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Molecular mapping of resistance and aggressiveness in the cereal/Fusarium head blight pathosystem

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Abbreviations

COS	Conserved ortholog set
DArT	Diversity array technology
DON	Deoxynivalenol
ELISA	Enzyme linked immunosorbent assay
FHB	Fusarium head blight
GC	Gas chromatography
h^2	Heritability
HPLC	High pressure liquid chromatography
LD	Linkage disequilibrium
mg kg^{-1}	Milligram per kilogram
NIV	Nivalenol
p_G	Proportion of genotypic variance
QTL	Quantitative trait loci
SNP	Single nucleotide polymorphic
TLC	Thin layer chromatography
ZEN	Zearalenone

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1. General introduction

1.1 Importance of triticale (X *Triticosecale* Wittmack) and wheat (*Triticum aestivum* L.)

Triticale (*X Triticosecale* Wittmack) is the intergeneric hybrid between the female parent wheat (*Triticum* spp.) and the male parent rye (*Secale* spp.). The first registration for the natural wheat-rye hybrids was at a research station in Russia (Oettler 2005, Guedes-Pinto et al. 1996). The possibility of obtaining such a hybrid between wheat and rye prompted breeders to make several artificial crosses between wheat and rye to obtain a fertile wheat-rye hybrid, the main goal was to assemble desirable traits from both parents. Specifically, winter hardiness and adaptation to inappropriate soils and climates of rye combined with the quality and productivity of wheat (Hao et al. 2013). First commercialized production was achieved at the end of 1980's (Oettler 2005). Nowadays triticale is synthesized by crossing rye with tetraploid wheat and doubling the chromosomes to convert the sterile hybrid to a fertile hexaploid triticale (Guedes-Pinto et al. 1966). Hexaploid triticale has proven to be the most successful type because of its superior vigor and yield stability, representing the basis of the current applied triticale breeding worldwide (Fox et al. 1990). Triticale cultivation areas have been significantly increased globally in the last two decades. Growing areas are concentrating in Central and Eastern Europe, especially Poland, Germany and France. The harvested area in Europe reached in 2013 around 3.8 million hectares (FAOSTAT 2015). Triticale is mainly used as animal feed due to the high content of amino acids and the good performance in all environments (McGoverin et al. 2011, Fernandez-Figares et al. 2008). Recently, triticale came into focus as a promising energy crop and many studies have reported the high potential of modern triticale cultivars for biogas and biofuel production (McGoverin et al. 2011, Gowda et al. 2011).

Wheat (*Triticum aestivum* L.) is the world's most widely cultivated crop, with a production area of more than 220 million ha in 2013 (FAOSTAT 2015). More than 30% of the wheat global production is located in Europe (FAOSTAT 2015). Wheat can be cultivated in a wide range of environments, representing the primary source of calories and protein for the majority of humans (Chaves et al. 2013). Therefore, the need to improve wheat production is essential to face the challenge of the growing human need for cereals (Curtis and Halford 2014). Increasing wheat production can be achieved by expanding cultivation area, improving the yield, and reducing pre and post harvest losses (Curtis et al. 2002). Currently, wheat breeders' efforts concentrate on increasing the grain yield per area, not only by improving the agricultural practices in the field, but also by enhancing grain yield and disease resistance like Fusarium head blight (FHB) and Septoria tritici blotch (STB) (Summers and Brown 2013).

1.2 Epidemiology of *Fusarium* species causing cereal head blight

Fusarium head blight (FHB) is a harmful disease of cereals, caused by several species within the *Fusarium* genus (Goswami and Kistler 2005). *Fusarium* spp. is well adapted to infecting all cereals in temperate and semi-tropical areas due to its ability to grow and spread over a wide range of temperatures (Arseniuk et al. 1999).

Fusarium graminearum Schwabe (teleomorph: *Gibberella zeae* (Schw.) Petch) and *Fusarium culmorum* (W.G. Smith) Sacc. (teleomorph: unknown) are reported as the most prominent species in cereals (Parry et al. 1995). Geographic distribution of *F. graminearum* and *F. culmorum* is directly connected with the climate. Whereas *Fusarium graminearum* more widespread in North America, Southern China, and Eastern Europe*, Fusarium culmorum* occurs more in cooler areas such as Western Europe (Wegulo 2012). *Fusarium graminearurm* and *Fusarium culmorum* are non-host specific, i.e. both species can infect a broad range of small-grain cereals and grasses (van Eeuwijk et al. 1995). However, colonized plant residue is the primary source of inoculum for FHB epidemics (Becher et al. 2013). Head blight disease is initiated by airborne spores landing on flowering spikelets. Warm and moist weather during cereal flowering-time in spring provides the optimal conditions to initiate FHB infection (Goswami and Kistler 2004). Infection initiates after macroconidia germinate and enter the plant via stomata or more susceptible interior floret tissues (Bushnell et al. 2003). The primary symptoms of FHB appear as slightly brown water-soaked lesions on the glumes, which progressively increase in size as the fungus spreads to neighboring spikelets (Fig. 1). Under prolonged wet weather, pink spore masses of the fungus become more evident on the infected spikelets, glumes and kernels (Rubella et al. 2004).

Figure 1: Fusarium head blight symptoms on spikelets (bleached spikelets) on wheat head.

Infected wheat heads with FHB often produce small and shriveled kernels (Wegulo et al. 2013) which are of a chalky white or pink color, low test weight and low protein quality (Jin et al. 2014). Infected kernels with *Fusarium* spp. are known as Fusarium-damaged kernels (FDK) (Cowger et al. 2009). FHB can lead to severe losses in yield quality and quantity due to the multifaceted damage of the grains, like the high percentage of FDK, low milling and baking quality, reducing the seed vitality and contamination with mycotoxins (Wegulo 2012). *F. graminearum* and *F. culmorum* produce the most important and common mycotoxins: deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN) (Bottalico and Perrone 2002, D'Mello and MacDonald 1997). Deoxynivalenol (DON) and its derivatives are the most dominant trichothecenes in wheat, barley and triticale (Miedaner et al. 2004, Sugiura et al. 1990, Tanaka et al. 1990). Food and feed contamination by DON has been associated with human and animal toxicity, DON disrupts regular cell function by inhibiting protein synthesis

which prevents signal transduction related to proliferation, differentiation, and apoptosis (Pestka and Smolinski 2005). The maximum allowable DON content in the EU is 1250 µg/kg for unprocessed cereals and 1750 µg/kg for unprocessed durum wheat, oats and maize when used for food (Commission Regulation (EC), 2007). Due to the high economic impact of mycotoxin contamination and its connection with accepting or rejecting shipments within global cereal trade, several technological approaches have been developed to quantify DON concentration in cereal grains. Chromatographical methods including GC (gas chromatography), HPLC (high performance liquid chromatography), and TLC (thin layer chromatography) require long time, high cost and complex treatments. In contrast, enzyme-linked immunosorbentassay (ELISA) is simple, fast and applicable to high throughput analysis (Goryacheva et al. 2009).

1.3 Fusarium head blight resistance in cereals

Continuous attempts to find effective practices to improve resistance for FHB in cereals have been made by breeders and researchers during the last two decades. Consequently, several studies found that genetic resistance represents the best strategy to provide economical and effective management for FHB and mycotoxin contamination (Anderson 2007, Miedaner 1997). Resistance to FHB is quantitatively inherited with a continuous distribution among the progeny in wheat, rye and triticale (Oettler et al. 2004, Buerstmayr et al. 1999, Miedaner and Geiger 1996, Bai and Shaner 1994). Mesterhazy et al. (1999) described five types of FHB resistance: type I: resistance to initial penetration of the pathogen; type II: resistance to disease spread within a spike; type III: resistance to kernel infection; type IV: tolerance and type V: resistance to accumulation of DON. Quantitative trait loci (QTL) for FHB resistance, mainly for type II resistance and reduced DON accumulation, received great attention of

breeders and have been used extensively in genetics studies due to their stability in field (Yu et al. 2007).

Most of the FHB-resistance research had focused on wheat as a direct result of the global economic importance of wheat and its high level of infection compared to other cereals (Foroud and Eudes 2009). In Europe, most of the wheat varieties are moderately resistant obtained due to accumulation of FHB resistance genes in adapted germplasm after numerous cycles of phenotypic selection (Chrpova et al. 2013). In the past few decades, the advent of molecular markers has made it feasible to generate linkage mapping and identify progenies possessing high FHB resistance in wheat (Liu et al. 2009, Löffler et al. 2009, Buerstmayr et al. 1999). The first major QTL for FHB resistance in wheat *Fhb1* was detected on chromosome 3BS in a Chinese variety called Sumai 3 using traditional RFLP markers (Waldron et al. 1999). Since then, an enormous number of QTL studies have been carried out to identify chromosomal regions associated with FHB resistance in wheat (Buerstmayr et al. 2009, Holzapfel et al. 2008). However, only a few of the many QTL reported for FHB resistance in wheat have been validated and found their way into commercial breeding programs (Miedaner and Korzun 2012).

In triticale, only a few studies concerning FHB resistance have been performed. Arseniuk et al. (1993) found that triticale is more resistant to FHB than wheat, and has resistance level comparable to rye. In addition, various levels of resistance to FHB were detected between triticale genotypes (Miedaner et al. 2004, Oettler and Wahle 2001). Miedaner et al (2006) tested five winter triticale populations in different environments, and reported a considerable genotype X environment interaction, similar to that found in wheat. Furthermore, additive gene effects were shown to be an important factor in FHB resistance in triticale, as is the case in wheat and rye (Oettler et al. 2004). To the best of our

knowledge, no previous QTL for FHB resistance in triticale was detected, which makes our work the first to detect FHB resistance QTL in triticale.

1.4 Association mapping for candidate genes of aggressiveness and DON production in *Fusarium graminearum*

Fusarium graminearum is the principal agent of FHB in wheat in Germany (Talas et al. 2011). Aggressiveness is a fundamental characteristic of *Fusarium graminearum* (Wu et al. 2005). Purahong et al. (2014) defined aggressiveness as a quantitative measurement of the level of the disease induced by the pathogen, reflecting its importance to explaining and understanding the interaction between host-pathogen in FHB-wheat system (Wu et al. 2005). Recently, several reports have studied the aggressiveness of *F. graminearum* and report a significant genetic variation for aggressiveness and deoxynivalenol production between different isolates (Von der Ohe et al. 2010, Gale et al. 2002, Xue et al. 2004). Understanding the aggressiveness and the toxin synthesis in *F. graminearum* by illustrating the components involved in these two traits, *i.e.* pathogenicity genes, became necessary (Liu et al. 2010). The full genomic sequence of *F. graminearum* published by the Broad Institute (http://www.broadinstitute.org) facilitates the introduction of new molecular approaches such as association analysis which is based on linkage disequilibrium (LD) to discover new useful allelic variation among different *F. graminearum* isolates (Talas et al. 2012).

Currently, genome-wide scanning and candidate gene approaches are commonly used for genetic dissection of complex and quantitative traits (Zhu and Zhao 2007). The principle of candidate gene association mapping is based on the assumption that nucleotide diversity in genes with known function is compatible with quantitative loci controlling studied traits

(Pflieger et al. 2001). Recently, Talas et al. (2012) suggested that this approach is a powerful method to reveal functional SNPs linked to aggressiveness or DON production for *F. graminearum* populations. Consequently, they identified for FHB aggressiveness one QTN (Quantitative trait nucleotide) in the *Erf2* gene with 13.1% of the explained genetic variance, and two QTN in the *MetAP1* gene explaining 25.6 and 0.5 % of the genetic variance, in addition to one QTN in the gene *Tri1* that explained around 4.4 % of the genetic variance of DON production.

1.5 Aims of this study

The major objective of this study was to evaluate the interaction between FHB and cereals by two models: (1) Screen different populations of triticale against one highly aggressive *Fusarium culmorum* isolate (FC46), (2) investigate the genetic background of aggressiveness and mycotoxin production in natural *Fusarium* populations and its effect on one wheat cultivar. The specific objectives were to study:

1. **Effect of rye dwarfing gene *Ddw1* in triticale (publication 1)**

a) localize and map *Ddw1* in one triticale population,

b) analyze the effect of *Ddw1* on FHB resistance, plant height, and heading stage.

2. **Genetic architecture of FHB resistance in triticale (publication 2)**

a) study the inheritance of FHB resistance in four triticale populations,

b) evaluate correlations between FHB severity and the agronomic traits plant height and heading stage in all populations.

3. **Correlation between FHB and DON content in triticale (publication 3)**

a) investigate the correlation between FHB severity and DON content in one triticale population.

4. **Association mapping between candidate genes and aggressiveness/mycotoxin production in *F. graminearum* (publication 4)**

a) screen seven selected candidate genes for their nucleotide diversity

b) evaluate the association of SNPs with aggressiveness and DON production in a *F. graminearum* population.

2. Publication 1: Effect of a rye dwarfing gene on plant height, heading stage, and Fusarium head blight in triticale (×*Triticosecale* Wittmack)

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Abstract

Hexaploid triticale (×*Triticosecale* Wittmack), an amphiploid hybrid between durum wheat and rye, is a European cereal mainly grown in Germany, France, and Poland for animal feeding. Dwarfing genes might further improve the genetic potential of triticale concerning lodging resistance and yield. However, they might have pleiotropic effects on other, agronomically important traits including Fusarium head blight. Therefore, we analyzed a population of 199 doubled haploid (DH) lines of the cross HeTi117-06 × Pigmej for plant height, heading stage, and FHB severity across 2 locations and 2 years. The most prominent QTL was detected on chromosome 5R explaining 48, 77, and 71 % of genotypic variation for FHB severity, plant height, and heading stage, respectively. Because the markers that detect dwarfing gene Ddw1 in rye are also in our population the most closely linked markers, we assume that this major QTL resembles *Ddw1*. For FHB severity two, for plant height three, and for heading stage five additional QTL were detected. Caused by the considerable genetic variation for heading stage and FHB severity within the progeny with the dwarfing allele, short-strawed, early heading and FHB-resistant lines can be developed when population size is large enough.

3. Publication 2: Genetic architecture of Fusarium head blight resistance in four winter triticale populations

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Abstract

Fusarium head blight (FHB) is a destructive and economically important disease of cereals like wheat, rye, and triticale. In triticale, knowledge of the genetic architecture of FHB resistance is missing but essential due to modern breeding requirements. In the present work, four doubled-haploid triticale populations (N = 120 to 200) were evaluated for resistance to FHB caused by artificial inoculation with *Fusarium culmorum* across four environments. All triticale lines were genotyped by DArT markers. For FHB resistance, seventeen quantitative trait loci (QTL) were detected across all populations; six of them were derived from rye genome and located on chromosomes 4R, 5R, and 7R, which are here reported for the first time. The total cross-validated ratio of the explained phenotypic variance for all detected QTL in each population was 41 to 68%. In all, 17 QTL for plant height and 18 QTL for heading stage were also detected across all populations; 3 and 5 of them, respectively, were overlapping with QTL for FHB. In conclusion, FHB resistance in triticale is quantitatively inherited with a predominantly additive gene action.

4. Publication 3: Correlation between Fusarium head blight severity and DON content in triticale as revealed by phenotypic and molecular data

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Abstract

Fusarium head blight (FHB) is a major problem in small cereals like wheat, rye and triticale. leads not only to reduced thousand-grain weight and grain yield, but also to contamination by mycotoxins, e.g., deoxynivalenol (DON). In this study we analysed the correlation between FHB severity and DON content in a DH population of 146 entries across environments. Additionally, Fusarium damaged kernel (FDK) rating, heading stage and plant height were recorded. Highly significant ($P < 0.001$) genotypic variances were found throughout, but also significant ($P < 0.001$) genotype–environment interaction variances occurred. Correlation between FHB severity and heading stage or plant height was low (r = 0.144 and r = -0.153, $P < 0.10$). A prediction of DON content from FHB severity or FDK rating is not possible caused by low correlations (r = 0.315 and 0.572, respectively, $P < 0.001$). A common quantitative trait locus (QTL) for all FHB-related traits was found on wheat chromosome 2A being of minor importance for FHB severity, but of high importance for DON content and FDK rating. Another QTL on rye chromosome 5R was more important for FHB severity. In conclusion, DON content has to be measured in triticale after selection for FHB severity to gain for healthy and mycotoxin reduced feed.

5. Publication 4: Candidate pathogenicity genes in *Fusarium graminearum* revealed significant associations to aggressiveness and deoxynivalenol production in wheat

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Candidate pathogenicity genes in *Fusarium graminearum* revealed significant associations to aggressiveness and deoxynivalenol production in wheat

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ABSTRACT

Fusarium graminearum sensu stricto is the most prominent pathogen causing Fusarium head blight (FHB) and mycotoxin contamination in wheat in Europe. Aggressiveness defined as the quantity of disease induced by a pathogenic isolate on a susceptible host and deoxynivalenol (DON) production are quantitatively inherited. Our objective was to use candidate gene association mapping of seven genes (*HDF1*, *RAS2*, *Gmpk1*, *Mgv1*, *FTL1*, *TRI6*, and *Erf2*) selected from literature to reveal associations among quantitative trait nucleotides (QTN) and the phenotypic traits in a population of 152 *F. graminearum* isolates sampled from naturally infected wheat. Two field experiments with four environments (location x year combinations) each were conducted with artificial inoculation of the isolates onto wheat heads. Phenotypic data revealed significant (P<0.001) genetic variation for mean FHB rating ranging from 10.7 to 48.9% and for DON production ranging from 0.3 to 13.1 mg kg^{-1} among the isolates. Four genes (*i.e., Gmpk1, Mgv1*, *TRI6*, and *Erf2*) were found to contain QTNs significantly (P < 0.01 using cross-validation) associated to mean FHB rating explaining in total 13.1 % of the genotypic variance. One QTN in gene *Mgv1* was significantly associated to DON production and explained 5.5 % of the total genotypic variance. This confirms the quantitative nature of both traits and the possibility to identify functional SNPs being associated with aggressiveness and DON production in natural *F. graminearum* populations.

Additional key words: DON, FHB, mycotoxins, QTN, SNP

Fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] is a devastating disease of bread wheat (*Triticum aestivum* L.) and other small-grain cereals (Goswami and Kistler 2004). Among all members of the *Fusarium graminearum* species complex (FGSC), *F. graminearum* sensu stricto (s.s., former lineage 7) is the most common species in Germany associated with bread wheat (Talas et al 2011) and will exclusively be analysed in this paper. This disease leads to significant yield losses (Gilbert and Tekauz 2000). Fusarium-damaged kernels are contaminated with mycotoxins which pose a significant risk to human and animal health (Pestka 2010). FGSC species mainly produce type B trichothecenes, including deoxynivalenol (DON) and its acetylated forms 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), but also zearalenone and other mycotoxins (Boutigny et al. 2011; Goswami and Kistler 2005). DON is the predominant and most economically important trichothecene detected in cereals in Europe (Cumagun and Miedaner 2004; Placinta et al, 1999). Therefore, the European Union has set a limit for DON in unprocessed bread wheat for human consumption of 1.25 mg/kg (EU 2006).

Aggressiveness, i.e., the quantity of disease induced by a pathogenic isolate on a susceptible host, and pathogenicity, i.e., the ability to cause disease, are two essential characteristics of *F. graminearum* (Miedaner et al. 2008; von der Ohe et al. 2010; Purahong et al. 2014). Several studies were conducted worldwide to analyze genetic variation of aggressiveness and DON production of *F. graminearum* in order to improve disease control strategies (Cumagun and Miedaner 2004; Goswami and Kistler 2005; Talas et al. 2012a; Wang et al. 2011). The toxic effect of trichothecenes including DON is mainly due to their ability to inhibit protein synthesis and to induce apoptosis (Rocha et al. 2005). DON production has an important role during head blight infection of wheat by *F. graminearum* enabling pathogen spread from infected florets into the wheat rachis (Jansen et al. 2005; Maier et al. 2006). Moreover, many reports revealed that DON plays a key role in *F. graminearum* aggressiveness (Boenisch and Schaefer 2011; Kazan et al. 2012; Walter et al. 2010) although DON production per unit mycelium cannot explain the differences in aggressiveness (Cumagun et al. 2004b). As a conclusion, a large variation has been found among *F. graminearum* isolates for both traits on very small spatial scales (Bai and Shaner 1996; Cumagun and Miedaner 2004; Miedaner and Schilling 1996; Miedaner et al. 2001; Talas et al. 2012a; Walker et al. 2001). Moreover, a quantitative inheritance of both, aggressiveness and DON production, could be clearly demonstrated by analyzing progeny of biparental crosses of genetically diverse *F. graminearum* isolates (Voss et al. 2010). However, until now no explanation for the tremendous genetic variation of both traits within the species *F. graminearum* is available. This might be caused by different alleles of structural and/or regulatory genes affecting phenotype.

Cumagun et al. (2004a) detected two neighboring QTL linked to *TRI5* locus explaining 51% and 29% of the observed variation for aggressiveness in a quantitative trait loci (QTL) study using 99 haploid progeny from a cross of *F. asiaticum* (former lineage 6, nivalenol producer) and *F. graminearum* s.s. (former lineage 7, deoxynivalenol producer). However, this result refers to an interspecific cross within FGSC and the *F. asiaticum* isolate had an extremely reduced aggressiveness being unique among the hundreds of isolates analyzed so far. In a more recent study, Talas et al (2012b) reported two significant SNPs associated with aggressiveness and explaining 25% and 13% of the genotypic variance in the candidate genes *MetAP1* and *Erf2* and one SNP significantly associated with DON production in gene *TRI1*. Most of the other trichothecene-encoding genes are located in the *TRI5*-gene cluster that contains up to 14 genes coding for proteins involved in production of DON and its acetylated derivatives (Kimura et al. 2007). With the respective *Tri5* gene, low amount of SNPs and no significant association with DON production was found in our population (Talas et al. 2012b)

indicating that this fundamental gene coding the first enzyme in the trichothecene pathway is not subject to many functional alterations/alleles.

Hundreds of genes have been characterized affecting host-fungus interaction in agricultural pathosystems, many of them were assigned to play a role in aggressiveness of *F. graminearum* (Kazan et al. 2012; Geng et al. 2014; Urban and Hammond-Kosack 2012). However, such experiments can only analyze the effect of a single gene on pathogenity and/or aggressiveness, but could not explain the quantitative differences among isolates. Candidate gene association approach appears as a promising and potentially powerful tool to detect functional SNPs causing differences in aggressiveness and DON production in *F. graminearum* populations. This got possible since the full sequence of the *F. graminearum* genome with four chromosomes and about 14,000 identified gene models is publicly available (Cuomo et al. 2007). Consequently, our objectives in this study were to (i) screen SNPs of seven candidate genes selected from literature for their association with aggressiveness and DON production in a *F. graminearum* population and identify causal quantitative trait nucleotides (QTNs), (ii) verify the previously identified SNPs in the yet uncharacterized gene *Erf2* (Talas et al, 2012b) with a larger population size (N=152 instead of N=77), (iii) estimate the proportion of explained phenotypic variance by each QTN in a *F. graminearum* population of 152 isolates.

MATERIALS AND METHODS

Fungal materials and field trials. All isolates were sampled from eleven agricultural fields in Germany. Collections were performed by picking arbitrarily 30 visually infected heads per field with 10 heads each from the front, middle, and end of the field. Sampling was done at milk-ripening stage where the typical bleaching of FHB could be clearly seen. Winter wheat (*Triticum aestivum* L.) heads with FHB symptoms were collected to establish a fungal population of *F. graminearum*. One isolate from each infected head was used to obtain a single-spore culture followed by morphological and molecular analysis for their species identity and chemotype as described previously in detail (Talas et al. 2011). In total, 152 single-spore isolates of *F. graminearum* sensu stricto (former lineage 7) were used for analysis for aggressiveness and DON production under field conditions.

Field experiments were conducted in two locations: Hohenheim (HOH, longitude 9° 11' 23" E, latitude 48° 42' 54" N, altitude 403 m) and Oberer Lindenhof (OLI, longitude 9° 18' 17", latitude 48° 28' 25" N, altitude 702 m). Phenotypic data of aggressiveness and DON production for the 152 isolates of *F. graminearum* were taken from two experiments. The first experiment was performed at HOH and OLI in 2009 and 2010 with 70 *F. graminearum* isolates and was reported previously (Talas et al, 2012b). The second experiment was performed with 69 new isolates in addition to 13 isolates repeated from the first experiments to facilitate the statistical analysis for the two experiments. Field trials were conducted in the same locations over two subsequent years, 2011 and 2012. Hence, each experiment took place in four location x year combinations (=environments). The first experiment was assigned to a randomized complete block design with three replications, the second experiment followed a design with incomplete blocks (α design) with two replications. Thirteen isolates were the same in both experiments. Additionally, standard isolates were used to fill up the design.

For both experiments a moderate susceptible spring wheat cultivar as host was used ('Taifun', KWS LOCHOW GMBH, Bergen, Germany). Plants were grown in two-rowed micro-plots of 1 m length and 0.417 m width. Each entry plot was separated by four plots of similar size that were planted with the

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. Control plots in all experiments were left without inoculation to monitor the natural infection level. For inoculation, spore suspensions with a concentration of 2×10^5 spores ml^{-1} were sprayed during full flowering, i.e., the most susceptible wheat stage for FHB infection, onto wheat heads of each plot. Inoculum was sprayed using a hand atomizer with constant air pressure of 3 bar from a tractor to ensure full coverage of all heads of the plot with the same dosage.

FHB aggressiveness was visually rated in each plot three times starting with the onset of symptom development. Rating was performed as percentage infected spikelets per plot (0-100%). This rating reflects the number of infected spikes per plot and the number of infected spikelets per spike and corresponds to FHB index. Wheat plots were harvested at full ripening by hand, threshed, cleaned, and ground with a 1mm sieve. Later the coarse meal was analyzed to quantify the amount of DON by a commercially available immunotest (R-biopharm AG, Darmstadt, Germany) as previously described in detail (Talas et al. 2012a).

Population structure assessment and genes selection. Population structure of the tested isolates in our study was performed by fingerprinting all isolates by 18 simple sequence repeat (SSR) markers dispersed throughout the whole genome following standard protocols (Talas et al. 2011). Candidate genes reported to have a role in the pathogenesis of *Fusarium* spp., aggressiveness and/or trichothecene biosynthesis were used in our study. Moreover, *Erf2* was reported in related study to contain one significant SNP associated with aggressiveness in *F. graminearum* (Talas et al. 2012b). Nucleotide sequences of these genes were obtained from *Fusarium graminearum* database FGDB (Wong et al. 2011). The selected genes were (Table 2): *HDF1* (FGSG_01353), *RAS2* (FGSG_07024), *Gmpk1* (FGSG_06385), *Mgv1* (FGSG_10313), *FTL1* (FGSG_00332), *TRI6* (FGSG_16251), and *Erf2* (FGSG_08531). Specific primers for each gene (Suppl. Table 1) were designed to amplify parts of these genes using the software CLC genomic workbench (CLC Bio version 6.0). A polymerase chain reaction (PCR) was conducted using the designed primers for each gene, following a standard protocol with different annealing temperatures accordingly (Suppl. Table 1). The sequence was performed once for each isolate, unless the alignment contains sequence noise. Expected sizes of PCR products were obtained from all isolates for all tested genes. Sequences of the genes *Erf2*, *Gpmk1*, *Mgv1* and *FTL1* stretched over two, four, five and six exons, respectively. The sequences were aligned, according to the initial matrix published in FGDB (*F. graminearum* PH-1) using CLC sequence viewer 6.3 (CLC Bio, Denmark) to identify single nucleotide polymorphisms (SNPs) among the 152 isolates.

Phenotypic and molecular data analyses. Phenotypic data of aggressiveness and DON production were obtained from two experiments in two locations across four years. Fusarium head blight data were subjected to arcsine square-root transformation, DON production to log transformation for all computations to reduce heterogeneity of variances. A two-step analysis was used to analyze the phenotypic data. Adjusted entry means (BLUEs) were calculated for each environment separately. The adjusted entry means from each location were used in a second step to estimate variance components. HOH 2009 was excluded due to an extreme high genotype x environment interaction produced by this environment for mean FHB rating.

Variance components were determined by the restricted maximum likelihood (REML) method. Heritability (H^2) was estimated on an entry-mean basis following Hallauer and Miranda (1988) following the formula:

$$H^2 = \sigma_G^2/\sigma_G^2 + \sigma_{GE}^2/e + \sigma_e^2/re$$

where σ_G^2 denotes the genotypic variance, σ_{GE}^2 the genotype × environment interaction variance, σ_e^2 the error variance, *e* and *r* are the numbers of environments and replications, respectively. All statistical analyses were performed using ASReml 3.0 (Gilmour et al. 2009) and R (R Development Core Team 2012).

Genetic relatedness among the 152 isolates was determined by applying principal coordinate analysis PcoA (Gower 1966) based on the pairwise Rogers' distance among the individuals (Wright 1978). Linkage disequilibrium (LD) between the selected SNPs was measured by squared coefficient of correlation (r^2). LD and PCoA computations based on modified Rogers' distance were performed with the R software package Plabsoft (Maurer et al. 2008).

Association mapping. For association mapping a two-step association analysis with a linear mixed model incorporating a kinship matrix as described previously was used (Talas et al. 2012b). In brief, the best linear unbiased estimates (BLUEs) across environments were used as input for the association analysis.

The used linear mixed model was:

$$y_{ijn} = \mu + a_p + Iso_i + Env_j + e_{ijp}$$

where a_p is the effect of allele *p*. The allele effect a_p was modeled as fixed effect while Iso_i and Env_j were regarded as random effects. The variance of the random genetic effect was presumed to be $Var(g) = 2K\sigma_G^2$, where σ_G^2 refers to the genotypic variance estimated by REML and K was a 152 × 152 matrix of kinship coefficients that define the degree of genetic covariance between all pairs of entries. Calculating of the kinship coefficient K_{ij} between isolates i and j on the basis of the SSR marker data was done following the suggestion of Bernardo (1993) as described by Talas et al (2012b). The total 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 (Utz et al. 2000). Fivefold cross-validation was done to assess unbiased estimates of the proportion of genotypic variance explained by the detected SNP as described previously (Würschum and Kraft 2014). Out of this analysis, we give the frequency of recovery, *i.e.*, the percentage of validation runs detecting the respective SNP. Only SNPs with minimum allele frequencies >5% were regarded in the analysis.

RESULTS

Phenotypic data. All 152 *F. graminearum* isolates successfully produced symptoms on the inoculated wheat spikes at all environments except HOH 2009 (Table 1). Hence, we deleted this environment from our analysis for FHB severity. The average of mean FHB infection among all isolates was 32.9% ranging from 10.7 to 48.9% and for DON production the overall mean was 7.3 mg kg^{-1} ranging from 0.3 to 13.1 mg kg^{-1} (Fig. 1). Genotypic variances across all environments were significantly (P<0.001) different from zero, with large effect of genotype x environment interaction. Thus moderate entry mean heritability estimates were achieved for mean FHB rating (0.64) and DON production (0.64, Table 1). The frequency of aggressiveness and DON production was continuous and followed a normal distribution of the best linear unbiased estimated means (BLUEs) for both traits (Fig. 1).

TABLE 1. Means and ranges of Fusarium head blight (FHB, arcsin transformation) and DON production (log transformation) in eight environments and estimates of variance components (σ_G^2 = genotypic, σ_{GxE}^2 = isolate-environment interaction, σ_e^2= mean error variances across all environments) and entry-mean heritabilities (h^2) after inoculation with *Fusarium graminearum* isolates

Parameter	FHB severity (%)	DON concentration (mg kg^{-1})
Mean and ranges[a]		
HOH 2009	-[b]	8.8 (1.5 – 16.4)
OLI 2009	27.3 (13.6 – 44.9)	11.5 (5.5 – 18.2)
HOH 2010	60.1 (23.7 – 76.3)	9.2 (1.6 – 21.9)
OLI 2010	31.1 (11.3 – 56.7)	12.9 (1.5 – 27.2)
HOH 2011	28.1 (12.4 – 43.8)	11.4 (1.4 – 29.5)
OLI 2011	23.6 (6.6 – 46.8)	3.8 (0.6 – 11.3)
HOH 2012	32.5 (15.6 – 49.4)	42.1 (3.0 – 82.8)
OLI 2012	26.4 (8.1 – 45.0)	14.3 (1.3 – 35.5)
Variances (σ^2) and heritabilities[c]		
σ_G^2	2.598×10^{-3} ***	1.886×10^{-2} ***
σ_{GxE}^2	4.002×10^{-3}***	3.243×10^{-2} ***
σ_e^2	2.671×10^{-3}	2.572×10^{-2}
h^2	0.64	0.64

*** Significant at P<0.001.
[a] Backtransformed data.
[b] Excluded due to extreme high genotype x environment interaction.
[c] Calculated with transformed data.

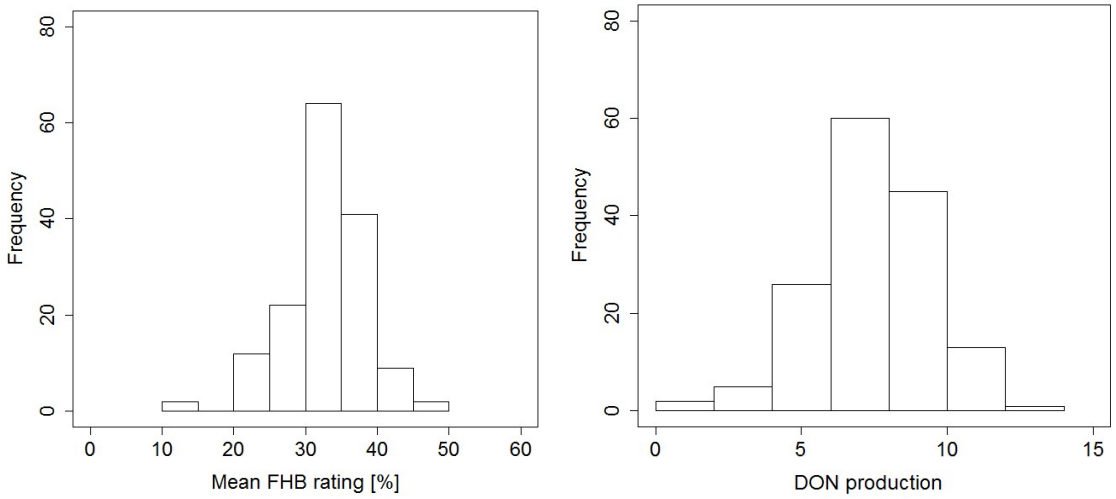

Fig. 1. Histograms of best linear unbiased estimates (BLUEs) for mean Fusarium head blight (FHB) rating and DON production for 152 *F. graminearum* isolates across seven (FHB severity) and eight (DON production) environments

Population structure and linkage disequilibrium. Principle coordinate analysis (PCoA) based on modified Rogers' distance revealed no distinct grouping pattern among *F. graminearum* isolates (Figure 2). High nucleotide diversity was found in most genes (Table 2). However, the percentage of polymorphic sites per sequenced region of each gene (without singletons) varied from 1.2% in *Gpmk1* to 9.6% in *Mgv1*. Generally low LD was detected within the genes over their physical distance (Fig. 3). As expected, two gene pairs situated on the same chromosome showed significant LD (*HDF1-Mgv1*, *Erf2 – RAS2*). However, in most cases LD was detected among genes from different chromosomes (Fig. 3). The genes *HDF1/Mgv1, Tri6, Gmpk1, Erf2/RAS2* showed strong LD with each other.

Association analysis for aggressiveness and DON content. Four genes (*i.e., Gmpk1, Mgv1*, *TRI6*, and *Erf2*) were found to contain SNPs significantly ($P < 0.01$ using cross-validation) associated to mean FHB rating explaining in total 13.1 % of the genotypic variance across all SNPs with significant association (Table 3). More specifically, three SNPs within the gene *TRI6* were significantly associated with mean FHB rating with an explained genotypic proportion of variance of about 5% for each SNP. Two SNPs positioned in *Erf2* and significantly detected as QTNs assisted to mean FHB rating explaining each about 3% of the genotypic variance. One SNP was found in gene *Gpmk1* and one SNP in *Mgv1* significantly associated with mean FHB rating explaining 8.3% and 9.7% of the genotypic variance, respectively. In contrast, just one SNP in *Mgv1* was significantly associated to DON production and explained 6.5 % of the total genotypic variance.

TABLE 2. Sequenced regions of candidate genes, number of single nucleotide polymorphisms (SNPs) detected and those used for analysis, and nucleotide diversity for all candidate genes

Gene ID[a]	Sequenced region relative to the ATG[b]	No. of SNPs detected	No. of SNPs without singletons	No. of SNPs used for analysis	Nucleotide diversity (%)[c]
HDF1 (FGSG_01353)	170-770	43	34	12	2.5
	850-1400				
RAS2 (FGSG_07024)	1-640	63	28	11	4.5
Gpmk1 (FGSG_06385)	1-580	14	8	7	1.2
Mgv1 (FGSG_10313)	34-610	77	62	20	9.6
TRI6 (FGSG_16251)	53-520	73	17	11	3.2
FTL (FGSG_00332)	820-1431	72	29	25	4.3
	1500-1860				
Erf2 (FGSG_08531)	1180-1900	75	49	10	7.4

[a]The given ID (FGSG) is the entry number of the MIPS *F. graminearum* genome database (Wong et al. 2011).
[b] The position is relative to the start codon (ATG).
[c]Nucleotide diversity is the frequency of SNPs (without singletons) relative to the total length of the sequenced gene region

DISCUSSION

Despite the high number of studies conducted on genetics of *F.graminearum* and the resistance to FHB in wheat, many regulatory mechanisms controlled by gene networks that may related to aggressiveness or DON biosynthesis are still uncharacterized (Liu et al. 2010). Association mapping approach based on candidate genes appears as a promising tool to identify specific functional variants (loci, alleles) linked to phenotypic variance of quantitative traits (Gomez et al. 2011). Recently, a first identification of a few quantitative trait nucleotides (QTNs) in candidate genes towards aggressiveness and DON production in a smaller *F.graminearum* population (Talas et al. 2012b) was the first proof of concept that paved the way for additional work. Overall aim is to identify functional nucleotide diversity in genes known for their pathogenicity function to explain the large quantitative variation of aggressiveness and DON production in *F. graminearum* poulations.

Evaluating phenotypic data. Analyzing the phenotypic variation of *F. graminearum* populations for aggressiveness and DON production is a prerequisite for association mapping. Significant genotypic ($P < 0.001$) variance for FHB severity and DON production among the 152 isolates across environments confirms results of previous studies on *F. graminearum* populations (Guo et al. 2008; Talas et al. 2012a). It should be noted that also the isolate by environment interaction played a major role and, therefore, testing in different locations and years is indispensable to get reliable results in *F. graminearum*. Sexual reproduction may explain the high genetic variation (Chen and Zhou 2009), especially when taking into account that our isolates were collected from different wheat fields (Talas et al. 2012b). Hence, this population of isolates is suitable for further molecular studies.

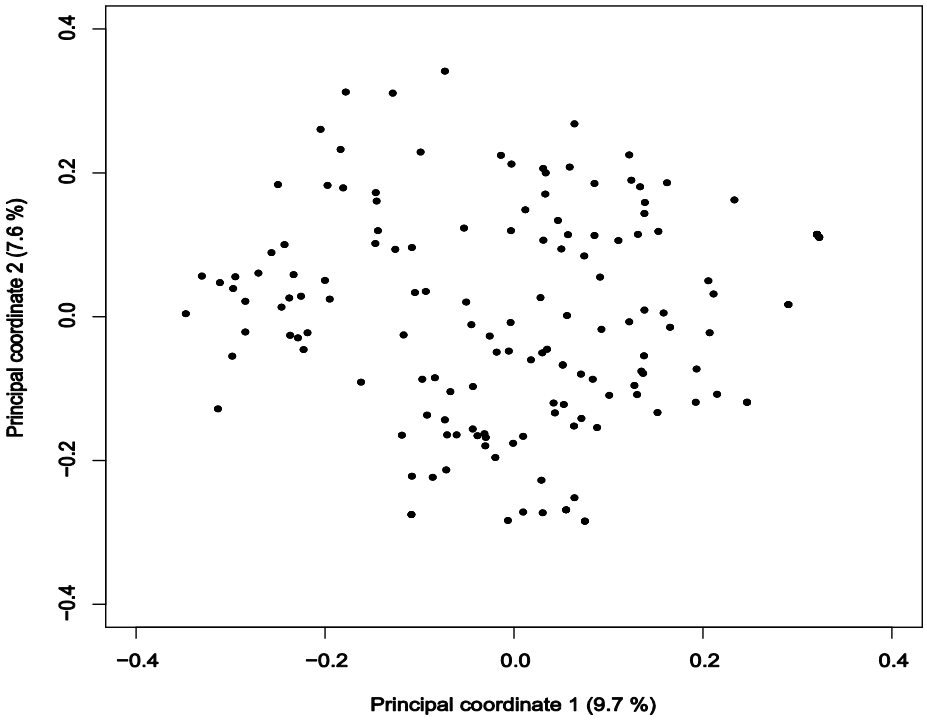

Fig. 2. Population structure and familial relatedness. Principal coordinate analysis of the 152 *F. graminearum* isolates, based on modified Rogers' distance. Number in parentheses refer to the proportion of variance explained by the first two principal coordinates.

Population structure. Taking into account the population stratification and cryptic relatedness in association mapping studies is essential to avoid the risk of inflating false-positive quantitative trait loci (QTL) (Sillanpää 2011; Würschum and Kraft 2014). According to PCoA analysis results, in our *F. graminearum* population no subpopulations were observed (Fig. 2). However, we conducted a correction for familial relatedness between isolates to avoid any possible false positive discoveries. Therefore, trait-SNP association was investigated in detail with the K model, which incorporated estimates of kinship coefficients based on SSR data. Additionally, we performed a cross-validation to filter out the most stable SNP-trait combinations (Würschum and Kraft 2014). In accordance to the high isolate x environment interaction variance we report only SNPs that showed significant association across all environments.

Nucleotide diversity in the *F. graminearum* population. Regions with high SNP density in *F. graminearum were reported to be* highly enriched with *genes* expressed *specifically* during plant infection (Cuomo et al. 2007). Most of these genes are of regulatory function such as major facilitators or transporters, whereas the conserved, so called housekeeping genes are outside the high nucleotide diverse regions. Finding that some regulatory genes such as *TRI6* and *Erf2* contained SNPs highly associated to aggressiveness in our candidate gene association approach, support the idea that regulatory genes are essential for quantitative variation in aggressiveness and mycotoxin production (Talas, *personal communication*).

In our data the highest nucleotide diversity was detected in *Mgv1* and *Erf2* genes (Table 2). However, high within-gene diversity is only a prerequisite, there seems to be no association between the magnitude of nucleotide diversity and number of detected functional SNPs. For example, three significant SNPs associated with FHB aggressiveness were found in *TRI6* although nucleotide diversity for this gene was low (3.2%). In conclusion, the nucleotide polymorphism within the genes rather indicates frequent intragenic recombination events.

TABLE 3. Single nucleotide polymorphisms (SNPs) in the candidate genes significantly associated with mean Fusarium head blight (FHB) and/or DON production and their characteristics (FreqCV = frequency of occurrence in cross validation procedure after 1.000 runs, p_G = proportion of explained genotypic variance)

Trait/ Candidate gene	SNP	Position[a]	Poly-morphism	Changes in amino acids	P-value	FreqCV	p_G (%)
Mean FHB rating (%):							
Gpmk1	SNP 1[b]	21	T/C	P/P	0.001	0.747	8.3
Mgv1	SNP 1[c]	513*	-/A	D/R	0.003	0.599	9.7
TRI6	SNP 1[c]	215	A/T	Y/F	0.009	0.334	5.3
	SNP 2[c]	217	T/C	S/P	0.009	0.334	5.3
	SNP 3[c]	393	A/G	E/E	0.009	0.334	5.3
Erf2	SNP 1[c]	1619	G/A	C/Y	0.006	0.459	2.8
	SNP 2[c]	1717	G/A	G/S	0.006	0.459	2.8
DON production (mg kg^{-1}):							
Mgv1	SNP1[b]	57	T/C	V/V	0.007	0.321	6.5

[a]The position is relative to the start codon (ATG).
[b]Minor allele frequency (MAF) > 5%
[c]Minor allele frequency (MAF) < 5%
*Insertion

Association mapping of genes underlying aggressiveness and DON production. In our study, we used association analysis to determine the relationship between detected nucleotide polymorphisms in seven candidate genes and the differences in aggressiveness and DON production for 152 isolates of *F. graminearum.* The genes were selected according to literature results. For three genes (*HDF1*, *RAS2*, and *FTL1*), no significant association to the phenotypic traits was detected in our study although they were reported to be important for pathogenicity in other plant-pathogenic fungi. Histone modifications have been involved in regulating genes essential for pathogenicity, stress response, and secondary metabolism in several pathosystems (Ding et al. 2009; Li et al. 2011). Genome sequence of *F. graminearum* indicate the presence of three genes belonging to class II histone modifications mediated by histone deacetylases (HDAC) genes. The major gene in this class is *HDF1* (Li et al. 2011). Deletion of *HDF1* significantly reduced the virulence in infection assays with flowering wheat heads and corn stalks, as well as it plays an important role for sexual reproduction in selfings of *F.graminearum* (Li et al. 2011). *FTL1* is also involved in histone deacetylation and may interact with *HDF1* (Ding et al. 2009; Li et al. 2011).

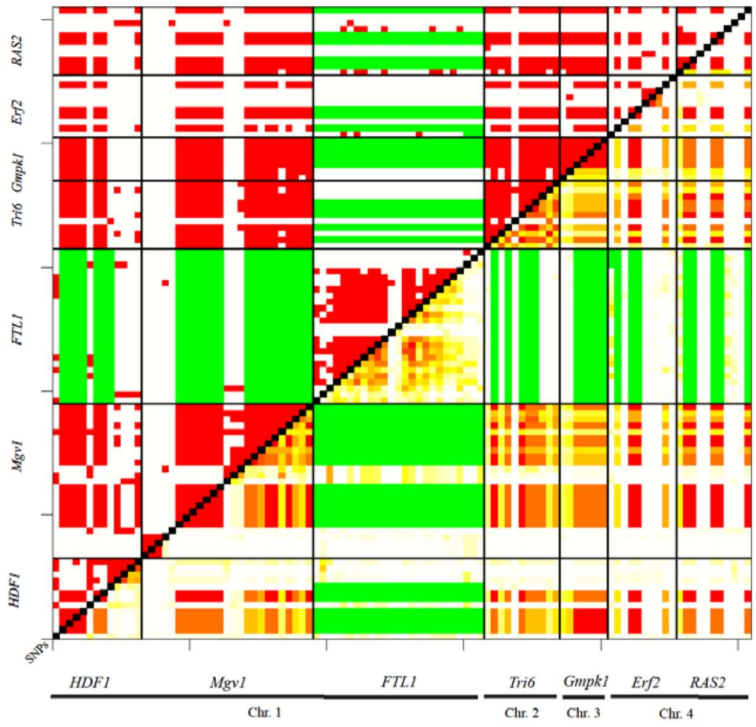

Fig. 3. Pairwise linkage disequilibrium (LD). The structure of LD within and among seven candidate genes for 152 *Fusarium* isolates. Significant LD ($P < 0.05$, above diagonal) and LD measured as r^2 between all pairs of selected SNP loci (below diagonal). The horizontal and vertical lines separate 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 $r^2 = 0$. Green indicates missing data.

In *F. graminearum, FTL1* was found to be involved in novel mechanisms regulating the fungal infection processes, but no role affecting DON production for this gene was found (Ding et al. 2009). Four genes showed significant associations to fungal aggressiveness and/or DON production. *Gpmk1* and *Mgv1* are two MAP kinases known to play a functional role in pathogenesis of *F. graminearum*. *Gpmk1* was reported to be essential for sexual reproduction and pathogenicity in *F. graminearum* (Bluhm et al. 2007; Jenczmionka et al. 2003; Urban et al. 2003), one SNP in *Gmpk1* found to be significantly associated with aggressiveness in our population. This is in accordance to Bluhm et al. (2007) who found that a *F. graminearum* isolate with *Gpmk1* gene knockout was significantly reduced in its virulence but with no change in DON production in inoculated wheat kernels. This could explain why no SNP was detected significantly associated with DON production in our data. The *Mgv1* gene was found to be necessary for female fertility during sexual reproduction (Hou et al. 2002). Vegetative growth of *Mgv1* deletion mutants of *F. graminearum* was reduced on solid media, additionally, the *Mgv1* mutants had an impaired cell wall. As a result, their aggressiveness was severely reduced (Hou et al. 2002). Accordingly, we found one SNP in this gene to be significantly

associated with aggressiveness and another SNP with DON production. The *Mgv1* gene was the only detected gene which contained significant SNPs associated with both traits.

A significant association between SNPs of the putative candidate gene *Erf2* and FHB aggressiveness in wheat was reported previously (Talas et al. 2012b). Although no direct role for this gene in FHB infection and development was reported in literature, *Erf2* is considered as a component of the processing that precedes palmitoylation of RAS2 genes which have known effect on the pathogenicity of *F. graminearum* (Bluhm et al. 2007). Astonishingly, our sequenced part of the *RAS2* gene had no impact on any of the traits. Two significant SNPs associated to mean FHB rating in *Erf2*, however, could be validated in this study with a larger population size supporting the role of *Erf2* in *F. graminearum* aggressiveness. This could be a starting point for functionally characterizing this gene.

In *F. graminearum*, *Tri* genes involved in trichothecene biosynthesis have been described and studied in detail (Kimura et al. 2007). Among all *Tri5* cluster genes involved in trichothecene biosynthesis, *TRI6* gene has particular position. *TRI6 gene is known to have a regulatory role in* trichothecene biosynthesis pathway. A *F. graminearum* isolate with a *TRI6* deletion mutant was reduced in pathogenicity and toxin production (Seong et al. 2009). In addition, *TRI6* affects the expression of several other genes that are related to housekeeping functions, secondary metabolism and pathogenesis (Seong et al. 2009). Recentally, Nasmith et al (2011) confirmed that *TRI6* is a global transcription regulator affecting the whole growth and developmental system in *F. graminearum*. Three significant SNPs were detected in the *TRI6* gene in our study, interestingly all of them were associated with FHB aggressiveness and no SNP was significantly associated with DON production. Our results are consistent to the fact that the *TRI6* gene has a great influence in the whole pathogenicity system of *F. graminearum* (Nasmith et al. 2011; Seong et al. 2009).
In conclusion, candidate gene association mapping is a powerful tool to detect genes responsible for phenotypically observed traits. It is a resource demanding method, but in contrast to genome-wide associations studies (GWAS), it directly detects functional genes that could be further analyzed by gene knock out, over expression or gene silencing techniques. Because aggressiveness and DON production are quantitatively inherited traits, each single gene has only a limited effect as illustrated by our small estimated values of the proportion of genotypic variance p_G (Lynch and Walsh 1998). However, by continuing and intensifying such studies, networks of functional genes quantitatively regulating aggressiveness and DON production should become visible in future.

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Supplemental Table 1. Names of tested genes, chromosomal localization, primer sequences and expected amplified DNA products relative to gene size at temperature (Ta)

Gene ID[a]	Chromo-some		Sequences of the primers	Ta (°C)	Expected products (bp)	Gene size (bp)
*HDF1** (FGSG_01353)	1	F	TCAGAACCATCATCCACAT	56	690	1509
		R	GCTCACCACGCTTTCAAT			
		F	GGGAAGAAGTTGTTTGGG	54.7	642	
		R	CGAGGAATCTGGCATCAC			
RAS2 (FGSG_07024)	4	F	CAACACACTCATACACCCTC	56	753	783
		R	GCTTCTCATTTTCGCTGT			
Gpmk1 (FGSG_06385)	3	F	GACAACCTTTCCACCTTCT	53.7	648	1246
		R	GAGATCACAGTTGGCGTT			
Mgv1 (FGSG_10313)	1	F	ACACCACCACACAAATAC	52.3	644	1541
		R	TAAAGGATTGGAAGTGGG			
Tri6 (FGSG_16251)	2	F	GAGGCCGAATCTCA	54.4	600	673
		R	ACCCTGCTAAAGACCCT			
*FTL** (FGSG_00332)	1	F	TTGCATAAATGGGAGGAGA	52	509	2377
		R	CCTTTACCTTGTTCACTTGT			
		F	CGACAAGTGAACAAGGTAA	45	664	
		R	GAGTGGAACCCAAGAGAC			
Erf2 (FGSG_08531)	4	F	GCATCTTTGTTGTTGT	52	750	2095
		R	GGTAATACGTGGGTTGT			

*Two primers were used for gene sequencing.

[a]The given ID (FGSG) is the entry number of the MIPS *F. graminearum* genome database (Wong et al. 2011).

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6. General Discussion

Breeding cereals for FHB resistance has received increasing attention from breeders during the last few decades. Additionally, enhancing FHB resistance in small-grain cereals like wheat, barley and triticale became an urgent task due to the great contribution to food and feed safety (FAO 2010). However, achieving an effective breeding system requires combining different FHB resistance sources and desirable agronomic performance by integrating multiple genes, considering that additive effects are a substantial component of FHB resistance (Bai et al. 2000). Another major challenge for cereal breeders is to screen the available genetic resources for promising genotypes that do not permit high mycotoxin accumulation. Consequently, cereal breeders aim to produce superior genotypes that maintain sustainable high productivity and resistance to FHB combined with no or minimum mycotoxin accumulation (Bai et al. 2001a).

6.1 Genotypic variance of triticale populations for FHB resistance

Fusarium head blight (FHB) caused by the ascomycete fungi *Fusarium graminearum* (Schwabe) and *F. culmorum* (W.G. Sm.) Sacc, is a widespread disease on different small-grain cereals (Miedaner et al. 2004). So far, wheat (*Triticum aestivum* L.) has received the most attention from breeders and researchers due to its high economic value and global increasing demand. However, triticale is also susceptible to FHB, and infection with this disease can lead to losses similar to that reported in wheat and rye (Oettler and Wahle, 2001). Only few studies were conducted on FHB resistance in triticale, and their results were limited to genetic components of variation, heritability, and correlations between FHB

symptoms and different agronomic traits (Veitch et al. 2008, Arseniuk et al. 1993, Oettler and Wahle 2001, Miedaner et al. 2006). Therefore, research is required on the molecular level as a basis for marker-assisted FHB resistance breeding.

In the present study, five biparental (DH) mapping populations were used to investigate FHB resistance in triticale, in addition to related agronomic traits in different locations and years. To optimize the experimental conditions in all field locations, several recommended techniques were followed in our work. A highly aggressive DON producing isolate of *F. culmorum* (FC46) was used as a source of inoculum at all environments. Using highly aggressive isolates is preferential to set a clear differentiation among the studied genotypes and discard the susceptible ones (Sip et al. 2011, Mesterhazy et al. 2003). Spray inoculations were conducted two to three times in all environments to ensure inoculation at mid-anthesis for each genotype, due to the wide variation of flowering time among triticale populations. Normally, symptoms develop within 14 days post inoculation, however weather conditions have a great impact on the development of FHB-symptoms in the field (Doohan et al. 2003). Thus, several ratings were performed in order to have a relatively unbiased score for FHB infection (Miedaner 1997). Even though the inoculation date was adjusted to the flowering time, some genotypes with late heading time showed a tendency to escape infection. Hence, heading date corrected means for FHB rating for each genotype were used for QTL analysis when necessary (Kalih et al. 2015, Emrich et al. 2008). All genotypes in all locations showed a successful disease development post inoculation and all populations revealed continuous distribution of FHB rating following a normal distribution.

Significant genotypic variance was observed among the populations, hence heritabilities for FHB estimates were relatively high > 0.80 (Kalih et al. 2015). This fits well with results from previous studies on triticale (Miedaner et al. 2006, Miedaner et al. 2004, Oettler and Wahle

2001), and supports the conclusion that resistance-breeding experiments in small-grain cereals require an accurate inoculation and rating method suitable for multiple environments in order to distinguish between the inherited resistance (i.e., our target as breeders) and the environmental adaptation that normally is referred to genotype x environment interaction. In general, precise estimation of the phenotypic data is the key factor to achieve substantial QTL identification in molecular based breeding experiments (Charcosset and Gallais 1996).

6.2 Association between FHB resistance and related agronomic traits in triticale

Plant height, heading date and flowering time have been reported to be highly associated with FHB severity in wheat (Liu et al. 2013, Klahr et al. 2007, Steiner et al. 2004). Highlighting the effect of these agronomic traits on the analysis of FHB resistance is quite important due to their role in passive resistance during disease infection and development in the field. For example, tall or late heading plants generally have lower FHB severity than the short or early heading ones (Buerstmayr et al. 2009, Mesterhazy 1995).

Flowering time is the most sensitive period during cereal growth, especially if it is accompanied by warm weather and high humidity which enhance infection (Buerstmayr et al. 2012). Variation in flowering date among wheat genotypes can potentially influence the initial infection and FHB development (Liu et al. 2013). In triticale, the period of flowering is longer compared to wheat, with significant variation between genotypes and strong effect of environmental conditions (Oettler 2005). In the present study, significant variation in the flowering time was also observed among genotypes. Flowering occurred over a period of

two weeks in colder locations (i.e., Oberer Lindenhof). In large breeding programs with a high number of genotypes and replications, flowering time assessment is difficult and time consuming. Therefore, we considered the heading time as another reliable trait in our study.

Heading stage (HS) in wheat is controlled by multiple genes and influenced by environmental conditions (Zhang et al. 2009). Generally, a negative correlation between heading time and FHB development/severity can be noticed under field conditions (Emrich et al. 2008). Similar correlations between heading stage and FHB in wheat have been reported in several studies (Schmolke et al. 2005, Paillard et al. 2004, Gervais et al. 2003). Heading stage of all five populations was normally distributed and possessed high heritabilities and significant genotypic variance across all environments (kalih et al. 2015, kalih et al. 2014, Miedaner et al. 2016). Correlation between heading stage and FHB was significant only in populations C and D. This can be explained by the relatively large effect of the detected QTL for heading stage in both populations compared to the other populations. Of the six QTL, four are major (R^2 explained phenotypic variance > 10%) in population C, and four major QTL for heading time were found in population D (Kalih et al. 2015).

Significant association between FHB severity and plant height was reported in previous studies dealing with FHB resistance in wheat (Miedaner and Voss 2008, McCartney et al. 2007, Gervais et al. 2003, Yan et al. 2011, Hilton et al. 1999). In general, taller cultivars tend to have a lower level of FHB infection, while shorter cultivars tend to develop more severe disease especially if these varieties carry the dwarfing allele *Rht-D1b* (Buerstmayr et al. 2000, Hilton et al. 1999, Mesterhazy 1995). With natural infection, shorter plants can receive more easily Fusarium spores coming from the debris on the soil surface which is the primary source of infection, due to the reduced distance between the spikes of short plants and the ground (Miedaner and Voss 2008). To avoid such differences between tall and short

genotypes in our experiments, artificial inoculation was performed on the heads (i.e., the inoculum had direct contact with the heads) two to three times during flowering time in all environments. All populations showed wide variation in plant height and normal distribution except the population D that showed bimodal distribution due to the effect of dwarfing gene *Ddw1* (Kalih et al. 2014). Significant negative correlations were observed between PH and FHB severity in four populations, which generally agreed with previous studies in wheat (Voss et al. 2008, Steiner et al. 2004, Buerstmayr et al. 2000). Only in population E the correlation was low, which is compatible with detecting no common QTL for FHB resistance and plant height in this population (Miedaner et al. 2016). However, one to five QTLs for plant height were reported in each triticale population in our study, two of them co-localized with QTL of FHB resistance (kalih et al. 2015, kalih et al. 2014) which strongly suggests direct or indirect effects of height differences *per se* (Chen et al. 2014).

6.3 Association of *Ddw1* gene with Fusarium head blight resistance in triticale

Controlling plant height is an important goal in small-grain cereal breeding programs due to the significant effect of plant height on lodging, grain yield, and grain quality (Griffiths et al. 2012). Identification of dwarfing genes (*Rht-B1b* and *Rht-D1b*) in wheat enables breeders to achieve significant shorter wheat in parallel with an increase in grain yield (Flintham et al. 1997). Other dominant dwarfing genes were suggested to be used in other species, like *Ddw1* in rye (Banaszak 2010). *Ddw1* was commonly used in Eastern Europe by introducing the dwarfing mutant EM-1 in rye breeding programs (Korzun et al. 1996). In general, major dwarfing genes are not difficult to transfer by crossing (Borojevic and Borojevic 2005). Polish breeders have widely introduced the *Ddw1* gene from rye into triticale lines during the

breeding process in order to improve lodging-resistance (Banaszak 2010). However, some dwarfing genes in wheat were unfortunately reported to be associated with an increased susceptibility to splash-dispersed pathogens like septoria tritici blotch and fusarium head blight (Miedaner and Voss 2008, Eriksen et al. 2003, Gervais et al. 2003). Several studies were conducted to explore the effect of dwarfing genes on FHB resistance in wheat cultivars and confirmed the negative relationship between plant height and FHB severity (Miedaner and Voss 2008, Hilton et al. 1999). Due to the important impact of the dwarfing gene (*Rht-B1b* and *Rht-D1b*) on increasing the wheat sensitivity towards FHB, similar investigation was required to explore the impact of dwarfing gene introduction in triticale breeding programs. Therefore, one major part of this work was conducted for further analysis on population D (HeTi117-06 X Pigmej) to detect the effect of the dwarfing gene *Ddw1* on FHB severity, plant height and heading stage (Kalih et al. 2014).

Reducing the influence of plant height *per se* was achieved by applying repeated spray inoculations in the field experiments. Segregating genotypes showed significant genotypic variance for plant height, heading stage and FHB severity combined with high heritability reaching 97% for plant height (Kalih et al. 2014). High quality of the phenotypic data combined with utilizing specific (COS) markers for detecting the *Ddw1* gene facilitate the understanding of the genetic architecture of plant height and the effect of the dwarfing gene *Ddw1* from the cultivar Pigmej on FHB resistance and other related agronomic traits in triticale. *Ddw1* was previously mapped on the long-arm of rye chromosome 5R (korzun et al. 1996). Börner et al. (2000) found in a greenhouse study a large effect of *Ddw1* on heading time, flowering time and plant height in rye, which corresponds to our finding (Kalih et al. 2014). Plant height varied widely in our population from 53 to 142 cm. On average, progenies possessing the mutated allele were 22 cm shorter than those with the wild type

allele (Kalih et al. 2014). A significant correlation between plant height and FHB severity was observed (r = −0.67) revealing that short progenies tended to be more susceptible to FHB, which perfectly fits with a previous report in wheat (Draeger et al. 2007). The most prominent QTL were detected on chromosome 5R and explained 48, 77, and 71 % of genotypic variation for FHB severity, plant height, and heading stage, respectively (Kalih et al. 2014). This result identified for the first time the pleiotropic effects of *Ddw1* gene on plant height, heading stage and FHB severity in triticale.

6.4 Correlation between FHB severity and DON content in triticale

The occurrence of DON in small-grain cereals is of worldwide concern due to the toxic effects on human and animal health (Sobrova et al. 2010). In triticale, which is mainly used in animal feeding, very strict limits of DON content are recommended ($\leq$ 0.9 mg DON kg^{-1}) for diets of sensitive animal like swine (Europian Commision 2006). Direct DON detection is not convenient in practical breeding because it is expensive and time consuming (Bai et al. 2001b). Generally, in wheat, the significant correlation between FHB ratings and DON levels (Bai et al. 2011b) has great advantage for indirectly selecting genotypes having low DON levels (Snijders and Perkowski 1990). In a previous work using 113 wheat and 55 triticale genotypes inoculated with the same isolate (FC46), low correlation between FHB rating and DON was found in triticale (r= 0.36) compared with that in wheat (r= 0.80) (Miedaner et al. 2004). In the same study, high correlation was found between FHB rating and FDK rating in triticale (r= 0.71) (Miedaner et al. 2004). This is consistent with our results (Miedaner et al. 2016) as the correlation between FHB severity and DON was significant, but low in size (r=0.31), whereas the correlation between FDK and FHB severity was significant and higher (r=0.57) (Miedaner et al. 2016). The relatively weak relatedness between visual ratings for

FHB in the field and DON content can be explained by the differences of the tissue used for disease assessment and the tissue used for measuring DON content (Paul et al. 2005). Taking into account that rating symptoms in triticale is difficult due to color variation of triticale heads (Miedaner et al. 2016). Moreover, QTL analysis detected one major QTL for FHB severity on same chromosome 5R without any similar effect for FHB severity or FDK rating which can explain the low correlation between these traits (Miedaner et al. 2016). Conversely, a common QTL on chromosome 2A was reported for the three traits, with a minor effect for FHB severity. Interestingly, for DON content and FDK rating this QTL was a major QTL with explained genotypic variances of 35% and 37%, respectively. In conclusion, low correlation between FHB severity and DON content in triticale makes the selection for low DON content challenging and inefficient in the field, but in same time FDK rating after harvest can serve as an alternative indicator for less DON accumulation in grain.

6.5 QTL detection for FHB resistance in triticale

Reducing the impact of FHB disease on yield and grain quality in triticale received a priority in triticale breeding programs (Oettler 2005). Preliminary studies of FHB in triticale showed that the genetic background of FHB resistance in triticale is similar to that in wheat and rye (Miedaner et al. 2004, Miedaner et al. 2001,). Therefore, different options for resistance selection used in wheat as marker-assisted selection and genomic selection are also possible in triticale. Moreover, performing molecular breeding programs in triticale for FHB resistance became facilitated by the availability of the genetic maps of triticale, which is considered as the basic step for any molecular study. Recently, Alheit et al. (2011) presented a consensus genetic linkage map for triticale depending on six mapping populations; four of them were used in our study as well. However, opposite orientation for triticale

chromosomes in this map was used comparing with the known position of *Ddw1* gene on chromosomes 5R in rye (Kalih et al. 2014).

To our best knowledge, our work was the first QTL study for FHB resistance in triticale facilitated by this integrated map. As expected, QTL results confirmed the quantitative inheritance of FHB resistance as illustrated by the detection of several QTLs, each explaining a part of the phenotypic variance in a similar way as in wheat (Buerstmayr et al. 2009, Bai and Shaner 2004, Gervais et al. 2003). In total, twenty two QTLs of FHB resistance in triticale were mapped across five populations distributed among twelve chromosomes (Fig. 1). Interestingly, nine QTLs were reported for the first time in the R genome of rye and all of them were major QTLs with high contribution to the genotypic variance of FHB resistance reaching up to 48% for the QTL located on chromosome 5R in population D (Kalih et al. 2014). Two major QTLs were detected on the wheat genome for FHB resistance. The first QTL was located on chromosome 6A with 30.8 % of the explained genotypic variance in population C which is comparable to QTL found previously in wheat (Schmolke et al. 2005, Anderson et al. 2001). Another QTL on chromosome 3B was often detected in two populations in our study, and located close to the region where also a QTL on chromosome 3B for FHB resistance in wheat was previously reported (Kalih et al. 2015, Buerstmayr et al. 2009). An overview of the detected resistance loci in triticale genomes A, B and R shows that the QTLs detected in the R genome constituted more than 60% of the total explained genotypic variance for FHB resistance in each population except for the population C (no QTL on the R genome was detected). This reveals the high value of rye as FHB-resistance donor to triticale.

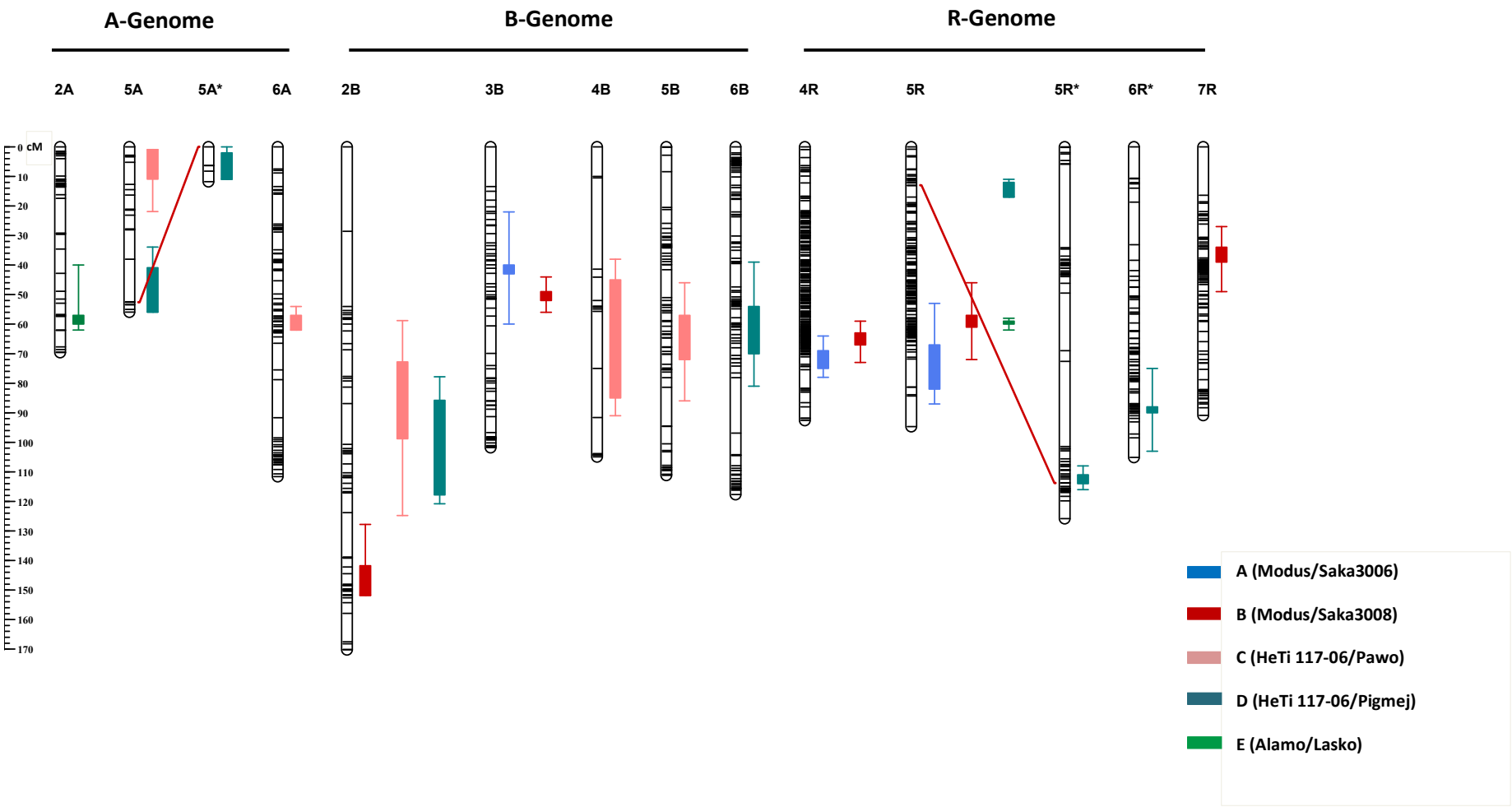

Figure 1: **Schematic illustration of all triticale chromosomes carrying QTL regions for Fusarium head blight (FHB) resistance in five populations**

*** Triticale chromosomes mapped according to kalih et al. (2014)**

6.6 Candidate genes associated with *Fusarium graminearum* aggressiveness and DON production in wheat

Although improving the disease resistance is based on identifying resistance sources in the host plants, knowing the corresponding mechanisms of the pathogen may enable us to disrupt pathogenicity pathways used for aggressiveness, fitness, or adaptation. Understanding the pathogenesis of *F. graminearum* by studying and revealing the components involved in the pathogenic system, (*i.e.* pathogenic genes) became recently the main subject in several reports by manipulating candidate genes (Liu et al. 2010, Bluhm et al. 2007, Kimura et al. 2007, Hou et al. 2002). However, a wide genetic variation of aggressiveness and DON production was reported among several *F. graminearum* populations (Talas et al. 2011, Fernando et al. 2006). Exploring the genetic background of important pathogenicity traits in *F. graminearum* such as aggressiveness and mycotoxin production was conducted by finding a correlation between phenotypic variance of these quantitative traits and single nucleotide changes of candidate genes. Candidate gene association study (CGAS) was recently demonstrated to be an effective approach (Talas et al. 2012). In the current CGAS, we assessed the association between seven candidate genes related to aggressiveness and/or DON production with the phenotypic variance of these traits among 152 isolates of *F. graminearum*.

One of the most challenging issues in association studies is the risk of having false discoveries (Sillanpää 2011). However, our model of association was corrected for the hidden population structure presented by kinship matrix that minimizes the effect of SNP identification due to the familial relationships. Accordingly, population structure assessment beside the cross-validation approach was applied to obtain realistic SNP-trait combinations.

Significantly associated SNPs were identified in four genes (*Gmpk1, Mgv1, TRI6*, and *Erf2*) for aggressiveness and DON production in our study (Kalih et al. in review).

MAP kinase genes (*Gmpk1*, *Mgv1*) have been demonstrated to be involved in fungal development, sexual reproduction and pathogenicity in *F. graminearum* (Bluhm et al. 2007, Jenczmionka et al. 2003, Urban et al. 2003, Hou et al. 2002). Therefore, it is not surprising to detect significant associations between the nucleotide polymorphisms of these genes and aggressiveness. One significant SNP associated with aggressiveness was reported for each gene (i.e., *Gmpk1*, *Mgv1*). Additionally, in *Mgv1* we detected another SNP associated with DON production, a result that agrees with those of Hou et al. (2002), who they reported a significant reduction in DON production by *Mgv1* deletion-mutants.

Although *Tri6* gene is a member of the *TRI5* gene cluster, it has an essential regulating function with significant influence on other genes with well-known pathogenicity functions (Seong et al. 2009). Hence, this might explain that we detected three significant SNPs associated with FHB aggressiveness, although no significant SNP for DON production was reported (Kalih et al. in review).

Additionally, a putative gene *Erf2*, was chosen to validate previously published results from Talas et al. (2012). Our new results confirmed the putative involvement of *Erf2* in quantitative variance of aggressiveness. Thus, this gene would be a good candidate for further functional analysis by gene knock out or gene silencing. A follow-up study is essential to validate the detected SNP in larger populations, and reveal more SNPs in other pathogenicity genes in *F. graminearum* to understand and improve FHB management in wheat.

6.7 Consequences for FHB resistance breeding in triticale

Different genomic loci were reported to be associated with FHB resistance in cereals. In order to successfully exploit these QTL for practical cereal breeding programs, selection for resistance genotypes should be accompanied with superior and stable yield and quality performance. Difficulties may arise with using exotic resistance sources that are usually poor in their agronomical performance. In our work, detecting QTL for FHB resistance in triticale was performed based on commercial elite materials having already adapted agronomic traits that can be directly used for breeding programs. However, wide range of resistance level was detected in triticale populations in our study, major and minor QTL for FHB resistance were found in all populations. Interestingly, several major QTLs came from rye genome located on the chromosomes 4R, 5R, and 7R. These QTLs could be used for marker-assisted selection (MAS) or genomic selection (GS) after validation.

In order to better exploit of the dwarfing gene *Ddw1* in triticale breeding programs, unfavorable effects for this genes on other traits like FHB resistance should be minimized by introducing and pyramiding several FHB resistant QTLs to reach acceptable resistance level in triticale.

Breeding for less DON content in triticale is more challenging compared to that in wheat due to the low correlation between FHB severity and DON content. Thus using FDK rating would form an indicator of a higher reliability for DON content evaluation and can be an alternative method to breed triticale for lower levels of DON.

7. References

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8. Summary

Fusarium head blight (FHB) is one of the most destructive fungal diseases in small-grain cereals worldwide causing significant yield losses and contamination of grain with mycotoxins e.g., deoxynivalenol (DON). This renders the grain unsuitable for human consumption and animal feeding. Exploring the genetic mechanism of FHB resistance is considered the key tool for modern cereal breeding activities. Triticale, the intergeneric hybrid between wheat and rye, is an important cereal crop in Poland and Germany. Resistance breeding using genetic mapping to identify quantitative-trait loci (QTL) associated with FHB resistance represents the best strategy for controlling the disease. In parallel, understanding the mechanism of aggressiveness and DON production of *F. graminearum* will be a significant contribution to improve FHB management.

The objectives of the present work were (1) identification of QTL related to FHB resistance in triticale, together with the analysis of the correlation of FHB severity with other related traits such as plant height and heading stage, (2) correlation between DON production and FHB severity, (3) mapping of dwarfing gene *Ddw1* in triticale and studying its effect on FHB resistance, plant height and heading stage, (4) detection of SNPs in candidate genes associated with aggressiveness and DON production of a large *Fusarium graminearum* population in bread wheat.

To study the genetic architecture of FHB resistance in triticale, five doubled-haploid (DH) triticale populations with 120 to 200 progenies were successfully tested under field conditions by inoculation with *Fusarium culmorum* (FC46) in multiple environments. All genotypes were evaluated for FHB resistance, plant height and heading stage. DArT markers were used to genotype triticale populations. Significant genotypic variances (*P*<

0.001) were observed for FHB severity in all populations combined with high heritability. Twenty-two QTLs for FHB resistance in triticale were reported with two to five QTL per population, thus confirming the quantitative inheritance of FHB resistance in triticale. The most prominent ($R^2 \geq 35\%$) QTLs were located on chromosomes 6A, 3B, 4R, and 5R. QTLs for plant height and heading stage were also detected in our work, some of them were overlapping with QTLs for FHB resistance.

Correlation between FHB severity, DON content and Fusarium damaged kernels (FDK) in triticale was studied in the population Lasko x Alamo. Significant genotypic variance was detected for all traits. However, low correlation between FHB severity and DON content (r=0.31) was found. Interestingly, correlation between FHB severity and FDK rating was considerably higher (r=0.57). For FHB severity, two QTLs were detected in this population. A QTL located on chromosome 2A with minor effect for FHB severity was also a common QTL for DON content and FDK rating and explained ≥34% of genotypic variance for these two traits. A second QTL on chromosome 5R was a major QTL but it has no effect on DON content or FDK rating.

For analyzing the rye dwarfing gene *Ddw1* derived from the father Pigmej, 199 (DH) progenies were genotyped with DArT markers and in addition with conserved ortholog set (COS) markers linked to the *Ddw1* locus in rye. QTL analyses detected three, four, and six QTLs for FHB severity, plant height and heading stage, respectively. Two specific markers tightly linked with *Ddw1* on rye chromosome 5R explained 48, 77, and 71 % of genotypic variation for FHB severity, plant height, and heading stage, respectively. This is strong evidence, that we indeed detected the rye gene *Ddw1* in this triticale population.

Another objective was to highlight the association between quantitative variation of aggressiveness and DON production of 152 *F. graminearum* isolates with single

nucleotide polymorphism (SNP) markers in seven candidate genes. One to three significant SNPs ($P < 0.01$ using cross-validation) were associated to FHB severity in four genes (i.e., *Gmpk1*, *Mgv1*, *TRI6*, and *Erf2*). For DON content, just one significant SNP was detected in the gene *Mgv1* explaining 6.5% of the total genotypic variance.

In conclusion, wide genetic variation in FHB resistance in triticale has been observed in five populations. QTL mapping analyses revealed twenty-two QTLs for FHB resistance derived from wheat and rye genomes. QTLs located on the rye genome were reported here for the first time and they are a new source for FHB resistance in triticale. In parallel, analysis of the diversity of four pathogenicity genes in *F. graminearum* is an important first step in inferring the genetic network of pathogenicity in this fungal pathogen.

9. Zusammenfassung

Ährenfusariosen (Fusarium head blight, FHB) zählen weltweit zu den bedeutendsten pilzlichen Krankheitserregern im Getreide, wobei eine Infektion erhebliche Ertragsverluste verursachen kann und zu einer Anreicherung von Mykotoxinen, wie z.B. Deoxynivalenol (DON), im Erntegut führen kann. Dies bedeutet, dass das Erntegut dann für die menschliche oder tierische Ernährung ungeeignet ist. Die Untersuchung der genetischen Grundlagen der Fusarium-Resistenz stellt eine wichtige Aufgabe in der modernen Getreidezüchtung dar. Triticale, eine Kreuzung aus Weizen und Roggen, ist eine bedeutende Getreideart in Frankreich, Polen und Deutschland. Die genetische Kartierung zur Identifizierung von so genannten *quantitative trait loci* (QTL) die mit Fusarium-Resistenz assoziiert sind, stellen eine wichtige VoraussetzungKontrolle dieser Getreidekrankheit. Gleichzeitig kann das Verständnis von Mechanismen, welche die Aggressivität und die DON-Produktion von *F. graminearum* steuern, zur Kontrolle von FHB beitragen.

Die Ziele der vorliegenden Arbeit waren (1) die Identifikation von QTL, die mit Fusarium-Resistenz assoziiert sind, die Analyse von Korrelationen zwischen FHB-Resistenz und weiteren Merkmalen wie z.B. Wuchshöhe oder Ährenschieben, (2) die Untersuchung der Korrelation zwischen DON-Produktion und FHB-Resistenz, (3) die Kartierung des Verzwergungsgens *Ddw1* in Triticale und die Untersuchung zum Effekt dieses Gens auf FHB-Resistenz, Wuchshöhe und Ährenschieben, (4) die Detektion von SNPs in Kandidatengenen, die mit Aggressivität und DON-Produktion in einer großen *F. graminearum*-Population in Winterweizen assoziiert sind.

Zur Untersuchung der genetischen Architektur der Fusarium-Resistenz in Triticale wurden fünf doppelhaploide (DH) Triticale-Populationen mit je 120 bis 200 Nachkommen in mehreren Umwelten unter Feldbedingungen erfolgreich mit *Fusarium culmorum* (FC46) inokuliert. Alle Genotypen wurden für Fusarium-Resistenz, Wuchshöhe und Ährenschieben bonitiert. Die Triticale-Populationen wurden mit DArT Markern genotypisiert. Es wurden signifikante genetische Varianzen (P <0.001) und hohe Heritabilitäten für Fusarium-Resistenz in allen Populationen beobachtet. Es wurden insgesamt 24 QTL für Fusarium-Resistenz in Triticale gefunden, von denen zwei bis fünf QTL je Populationen kartiert wurden, was die quantitative Vererbung von Fusarium-Resistenz in Triticale bestätigt. Die wichtigsten QTL (R^2 ≥ 35%) waren auf den Chromosomen 6A, 3B, 4R und 5R lokalisiert. Außerdem wurden QTLs für Wuchshöhe und Ährenschieben in dieser Studie gefunden, wobei manche dieser QTL überlappend mit QTL für Fusarium-Resistenz waren.

Die Korrelationen zwischen FHB-Anfälligkeit, DON-Gehalt und Fusarium-infizierte-Körnern (FDK, *Fusarium damaged kernels*) wurden in der Triticale-Population Lasko x Alamo untersucht. Es wurden signifikante genotypische Varianzen für alle Merkmale gefunden. Trotzdem konnte nur eine geringe Korrelation zwischen Fusarium-Resistenz und DON-Gehalt (r=0.31) festgestellt werden. Interessanter-weise war die Korrelation zwischen Fusarium-Resistenz und der FDK-Bonitur erheblich höher (r=0.57). Es wurden zwei QTL für Fusarium-Resistenz in dieser Population gefunden. Ein QTL auf Chromosom 2A hatte einen geringen Effekt auf die Fusarium-Resistenz, erklärte aber DON-Gehalt und FDK je 34% der genotypischen Varianz. Ein weiterer QTL auf Chromosom 5R war ein Haupt-QTL aber hatte keinen Effekt auf den DON-Gehalt oder FDK.

Zur Analyse des Roggen-Verzwergungsgens *Ddw1*, welches vom Vater Pigmej stammte, wurden 199 DH-Nachkommen mit DArT-Markern und zusätzliche mit COS (conserved ortholog set) Markern genotypisiert, welche mit dem *Ddw1*-Locus in Roggen gekoppelt sind. Durch die QTL-Analyse wurden drei QTL für Fusarium-Resistenz, vier QTL für Wuchshöhe und sechs QTL für Ährenschieben gefunden. Zwei Marker, die eng mit *Ddw1* auf dem Roggenchromosom 5R gekoppelt waren, erklärten für Fusarium-Resistenz, Wuchshöhe und Ährenschieben jeweils 48, 77 bzw. 71 % der genotypischen Varianz. Dies ist Hinweis, dass wir das Roggengen *Ddw1* in dieser Triticale-Population nachweisen konnten. Ein weiteres Ziel der Arbeit war die Untersuchung zur Assoziation zwischen SNP (*conserved ortholog set*)-Markern in sieben Kandidatengenen und der Aggressivität sowie DON-Produktion von 152 *F.graminearum* Isolaten. Ein bis drei signifikante SNPs ($P < 0.01$, kreuzvalidiert) waren mit Fusarium-Resistenz in vier Genen assoziiert (*Gmpk1*, *Mgv1*, *TRI6*, und *Erf2*). Für den DON-Gehalt wurde jedoch nur ein signifikanter SNP im Gen *Mgv1* identifiziert, welcher 6,5 % der genotypischen Varianz erklärte.

Zusammenfassend haben wir eine große genetische Variation für FHB-Resistenz in fünf Triticale-Populationen gefunden. Durch die QTL-Kartierung konnten 24 QTLs für Fusarium-Resistenz gefunden werden. Die in dieser Studie auf dem Roggen-Genom detektierten QTLs für Fusarium-Resistenz wurden hier zum ersten Mal b berichtet. Diese stellen eine neue Quelle für Fusarium-Resistenz in Triticale dar. Zusätzlich legt unsere Analyse von vier Pathogenitätsgenen in *F. graminearum* eine erste Grundlage zur Untersuchung der quantitativ vererbten Aggressivität dieses pilzlichen Erregers.

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