, Mark, Sylvia, Gabi, Marlene, Jochen, and Bianca for the strong company and all technical field and laboratory assistance.
- Prof. Dr. Chris-Carolin Schön, PD Dr. Jochen C. Reif, Dr. Volker Hahn, Dr. Christof I. Kling, Dr. Ulrich K. Posselt, Dr. Tobias Würschum, Lore Handt, G. Hartmann, M. Stierle, D. Wahnelt, U. Schrader, and all members of the State Plant Breeding Institute for their scientific and organisational assistance, not to forget the exhilarant coffee breaks.
- Katharina Alheit, Sankalb Bhosale, Christoph Bolduan, Christine Beuter, Maren Fautz, Dr. Christian Flachenegger, Christoph Grieder, Dr. Martin Heckenberger, Marlen Hübner, Delphine van Inghelandt, Martin Löffler, Dr. Friedrich Longin, Matthias Martin, Dr. Vilson Mirdita, Greta Mittweg, Bettina Müller, Dr. Elena Orsini, Peter Risser, Dr. Tobias Schrag, Jana Steinhoff, Dr. Benjamin Stich, Alexander Strigens, Firas Talas, and Thilo Wegenast for creating the best imaginable working environment, and all the analytical expertise, controversial discussions and professional advices during the midday walks.
- My family for always keeping me grounded and continuously encouraging and supporting me during my time as Ph.D. student in Hohenheim.
- The Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP) for the financial support of the project.

Curriculum vitae

Name	Hans-Henning Voß
Birth	5th July 1978 in Bad Gandersheim
Civil Status	unmarried
Citizenship	German

Professional employment

06/2008-09/2009	Head of wheat breeding and assistant in hybrid-rye breeding at Dieckmann Seeds, Nienstädt

University education

04/2005-04/2008	Ph.D. student at the State Plant Breeding Institute, University of Hohenheim, Advisor Prof. Dr. Miedaner Title: Inheritance of quantitative resistance and aggressiveness in the wheat/*Fusarium* pathosystem with emphasis on *Rht* dwarfing genes
10/1998-03/2004	Master of Science in Agriculture specialized in plant production, Georg-August-University Göttingen Master thesis at the Institute for Sugar Beet Research, Göttingen, Advisors Prof. Dr. Märländer, Dr. Mäck Title: Phenotypic characterization of chronological and organ-specific development of components of harmful nitrogen in different sugar beet genotypes

Stay abroad

06/2004-12/2004	Educational journey through Australia, New Zealand, USA and Canada
09/2001-02/2002	Exchange semester, University of Nottingham, School of Biosciences, Sutton Bonington Campus, England

Agricultural experiences

05/2004	Institute for Sugar Beet Research, Göttingen
08/2002-09/2002	State Plant Breeding Institute, working group: rye, University of Hohenheim
07/2000-10/2000	Agricultural farm of Fritz Engelke, Kaltenweide, Hannover
07/1999-10/1999	KWS SAAT AG, working groups: sugar beet, maize and rape seed, Einbeck

Basic military service

09/1997-06/1998	Yorck-Kaserne, Stadtoldendorf

School education

07/1997	Final secondary school examination
1990-1997	Roswitha-Secondary school in Bad Gandersheim
1984-1990	Primary school and Middle School in Bad Gandersheim