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**Molecular and genetic analyses of aggressiveness in *Fusarium*
graminearum populations and variation for *Fusarium* head blight
resistance in durum wheat**

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Abbreviations

15-ADON	15-acetyldeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BIR	Birkach
BLUE	Best linear unbiased estimates
BOL	Bohlingen
bp	Base pair
DON	Deoxynivalenol
ELISA	Enzyme linked immunosorbent assay
ENT	Entringen
FGDB	Fusarium graminearum genome database
FHB	Fusarium head blight
F_{st}	Fixation index
GCSAR	General commission for scientific agriculture
h^2	Heritability
HER	Herrenberg
HOH	Hohenheim
ICARDA	International center for agriculture research
K matrix	Kinship matrix
KEL	Kehl
LD	Linkage disequilibrium
LSD	Least significant difference
mg kg ⁻¹	Milligram per kilogram
NIV	Nivalenol
Nm	Gene flow
NUF	Nufringen
PCoA	Principle coordinate analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
p_G	Proportion of genotypic variance
PLN	Plieningen
QTL	Quantitative trait loci
QTN	Quantitative trait nucleotide
r^2	Squared value of the correlation coefficient
SCHICK	Schickelsheim
SNA	Synthetic nutrient agar
SNP	Single nucleotide polymorphic
SSR	Single sequence repeats
Ta	Annealing temperature
TUB	Tübingen
U.V.	Ultraviolet
WET1	Wetze (2006)
WET2	Wetze (2009)

1. General Introduction

1.1. Economic value of wheat

Bread wheat (*Triticum aestivum* L.) is the most important crop in the world (Table 1) due to the continuous increase of demand particularly for the bread and baking industry. Durum wheat (*Triticum turgidum* L. var. *durum*) is the only widely grown tetraploid species of wheat. The high protein content and gluten strength makes it ideal for premium pasta products, couscous and durum breads. Wheat adapts well in harsh environments and grows best at intermediate temperatures. Wheat originates from south-west Asia with some of the earliest remains of the crop found in Syria, Jordan, and Turkey dating back 10,000 years (Heun et al. 1997).

Table 1. Production, cultivated area, and productivity of bread and durum wheat compared to two other major crops, maize and rice (FAOSTAT 2011)

Crop	World production (MT) ^c	Area Harvested (Mha) ^d	Yield (Ton/ha)
Bread wheat ^a	682	225	3.02
Durum wheat ^b	29	14	-
Rice ^a	679	161	4.20
Maize ^a	817	160	5.12

^a Estimated from year 2009, ^b Estimated from year 2003, ^c Million tons, ^d Million hectares

1.2. Taxonomy, life cycle, and epidemiology of *Fusarium graminearum*

Fusarium head blight (FHB) is one of most destructive diseases of wheat, maize, and other small grain cereals. The high economic impact is caused by losses in quantity and quality, the latter due to contamination with mycotoxins including deoxynivalenol (DON). The European Union has established standard levels of maximum DON content that is allowed in nutritional products: 1.25 mg kg⁻¹ in unprocessed cereals except durum wheat, 1.75 mg kg⁻¹ in durum wheat, 0.75 mg kg⁻¹ in cereals' flour and pasta, and 0.50 mg kg⁻¹ in bread (Anonymous 2007). FHB is mainly caused by *Fusarium graminearum* and *F. culmorum* along with other species causing disease on a lesser scale. The genus *Fusarium* belongs to Kingdom: *Fungi*, Phylum: *Ascomycota*, Class: *Sordariomycetes*, Family: *Nectriaceae*. *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schw.) Petch] is a haploid homothallic fungus, and has both, a sexual and asexual life cycle. The asexual cycle starts during wheat flowering when macroconidial spores land on the wheat flowers and cause infection manifesting as water-soaked lesions in the beginning, which later turn to a yellowish-red color. Orange spots

formed on the infected spikelet's surface, called sporodochium, contain many macrospores. Besides the internal growth of mycelium in host tissues, dispersal of macrospores can also initiate secondary infection on the same plant or neighboring plants. The sexual life cycle starts from overwintered mycelium in the plant debris forming small bluish-black round bodies on the crop debris called perithecia. Perithecia (also called fruiting bodies) are fungal structures containing several asci each contain eight ascospores, each having up to three septa. The ascospores are released in spring which coincides with wheat flowering, thus, starting another life cycle (Agrios 2004).

Epidemics caused by FHB are influenced by several factors. The most apparent is the effect of high temperature and high wetness of plant tissues especially during anthesis (Osborne and Stein 2007). Other factors are also very important for epidemics such as the level of resistance in the host plant, and aggressiveness of the dominant *F. graminearum* population in a given location. Further, agricultural practices in a given area play an important role. Growing wheat on the maize stubble can increase the severity of FHB since perithecia are more frequently produced on maize debris than on wheat (Sutton 1982). Genetic variation is mirrored by the phenotypic variation, hence a high variation of aggressiveness through out symptom development and type and amount of secreted mycotoxins in the host plant is detected (Miedaner et al. 2000, Dejardins et al. 2004). Sufficient rainfall is needed for sporulation (ascospores and macroconidia) and for efficient spore dispersal (Xu 2003). Optimum temperature for mycelium growth is 25 °C, and for sporulation 32 °C (Osborne and Stein 2007). Moreover, low soil moisture (<30%) inhibits ascospores production, while high soil moisture (>80%) maximizes ascospore production (Xu 2003).

1.3. Fusarium head blight (FHB) resistance

Deployment of FHB-resistant cultivars is considered the most effective and cost efficient strategy to combat this disease (Miedaner 1997). Wheat breeding towards FHB resistance has become one of the major initiatives of wheat breeders. Combining high and, stable grain yields and good quality with resistance to pests and diseases including FHB in one cultivar is challenging (Buerstmayer et al. 2009). Wheat resistance to *F. graminearum* is a quantitative trait induced by several genes and affected by environment (Bai et al. 2000). Therefore, a quantitative trait loci (QTL) approach has been applied extensively using molecular markers. However, durum wheat sources with effective FHB resistance have not yet been found (Chen et al. 2007; Burrows et al. 2008). Buerstmayer et al. (2009) summarized the studies that

reported resistance in wheat: 46 different QTLs were identified for FHB resistance in bread wheat, only four QTL were detected in durum wheat and two in related species.

1.4. Population structure of *F. graminearum*

Population structure is the result of evolutionary forces acting on a population over time and space such as mutation, reproductive mode, migration, random drift and selection affecting the genetic variation of a population (McDonald and Linde 2002).

F. graminearum is a member of a complex group causing FHB disease. According to phylogenetic analysis of 16 kb (kilo base pairs) of unique DNA sequence data coding for different proteins, 14 cryptic species within the *F. graminearum* clade were identified (Yali-Mattila et al. 2009). *F. graminearum* sensu stricto (s.s.) is the dominant species in U.S.A. (Zeller et al. 2003). More recent studies reported highly divergent populations proving that *F. graminearum* s.s. in U.S.A. does not consist of a single homogeneous population (Gale et al. 2007). This is in contrast with the previous concept of a large single *F. graminearum* s.s. population existing in the U.S.A. (Zeller et al. 2003). Two chemotypes mainly dominate the U.S. *F. graminearum* s.s. populations, *i.e.*, 15-ADON and 3-ADON (Gale et al. 2007). Very recently, Gale et al. (2011) identified a new population of *F. graminearum* in south central Louisiana that predominantly contained the NIV chemotype (93.6%). Moreover, *F. asiaticum* was reported for the first time in the U.S.A. having a low genetic diversity that suggests its origin from abroad (Gale et al. 2011). Changes in the percentages of chemotypes over the time were reported from Canada. Although 15-ADON isolates are still predominant in Manitoba, the 3-ADON chemotype showed increasing percentage in southern Manitoba (Ward et al. 2008). A possible explanation for this variation is the introduction of the 3-ADON chemotype into a new area that may have shown greater fitness allowing it to dominate the population of *F. graminearum* in that area (Guo et al. 2008). This dynamic change in *F. graminearum* populations is possible because of the high genetic diversity in these populations reported from several regions in the world, *e.g.*, Canada, U.S.A., Germany, and Japan (Guo et al. 2008; Gale et al. 2011; Miedaner et al. 2008; Karugia et al. 2009, respectively).

1.5. Genetic basis of aggressiveness

Aggressiveness in *F. graminearum* denotes the quantity of disease induced by a pathogenic isolate on a susceptible host in this non-race specific pathosystem (Vanderplank 1968) and is measured quantitatively (Cumagun et al. 2004). Aggressiveness is usually evaluated by direct rating of the general epidemic effect on the plants (Cumagun and Miedaner 2003).

Additionally, several quantitative traits derived from the host-pathogen interaction called aggressiveness components, *i.e.*, infection efficiency, latent period, spore production rate, infectious period, and lesion size may be recorded (Pariaud et al. 2009).

Heredity of aggressiveness strongly depends on the genetics of the host-pathogen interaction (Pariaud et al. 2009). Several genes are involved in aggressiveness but the exact number of genes and their interaction are still to be determined in *F. graminearum*. Two QTLs for aggressiveness were identified on linkage group I using simple interval analysis (Cumagun et al. 2004a).

Few genes in *F. graminearum* are reported to be involved directly with aggressiveness. It has been demonstrated that mitogen-activated pathways plays an important role in the pathogenicity of many filamentous fungi (Mayorga and Gold 1999; Xu et al. 1998; Zheng et al. 2000). *MAP* kinase genes (including *Gpmk1*) regulate the production of cell wall degrading enzymes, *i.e.*, regulating the early induction of extracellular endoglucanase, xylanolytic, and proteolytic activities (Jenczmionka and Schäfer, 2005). The gene *RAS2* has an essential role in regulating pathogenicity, fungal growth, and sporulation, in addition to regulating and controlling aggressiveness of *F. graminearum* by regulating the *Gpmk1* (Bluhm et al. 2007).

1.6. Mycotoxins as aggressiveness component

Mycotoxins are fungal secreted substances, which can contaminate agricultural products. Trichothecenes are a major group of mycotoxins produced as secondary metabolites by certain species of the genera *Fusarium* (Bennett and Klich 2003). Trichothecenes are grouped into four types (A, B, C, and D). *F. graminearum* produces type B Trichothecenes, which include deoxynivalenol (DON), nivalenol (NIV), and its acetylated derivatives (Kimura et al. 2007). Isolates of *F. graminearum* produce three trichothecene chemotypes (Miller et al. 1991, Ward et al., 2002): (i) NIV chemotype producing nivalenol and acetylated nivalenol derivatives, (ii) 3-ADON chemotype producing deoxynivalenol and predominantly 3-acetyldeoxynivalenol, (iii) 15-ADON chemotype producing deoxynivalenol and predominantly 15-acetyldeoxynivalenol. DON is produced during infection and colonization irrespective whether it is needed for aggressiveness or not (Maier et al. 2006). Although DON biosynthesis is not the only factor controlling aggressiveness in *F. graminearum*, it is considered as an important agent for disease severity (Proctor et al. 1995).

In general, mycotoxin secretion is considered as an aggressiveness component. Cumagun and Miedaner (2003) found a positive correlation ($r = 0.69$, $P < 0.01$) between aggressiveness and DON content in a field experiment with 50 isolates of *F. graminearum*. Several genes are involved in the trichothecene (*TRI*) biosynthetic pathway (Kimura et al. 2003). Most of *TRI*

genes were found in a 25 kb *TRI5* cluster (*i.e.* *TRI3* to *TRI14*). DON is produced through several steps starting with the cyclization of farnesyl pyrophosphate transferring into trichodiene by means of trichodiene synthases (encoded by *TRI5* gene, Kimura et al, 2007) followed by several steps encoded by previously mentioned genes (Dyer et al. 2005).

The genes *TRI6* and *TRI10* regulate the expression of all TRI genes, both located within the core of *TRI5* cluster (Seong et al. 2009). Full expression of at least two TRI genes from the cluster (*TRI4* and *TRI5*) depend on *TRI6* and *TRI10* (Proctor et al. 1995b; Peplow et al. 2003). *TRII* encodes a cytochrome P450 monooxygenase that catalyzes hydroxylation of the C-8 position during trichothecene biosynthesis (Meek et al. 2003).

1.7. Association between phenotypic traits and nucleotide variation

Populations of *F. graminearum* like other natural populations, harbor a stunning diversity of phenotypic variation (Mackay et al. 2009) for morphology, physiology, aggressiveness or mycotoxin production. The phenotypic variation is typically due to underlying genetic complexity from multiple loci with allelic effects that are sensitive to the environment. Understanding the relationship between DNA sequence variation and variation in phenotypes for quantitative traits will result insights that are important for predicting adaptive evolution within the host-pathogen system. Association mapping using diverse genotypes or isolates is a promising and powerful approach could also that yield a useful insights in identifying the functional variation in both known and unknown genes associated with important traits in filamentous fungi (Yu and Buckler 2006, Hall et al. 2011) including *F. graminearum*.

The resolution of association mapping depends on the extent of linkage disequilibrium (LD), *i.e.* the non-random association of alleles present in a species (Wilson et al. 2004). Low LD was reported in *F. graminearum* estimated by different types of genetic makers such as VNTR, AFLP, RFLP (Karugia et al. 2009; Zeller et al. 2004; Gale et al. 2002, respectively). LD is species and population specific and consequently has to be determined before conducting an association mapping study. The breakdown of linkage disequilibrium (LD) across the genome of an organism play a major role in affecting the precision and accuracy of association mapping, and in turn affected by many genetic and non genetic factors including recombination, drift, and selection (Yan et al. 2009).

The published chromosomal sequence of *F. graminearum* facilitate the identification of the potential candidate genes responsible for the aggressiveness or DON production, which can result on identifying important SNPs markers useful to employ in a genome wide association studies.

2. Objectives of this study

The main objective of this work was to gain a specific understanding of molecular components in the wheat/Fusarium pathosystem, focusing on the analyses of *F. graminearum* populations including the genetic, molecular and chemotype structure and its reflection on FHB severity, and the amount of secreted trichothecenes. Specific objectives of this research were:

1. Screen Syrian landraces for resistance to Fusarium head blight under artificial infection in the field
2. Study the population structure of *F. graminearum* in Germany:
 - 2.1. Identify the dominant species
 - 2.2. Determine the dominant chemotype among *F. graminearum* populations
 - 2.3. Evaluate the genetic diversity within and between populations using molecular markers
 - 2.4. Determine the structuring pattern in *F. graminearum* populations
3. Study the variation of *F. graminearum* populations in individual naturally infected wheat fields:
 - 3.1. Evaluate the phenotypic variation of aggressiveness and DON content
 - 3.2. Compare the genetic variance partitioning of phenotypic traits in comparison with molecular variation partitioning
4. Association mapping between single nucleotide polymorphism (SNPs) in candidate genes and phenotypic variation of aggressiveness and DON production in *F. graminearum*:
 - 4.1. Evaluate linkage disequilibrium (LD) within and between candidate genes
 - 4.2. Identify possible associations between SNPs within candidate genes and aggressiveness
 - 4.3. Identify possible associations between SNPs and genes for trichothecene biosynthesis

3. Submitted Papers

3.1. Resistance to FHB in durum wheat

Sources of resistance to Fusarium head blight within Syrian durum wheat landraces

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Abstract

Fusarium head blight (FHB) is one of the most destructive diseases of wheat cultivation all over the world. FHB causes high economic losses for durum wheat because of the lack of resistance sources. Initially, sixty-eight Syrian landraces were tested in field conditions after inoculation to discriminate the most resistant and environmentally stable landraces. Further tests across a total of four environments revealed four landraces with a low mean FHB rating (19–25%) compared to common German durum varieties (56–60%) and an environmentally stable resistance. They are considered as promising resistance sources to FHB for introgression in the adapted durum wheat gene pool.

3.2. Population structure of *F. graminearum*

Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany

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European Journal of plant pathology 131: 39-48 (2011).

The original publication is available at <

<http://www.springerlink.com/content/g358j1806p31423n/>>

Abstract

Fusarium graminearum Schwabe (teleomorph: *Gibberella zeae*) is an important cereal pathogen worldwide. The fungus causes Fusarium head blight (FHB) on wheat and other cereals. Twelve small commercial wheat fields (size 1–3 hectares) were sampled in Germany for *Fusarium* populations at three spots per field with 10 heads each. PCR assays using generic primers confirmed 338 isolates as *F. graminearum* sensu stricto (s.s.) (64.9%) out of 521 *Fusarium* spp. that were further analyzed. Populations of *F. graminearum* s.s. in Germany contain three types of tricothecenes with a dominance of 15-acetyldeoxynivalenol chemotype (92%) followed by 3-acetyldeoxynivalenol chemotype (6.8%) and a few isolates of nivalenol chemotype (1.2%). All these isolates were genotyped using 19 microsatellite loci. The 12 populations showed a high genetic diversity within the small scale sampling areas resulting in 300 different haplotypes. Genetic diversity within populations (71.2%) was considerably higher than among populations (28.8%) as shown by analysis of molecular variance. Gene flow (Nm) between populations ranged from 0.76–3.16. Composition of haplotypes of one population followed over 2 years changed considerably. No correlation between genetic and geographical distance was found. In conclusion, populations of *F. graminearum* s.s. in Germany display a tremendous genetic variation on a local scale with a restricted diversity among populations.

3.3. Variation of aggressiveness and mycotoxin secretion of *F. graminearum*

Within-Field Variation of *Fusarium graminearum* Isolates for Aggressiveness and Deoxynivalenol Production in Wheat Head Blight

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Phytopathology (in press).

The original publication is available at < <http://apsjournals.apsnet.org> >

Abstract

Fusarium graminearum Schwabe (teleomorph: *Gibberella zeae*) is an important cereal pathogen worldwide. The fungus causes Fusarium head blight (FHB) on wheat and other cereals with a tremendous yield losses worldwide annually. Variation of aggressiveness of isolates from individual field populations in terms of FHB infection and deoxynivalenol (DON) concentration in the host are important population parameters reflecting parasitic ability. Our main objective was to estimate the variation of both traits within three populations of *F. graminearum* s.s., each consisting of 30 single-spore isolates collected from small wheat fields in Germany, and to compare it with 11 isolates of a collection (*F. graminearum* collection) from four countries. The same isolates were characterized using 19 single-sequence repeat markers. All isolates were spray inoculated on a moderately resistant spring wheat cultivar at two field locations over 2 years (i.e., in four environments). The genotypic proportion of phenotypic variance (σ^2_G) within populations was significant ($P < 0.01$) for both traits, and the $\sigma^2_G \times$ environment interaction was even more important for mean FHB severity. Ranges in mean FHB severity and DON concentration in the host were only slightly smaller for the field populations than for the *F. graminearum* collection. Both traits were significantly ($P < 0.05$) correlated within and across populations. A further partitioning of σ^2_G revealed 72% of σ^2_G within and 28% of σ^2_G across populations for both traits. Molecular variance of the three populations was similarly distributed (73.6% within versus 26.4% between populations). In view of this high within-field variation for traits of parasitic ability and selection of neutral molecular markers, multiple resistance genes of different origin should be employed in wheat breeding programs to obtain a long-term stable FHB resistance.

3.4. Association between SNPs in candidate genes and phenotypic traits

Association of single nucleotide polymorphic sites in candidate genes with aggressiveness and deoxynivalenol production in *Fusarium graminearum* causing wheat head blight

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BMC Genetics (in review)

Abstract

Background: *Fusarium graminearum* sensu stricto (s.s.) is an ubiquitous pathogen of cereals. The economic impact of Fusarium head blight (FHB) is characterized by crop losses and mycotoxin contamination. Our objective was to associate SNP diversity within candidate genes with phenotypic traits. A total of 77 *F. graminearum* s.s. isolates was tested for severity of fungal infection (=aggressiveness) and deoxynivalenol (DON) production in an inoculated field experiment at two locations in each of two years. For seven genes known to control fungal growth (*MetAP1*, *Erf2*) or DON production (*TRII*, *TRI5*, *TRI6*, *TRI10* and *TRI14*) single nucleotide polymorphic sites (SNPs) were determined and evaluated for the extent of linkage disequilibrium (LD). Associations of SNPs with both phenotypic traits were tested using linear mixed models.

Results: Decay of LD was in most instances fast. Two neighboring SNPs in *MetAP1* and one SNP in *Erf2* were significantly ($P < 0.05$) associated with aggressiveness explaining proportions of genotypic variance (p_G) of 25.6%, 0.5%, and 13.1%, respectively. One SNP in *TRII* was significantly associated with DON production ($p_G = 4.4$).

Conclusions: We argue that using the published sequence information of *Fusarium graminearum* as a template to amplify comparative sequence parts of candidate genes is an effective method to detect quantitative trait loci. Our findings underline the potential of candidate gene association mapping approaches to identify functional SNPs underlying aggressiveness and DON production for *F. graminearum* s.s. populations.

Background

Fusarium head blight (FHB) is a destructive disease to cereals including wheat and barley. *Fusarium graminearum* (teleomorph *Gibberella zeae*) is considered to be the main causal agent of this disease in addition to other species such as *F. culmorum* [1]. *F. graminearum* complex has been subdivided into several cryptic species [25], the main member in Germany is *F. graminearum* sensu stricto (s.s.) [32]. FHB disease leads to prematurely bleached spikes in infected plants [1] with considerable yield losses and contamination by mycotoxins such as deoxynivalenol (DON) [24]. DON is the most common food and feed contaminant in Europe and the maximum permissible level in unprocessed wheat used for food is 1.25 mg kg⁻¹ [11].

The quantitative ability of an isolate to cause disease on a susceptible host plant in a non-race specific pathosystem is defined as aggressiveness [35]. Aggressiveness is an important factor determining the potential ability of an isolate to cause yield losses. Large genetic variation of aggressiveness, type of mycotoxin, and DON production was found among isolates sampled in the same country or even the same field [23, 12, 45, 37, 33]. The causes of this tremendous variation in aggressiveness and DON production in *F. graminearum* are still unclear. To date, only one QTL (quantitative trait locus) study from a single cross was reported detecting at least two QTL for aggressiveness [6]. It was shown before, that a large amount of molecular variation was found within field populations of *F. graminearum* s.s. using SSR (simple sequence repeat) markers. Comparing 12 individual field populations each consisting of 30 isolates, Talas et al. [32] reported that 71% of the molecular variation was assigned within populations and 29% between populations. With the availability of the full genomic sequence of *F. graminearum* [8], it is now possible to deeper analyze this variation by a candidate gene approach. An array of candidate genes, including components of transcription, signal transduction, host-specific nutrition, host infection/colonization and trichothecene biosynthesis is available in the version 3.2 of the Pathogen-Host Interactions database [40]. The candidate genes we were aiming for are known to control DON

biosynthesis and/or aggressiveness (*TRII*, *TRI5*, *TRI6*, *TRII0*, *TRII4*) [17, 9] or are expected to have a link to aggressiveness but are yet uncharacterized for *Fusarium* spp. (*MetAPI*, *Erf2*) [5, 2]. Candidate gene association mapping is a sensitive tool if the mapping resolution is high enough [44]. The resolution of association mapping depends on the extent of linkage disequilibrium (LD), *i.e.* the non-random association of alleles present in a species [39]. Low LD was reported in *F. graminearum* estimated by different types of genetic makers such as VNTR, AFLP, RFLP [16, 45, 12], but no data on LD within and among genes is available. Because LD is species and population specific, it should consequently be determined before conducting an association mapping study.

The specific objectives of our study were to: (i) investigate the nucleotide diversity on gene level, (ii) investigate the extent of LD between single nucleotide polymorphic sites (SNPs) within and among candidate genes, (iii) identify SNPs of candidate genes *Erf2* and *MetAPI* for testing whether they affect the quantitative variation for aggressiveness, and (iv) evaluate associations of SNPs in *TRII* and other four genes of the *TRI5* cluster with variation in DON production.

Results

Phenotypic analysis

Phenotypic data were analyzed in detail in a companion study [33]. All 77 *F. graminearum* s.s. isolates produced symptoms of FHB disease in all environments. Briefly, genotypic proportion of phenotypic variance of the isolates was significant ($P < 0.01$) for both traits: Aggressiveness measured as FHB rating on scale from 0 to 100 and DON production measured as DON concentration in wheat kernels in mg kg^{-1} . Isolate \times environment interaction variance was significant ($P < 0.01$) only for mean FHB rating but not for DON production. Entry-mean heritability was moderate for mean FHB rating (0.55) and DON production (0.62).

Histograms based on best linear unbiased estimators (BLUEs) followed a normal distribution for both traits (Fig. 1). The mean of FHB infection among all isolates was 30.6% ranging from 18.4 to 38.9%. Average DON production was 12.6 mg kg⁻¹ ranging from 3.5 to 21.6 mg kg⁻¹. One isolate (WET24) produced a very low concentration of DON (0.3 mg kg⁻¹) and was found to be a nivalenol producer by chemotype-specific primers.

Analysis of population structure and diversity

Principal coordinate analysis (PCoA) based on modified Rogers' distances between all isolates did not show a distinct separation of the isolates sampled from different locations (Fig. 2A). Explained variance gradually decreased according to the first ten principal coordinates (Fig. 2B). The violin plot (Fig. 2C) had a continuous density of distribution over all ten principal coordinates without any division within the principal coordinates. Genetic similarity ranged from 0.057 to 1.0 with a mean value of 0.31 (Fig. 2D).

Estimation of nucleotide diversity and linkage disequilibrium

Percentage of polymorphic sites per total sequenced region of each gene (without singletons) varied from 0.9% (5/513) on *TRII0b* to 8.8% (65/734) on *MetAPI* indicating a high nucleotide diversity in most tested genes (Table 2). LD of SNPs within the gene *MetAPI* decayed rapidly within 200 bp of physical distance, *i.e.*, the robust locally fitted regression of r^2 values has a trend to decay from $r^2 = 0.35$ to $r^2 < 0.1$, whereas LD within the gene *Erf2* had r^2 values ranging from 0.8 to 0.2 and the regression of r^2 trends to decay already after 150 bp (Fig. 3). LD with r^2 values higher than 0.1 were detected between all allele combinations within the tested genes followed by a rapid decay negatively correlated with the physical distance in base pair (Fig. 4). In line with the rapid decay, LD between genes located on the same chromosome (*i.e.*, *TRII* and *MetAPI*) is low ($r^2 < 0.1$). Interestingly, 48%, 19%, and 45% of the SNP pairs between the genes *TRII0/MetAPI*, *MetAPI/TRI5* and *MetAPI/Erf2*, respectively, have higher values of r^2 than 0.1, although they are located on different chromosomes. Low r^2 values (< 0.1) were observed for SNPs of gene pairs *TRII0/Erf2* and

TRI5/Erf2, whereas r^2 values of 0.2 were detected between SNP pairs of *TRI10b* and *TRI5*, followed by a rapid decay of LD.

Association analysis for aggressiveness and mycotoxin

Two adjacent SNPs of the gene *MetAP1* were significantly associated ($P < 0.05$ using Bonferroni-Holm correction) [15] with mean FHB rating with an explained genotypic proportion of variance $p_G=25.6$ and 0.5, respectively (Table 3, Fig. 5A). Additionally, one SNP in the gene *Erf2* was significantly associated with this trait showing a p_G of 13.1% (Table 3, Fig. 5A). A single SNP significantly associated with DON production was identified in the *TRII* gene explaining 4.4 % of the genotypic variance of DON production (Table 3, Fig. 5B). All detected SNPs that were associated to the mentioned phenotypic traits were non-synonymously substituted (Table 3).

Discussion

Association mapping based on candidate genes is a promising tool for high-resolution mapping of genes contributing to quantitative traits [19]. Nevertheless, it has not yet applied to investigate the basis of quantitative variation in aggressiveness and/or DON production in *F. graminearum*. This is a totally different approach than using knock-out mutants [27], because we are aiming for the analysis of quantitative differences produced by single nucleotide changes of the respective genes in a set of 77 isolates.

Choice of candidate genes

The genes for deoxynivalenol (DON) biosynthesis reside primarily in a 25 kb cluster (*TRI5* cluster), *TRII* gene belong to a second smaller cluster that has a different chromosomal localization [17]. *TRII*, *TRI5*, *TRII4* have a direct effect on the production of DON or acetylated DON, *TRI6* and *TRII0* are regulating this pathway. *TRII4* export the deoxynivalenol outside the mycelia in addition to its major role in aggressiveness [9].

The function of *MetAP1* is still not described in *Fusarium* spp., a deletion, however, reduces growth in *Saccharomyces cerevisiae* [18, 5, 10]. In both, prokaryotes and eukaryotes the N-terminal methionine is often cleaved by methionine aminopeptidase encoded by the gene *MetAP1* [5]. *Erf2* gene is a component of the *RAS* protein subcellular localization pathway in yeast [2]. *RAS2* gene is known to affect the pathogenesis of *F. graminearum* by regulation of hyphal growth and expression of hydrolytic enzymes [4]. Failing of palmitoylation mainly controlled by the *Erf2* gene in *S. cerevisiae* reduces amount of RAS protein at the plasma membrane and lead to poor growth of yeast [2]. Both genes are not indispensable for fungal growth because their deletion does not prevent any growth at least in *S. cerevisiae*. And the 77 isolates analyzed in this study, all had a similar growth *in vitro*, produced enough spores for inoculation and were able to infect the host in the field. Significant, quantitative differences for aggressiveness and DON production, however, were found illustrating those genes with regulatory functions might be involved. Implementing

associations between new candidate genes and important phenotypic traits may provide a useful tool to rapidly check a possible contribution of these genes to QTL of DON production or aggressiveness in *F. graminearum* as shown previously in mammal research [19]. If an association is found, a more detailed analysis of the function of the respective genes should, of course, follow.

Molecular diversity of the 77 isolates

All isolates were checked by respective primers for their designation to *F. graminearum* s.s. Nucleotide diversity within candidate genes of this study ranged from 1 to 9 per 100 bp being high compared to 0 to 2 SNPs per 100 bp reported by Cuomo et al. [8]. This was not unexpected since Cuomo et al. [8] used just two isolates (PH-1 and GZ3639) while in this study 77 isolates were analyzed. Many genes in *F. graminearum* are localized in regions of high SNP density, *i.e.* highly variable regions, especially those expressed during plant infection. Moreover, it is known that the density of SNPs is biased in *F. graminearum* in a way that 50% of the SNPs are within 13% of the genome sequence, the highest SNP density was reported in some regions of chromosome II, where most of the *TRI* genes are located [8]. The high nucleotide diversity of these groups of genes suggests that the fungus has a great capacity for adaptability and genetic change during its interaction with the host plant [8]. Despite this, we found much more SNPs with allele frequencies >0.1 in the *MetAPI* and *Erf2* genes than in the *TRI* genes analyzed. This was not the case, when just the number of detected SNPs is regarded, indicating that nucleotide diversity may be similar, but more rare haplotypes occur in the *TRI* genes. Moreover, high SNPs density was detected in one or two large interstitial regions on chromosomes I, II, and IV [8].

Population structure and consequences for association mapping

Correcting for population and/or family structure is essential for association mapping to decrease the number of false positive QTL [28]. An appropriate statistical model should provide an excellent compromise between correcting for population stratification to decrease

the probability of detecting false positive SNP-trait associations but still retaining enough information within the SNPs for QTL detection [43]. Although some grouping according to the sampling location might be seen in the PCoA, the violin plots did not show any separated knots on the first ten principle coordinates (Fig. 2). Thus, no distinct subpopulations were identified and correction for familial relatedness between isolates should be sufficient. Therefore, trait-SNP association was investigated in detail with the K model, which incorporated estimates of kinship coefficients based on SSR data.

Extent of linkage disequilibrium and resolution of association mapping

Rapid decay of regression parameter r^2 in a short physical distance of 200 bp within two genes was presented in this study (Fig. 3), providing a high resolution of association mapping. Generally, low LD was reported in *F. graminearum* populations using selection-neutral markers (VNTR, AFLP, or RFLP) especially in the region that includes the *TRI5* gene cluster [45]. Moreover, the weak correlation between r^2 and physical distance in addition to the large proportion of unlinked SNP with significant LD, such as SNPs between gene pairs *TRI5/MetAP1*, *TRI10/MetAP1*, or *MetAP1/Erf2* (Fig. 4), suggest the presence of other forces generating LD between unlinked SNPs. The values of r^2 between most pairs of SNPs in *TRI5/TRI10b* are >0.1 , presenting these two genes in LD, that might refer to the controlling role of *TRI10* on *TRI5* gene cluster [27, 26, 29]. That *TRI10* and *TRI5* are located close to each other on the same chromosome play a minor role hence the detected LD decay is occurring within 200 bp. Selection acting on oligo- or polygenic traits such as aggressiveness and DON production might be responsible for this [30]; an alternative explanation would be the involvement in the same trait network with other physiologically important genes.

Association mapping of genes underlying aggressiveness and DON production

We identified three SNPs related to aggressiveness and one SNP related to DON production using the fairly conservative Bonferroni-Holm correction. Setting the threshold for detection of trait associations to an allele frequency of 0.1 might underestimate the number of

QTL detected but is due to the restricted population size. Association between SNPs of candidate gene *Erf2* and phenotypic data revealed a significant association to mean FHB rating, but not to DON content. This might be explained by the fact that the *Erf2* gene is involved in *RAS2* processing or trafficking that precedes palmitoylation of *RAS2* genes [2]. *RAS2* is known to regulate the aggressiveness through affecting fungal growth and regulating other pathogenicity genes, e.g., *Gmpk1*, which controls the induction of extracellular enzymes required for pathogenesis [4]. Further study is needed to understand how *Erf2* affects differences in aggressiveness of *F. graminearum* isolates.

Two SNPs in *MetAPI* gene significantly associated with aggressiveness were located adjacent to each other forming a collinearity pattern. The role of *MetAPI* was reported in *S. cerevisiae* as reducing cell growth by N-terminal protein modification. A similar role might be expected for *MetAPI* in *F. graminearum*, hence significant positive correlations between aggressiveness and fungal biomass ($r = 0.7$, $P=0.01$), and fungal biomass and DON content ($r = 0.8$, $P=0.01$) were reported among 50 *F. graminearum* isolates [7]. Nevertheless, analyzed isolates were sampled from visually diseased spikelets, thus all were able to infect wheat ears and induce symptoms, non-aggressive isolates were not included in our *F. graminearum* population sample.

A single SNP detected in *TRII* was associated with DON production in *F. graminearum*. *TRII* encodes a cytochrome P450 monooxygenase that catalyzes hydroxylation of C-8 position during trichothecene biosynthesis [21]. This confirmation change obviously does not affect aggressiveness, because the respective SNP is just associated with DON content but not with aggressiveness.

Conclusions

This is the first candidate gene association mapping study provided insights on some genes involved in aggressiveness and DON concentration of *F. graminearum* s.s. The described

associations should be validated using a larger number of isolates and different environments. The validated genes are an important starting point for further functional analyses.

Materials

Fungal material

Ears of winter wheat (*Triticum aestivum* L.) showing symptoms of FHB were sampled from three commercial fields in Germany to establish a fungal population of *F. graminearum* s.s. : Stuttgart-Hohenheim in southwest Germany in 2008, Wetze and Schickelsheim in Lower Saxony in 2006 and 2007, respectively. From each infected head, one isolate was recovered, transferred onto a fresh SNA plate and placed under permanent UV light for induction of sporulation as described recently [32]. From each isolate, one single spore was picked out under the microscope and transferred onto a fresh SNA plate to establish a single-spore culture. The single-spore isolates were checked morphologically and analyzed for their species specificity and chemotype by different PCR-based assays as described recently in detail [32]. In total, 77 single-spore isolates of *F. graminearum* s.s. (former lineage 7) were inoculated on the moderately resistant German spring wheat cultivar Taifun (KWS LOCHOW GMBH, Bergen, Germany) for analyzing aggressiveness and DON production in the field.

Design of field studies

Field experiments were planted at each of two locations in 2009 and 2010: Hohenheim (HOH, longitude 9° 12' 58'', latitude 48° 42' 50'', altitude 400 m) and Oberer Lindenhof (OLI, longitude 9° 18' 12'', latitude 48° 28' 26'', altitude 700 m), resulting in four environments (location×year combinations). Mean annual temperatures at HOH and OLI were 10.1°C and 9.0°C, respectively, mean annual precipitations were 644 mm and 723 mm, respectively, across 2009 and 2010. Plants were grown in two-rowed micro-plots of 1 m length and 0.42 m width. Plots were arranged in a chessboard-like design, *i.e.*, each plot with a wheat entry was bordered by four plots of similar size that were planted with a long-strawed spring triticale cultivar Nilex (NORDSAAT GmbH, Halberstadt, Germany) to reduce inter-

plot interference caused by drifting of inoculum during spraying or secondary distribution of spores. The experiment was arranged in a split-plot design with two replications. The main plot factor was the *F. graminearum* s.s. population, the subplot factor the isolate. Both factors were assigned to a randomized complete block design. Eight plots per replication included in the subplot factor were not inoculated to estimate the degree of natural infection.

Inoculum was sprayed with a concentration of 2×10^5 spores ml⁻¹ onto the wheat heads of each plot. Inoculation was performed at full flowering time of the wheat cultivar to ensure maximum susceptibility of wheat to *F. graminearum*. Fusarium head blight (FHB) aggressiveness was rated visually four times as the percentage of infected spikelets per plot (0-100%). This rating included both the number of infected spikes per plot and the number of infected spikelets per spike. To compare FHB reactions, the arithmetic means of four ratings were used and assigned as mean FHB rating throughout the paper. All plots were harvested by hand, threshed, and the grain analyzed to quantify the amount of DON by a commercially available immunotest (R-biopharm AG, Darmstadt, Germany) as previously described in detail [33]. This test cannot differentiate between DON and 3-ADON, so the results include both mycotoxins. Generally, however, the amount of 3-ADON is only 2-3% of total DON content [36]. In each of the four environments, the natural infection rate was very low ranging from 0 to 3% FHB rating and from 0 to 0.34 mg kg⁻¹ DON concentration. Prediction of NIV chemotype was done using the primers N11, 15D11, 3D11, and 11R in a multiplex polymerase chain reaction (PCR) as designed by Zhang et al. [46].

Detection of population structure and gene sequencing

In order to analyze the population structure, all isolates were fingerprinted with 19 simple sequence repeat (SSR) markers dispersed throughout the whole genome following standard protocols [32].

Five candidate genes were chosen according to their role in trichothecene biosynthesis and/or aggressiveness (*TR11*, *TR15*, *TR16*, *TR110*, and *TR114*). Two other, yet uncharacterized

candidate genes in *Fusarium* spp. (*Erf2*, *MetAPI*) were tested for a possible association with aggressiveness. Nucleotide sequences of these genes were imported from *Fusarium graminearum* database FGDB [41]. The selected genes were (Table 1): *TRII* (FGSG_00071), *TRI5* (FGSG_03538), *TRI6* (FGSG_16251), *TRII0* (FGSG_03538), *TRII4* (FGSG_03543), *MetAPI* (FGSG_01397), and *Erf2* (FGSG_08531). Specific primers were designed (see supplemental data) to amplify parts of these genes using the software Primer Premier 4.0 (Premier Biosoft International, CA, U.S.A.).

Polymerase chain reaction (PCR) was performed using the designed primers related to each gene separately following standard protocols, however, with a different annealing temperature for each gene (Table 1). Purification of PCR products was performed by precipitating the DNA with 10% (v/v) of 3M Sodium Acetate and 25% (v/v) of absolute cold ethanol overnight. Precipitated DNA was cleaned twice with 70% ethanol and finally diluted in 10 μ l ddH₂O. Purified PCR products were sequenced (QIAGEN[®] Sequencing Services, Hilden, Germany). Expected sizes of PCR products were obtained for all tested genes. All sequences of *TRI6* and *TRII4* were located in the coding region of the genes (exon, see supplemental data). Sequences of the genes *TRI5*, *MetAPI*, *Erf2*, and *TRII0* (two parts) were stretched over two exons. Sequence of *TRII* was located over four exons according to the reference sequence. Number of identified SNPs was high over all sequenced regions of candidate genes (Table 2). The sequences were aligned using CLC sequence viewer 6.3 (CLC-bio, Aarhus, Denmark) to identify single nucleotide polymorphisms (SNPs) among the 77 isolates.

Phenotypic data analyses

The following linear mixed model was used to estimate variance components: $y_{ijn} = \mu + Iso_i + Env_j + (Iso \times Env)_{ij} + Rep_n + e_{ijn}$, where μ is the population mean, Iso_i the genetic effect of the i th isolate, Env_j the effect of the j th environment, $Iso \times Env$ the isolate times environment interaction effect, Rep_n the effect of the n th replication, and e_{ijn} the residual error.

Variance components were determined by the restricted maximum likelihood (REML) method using the software ASReml 2.0 (VSN International Ltd, Hemel Hempstead, U.K.). Significance for variance component estimates was tested by model comparison with likelihood ratio tests where the halved P values were used as an approximation [31]. Heritability (h^2) on an entry-mean basis was estimated as the ratio of genotypic to phenotypic variance according to Melchinger et al. [22]. Furthermore, genotypes were regarded as fixed effects and best linear unbiased estimates (BLUEs) were determined for all isolates and traits.

Genetic relatedness among the 77 isolates was determined by applying principal coordinate analysis (PCoA) [13] based on the modified Rogers' distances of the isolates [42]. Linkage disequilibrium (LD) between the selected SNPs was assessed by the LD measure r^2 [38] and significance of LD was tested with Fisher's exact tests [14]. LD and PCoA computations were performed with the software package Plabsoft [20].

Association analysis

A two-step association approach was applied in this study and the BLUEs per environment were used as input for the association analysis. The linear mixed model for the association approach was: $y_{ijp} = \mu + a_p + Iso_i + Env_j + e_{ijp}$, where a_p is the effect of allele p . The allele effect a_p was modeled as fixed effect whereas Iso_i and Env_j were regarded as random effects. We assumed that the variance of the random genetic effect was $Var(g) = 2K\sigma_g^2$, where σ_g^2 refers to the genetic variance estimated by REML and K was a 77×77 matrix of kinship coefficients that define the degree of genetic covariance between all pairs of entries. We followed the suggestion of Bernardo [3] and calculated the kinship coefficient K_{ij} between isolates i and j on the basis of the SSR marker data as $K_{ij} = 1 + (S_{ij}-1)/(1-T_{ij})$, where S_{ij} is the proportion of marker loci with shared variants between isolates i and j , and T_{ij} is the average probability that a variant from one isolate i and a variant from one isolate j are alike in state, given that they are not identical by descent. The coefficient T_{ij} was estimated separately for each gene and trait using a REML method setting negative kinship values between isolates to

zero. SNPs with allele frequencies <0.1 were not considered in the association analysis or LD estimation. The obtained optimum T values were for DON content: *TR11* (0.025), *TR15* (0.575), *TR110* (0.375), *MetAPI* (0.300), and *Erf2* (0.400). For mean FHB rating: *TR11* (0.600), *TR15* (0.150), *TR110* (0.200), *MetAPI* (0.075), and *Erf2* (0.275). For the detection of main effects of the candidate gene SNPs, these were fitted as fixed effects in the mixed model and their significance was tested by a Wald F test. Based on the Wald F statistic, we performed tests for the presence of marker-phenotype associations with a significant ($P < 0.05$) effect on DON content and mean FHB rating applying the Bonferroni–Holm procedure [15] to correct for multiple testing.

The proportion of genotypic variance (p_G) explained by the detected SNP was calculated by fitting each SNP in a linear model to obtain R^2_{adj} . The ratio $p_G = R^2_{adj} / h^2$ yielded the proportion of genotypic variance [34]. In the case of *MetAPI*, where two SNPs were detected for mean FHB rating, both were simultaneously fitted in the linear model in the order of their P values to correct for collinearity. All mixed model calculations were performed using the software ASReml 2.0 (VSN International Ltd, Hemel Hempstead, U.K.).

Authors' contributions

FT carried out the phenotypic and molecular analyses, performed parts of the statistical analyses and drafted the manuscript. TW performed parts of the statistical analyses and helped to draft the manuscript, JCR edited the manuscript, HKP[†] participated in the design of the study and supported the technical realization. TM participated in the design of the field study and helped to draft the manuscript. All authors read and approved the final manuscript.

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Table 1. Sequenced regions in the candidate genes

Gene ID ^a	Sequenced region relative to the ATG	No. of SNPs detected	No. of singletons	Nucleotide diversity (%) ^b	No. of SNPs with allele frequency >0.1
<i>TR11</i> (FGSG_00071)	311 – 1065	73	54	2.5	3
<i>TR16</i> (FGSG_16251)	35 – 558	36	29	1.3	0
<i>TR15</i> (FGSG_03537)	479 – 1080	70	38	5.3	4
<i>TR110 a</i> (FGSG_03538)	31 – 678	123	80	6.6	0
<i>TR110 b</i> (FGSG_03538)	760 – 1273	34	29	0.9	1
<i>TR114</i> (FGSG_03543)	277 – 976	43	23	2.9	0
<i>MetAPI</i> (FGSG_01397)	200 – 934	106	41	8.8	25
<i>Erf2</i> (FGSG_08531)	1193 – 1825	80	42	6.0	11

^a The given gene ID is the entry number of the MIPS *F. graminearum* genome database (FGDB; Wong et al. 2011).

^b Nucleotide diversity is the frequency of SNPs (without singletons) relative to the total length of the sequenced gene region.

Table 2. Associated SNPs with FHB rating or DON content, its position, and related amino acids

Trait / Candidate gene	SNP #	Position ^b	Polymorphism	Changes in amino acids ^a	$p_G(\%)^c$
Mean FHB rating (%)					
<i>MetAP1</i>	SNP 05	904	A, G	H, R	25.6
	SNP 03	909	A, C	T, P	0.5
<i>Erf2</i>	SNP 47	1424	C, T	Q, Stop	13.1
DON production (mg kg ⁻¹)					
<i>TRII</i>	SNP 23	851	A, G	R, Q	4.4

^a Different amino acids coded by SNPs located in exon regions (non-synonymous SNPs).

^b The position is relative to the start codon (ATG).

^c The proportion of explained genotypic variance in percent (p_G) after Bonferroni-Holm procedure.

Table 3. Name of tested genes, chromosomal localization, primer sequence, and the expected amplified DNA products

No.	Gene ID ^a	Chromosome		Sequences of the primers	T _a (°C)	Expected products (bp)	Gene size (bp)
1	<i>TRI1</i> (FGSG_00071)	I	F	CACCAGTTTGCAGGATGT	53.2	850	1980
			R	AATGGGAGTGATTAGTTCG			
2	<i>TRI5</i> (FGSG_03537)	II	F	TGGCGGATCTATCTATTCA	57.1	750	1129
			R	CTTCTTGGCGTCCTCTGT			
3	<i>TRI6</i> (FGSG_1625)	II	F	ATGGAGGCCGAATCTCAC	54.4	600	673
			R	CCACCCTGCTAAAGACCCT			
4	<i>TRI10 a</i> (FGSG_03538)	II	F	ATTTCCCAAAGCCTAGACAA	54.2	700	1264
			R	GGCCGTAATCTTCAAATGGT			
5	<i>TRI10 b</i> (FGSG_03538)	II	F	GCACCATTTGAAGATTACGG	54.2	550	
			R	CGTCAAGTCTTCCCATCTCA			
6	<i>TRI14</i> (FGSG_03543)	II	F	AGCCCGAACCCGCTACAT	57.3	750	1116
			R	CGAACCTGCTGCTCTTAC			
7	<i>MetAP1</i> (FGSG_01397)	I	F	AACGATGCCGGATCGTCA	55.3	800	1403
			R	TTGCCAAACTCTCGGATCAA			
8	<i>Erf2</i> (FGSG_08531)	IV	F	AGGCATCTTTGTTGTTGTG	52.4	750	2040
			R	TTGGTAATACGTGGGTTGT			

^a The given gene ID is the entry number of the MIPS *F. graminearum* genome database (FGDB; Wong et al. 2011).

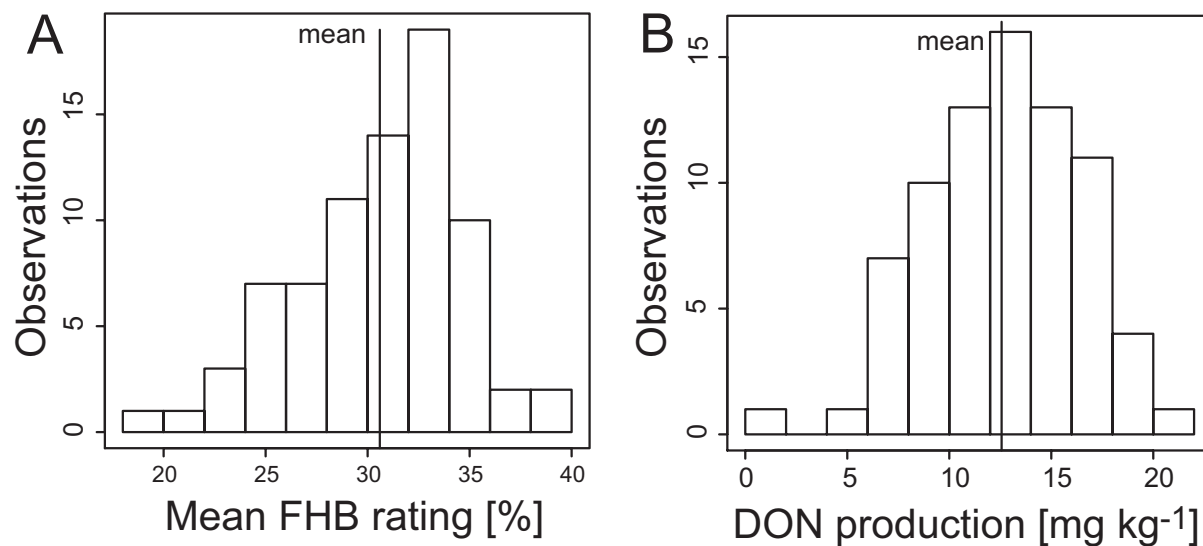


Fig. 1. Histograms of the best linear unbiased estimates (BLUEs). A. mean FHB rating and B. DON content calculated for 77 *F. graminearum* s.s. isolates of the two analyzed traits across four environments (location × year combination)

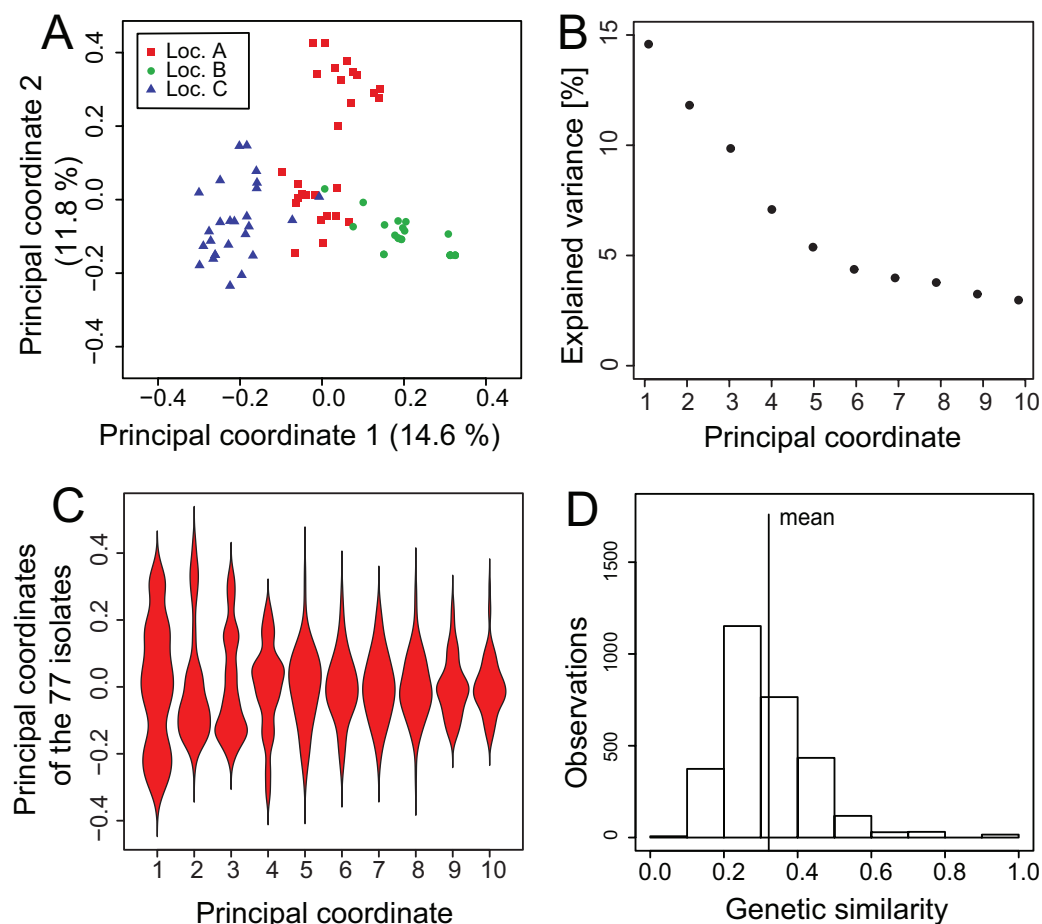


Fig. 2. Population structure and Familial relatedness. (A) Principal coordinate analysis of the 77 isolates, based on modified Rogers' distance estimates whereas location a=Hohenheim, location b= Schickelsheim, and location c=Wetze, (B) Explained genotypic variance of the first ten principal coordinates, (C) Violin plot showing the density distribution of the first ten principal coordinates, (D) Histogram of the genetic similarities among the 77 isolates

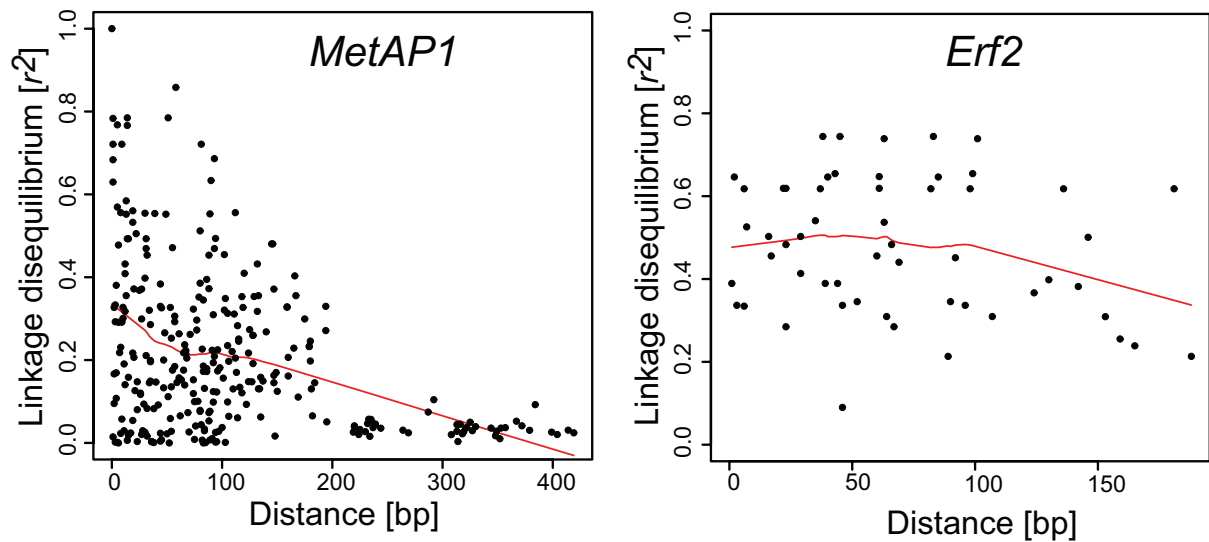


Fig. 3. Linkage disequilibrium (LD) decay. Robust locally fitted regression of linkage disequilibrium over base-pair distance within the two candidate genes, *MetAP1* (25 SNPs) and *Erf2* (11 SNPs)

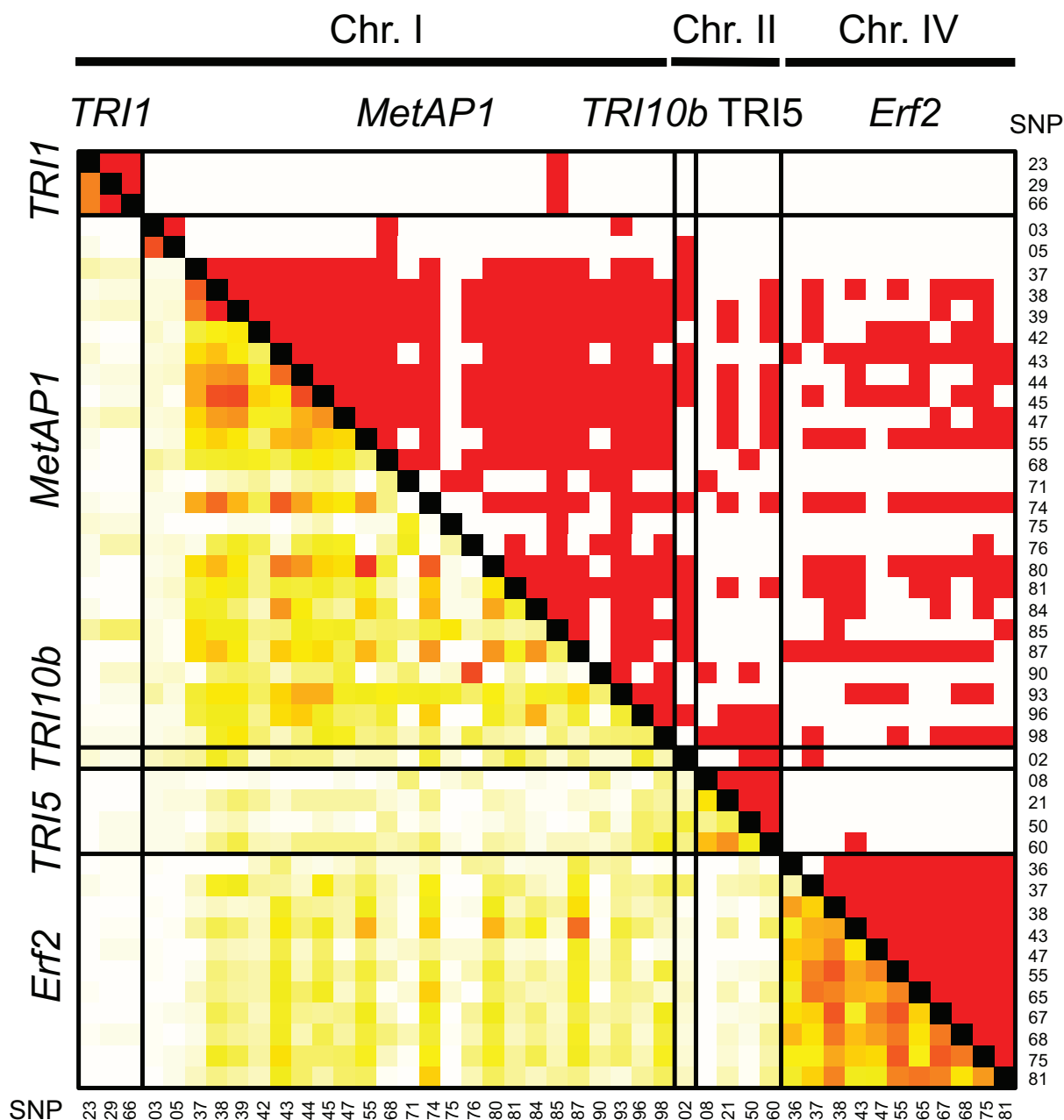


Fig. 4. Pairwise Linkage disequilibrium (LD). The structure of LD within and among five candidate genes for 77 *Fusarium* isolates. Significant LD ($P < 0.05$, above diagonal) and LD measured as r^2 between all pairs of selected SNP loci (below diagonal). The horizontal and vertical lines separate the candidate genes, red coloring indicates significant LD and the higher the r^2 values ($r^2 \geq 0.1$), the darker is the used color, white indicates non-significant LD or $r^2 = 0$

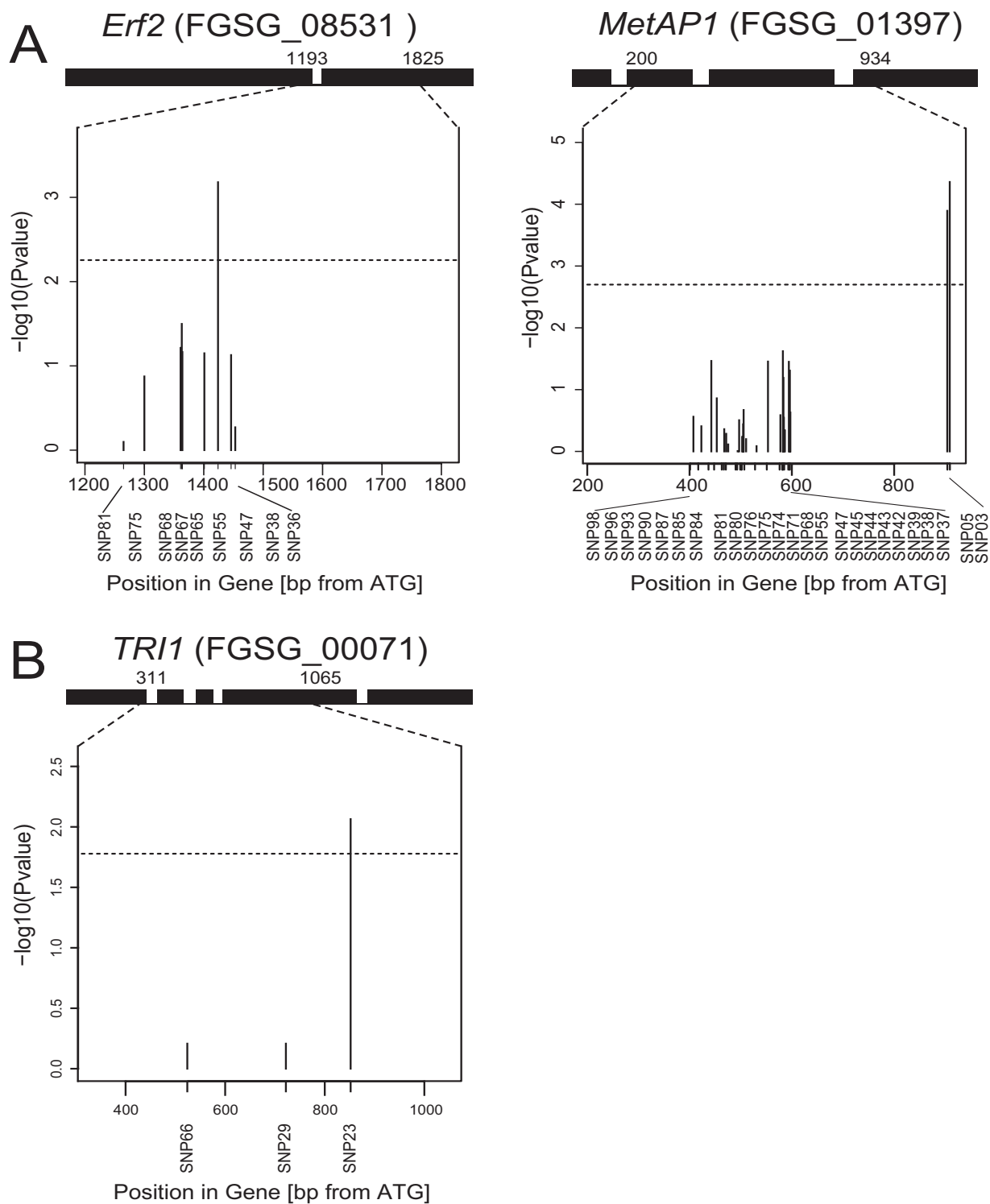
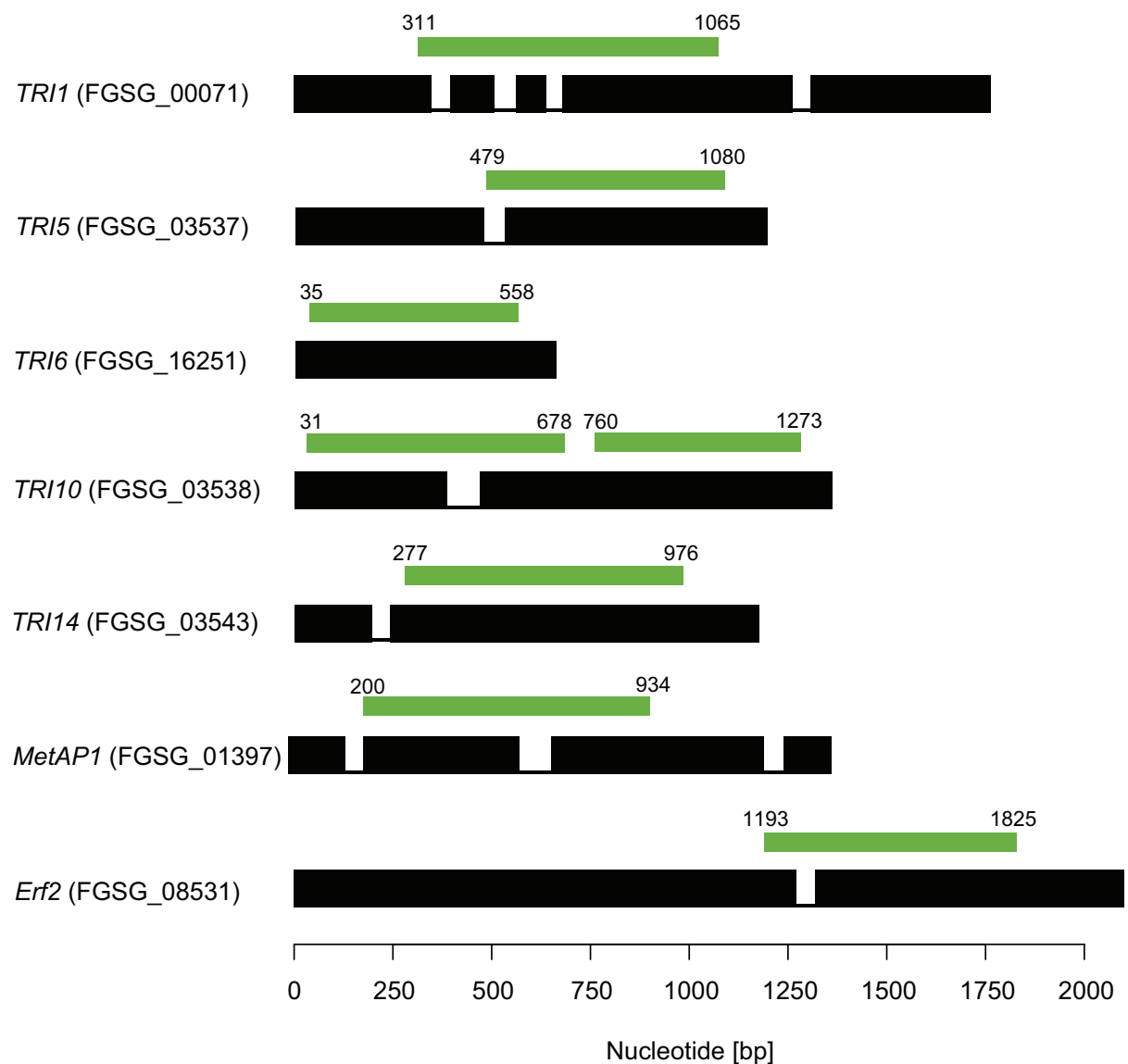


Fig. 5. Significant associations in the candidate genes. (A) *Erf2* and *MetAp1* for mean FHB rating and (B) *TRI1* for DON content. The dashed horizontal line indicates the threshold (Bonferroni-corrected $P < 0.05$).



Additional file 1: Genomic organizations of the seven candidate genes with exons in black color. The sequenced region is shown as a green bar above each gene

4. General discussion

The main target of wheat breeding programs worldwide is to achieve optimum grain production with only the minimum amount of contaminating mycotoxins (Buerstmayr et al. 2009). Fusarium head blight (FHB) is a destructive disease, and difficult to control, thus all control strategies should ensure the lowest hazards. The EU applied standard maximum allowed levels of DON for several kinds of wheat products that should not be exceeded (Anonymous 2007). Many methods can potentially be applied to control FHB, e.g., host resistance, agricultural methods, chemical control, and biological control. Breeding for resistance varieties is the most effective method to control FHB in wheat (Miedaner 1997). Nonetheless, understanding the population dynamics of FHB causal agents may enable wheat breeders to set up long-term strategies to develop stable resistance to FHB (McDonald and Linde 2002). Moreover, many studies were focused on understanding the genetic basis of *F. graminearum* aggressiveness and the mycotoxin production (Kimura et al. 2007; Proctor et al. 1995a). Hence the biology and molecular basis of *F. graminearum* populations are well understood, it could be possible to interrupt its life cycle or overcome its aggressiveness with long-term stable and resistant genotypes of wheat.

4.1. Host resistance (phenotypic variation)

Different levels of FHB resistance were previously reported in wheat (Mesterhazy 1995; Miedaner 1997). Breeding for FHB in bread wheat succeeded in obtaining remarkable resistant varieties such as Sumai 3, Ning 7840, and Ning 8331 (Buerstmayr et al. 2009). In contrast, current durum wheat varieties are still highly susceptible to FHB. Introgression of FHB resistance from hexaploid wheat into tetraploid wheat was unsuccessful. Thus, screening for resistance sources of FHB within cultivated durum wheat may be more effective for use in breeding programs. Four Syrian landraces (ICDW95842, ICDW92330, ICDW96165, Chahba) were identified as potential carriers of FHB resistance. Selection of these genotypes was based on disease severity and resistance stability of these genotypes under field conditions in different environments. The mean value of FHB rating for these genotypes reflects the ability of the same isolate of the pathogen to distinguish different levels of resistance. Values of mean FHB ratings on the candidate genotypes (18.94, 23.75, 24.95, and 27.40 %, respectively) might be promising for durum wheat resistance compared to the standard cultivars. However, in bread wheat these values are far greater than those of resistant

genotypes, *e.g.*, mean FHB rating of a resistant genotype was 4.4% in a bread wheat experiment conducted under the same conditions like our experiment (Von der Ohe and Miedaner 2010). Very limited variation in FHB resistance has been reported in durum wheat or its relatives, *e.g.*, *Triticum dicoccum* and *Triticum dicoccoides* that could provide some resistant genetic sources (Oliver et al. 2008). Further field testing is needed to determine potential capacity towards FHB resistance and characterize agronomic features of Syrian landraces.

4.2. Molecular variation of *Fusarium graminearum* populations

4.2.1. Role of *F. graminearum* in FHB and related trichothecenes

Primary evaluation of natural populations of FHB causal agents in our study revealed that *F. graminearum* s.s. is the dominant species (64.9%) in Germany as reported from other parts of Europe, U.S.A. and Canada (Jennings et al. 2004; Zeller et al. 2004; O'Donnell et al. 2000; Talas et al. 2011). Although the occurrence of three chemotypes (3-ADON, 15-ADON, and NIV) was reported previously in Germany, this study is, to our knowledge, the first attempt to evaluate systematically the natural occurrence of the three chemotypes in Germany that have been found in frequencies of 92%, 6.8%, 1.2%, respectively. Percentage of NIV chemotypes among *F. graminearum* s.s. populations in Germany is much lower than that reported in the UK of 25% (Jennings et al. 2004). Nonetheless, 15-ADON chemotype also dominates the DON-producing isolates in the UK (95% of DON chemotype), which mirrors our result. 15-ADON chemotype also dominates the province of Manitoba, Canada (Guo et al. 2008); however, percentage of 3-ADON chemotype is shown to be increasing in southern Manitoba due to the introduction of the 3-ADON chemotype into the new area through seed or straw transportation. The same pattern was evident in North Dakota, U.S.A., whereas percentage of the 3-ADON chemotype increased 15 fold between the old collection (1989-2000) and a new collection (2008), due to the higher fitness of the newly emerging population of 3-ADON chemotype (Puri and Zhong 2010). Monitoring the population of *F. graminearum* in Germany is necessary to be repeated regularly to detect any possible shift of the dominating chemotype. Taking into account the suggestion of Puri and Zhong (2010) of the fitness advantage of 3-ADON over the prevalent 15-ADON population, a kind of new selection forces would be applied in U.S.A. and Canada, but not in Europe. Selection forces, generally comes through cultivation of more resistant varieties or by applying a new fungicide (new mode of action) in addition to the other environmental changes.

4.2.2. Population structure

Genetic variance within and among populations and gene migration between populations or subpopulations are essential parameters to understand population ecology (Hartl and Clark 2007). Our results showed a higher percentage of molecular variation within populations than among populations (71.2%, and 28.8%, respectively). Genetic distances between populations were not correlated with geographical distance, since there are many factors affecting the population structure. Principally, gene flow of single direction can increase the genetic variation of the receptor population. Although, differences between populations are expressed by high F_{st} values, these differences are minor ones compared to the variation within populations. Moreover, these differences reflect the required time for alleles to diffuse across the distance separating the different populations (Zeller et al. 2004). A Bayesian clustering approach implemented in the STRUCTURE software package (Pritchard et al. 2000) was used to determine the population structure using multilocus genotypes probabilistically to identify groups (K) being characterized by specific sets of markers and allele frequencies. In exploratory runs, we allowed values of K from 1 to 10, setting burn-in length of 2000, with 5000 Markov Chain Monte Carlo (MCMC) iterations (Haseneyer et al. 2009). To help detect the true number of groups, we applied the formula given in Evanno et al. (2005) with 10 independent runs of $K=1$ to $K=10$. STRUCTURE clustered our isolates into two groups (Fig. 1) with an optimum value $K=2$ (data not shown).



Fig. 1. Population structure of *Fusarium graminearum* s.s. inferred from haplotypes genotyped with simple sequence repeats (SSR) markers (see Talas et al. 2011b) using STRUCTURE 2.3.2. Vertical axis denotes estimated membership fraction in one of two groups, including (HOH, PLN, KEL, WET1, WET2, and SCHICK) in red, and (BIR, TUB, NUF, ENT, and HER) in green. The horizontal axis consists of a single vertical bar for each of 338 isolates, arranged according to membership fraction. Isolates were assigned to a specific population when membership fraction ≥ 0.8 .

Our results reveal a high gene flow between populations in the red group, in particular HOH, WET1, WET2 and KEL. For WET1 and WET2 this is not astonishing because they belong to

same sampling field of two years difference, but it is so for HOH/KEL and HOH/WET because these locations are about 150 and 500 km, respectively, away from each other. Although the direction of gene flow in natural populations could not be that clear, rather a complex network. Out of our data we hypothesized that gene flow from northern populations (WET, SCHICK) transferred to the sampling locations of HOH and PLN, because the genetic structure of these populations seems to be different from that of the other southern populations (green group) and closely related to the northern ones (red group) (Fig. 2). Exchanging plant material between experimental stations in these locations (northern vs. southern) may have a significant effect on the population structure at the receptor location. Similar change in the populations' structure of *F. graminearum* was reported in Canada (Guo et al. 2008). The genetic distances between sampled populations were not correlated with the geographical distance of sampling locations. Nei's unbiased genetic distances were calculated for all population pairs (Nei 1972, 1978). The resulted matrix was separated into its principal coordinates and the values of first three coordinates were plotted using Proc gplot in SAS 9.1. Similar pattern as in STRUCTURE software was obtained with unbiased Nei's distances, thus we give the color to refer the same groups as in previous figure. Gene flow might be restricted by forested uplands in southwest Germany (green group) that maintain the original genetic variation without foreign alleles (Fig. 2).

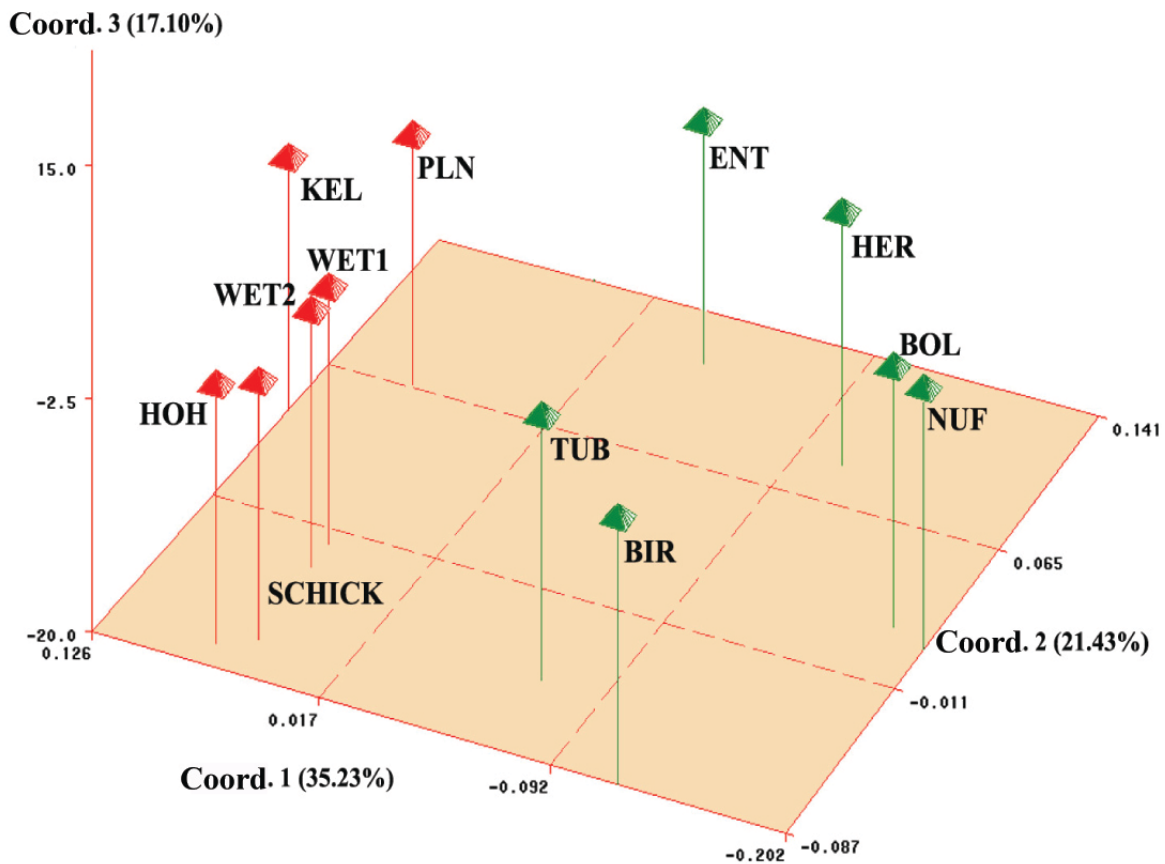


Fig. 2. Pairwise unbiased Nei's genetic distance between 12 populations of isolates from different locations in Germany, the three coordinates explain cumulatively 73.76% of total existing variance. The color-coding matches with Fig.1

However, this structure of genetic distances between field-populations might not be stable over the time, because the population structure is subjected to many other factors, such as natural air- borne spores, recombination system and human activities. Recently, Gale et al. (2011) reported for the first time a *F. asiaticum* population in U.S.A. Its low genetic diversity suggests that it is an introduced population. To conclude, gene flow can affect the genetic structure of the receptor location for a short time, *i.e.*, HOH/WET1,2/SCHICK. HOH and BIR populations seem to be genetically different (see Fig.2) although the geographical distance between them is short (about 5 km). A cause for this might be the migration of foreign alleles to one of these populations. That, the geographical distances between the three field-populations are short, and a different genetic structures are detected between HOH and PLN on one side and BIR on the other side, it might be that a large meta-population will be formed through allele diffusion from HOH, PLN, and BIR populations by means of (at least) airborne

spores. A relatively long time (2 – 3 seasons) is needed for alleles from previously mentioned locations to diffuse with each other, if there is no further migration occurs from abroad affecting the current genetic structure again.

Absence of clones common in pairwise comparisons of populations may suggest rearrangement (outcrossing between the haplotypes) of the alleles in one location creating new allele combinations (new haplotypes structure). This can be faster (over seasons) when genetic migration takes place since it adds an additional set of alleles to the allele's pool in the receptor population. The highest average number of alleles were detected in HOH and PLN followed by KEL, WET1,2. We propose that long distance genetic migration increased primarily the number of alleles at these locations. Together with outcrossing, new haplotypes would be created after several agricultural seasons. The molecular composition of the new haplotypes is expected to be partially similar to each other (original populations' sources) since a high or low percentage of the initial alleles are common.

4.3. Relation between molecular and phenotypic variation

A previous study has reported a high variation of FHB causal agents within one field considering the variation in species and produced mycotoxins (Xu et al. 2008). In our results, we report a high molecular variation within the populations from one side. On the other side, we evaluated two phenotypic traits (aggressiveness and DON production) in three populations of our collection. Individual populations from a naturally infected wheat field, showed a high variation in FHB rating (18-38%) and deoxynivalenol (DON) production (0.2-22 mg kg⁻¹). The lowest DON concentration (0.2 mg kg⁻¹) was produced by an isolate with the NIV chemotype. NIV chemotype isolates are among those isolates with lowest FHB ratings, which confirm the low aggressiveness of NIV isolates (Muthomi et al. 2000). Variation of aggressiveness and DON production within individual field-population (HOH, SCHICK, and WET) equals that of the tested international collection of *F. graminearum* (*Fg* coll.). Thus, wide genotypic variation (σ^2_G) of *F. graminearum* isolates causes wide phenotypic variation of these isolates. The genotypic proportion of phenotypic variance (genotypic prop.) within populations was significant (P<0.01) for both traits, genotypic prop. × environment interaction was even more important for mean FHB severity. Variance partitioning of genotypic prop. revealed 72% for within and 28% across populations for both traits. Accordingly, analysis of molecular variance (AMOVA) tests showed that molecular variation within these three populations and the *Fg* coll. was considerably higher than among

populations. Analysis of phenotypic trait variation (ANOVA) was almost equal to the molecular genetic variation (AMOVA) (Table 2).

Table 2. Estimates of variance components and its partitioning for aggressiveness (mean FHB rating in %), deoxynivalenol production (DON in mg kg⁻¹) and molecular markers with each of 30 isolates from three populations

Source of variation	Phenotypic variation		Molecular variation
	Aggressiveness	DON	
Population (P)	2.86	2.66	2.64
Isolates (I) within P	7.20	6.83	7.36

4.4. Genetic variation within candidate genes (Association mapping)

High genetic variation of *F. graminearum* haplotypes can be detected on a finer scale, when individual genes affecting aggressiveness and DON production in *F. graminearum* are analyzed for their nucleotide variation.

Nucleotide diversities were high in almost all genes in this study (from 9 to 88 SNPs per kb), comparing to the range of 0 to 17 SNPs per kb reported by Cuomo et al. (2007). The high degree of polymorphism was not unexpected for trichothecene cluster genes or genes related to aggressiveness. Hence these genes are highly enriched in the high SNP density regions (Cuomo et al. 2007). Moreover, it is known that the density of SNPs is biased in *F. graminearum*, because 50% of SNPs are within 13% of the genome sequence. Sharp LD decay after 200 bp was detected in two genes of our dataset (*MetAP1*, and *Erf2*). Large proportion of unlinked SNPs in significant LD such as SNPs in gene pairs *TR15/MetAP1*, *TR110/MetAP1*, or *MetAP1/Erf2* suggest the presence of other forces generating LD between unlinked SNPs. Selection acting on oligo- or polygenic traits such as the ones discussed in this work (aggressiveness and DON production) generate LD not only between linked genes but also between unlinked genes coding for the same trait (Stich et al. 2005) or involved in the same trait network. The rapid decay of LD allows a high resolution of association mapping. Consequently, it is necessary to use a higher SNPs marker density in further studies to facilitate genome wide association mapping.

Fungal enzymes like proteases, lipases and carbohydrate-degrading enzymes work together to facilitate cell colonization by extracting plant components and assist pathogen nutrition, e.g.,

amino acids, sugars, fatty acids and irons (Divon and Fluhr 2007). Deoxynivalenol (DON) of *Fusarium* damages the plasma membranes, chloroplasts and ribosomes in planta (Miller and Ewen 1997). DON triggers hydrogen peroxide production and causes cell death in cereals (Walter et al. 2010). Several genes in *F. graminearum* are related to aggressiveness (Bluhm et al. 2007; Mayorga et al. 1999) or to trichothecene production (Kimura et al. 2007). Production of trichothecenes enhances *F. graminearum* aggressiveness during the infection (Proctor et al. 1995a, Maier et al. 2006).

We identified a significant association between SNPs in genes *MetAP1*, *Erf2*, and *Tri1* for aggressiveness and DON production. *MetAP1* and *Erf2* are two genes identified in *F. graminearum* without further studies on their function in the wheat/*Fusarium* pathosystem till date. Our study detected significant association of aggressiveness, with proportion of genotypic variance (p_G) of 25.6 and 13.1 % for *MetAP1* and *Erf2*, respectively. Nonetheless, one SNP identified in *Tri1* and its association with DON production seems to be logically related to the gene function encoding cytochrome P450 monooxygenase that catalyzes hydroxylation of c-8 position during trichothecene biosynthesis (Meek et al. 2003). The challenge now is to understand the causative and correlative effects of the genes *MetAP1* and *Erf2* on aggressiveness networks and their downstream effects on *Fusarium*/wheat interaction. Understanding the DNA sequence variation and the quantitative variation of aggressiveness and DON production in *F. graminearum* would provide us with important insights for predicting the potential risk of this pathogen on wheat production. These insights can increase our understanding of a population's dynamics and development, and potentially speed up breeding to improve wheat resistance to FHB.

5. Conclusions

Two major types of FHB resistance were identified in wheat: resistance to the initial infection (type I) and resistance to spread within the ear (type II) (Schroeder and Christensen 1963). All studies and research done to improve wheat resistance did not achieve an immune variety to FHB (Rudd et al. 2001). However, the pathogen may overcome the host resistance if its aggressiveness increases on a population scale with respect to physiological, morphological, and biochemical characteristics of individuals. A major goal of wheat breeders is to increase the resistance to FHB with combining multiple QTLs in a single wheat genotype (Miedaner et al. 2006). This incremental approach of increasing disease resistance might result in a selective pressure consequently increasing the aggressiveness of pathogen populations (Voss

et al. 2010). Continuously increase the resistance sources (additional QTLs for resistance) in the cultivated wheat varieties definitely form unspecific selection pressure on *F. graminearum* populations to develop a genetic drift towards forming a much aggressive subpopulation. Our field data, which resulted from three populations, showed a broad spectrum of variation in aggressiveness and DON production among individual field-population. Monoculture of the improved wheat cultivars provide an open source for multiplication of the aggressive genotypes (those succeed to overcome resistance level of the cultivated variety) in *F. graminearum* populations, thus for a tremendous increase of these genotypes' frequencies over consequent seasons and generations. The balanced introduction of resistance factors (new QTLs of resistance) into the cultivated varieties, insure equilibrium between the high and low aggressive individuals. In this case, the favorable environmental conditions (for FHB infection) encourage both high and low aggressive individuals, and unfavorable conditions encourage the high aggressive ones without forming a continuous pressure. Hence, the continuous selection forces (varieties resistance) lead to a faster genetic drift towards aggressiveness than the intermittent pressure caused by the environmental conditions. High variation in aggressiveness suggests that the wheat/*Fusarium* pathosystem in Germany is subject to low selection pressure. Consequently, the level of resistance introduced into the recently cultivated wheat varieties in Germany (at least) seems to maintain high yield of wheat with the lowest selection pressure on the causal agents of FHB resulting no genetic drift into a higher aggressiveness. Regular monitoring of *F. graminearum* populations is required to detect possible changes in the structure that may affect aggressiveness or trichothecene production and aid development of a suitable wheat breeding strategy.

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newly discovered cereal head blight pathogen from Russian Far East. *Mycologia* 101: 841-852.

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7. Summary

Fusarium head blight (FHB) is a devastating disease of wheat, barley and other cereals, which affects all wheat-growing areas of the world. The most prevalent species are *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zae* (Schweinitz) Petch) and *Fusarium culmorum* (W. G. Smith) Saccardo. Wheat breeding for FHB resistance has become the most effective and cost efficient strategy to combat this disease. Assisting long term stable breeding programs need a better understanding of the biology and dynamic changes of the population structure. Deoxynivalenol (DON) has the most economical impact among the other mycotoxin secreted by this fungus. Several chemotypes characterizes *F. graminearum* isolates. All chemotypes (3-ADON, 15-ADON, and NIV) were detected in Europe. The prevalent chemotype in Germany and UK is 15-ADON. Population structure is the result of evolutionary forces acting on the population in time and space together with mutation, recombination, and migration enhancing the genetic variance of a population, random drift and the selection reducing it. Aggressiveness in *F. graminearum* denotes the quantity of disease induced by a pathogenic isolate on a susceptible host in a non-race specific pathosystem, and is measured quantitatively. The quantitative traits such as aggressiveness and DON production mirror both the environmental changes and the genetic variation. Several genes are responsible for DON production; majority of these genes are grouped in TRI5 cluster. Few genes are known to be associated with *F. graminearum* aggressiveness such as MAP kinase genes, *RAS2*, and *TRII4*. Association between single nucleotide polymorphism and genetic variation of aggressiveness and DON production traits provide a clear identification of quantitative participation of different SNPs in expressing the trait. Also, this approach provides a good method to test the association between candidate genes and the traits.

The objectives of this research were to (1) screen some durum wheat landraces for FHB resistance; (2) determine the genetic and chemotypic structure of natural population of *F. graminearum* in Germany; (3) determine the phenotypic variation in Aggressiveness and DON production, which come out one farmer wheat field; (4) compare the phenotypic variation and genetic variation occurring in one wheat field; and (5) associate the phenotypic traits with SNPs in candidate genes.

Screening for FHB resistance was performed on sixty-eight entries form the Syrian landraces. The main characters of selection for resisting FHB disease are low mean value of infection

and stability in different environments. Four genotypes (ICDW95842, ICDW92330, ICDW96165, Chahba) had small mean FHB value, small value of deviation from regression, and regression coefficient close to zero. These genotypes were considered as candidate resistant sources of FHB for further agronomic performance analysis through backcrossing generation.

The causal agent of FHB in Germany is *F. graminearum* s.s. with a dominating rate of 64.9 % (out of 521 *Fusarium spp.* isolates). Nonetheless, the three chemotypes were detected in Germany and some times within one wheat field. The 15-ADON chemotype dominated the populations of *F. graminearum* s.s. in Germany followed by 3-ADON then NIV chemotype (92, 6.8, and 1.2%, respectively). High genetic diversity (Nei's gene diversity ranged from 0.30 to 0.58) was detected on a single wheat field scale. Analysis of molecular variance (AMOVA) revealed a higher variance within populations (71.2%) than among populations (28.8%). Populations of *F. graminearum* s.s. in Germany display a tremendous genetic variation on a local scale with a restricted diversity among populations. Surprisingly the phenotypic variation of aggressiveness and DON production revealed a similar partitioning scale as the genetic variation. In other words, analyses of variance (ANOVA) revealed a higher variance within populations (72%) than between (28%) populations. The wide spectrum of aggressiveness (i.e., from 18 to 39%) and DON production (from 0.3 to 23 mg kg⁻¹) within single wheat field simulate the global variation in both traits.

Consequently, associating the observed variation of aggressiveness and DON production with detected single nucleotide polymorphism (SNPs) in some candidate genes revealed few but significant associations. According to Bonferroni-Holm adjustment, three SNPs were associated significantly with the aggressiveness, two in *MetAPI* and one in *Erf2* with explained proportions of genotypic variance (p_G) of 25.6%, 0.5%, and 13.1%, respectively. One SNP in *TRII* was significantly associated with DON content on *TRII* ($p_G=4.4$). The rapid decay of the LD facilitate a better high resolution of the association approach and is in turn suggest the need of higher number of SNP marker to facilitate a genome wide association study. The linkage disequilibrium between unlinked genes suggests the involvement of these genes in the same biosynthesis network.

In conclusion, building wheat breeding program for FHB resistance depend initially on identifying sources of resistance among wheat varieties or wild relatives. Moreover, understanding the population structure of the pathogen and the selection forces causing genetic alteration of the population structure enable us employ a sufficient increase of the host resistance. Keeping such a balanced equilibrium between increasing host resistance and

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changes occur in genetic structure of *F. graminearum* population would insure no application of additional selection pressure. Further association of candidate genes with aggressiveness can provide effective information of the population development. Continuous observation of Fusarium population's development is needed to insure a stable management of Fusarium head blight disease.

8. Zusammenfassung

Ährenfusarium gilt als schwerwiegende, ertrags- und qualitätsmindernde Krankheit, welche Weizen, Gerste und anderen Getreidearten befällt. Sie tritt weltweit in sämtlichen Anbaugebieten von Weizen auf und wird durch unterschiedliche Schimmelpilze verursacht. Die häufigsten Gattungen hiervon sind *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schweinitz) Petch) und *Fusarium culmorum* (W. G. Smith) Saccardo. Da andere Maßnahmen zur Krankheitsbekämpfung nicht ausreichend sind, gilt heute die Resistenzzüchtung als effektivste und kosteneffizienteste Herangehensweise um der Anfälligkeit von Weizen gegenüber Ährenfusarium entgegenzutreten. Für die Etablierung langfristiger und stabiler Zuchtprogramme sind jedoch mehr Kenntnisse über die Biologie und Populationsdynamik der *Fusarium* Gattungen notwendig. Neben Ertrags- und Qualitätsverlusten führen Ährenfusariosen zusätzlich zur Belastung der Körner mit Mykotoxinen, welche von den Pilzen gebildet werden. Die größte wirtschaftliche Bedeutung unter den Mykotoxinen besitzt Deoxynivalenol (DON). Charakteristisch für Isolate dieses Mykotoxins aus *F. graminearum* ist das Vorkommen verschiedener Chemotypen. In Europa wurden alle Chemotypen (3-ADON, 15-ADON und NIV) nachgewiesen, wobei 15-ADON als häufigster in Deutschland und Großbritannien gilt. Populationsstrukturen sind bedingt durch Evolution in einem gewissen zeitlichen und räumlichen Rahmen in Verbindung mit Mutation, Rekombination und Migration, welche die genetische Variation einer Population erhöhen und zufälliger genetischer Drift und Selektion, welche sie verringern. Die Aggressivität von *F. graminearum* wird beschrieben als Ausmaß der Krankheit induziert durch ein pathogenes Isolat auf einer anfälligen Wirtspflanze in einem nicht rassenspezifischen Pathosystem und wird quantitativ bestimmt. Quantitative Merkmale wie Aggressivität und DON Bildung spiegeln sowohl Veränderungen der Umwelt als auch genetische Variation wider. Die Bildung von DON ist durch mehrere Gene beeinflusst, wobei die Mehrheit der Gene in TRI5 Clustern gruppiert ist. Es ist bekannt, dass einige Gene wie MAP Kinase Gene, *RAS2* und *TRII4* eine Verbindung mit der Aggressivität von *F. graminearum* aufweisen. Assoziationen zwischen SNPs (single nucleotide polymorphisms) und der genetischen Variation der Aggressivität, wie auch Merkmalen, die mit der Bildung von DON in Verbindung stehen, ermöglichen eine Identifizierung der quantitativen Beteiligung verschiedener SNPs an der Merkmalsausprägung. Zusätzlich bietet dieser Ansatz

eine gute Methode um die Assoziation zwischen Kandidatengenomen und den Merkmalen von Interesse zu testen.

Die Ziele der vorliegenden Arbeit waren (1) mehrere Hartweizen Landrassen auf Ährenfusarium-Resistenz zu überprüfen, (2) die genetische und chemotypische Struktur einer in Deutschland natürlich vorkommenden *F. graminearum* Population zu bestimmen, (3) die phänotypische Variation der Aggressivität und DON Bildung zu bestimmen, die auf dem Weizenfeld eines Landwirts vorkamen, (4) die phänotypische und genetische Variation, die auf einem Weizenfeld vorzufinden war, zu vergleichen und (5) die phänotypischen Daten mit SNPs aus Kandidatengenomen zu assoziieren.

Das Screening auf Resistenz gegen Ährenfusarium wurde an 68 Prüfgliedern von Landrassen aus Syrien durchgeführt. Die Hauptkriterien der Selektion auf Ährenfusarium-Resistenz sind ein geringer Mittelwert in Bezug auf Infektion sowie Stabilität in verschiedenen Umwelten. Vier Genotypen (ICDW95842, ICDW92330, ICDW96165, Chahba) zeigten einen geringen mittleren Wert für Ährenfusarium, eine geringe Abweichung von der Regression, und einen Regressionskoeffizienten nahe null. Diese Genotypen werden als potentielle Resistenzquellen gegen Ährenfusarium im Backcross-Verfahren angesehen und auf agronomische Leistung getestet.

Der Haupterreger von Ährenfusarium in Deutschland ist mit einer Rate von 64,9% (aus 521 *Fusarium spp.* Isolaten) *F. graminearum* s.s. Nichtsdestotrotz wurden in Deutschland alle drei Chemotypen, teilweise sogar innerhalb eines Weizenfeldes, nachgewiesen. Die *F. graminearum* s.s. Populationen in Deutschland wurden vom 15-ADON Chemotyp dominiert, gefolgt von 3-ADON und NIV mit einem jeweiligen Anteil von 92, 6,8 beziehungsweise 1,2%. Innerhalb eines einzelnen Weizenfeldes wurde eine hohe genetische Diversität festgestellt (die genetische Diversität nach Nei reichte von 0,3 bis 0,58). Die Analyse der molekularen Varianz (AMOVA) zeigte eine höhere Varianz innerhalb der Populationen (71,2%) als zwischen den Populationen (28,8%). *F. graminearum* Populationen in Deutschland zeigen eine große genetische Variation auf lokaler Ebene mit eingeschränkter Diversität zwischen Populationen. Überraschenderweise zeigte die phänotypische Variation der Aggressivität und DON Bildung eine ähnliche Verteilung wie die genetische Variation. Die Varianzanalyse zeigte eine höhere Varianz innerhalb der Populationen (72%) als zwischen den Populationen (28%). Das große Spektrum der Aggressivität (z.B. von 18 bis 39%) und DON Bildung (von 0,3 bis 23 mg kg⁻¹) innerhalb eines einzelnen Weizenfeldes bildet die globale Variation beider Merkmale nach.

Infolgedessen zeigte eine Assoziation der beobachteten Variation der Aggressivität und DON Bildung mit den ermittelten SNPs in machen der Kandidatengene geringe aber signifikante Verbindungen. Nach der Bonferroni-Holm Korrektur waren drei SNPs signifikant mit der Aggressivität gekoppelt (zwei in *MetAPI* und eins in *Erf2*) mit erklärten genotypische Varianzen (p_G) von je 25,6, 0,5 und 13,1%. Ein SNP in *TR11* war signifikant mit dem DON Gehalt auf *TR11* gekoppelt ($p_G=4,4$). Der schnelle Abfall des LD ermöglicht eine bessere Feinauflösung des Assoziationsansatzes, deutet aber wiederum auf die Notwendigkeit einer größeren Anzahl SNP Marker hin, um eine genomweite Assoziationsstudie durchführen zu können. Das Gametenphasenungleichgewicht zwischen ungekoppelten Genen legt die Beteiligung dieser Gene am gleichen Biosynthese-Netzwerk nahe.

Zusammenfassend ist zu sagen, dass der Aufbau eines Weizenzuchtprogramms für Ährenfusarium-Resistenz zunächst von der Identifikation von Resistenzquellen aus Weizen-Sorten oder Wildtypen abhängt. Zusätzlich ermöglicht das Verständnis der Populationsstruktur des Pathogens und die Selektionskräfte, die genetische Veränderungen der Populationsstruktur bewirken, einen ausreichenden Anstieg der Resistenz der Wirtspflanze zu erzielen. Wird dieses Gleichgewicht zwischen ansteigender Resistenz der Wirtspflanze und Änderungen in der genetischen Struktur von *F. graminearum* gehalten, würde das keinen zusätzlichen Selektionsdruck bedeuten. Weitere Assoziation von Kandidatengenen mit der Aggressivität kann nützliche Informationen zur Entwicklung der Population liefern. Eine kontinuierliche Beobachtung der Entwicklung von Fusarium Populationen ist notwendig um ein dauerhaftes Krankheitsmanagement von Ährenfusarium zu gewährleisten.

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Erklärung

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

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Stuttgart, Juni 2011

Firas Talas

