Breeding for resistance to Fusarium ear diseases in maize and small-grain cereals using genomic tools

Dissertation to obtain the doctoral degree of Agricultural Sciences (Dr. sc. agr.)

Faculty of Agricultural Sciences

University of Hohenheim

State Plant Breeding Institute (720)

apl. Prof. Dr. Thomas Miedaner

Submitted by

Master of Philosophy

David Sewordor Gaikpa

Born in Donkorkrom, Ghana

Stuttgart-Hohenheim

2020

Printed and/or published with the support of the German Academic Exchange Service (DAAD)

This thesis was accepted as a doctoral dissertation in fulfillment of the requirements for the degree “Doktor der Agrarwissenschaften” (Dr. sc. Agr. / PhD. in Agricultural Sciences) by the Faculty of Agricultural Sciences at the University of Hohenheim on 14th October, 2020.

Day of oral examination: 02.02.2021

Examination Committee:

Head of Committee: Prof. Dr. Uwe Ludewig

1st examiner and reviewer: apl. Prof. Dr. Thomas Miedaner

2nd examiner and reviewer: Prof. Dr. Hermann Bürstmayr

3rd examiner: Prof. Dr. Ralf Thomas Vögele

Table of Contents

Publications

This thesis consists of four published articles:

[1]Gaikpa, D. S., Lieberherr, B., Maurer, H.P., Longin, C.F.H., Miedaner, T. (2020). Comparison of rye, triticale, durum wheat and bread wheat genotypes for Fusarium head blight resistance and deoxynivalenol contamination. Plant Breeding 139 (2):251–262. https://doi.org/10.1111/pbr.12779

[2]Gaikpa, D.S., Koch, S., Fromme, F.J., Siekmann, D., Würschum, T., Miedaner, T. (2020). Genome-wide association mappingand genomic prediction of Fusarium head blight resistance, heading stage, and plant height in winter rye (*Secale cereale* L.). Plant Breeding 139 (3):508–520. https://doi.org/10.1111/pbr.12810

[3]Gaikpa, D.S., Miedaner, T. (2019). Genomics-assisted breeding for ear rot resistances and reduced mycotoxin contamination in maize: methods, advances and prospects. Theoretical and Applied Genetics 132(10): 2721-2739. https://doi.org/10.1007/s00122-019-03412-2

[4]Gaikpa, D.S., Kessel, B., Presterl, T., Ouzunova, M., Galiano-Carneiro, A.L., Mayer, M., Melchinger, A.E., Schön, C.C., Miedaner, T. (2020) Exploiting genetic diversity in two European maize landraces for improving Gibberella ear rot resistance using genomic tools. Theoretical and Applied Genetics. https://doi.org/10.1007/s00122-020-03731-9

Abbreviations

CG	candidate gene
DON	deoxynivalenol
ER	ear rot
FHB	Fusarium head blight
FER	Fusarium ear rot
GER	Gibberella ear rot
GP	genomic prediction
GS	genomic selection
GWAS	genome-wide association study
KE	Kemater Landmais Gelb
MABC	marker-assisted backcrossing
MAS	marker-assisted selection
PE	Petkuser Ferdinand Rot
QTL	quantitative trait loci
RR-BLUP	ridge regression-best linear unbiased prediction
SNP	single nucleotide polymorphism
wRR-BLUP	weighted ridge regression-best linear unbiased prediction
ZON	zearalenone

1 General introduction

Maize and small-grain cereals

Globally, cereal crops like maize (*Zea mays* L.) and small-grain cereals, such as rye (*Secale cereale* L.), triticale (x*Triticosecale*), durum wheat (*Triticum turgidum* ssp. *durum*) and common (bread) wheat (*Triticum aestivum* ssp. *aestivum*) serve as indispensable sources of food for humans and feed for livestock. Indisputably, cereals provide more than half of global caloric intake (FAO, 2020). The consumption of cereals is expected to increase at 1.2 % per year, between 2019 and 2028, with increasing demand in Asia and Africa (OECD/FAO, 2019). The production of cereal crops also provides employment for millions of people throughout the world.

Maize is a diploid (2n = 20) cross-pollinating species and has a variable genome size of 2106 - 2500 Mbp (Díez et al., 2013; Jiao et al., 2017; Schnable et al., 2009). In the year 2018, the quantity of maize produced worldwide (about 1.1 billion metric tons) exceeded all cereal crops and it is ranked among the topmost consumed cereal crops (FAO, 2020; Chaudhary et al., 2014). Europe produced 11.21 % of the world maize production in 2018. Maize is a staple food for billions of people, especially in Africa, where it accounts for approximately 60 % of dietary calories (FAO, 2020). Currently, maize constitutes 19.5 % of global caloric intake (Pariona, 2019, June 7) and demand is expected to increase by 189 million metric tons, mainly driven by expanding animal production (OECD/FAO, 2019). In Germany, the largest proportion of the maize produced is used to feed livestock (Federal Ministry of Food and Agriculture, 2019).

Rye is a diploid (RR, 2n = 2x = 14) and allogamous small-grain cereal crop. It belongs to the *Triticeae* group and has a large genome size of ~7.9 Gbp (Bartoš et al., 2008). Rye is the male parent of triticale (Ammar et al., 2004). About 11.3 million metric tons of rye was produced worldwide in 2018, about 74 % being produced by Germany, Poland, Russia, Finno-

Scandinavia, Belarus and Ukraine together (FAO, 2020). The grains are used to make bread and livestock feed. Similar to other cereal crops produced in Germany, close to 60% of rye is used as animal feed (Federal Ministry of Food and Agriculture, 2019). Rye is more tolerant to biotic and abiotic stresses compared to triticale and wheat (Arseniuk et al., 1999; Bartoš et al., 2008; Miedaner et al., 2001; Myśków et al., 2018; Villareal et al., 1998). As a result, some desirable agronomic and resistance traits have been transferred from rye into wheat (Crespo-Herrera et al., 2017; Kim et al., 2004). Modern hexaploid triticale (AABBRR, 2n = 6x = 42) is an artificially produced small-grain cereal obtained from a cross between durum wheat and rye (Ammar et al., 2004). Unlike rye, it is self-pollinating. Out of approximately 12.8 million metric tons of triticale produced in 2018 worldwide, 90 % was produced in Europe alone (FAO, 2020). Triticale grains are used exclusively to feed animals because of poor baking quality of the flour (Tsen et al., 1971). Durum wheat is a tetraploid species (AABB, 2n = 4x = 28) of wheat and the female parent of modern hexaploid triticale (Ammar et al., 2004). It is self-pollinating. It accounts for up to 8 % of global wheat production, and is primarily used for making pasta because of its hard kernels, and for animal feed (Boyacioglu, 2017, October 23). Bread wheat is an allohexaploid (AABBDD, 2n = 6x = 42) and autogamous species of *Triticeae* tribe with a large genome size of ~17 Gbp (The International Wheat Genome Sequencing Consortium, 2014). On a global-scale, the quantity of wheat produced in 2018 (734 million metric tons) closely followed that of rice (782 million metric tons), making it among the three most important cereal crops. About 33 % of the world wheat produced occurred in Europe (FAO, 2020). Common (bread) wheat is widely used in making bread, biscuits, pies, cakes, pizzas, muesli, etc. and for animal feed.

Maize and small-grain cereal crops production must be scaled up in order to feed the world's rapidly growing population. Undeniably, cereal crops can be seen as the foundation for achieving sustainable global food security, eliminating hunger by 2030 (United Nations, 2015).

Therefore, it is highly imperative to tackle the risk factors, such as biotic and abiotic stresses that mitigate against large production and utilization of quality cereal grains.

Fusarium ear infections and management

Fusarium species are important fungal species that cause many diseases leading to yield loss and mycotoxin contaminations in cereal grains. The commercially most important Fusarium diseases are ear rots (ER) in maize and Fusarium head blight (FHB) or scab in small-grain cereals such as rye, triticale, durum wheat and bread wheat.

In maize, there are different types of toxigenic ER caused by *Fusarium* spp. depending on the geographical location and climate or weather. Gibberella ear rot (GER) is caused by *Fusarium graminearum* (telemorph/sexual stage: *Gibberella zeae*) species complex, and it is the major type of ER found in cooler regions like Europe, northern United States, Canada, South America, and higher altitudes in Africa (Fingstag et al., 2019; Mouton, 2014; Pfordt et al., 2020; Wise et al., 2016). However, *Fusarium* ear rot (FER) caused by *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheldon) and related species such as *F. proliferatum*, *F. subglutinans*, *F. temperatum* sp. nov may prevail in warmer years also in Germany and the United States (Pfordt et al., 2020). FER is one of the most predominant ERs found in Africa because of the prevailing climate. In small-grain winter cereal crops such as rye, triticale, durum wheat and bread wheat, *F. graminearum and F. culmorum* are among the major *Fusarium* spp. that cause FHB in Europe. Typical symptoms of GER, FHB and Fusarium-damaged kernels (FDK) in maize and small-grain cereals are shown in Figure 1.

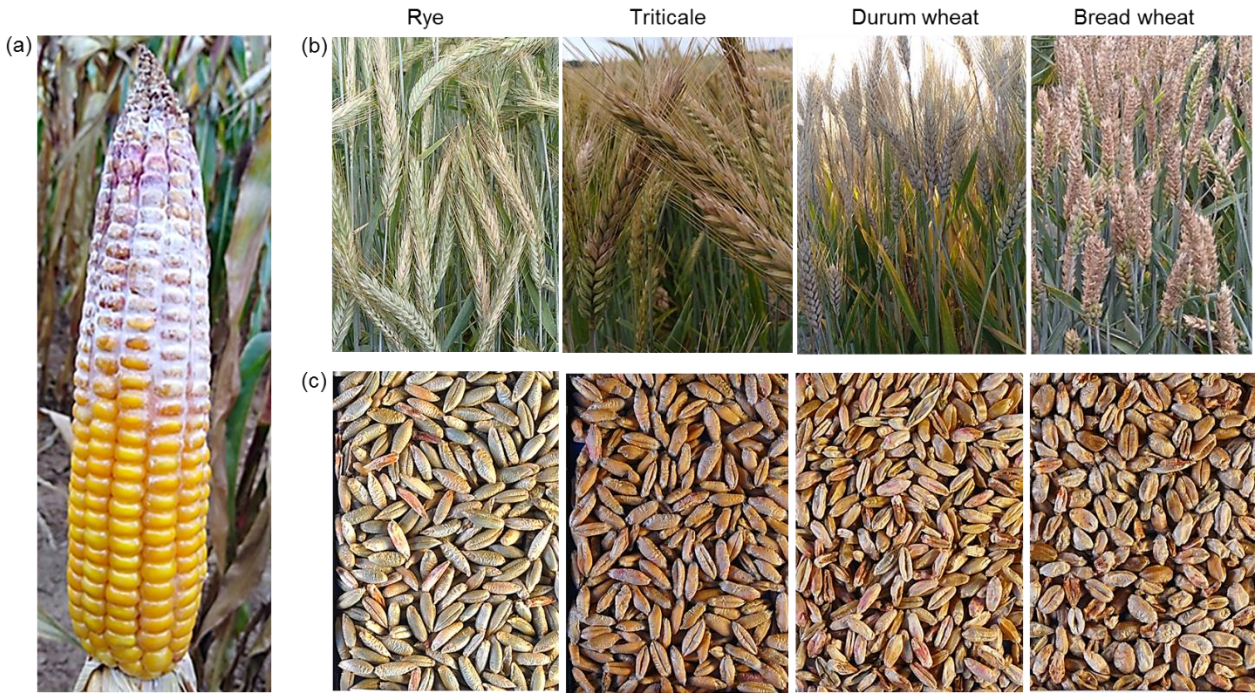


Figure 1. Typical symptoms of (a) Gibberella ear rot on a maize ear (whitish to reddish or pinkish mold), (b) Fusarium head blight (blighted spikelets), and (c) Fusarium-damaged kernels (whitish, pinkish or dark-red, shrivelled kernels) in rye, triticale, durum wheat and bread wheat

F. graminearum and *F. culmorum* are hemibiotrophic fungi and produce dangerous mycotoxins namely, zearalenone (ZON) and deoxynivalenol (DON) in maize and small-grain cereals during their necrotrophic stages (Bolduan et al., 2009; Martin et al., 2012; Miedaner et al., 2010; Pasquali et al., 2016; Suchowilska et al., 2010; Trail, 2009). These toxins can cause serious reproductive and other health problems among animals and humans. DON causes abdominal pain, diarrhea, acute nausea, vomiting, kidney disorders, equine leucoencephalomalacia, fever, poor growth rate, etc. and ZON causes infertility, abortion and premature puberty, especially among livestock (Massart et al., 2008; Pinton & Oswald, 2014; Zhou et al., 2018). The role of DON synthesis as a virulence factor for increasing severity of *Fusarium* diseases in cereals has been reported (Desjardins et al., 1996; Gunupuru et al., 2017; Harris et al., 1999). Studies with artificial infection showed high positive genotypic correlations between GER severity and *Fusarium* mycotoxin contaminations, DON and ZON (r = 0.73 – 0.98) in maize and small-grain cereals (Bolduan et al., 2009; Martin et al., 2011; Miedaner et

al., 2004). Mycotoxins may still be present among kernels having no visible ER or FHB symptoms. National and international regulatory bodies have set recommended limits for mycotoxins in cereals and cereal products because of the adverse health and economic effects associated with them (FAO, 2003; Foroud et al., 2019; The Commission of the European Communities, 2006). For example, in the European Union, the limit imposed on DON is 1.75 mg kg^{-1} in maize, durum wheat and oats, and 1.25 mg kg^{-1} in other small-grain cereals meant for human consumption. The allowable limits of DON in animal feed are 0.90 -12.00 mg kg^{-1}, depending on the type and age of animals (The Commission of the European Communities, 2006).

The negative effect of *Fusarium* spp. on maize and small-grain cereals is increasing due to climate change and changing farming methods such as mono-cropping, narrow rotations, and reduced soil tillage. In addition, the genomic structure of *Fusarium* spp. relating to pathogenicity is evolving (Lofgren et al., 2018; Sperschneider et al., 2015) and there is large seasonal plasticity in the occurrence and aggressiveness among and within *Fusarium* spp. (Castiblanco et al., 2020; Pfordt et al., 2020). These factors make management of Fusarium diseases more complicated. Fusarium diseases and mycotoxins can be controlled using chemicals, biocontrol agents such as some species of *Trichoderma*, *Bacillus*, *Lysobacter* and *Pseudomonas*, crop rotation and soil tillage (Anderson et al., 2017; Fingstag et al., 2019; Mielniczuk & Skwaryło-Bednarz, 2020; Pfordt et al., 2020). Individual methods such as chemical and biological control may be ineffective because of the negative effects of environmental conditions and high disease pressure (Anderson et al., 2017). Besides, fungicide application on cereal crops after certain growth stages is strictly regulated in some countries like the European Union. Integration of resistant cultivars into Fusarium disease management methods is the most sustainable, efficient and ecologically beneficial way to reduce the negative impact of Fusarium ear diseases and mycotoxin accumulations in maize and small-

grain cereals, especially in endemic regions and seasons. Therefore, genetic improvement of cultivars for GER and FHB resistances across multi-environments is very crucial.

Pure line breeding (either by single cross, three-way cross or four-way/double cross) is the major method used to produce wheat and triticale cultivars because of their self-pollinating nature. However, there are attempts to introduce hybrid wheat and triticale breeding in some countries by exploiting the advantage of genetic or chemically induced male sterility (Baenziger, 2016; Ayalew et al., 2018). Hybrid breeding is commonly used in rye and maize breeding programs. In rye, hybrid cultivars are composed of each of two inbred lines developed from two heterotic groups that are crossed for seed production by cytoplasmic-male sterility (Miedaner and Laidig, 2019). For maize, homozygous inbred lines are generated by self-pollination (controlled) or DH technology and the superior hybrid combinations selected to produce hybrid cultivars. The inbred lines are first evaluated for line per se and testcross performance. Historically, maize was introduced in Europe after Columbus discovered the new world. The European flint maize landraces were introduced in Europe from South and North America around the 16^{th} to 17^{th} century (Rebourg et al., 2003; Tenaillon and Charcosset, 2011). According to Rebourg et al. (2003), the adaptation of maize in Europe should be attributed to the cross-pollination events that occurred between the South and North American flint germplasms.

Genetics for Fusarium resistances and genomics-assisted breeding

The genetic architecture of ER and FHB resistances is complex, affected by multiple loci, the environment and genotype-environment interaction (G x E) (Becher et al., 2013; Martin et al., 2012). Significant genotype-isolate interaction was reported for ER severity and mycotoxin concentrations among elite maize lines inoculated with eight isolates each of *F. graminearum* and *F. verticicillioides* (Miedaner et al., 2010). However, they did not find change in ranking of the genotypes under the different isolates. Studies showed additive, dominance, digenic

(dominance x dominance) and epistatic genetic effects on the inheritance of Fusarium ear disease resistances in cereals with additive gene action being the most predominant effect (Butrón et al., 2015; Chungu et al., 1996; Fakhfakh et al., 2011; Martin et al., 2012; Miedaner & Geiger, 1996). In previous research, molecular analyses confirmed the important role of additive and epistatic genetic control of Fusarium resistance in maize and small-grain cereals (Han et al., 2018; Ma et al., 2006; Martin et al., 2012; Martin et al., 2011). Maternal effects might play no important role on resistance to Fusarium ear infections in cereals (Buerstmayr et al., 2000; Pereira et al., 2017), making the choice of a pollen donor or a female parent from among selected candidates in a breeding program non-problematic.

Over the past years, genomic tools such as quantitative trait loci (QTL or linkage) mapping, genome-wide association studies (GWAS), transcriptomics, proteomics and metabolomics have been used to decipher the molecular mechanisms for Fusarium ear disease resistances in maize and small-cereals (Chapter 4; Buerstmayr et al., 2019; Kazan & Gardiner, 2018; Ma et al., 2020). In maize, >100 QTLs scattered across the 10 chromosomes have been reported for ER resistances, of which 87 were incorporated into a meta-QTL map to derive 29 meta-QTLs (Xiang et al., 2010). About 198 candidate genes (CGs) have been reported for *F. graminearium* resistance in maize using transcriptomics and proteomics (Kebede et al., 2018; Mohammadi et al., 2011; Yuan et al., 2020). However, it has been difficult to employ these multiple QTLs or CGs in marker-assisted selection (MAS) to improve GER resistance in maize.

In small-grain cereals, many QTLs were reported for FHB resistance in durum wheat (Buerstmayr et al., 2019; Miedaner et al., 2017), bread wheat (Arruda et al., 2016; Buerstmayr et al., 2019; Venske et al., 2019), and triticale (Dhariwal et al., 2018; Galiano-Carneiro et al., 2019; Kalih et al., 2015). For bread wheat alone, ~550 FHB QTLs were found across the entire genome (i.e A, B, D) and have been reduced to 65 meta-QTL (Venske et al., 2019). Most of the QTLs contributed only small proportions of the genotypic variance for FHB resistance.

Attempts are being made to introduce a few major QTLs of Chinese origin (e.g., *Fhb1, Fhb5, Fhb6*) into wheat and triticale breeding materials for FHB resistance across the globe (Bai et al., 2018; Ma et al., 2020; Miedaner et al., 2019a; Ollier et al., 2020; Prat et al., 2017). Individual QTLs with large impact on FHB resistance in wheat have been successfully applied in MAS in US and China (Ma et al., 2020). However, QTL analyses for FHB resistance in rye are missing in literature. In the meantime, previous studies involving triticale, a progeny of wheat x rye crosses, found QTLs on chromosome 3R, 4R, 5R and 7R originating from rye (Dhariwal et al., 2018; Galiano-Carneiro et al., 2019; Kalih et al., 2015).

A large majority of QTLs detected in the past decades remain unutilized for MAS in practical breeding for GER and FHB resistances because of low validation rate, high cost and the tendency of fixing large portions of the genome (Brauner et al., 2017; Miedaner & Korzun, 2019). Therefore, genomic selection (GS) has been proposed as an option to facilitate the application of genomics in crop improvement. Genomic prediction (GP) involves using genome-wide high-density marker profiles to estimate the genomic breeding values of individuals to be selected. Once the effects of markers are estimated in GP models, non-tested genotypes can be predicted and selected. This strategy reduces large-scale phenotyping and enhances selection gains (Edwards et al., 2019; Wallace et al., 2014). Larger proportion of genetic variation may be captured in GS than in MAS, especially when the trait is mainly controlled by a multitude of rare additive alleles (Newell & Jannink, 2014). Factors, limitations and prospects of GS for complex traits have been extensively reviewed (Goddard & Hayes, 2007; Leng et al., 2017; Robertsen et al., 2019). As a result, GP has been used to predict resistance of maize to lethal necrosis (Gowda et al., 2015), Diplodia ear rot (dos Santos et al., 2016) and Northern corn leaf blight (Technow et al., 2013). Two studies have suggested that GS might accelerate breeding for GER resistance in maize (Han et al., 2018; Riedelsheimer et al., 2013). Furthermore, the prospects of genomic selction for FHB resistance breeding in

triticale (Galiano-Carneiro et al., 2019), durum wheat (Miedaner et al., 2017; Moreno-Amores et al., 2020) and bread wheat (Arruda et al., 2016; Mirdita et al., 2015; Rutkoski et al., 2012) have been studied. However, the potential of GS for breeding against FHB resistance in rye is unknown. Maize landraces are genetically diverse populations which habour many locally adapted traits (Böhm et al., 2017; Hölker et al., 2019; Mayer et al., 2017; Strigens et al., 2013). In order to exploit new sources of resistance, it is worthwhile to tap the wide diversity in maize landraces for GER resistance breeding using integrated genomic methods.

Objectives of the study

The main objective of this research was to analyze four winter small-grain cereals and two European maize landrace populations for resistance to Fusarium ear diseases, using genome-based approaches. The specific objectives were to:

1. Compare rye, triticale, durum wheat, and bread wheat for their FHB resistance and DON accumulation
2. Identify QTLs for FHB resistance in rye using GWAS and assess the potential of genomic prediction
3. Conduct a state-of-the-art literature review on QTLs, candidate genes and genomic selection for ER resistances and reduced mycotoxin contaminations in maize
4. Analyze phenotypic and genotypic data for GER resistance, across and within two European maize landraces, “Kemater Landmais Gelb” (KE) and “Petkuser Ferdinand Rot” (PE), to be used for multi-locus GWAS
5. Compare MAS and genomic selection for GER resistance in combined (COMB), between and within KE and PE DH libraries

2 FHB resistance and deoxinivalenol accumulation in four winter small-grain cereals

Received: 5 April 2019 | Revised: 20 September 2019 | Accepted: 28 September 2019

DOI: 10.1111/pbr.12779

ORIGINAL ARTICLE

Plant Breeding WILEY

Comparison of rye, triticale, durum wheat and bread wheat genotypes for Fusarium head blight resistance and deoxynivalenol contamination

David Sewordor Gaikpa | Bärbel Lieberherr | Hans Peter Maurer | C. Friedrich H. Longin | Thomas Miedaner

State Plant Breeding Institute, University of Hohenheim, Stuttgart, Germany

Correspondence
Thomas Miedaner, State Plant Breeding Institute, University of Hohenheim, Fruwirthstr. 21, 70599 Stuttgart, Germany.
Email: miedaner@uni-hohenheim.de

Funding information
German Academic Exchange Service (DAAD, Bonn, Germany), Grant to David S. Gaikpa, University of Hohenheim, grant number 91650671.

Communicated by: Hermann Buerstmayr

Abstract

Small-grain winter cereal crops can be infected with Fusarium head blight (FHB) leading to mycotoxin contamination and reduction in grain weight and quality. Although a number of studies have investigated the genetic variation of genotypes within each small-grain cereal, a systematic comparison of the winter crops rye, triticale, durum and bread wheat for their FHB resistance, *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) contamination across species is still missing. We have therefore evaluated twelve genotypes each of four crops widely varying in their FHB resistance under artificial infection with one DON-producing *F. culmorum* isolate at constant spore concentrations and additionally at crop-specific concentrations in two environments. Rye and triticale were the most resistant crops to FHB followed by bread and durum wheat at constant and crop-specific spore concentrations. On average, rye accumulated the lowest amount of DON (10.08 mg/kg) in the grains, followed by triticale (15.18 mg/kg) and bread wheat (16.59 mg/kg), while durum wheat had the highest amount (30.68 mg/kg). Genotypic variances within crops were significant ($p \leq .001$) in most instances. These results underline the differing importance of breeding for FHB resistance in the different crops.

KEYWORDS

deoxynivalenol concentration, *Fusarium culmorum*, Fusarium head blight resistance, small-grain cereals

1 | INTRODUCTION

Fusarium species cause diseases, reduce yield and produce several mycotoxins in all cereal crops, for example stalk and ear rots in maize and Fusarium head blight (FHB) or scab in small-grain cereals such as rye, triticale, durum wheat and bread wheat. These cereals provide 72% of the total small-grain production in the European Union with 7.4, 11.7, 8.8 and 141.5 million tons harvested in 2017, respectively (FAO, 2019). Modern hexaploid triticale is a cross between tetraploid durum wheat (2n = 28 = AABB, seed parent) and diploid rye (2n = 14 = RR, pollen parent; Ammar, Mergoum, & Rajaram, 2004).

Fusarium culmorum is one of the major *Fusarium* species in Europe causing FHB among small-grain cereals and contaminating the grain with deoxynivalenol (DON) or nivalenol and zearalenone

in cooler areas of Northern, Central and Western Europe (Pasquali et al., 2016; Suchowilska, Kandler, Sulyok, Wiwart, & Krska, 2010). Among these mycotoxins, DON is of high public health concern as the strict EU regulations only allow 1.75 mg/kg in durum wheat and oats and 1.25 mg/kg in the other small-grain cereals for human consumption (The Commission of the European Communities, 2006).

In Central Europe, rye is used for bread making, bioenergy production and animal feed. Triticale is used for both animal feed and bioenergy production, durum wheat for pasta production and bread wheat mainly for bread making and feeding livestock. New wheat varieties can be registered in Germany only if they are at least moderately resistant to FHB infection (Miedaner, Schulthess, Gowda, Reif, & Longin, 2017) because the *Fusarium* mycotoxins produced in grains pose serious health problems to humans and animals when ingested (Pierron, Alassane-Kpembi, & Oswald, 2016).

High demand for uncontaminated grains for food and feed calls for a continuous research into durable and effective ways of reducing FHB infection and DON contamination in small-grain cereals. The use of host–plant resistance is an effective and ecologically safe strategy of reducing *Fusarium* head infection and mycotoxin content in grains. FHB resistance in small-grain cereals can be divided into active or passive resistances (Mesterházy, 1995). The active resistance is made up of five types: type 1, which is resistance to initial pathogen infection; type 2, which involves resistance to the spread of infection within infected spikes; type 3, resistance to kernel infection; type 4, tolerance to infection; and type 5, resistance to mycotoxins. The passive resistance involves the role of agro-morphological traits, such as plant height, flowering time, presence or absence of awns and spikelet density (Mesterházy, 1995). In most cereals, genotypes are evaluated for active resistance types 1 and 2 (Burlakoti, Mergoum, Kianian, & Adhikari, 2010). Resistance to FHB disease is quantitatively inherited and influenced by multiple genes, the environment and genotype × environment (G × E) interaction in all cereals (Becher, Miedaner, & Wirsel, 2013). Hundreds of quantitative trait loci (QTL) have been identified in all genomes (i.e., A, B, D and R) of small-grain cereals (e.g., Arruda et al., 2016; Galiano-Carneiro, Boeven, Maurer, Würschum, & Miedaner, 2019; Ruan et al., 2012), mainly with low effects. Only a few QTL from Chinese origin have a higher impact on FHB resistance in bread wheat (e.g., *Fhb1*, *Fhb5*, *Fhb6*; Bai, Su, & Cai, 2018), and efforts are being made to introgress these QTL into European breeding materials. In durum wheat, individual QTL detected across the A and B genomes explained about 1%–19% of the genotypic variance for FHB resistance (Miedaner, Sieber, et al., 2017; Prat et al., 2017; Ruan et al., 2012; Zhao, Leng, Chao, Xu, & Zhong, 2018). In triticale, single QTL explained between 0.3% and 42% of the genotypic variance for FHB resistance (Dhariwal et al., 2018; Galiano-Carneiro et al., 2019; Kalih, Maurer, & Miedaner, 2015). There are no studies on QTL for FHB resistance in rye. However, previous studies in triticale identified FHB QTL on chromosomes 3R, 4R, 5R and 7R donated by rye (Dhariwal et al., 2018; Galiano-Carneiro et al., 2019; Kalih et al., 2015). The reduced height (*Rht*) genes *Rht-B1* and *Rht-D1* used globally in bread and durum wheat breeding programmes have been associated to higher FHB susceptibility (Draeger et al., 2007; Lu et al., 2013; Miedaner, Sieber, et al., 2017; Miedaner & Voss, 2008; Srinivasachary et al., 2009). In rye and triticale, much less work has been done on the effect of dwarfing genes, but the effect of one of these genes, *Ddw1*, is also increasing FHB severity (Kalih, Maurer, Hackauf, & Miedaner, 2014).

The role of DON as aggressiveness factor in wheat FHB was originally proved by Proctor, Hohn, and McCormick (1995) and shortly after confirmed by several authors (Bai, Desjardins, & Plattner, 2001; Desjardins et al., 1996). Although DON does not support initial infection (type 1 resistance), it is an important factor for the fungus to spread into the wheat head (type 2 resistance). Later on, Langevin, Eudes, and Comeau (2004) demonstrated that this effect is dependent on the cereal species. While in rye, triticale and bread wheat the DON-deficient isolate could not spread within the ear, a limited spread occurred in durum wheat.

Previous studies indicated differing associations between FHB symptoms, FDK rating and DON concentration in small-grain cereals, depending on the environment and the crop species (Góral & Ochodzki, 2017; Góral et al., 2018; Miedaner, 1997; Miedaner & Perkowski, 1996). Usually, genotypes with more symptoms have higher toxin contents. For example, a high correlation (r = .77) between DON and FHB symptoms in wheat was reported (Miedaner et al., 2004). Burlakoti et al. (2010) also observed a positive correlation (r = .67) between FHB severity and DON concentration across 113 F_9 recombinant inbred lines evaluated for two years under greenhouse conditions. In a meta-study, FDK rating had the strongest average association with DON content (r = .73, Paul, Lipps, & Madden, 2005). In triticale, however, a low association (r = .32, p < .001) between FHB severity and DON concentration was detected (Miedaner, Kalih, Großmann, & Maurer, 2016).

Studies have been conducted to separately evaluate rye (Miedaner & Geiger, 1996), triticale (Boeven, Würschum, Weissmann, Miedaner, & Maurer, 2016; Miedaner et al., 2016), durum (Miedaner & Longin, 2014) and bread wheat (Góral et al., 2018; Miedaner et al., 2004) for FHB resistance and mycotoxin accumulation in the grains. Durum wheat was generally described as highly susceptible to FHB infection (Langevin et al., 2004). A previous comparison of winter triticale, bread wheat and rye in field experiments used highly varying numbers of cultivars for each species that were not prescreened for their FHB resistance (Arseniuk, Foremska, Góral, & Chełkowski, 1999). To allow a fair comparison of the winter cereals rye, triticale, durum wheat and bread wheat for resistance to *F. culmorum* head infection (FHB severity, FDK rating) and DON accumulation under field conditions, in our study twelve cultivars per crop with a highly differing resistance level were inoculated with one *F. culmorum* isolate at constant and crop-specific inoculum concentrations. Our objectives were (a) to determine the difference in susceptibility between small-grain cereals; (b) to measure FDK rating and DON content; and (c) to analyse whether there is a difference in ranking of crops when different inoculum concentrations are used.

2 | MATERIALS AND METHODS

2.1 | Plant materials, field design and inoculation

Plant materials used for this study consisted of 12 cultivars or advanced genotypes of winter rye (*Secale cereale* L.), winter triticale (X *Triticosecale* Wittmack), winter durum (*Triticum turgidum* ssp. *durum*) and winter bread wheat (*Triticum aestivum* ssp. *aestivum*) selected on the basis of their known resistance to FHB and representing the maximum genetic variation within one crop in Germany (Table 1). All crops were mechanically sown at once in all environments. The same standard agronomic practices were carried out for the four winter cereal crops in all environments. Weeds were controlled two times by herbicides, first with Herold SC (400 g/L flufenacet, 200 g/L diflufenican) at BBCH 13 (Meier et al., 2009) and second with Ariane C (2.5 g/L florasulam, 100 g/L fluroxypyr, 80 g/L clopyralid) at BBCH 32–39 depending on the year. Insecticide Karate Zeon (250 g/L Lambda-Cyhalothrin) was sprayed two times at BBCH 49–65 to avoid insect damage. To prevent infection by fungal pathogens and complex interactions between *F. culmorum* and other fungi (Miedaner, Reinbrecht, Lauber, Schollenberger, & Geiger, 2001), the fungicides Bravo 500 (500 g/L chlorothalonil) and Acanto (250 g/L picoxystrobin) were sprayed at BBCH 31/33 in 2017. In the 2018 trials, the fungicides Capalo (75 g/L metrafenone, 62.5 g/L epoxiconazole, 200 g/L fenpropimorph) at BBCH 30 and Adexar (62.5 g/L epoxiconazole, 62.5 g/L fluxapyroxad) at BBCH 39 were used. Plant growth regulators CCC + Moddus (chlormequat chloride 66% +250 g/L Trinexapac-ethyl) were applied at BBCH 31 with 1,000 + 400 ml/ha in both years and additionally Camposan (660 g/L Ethephon) at BBCH 37 with 200 ml/ha in 2018 to prevent preharvest lodging.

The highly aggressive single-spore and DON-producing isolate of *F. culmorum* FC46 (=IPO 39-01; Snijders & Perkowski, 1990) was used for inoculum production. Inoculum was prepared on autoclaved wheat kernels as described in detail by Miedaner, Gang, and Geiger (1996). At 75% flowering (according to anther extrusion), inoculation was done separately for each genotype by spraying the heads from above with inoculum using a motor-driven backpack sprayer. Each plot was inoculated with about 100 ml/m^2 inoculum. Two experiments were conducted to evaluate the four winter small-grain cereal crops.

2.1.1 | Experiment 1

In Experiment 1, all four winter cereals with 12 genotypes each were inoculated with a constant spore concentration of 4×10^5 spores/ml. They were evaluated in three environments at the experimental stations of the University of Hohenheim, Germany, in Hohenheim (HOH) near Stuttgart in 2017 and 2018, and Oberer Lindenhof near Reutlingen (OLI) in 2018. To compare the cereal species, the experiment was randomized in a split-plot design with two replications. Cereal crop species were assigned to the main plots and randomized as a complete block design, and genotypes within species were assigned to the subplots randomized as 4 × 3 alpha-lattice design. Each subplot was 0.6 m^2 in size in HOH and 0.4 m^2 in OLI. The HOH location is at Heidfeldhof (9°12′58″E, 48°42′50″N; 400 m above sea level) with 717.9 mm (2017) and 543.7 mm (2018) precipitation per annum (p.a.) and 10.5°C (2017) and 11.5°C (2018) average temperature p.a. OLI is located on the Swabian Alb in Germany, (9°18′12″E, 48°28′26″N; 700 m above sea level) with average annual precipitation and temperature of 612.2 mm and 9.1°C, respectively, in 2018. Temperatures and sums of precipitation during the inoculation periods of the different crops are given in Table S1.

2.1.2 | Experiment 2

In Experiment 2, 12 genotypes each of the four winter cereals were inoculated with a crop-specific spore concentration of 1×10^6 spores/ml for rye, 7.5×10^5 spores/ml for triticale (Boeven et al., 2016) and 2×10^5 spores/ml for durum and bread wheat (Miedaner, Schulthess, et al., 2017) according to the observed differences of FHB susceptibility between the crops in the previous year. Experiment 2 was carried out in 2018, adjacent to experiment 1, in two locations, HOH and OLI. The experimental design was the same as described for experiment 1.

2.2 | Traits recorded

Number of days to heading (HD), plant height (PH) and FHB severity (%) were assessed visually and plotwise for both experiments. HD was recorded when 75% of crop heads emerged from the top leaves. PH was measured from the ground level to the tip of the heads in cm after flowering. FHB severity was assessed on a scale of 0%–100% of infected spikelets per plot at the onset of the first FHB symptoms, 13 days after inoculation (DAI) in OLI 2018 and 18 DAI in HOH 2017 and HOH 2018. Subsequently, the FHB ratings were done at constant intervals of 2 days in HOH and three days in OLI until the start of the yellow ripening. The system of disease rating employed took into account both type 1 and type 2 FHB resistance. The rating was adjusted to the same time interval after inoculation date for each crop to allow a comparison and yielded in total five ratings. Inoculation and disease rating periods for the four crop species are shown in Table S2.

After full ripening, all heads from each plot from experiment 1 were manually harvested with a sickle and threshed with a thresher at low wind speed to reduce loss of infected kernels with low weight. The grains were later cleaned by a machine with a low amount of forced air to get also small, light-weighted kernels into the sample fraction. All fragments of glumes and rachis were carefully removed manually. Rating of *Fusarium*-damaged kernels (FDK) was assessed using a linear scale of 1–9, where 1 = no FDK in the sample and 9 = 100% FDK rating. Kernels which were soft, shrivelled, shrunken and/or possessing white, pink or dark-red discoloration were regarded as FDK (Mesterházy et al., 2005).

TABLE 1 Adjusted means of FHB, FDK and DON concentration for tested rye, triticale, durum and bread wheat cultivars with the indication of the presence of a dwarfing (*dw*) locus across the three environments after inoculation with constant spore concentration

Crop	Cultivar	*dw* locus	FHB (%)	FDK (1–9)	DON (mg/kg)
Rye	Amilo	None	4.04	3.27	8.76
	Conduct	None	4.99	3.32	11.09
	Dank. Diament	None	5.67	3.65	6.82
	Dukato	None	6.48	3.49	7.64
	Helltop	None	5.68	4.76	11.91
	Inspector	None	4.06	3.02	8.56
	KWS Bono	None	6.69	3.54	11.73
	KWS Daniello	None	4.64	3.52	13.46
	KWS Gatano	None	3.32	3.49	9.85
	SU Cossani	None	4.01	3.57	10.88
	SU Performer	None	7.06	3.84	10.33
	SU Santini	None	4.47	4.20	10.70
	$LSD_{5\%}$		1.58	0.46	2.42
Triticale	Adverdo	NA	7.59	3.86	20.34
	Agostino	NA	4.52	3.47	13.44
	Fredro	NA	10.32	4.52	21.08
	Lombardo	NA	8.03	3.62	25.45
	Partout	NA	3.75	3.25	12.10
	Remiko	NA	4.36	2.80	12.03
	Rhenio	NA	4.88	4.02	8.10
	Securo	NA	4.31	3.01	11.83
	SU Agendus	NA	5.73	6.17	18.23
	Tantris	NA	8.27	4.11	15.72
	Team PZO	NA	5.61	3.21	13.53
	Vuka	NA	6.72	4.81	15.40
	$LSD_{5\%}$		2.14	0.84	6.66
Durum wheat	W11010-133-233	*Rht-B1b*[a]	18.73	3.88	13.71
	W11014-120-220	*b*	30.21	6.71	25.92
	W10029-207-305	*b*	28.76	6.55	23.75
	W10021-204-307	*b*	50.73	8.81	78.81
	Cliodur	*b*	28.37	7.79	52.84
	W09026-113-212	*b*	28.02	6.14	23.19
	W09028-114-213	*b*	21.28	5.67	17.00
	6.009/03/03	*b*	21.99	4.98	10.79
	6.040/05/01	*b*	27.99	5.47	10.62
	Lupidur	*b*	38.68	7.05	55.56
	Tempodur	*b*	19.94	6.66	41.56
	Wintergold	*b*	16.93	4.46	14.35
	$LSD_{5\%}$		3.98	0.76	10.12

(Continues)

For measuring DON concentration, grains of the twelve genotypes of each crop species from experiment 1 were analysed using a commercially available enzyme-linked immunosorbent assay (ELISA, RIDASCREEN FAST DON, R-Biopharm AG). About 200 g of the cleaned grains from each genotype was milled to a particle size of approximately 1 mm with a laboratory mill and stored frozen (−20°C) until analysis. DON was extracted from 5 g of each milled sample and quantified following the guidelines provided by the manufacturer. A microtiter plate spectrophotometer (Spectra Basic, TECAN Deutschland GmbH) was used to measure the absorbance at

TABLE 1 (Continued)

Crop	Cultivar	*dw* locus[a]		FHB (%)	FDK (1–9)	DON (mg/kg)
		Rht-B1	*Rht-D1*			
Bread wheat	Anapolis	a[b]	b	14.47	5.15	10.27
	Bernstein	a	a	33.95	6.63	25.75
	Elixer	b	a	28.04	5.61	19.18
	Franz	a	b	54.12	8.47	21.92
	Helmond	a	a	12.16	3.55	9.57
	Inspiration	a	b	50.97	8.02	22.83
	KWS_Maddox	a	b	49.24	8.46	21.23
	Moschus	a	b	28.79	5.42	8.86
	RGT_Reform	a	b	29.21	6.21	17.28
	Spontan	a	a	23.06	4.59	9.13
	Tobak	b	a	57.23	8.79	26.30
	Toras	a	b	21.86	3.94	11.65
	$LSD_{5\%}$			4.50	0.68	6.16

Abbreviations: DON, deoxynivalenol; FDK, *Fusarium*-damaged kernels; FHB, Fusarium head blight; $LSD_{5\%}$, least significance difference at 0.05 probability level; NA, not available.

[a]Personal information by E. Ebmeyer, KWS LOCHOW GmbH.

[b]"a" refers to the tall allele, "b" refers to the short allele of the respective reduced height (*Rht*) gene.

450 nm. A special software package (the RIDA SOFT Win.net) provided by the manufacturer was used to evaluate the immunoassays. The limit of detection for DON was 0.2 mg/kg (ppm).

2.3 | Data analysis

The five ratings of FHB severity were averaged for each genotype and used for analysis of variance. ASReml package (Butler, 2009) within the statistical software R (R Core Team, 2018) was used to estimate means and variance components for each recorded trait. Adjusted means of each trait per crop (main plot) and genotypes within crops (subplots) were calculated based on best linear unbiased estimation (BLUE) while variance components were estimated based on best linear unbiased prediction (BLUP). Akaike information criterion was used to select the best model and to make assumption on heterogeneous residual variance across environments or otherwise. The following hierarchical model with genotypes nested in the cereal crop species was used to estimate means and variance components:

$$Y_{ijklm} = \mu + E_i + C_j + (\mathrm{CE})_{ij} + G{:}C_{jm} + (G{:}\mathrm{CE})_{ijm} + B_{ijkl} + R_{ijk} + e_{ijklm},$$

where Y_{ijklm} = observed phenotypic value for genotype m nested in cereal crop j in environment i, replication k, incomplete block l, and, μ = common mean, E_i = effect of the ith environment, C_j = effect of the jth crop species j, $(\mathrm{CE})_{ij}$ = environment by crop species interaction, $G{:}C_{jm}$ = effect of mth genotype within jth crop species, $(G{:}\mathrm{CE})_{ijm}$ = environment by genotype interaction within jth crop species, B_{ikl} = effect of lth incomplete block, R_{ijk} = effect of kth replication in ith environment and e_{ijklm} = error. Variance components were determined by the restricted maximum likelihood (REML) method assuming a full random model. Best linear unbiased estimates were estimated across environments assuming fixed effects for the genotype, environment and crop species.

Heterogeneous residual variance and equal distribution of means across replication and blocks were assumed for crop species main effects. To estimate the means of cultivars within each crop species, the same model was used as above with omitting factor C and assuming homogeneous residual variance. The "Wald test" in ASReml R package was applied to establish the statistical significance of fixed effects at 5% significance level. Significance of variance components was determined using the likelihood ratio test. Significance differences between means of each trait across crop species as well as FHB severity under constant and crop-specific concentration were determined by using Tukey's method of multiple mean comparison (p = .05). Broad sense (entry-mean) heritability (H^2) was estimated by using modified equations proposed by Hallauer, Carena, and Filho (2010) for each crop species separately:

$$H^2 = \sigma^2_G / (\sigma^2_G + \frac{\sigma^2_{GE}}{E} + \frac{\sigma^2_e}{\mathrm{ER}}),$$

where, σ^2_G = genotypic variance, σ^2_{GE} = genotype × environment interaction variance, σ^2_e = residual error, E = number of environments and R = number of replications per environment. The phenotypic association between recorded traits was estimated by Pearson correlation tests using the "cor.test" function in R statistical software (R Core Team, 2018).

3 | RESULTS

Even though all crop species were planted at the same time, flowering time differed and, hence, also the inoculation periods and the corresponding mean temperatures and sums of precipitation (Table S1).

3.1 | Experiment 1

Among the four winter crops, rye and triticale showed significantly lower FHB severities and FDK ratings than durum and bread wheat (Figure 1, Table 1, Table S3). For DON concentration, bread wheat had a mean value similar to rye and triticale; durum wheat had a significantly higher content. Variation among genotypes was lowest in rye and highest in bread or durum wheat, depending on the trait (Table 1). For HD, rye cultivars were the earliest among crops followed by triticale, durum and bread wheat cultivars (Table S3). The largest variation of average HD was found in triticale, followed by durum wheat and bread wheat. The average HD in rye was less variable. Durum wheat was the shortest crop, followed by bread wheat and triticale while rye was by far the tallest.

The variance among crop species and the genotypic variances within crops were significantly ($p \leq .001$) different from zero in most instances (Table 2). Crop × environment and genotype × environment interaction variances in durum and bread wheat were also significant in most instances ($p \leq .001$). In rye and triticale, the variances varied from non to highly significant depending on the trait (Table 2). Heritabilities were moderate to high with the exception of FHB severity in triticale.

Associations among FHB severity, FDK rating and DON concentration revealed stronger relationships between the resistance traits and DON concentration in durum and bread wheat than in rye and triticale (Table 3). Highly significant correlations ($p = .001$) were detected between FHB and DON in all crop types with the exception of rye. FDK rating and DON concentration as well as FHB severity and FDK rating correlated significantly only for durum and bread wheat due to their larger variation between genotypes (Figure 2). Coefficients of correlation between FHB severity and heading date ranged from $r = -.04$ to $r = .47$ in rye, triticale and bread wheat and were significant only in durum wheat ($r = .72$, $p \leq .01$). Between FHB severity and plant height, no significant ($p \leq .1$) correlation occurred ($r = -.04$ to $-.48$).

3.2 | Experiment 2

A constant spore concentration did not lead to a significantly ($p > .05$) different FHB severity than the inoculation with a crop-specific spore concentration that was adapted to the basic susceptibility of a crop (Table 4). Rye and triticale had a slightly higher FHB severity when inoculated with crop-specific, that is higher spore concentrations, and durum and bread wheat a slightly lower FHB severity because they got lower spore concentrations (Table 4).

4 | DISCUSSION

In this study, we wanted to compare four winter cereal crops important for Central and Northern Europe for their FHB severity, FDK rating and DON concentration: rye, triticale, durum wheat and bread wheat. To allow a fair comparison, we selected 12 cultivars and advanced genotypes per crop with a maximum range of FHB severity on the basis of the official trials for wheat (BSL, 2017) and own experiments for the other cereals. Further, we used the same time interval between inoculation date and rating date for each crop.

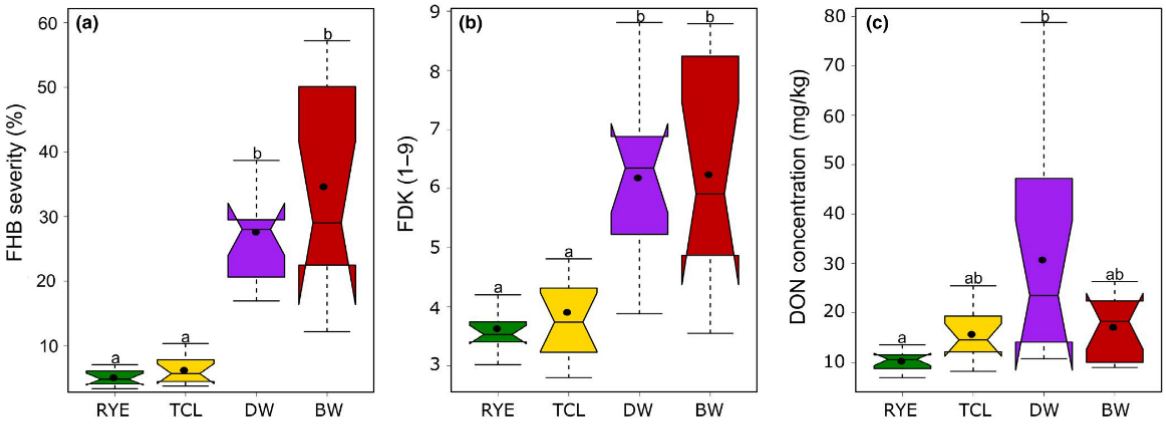


FIGURE 1 Box plots showing ranking of rye, triticale, durum and bread wheat for (a) Fusarium head blight severity, (b) *Fusarium*-damaged kernels and (c) deoxynivalenol concentration after inoculation with constant spore concentration. Black dots and horizontal lines in boxes represent the means and medians, respectively. Boxes sharing the same letters are not significantly different ($p \geq .05$) according to Tukey's test, BW, bread wheat; DW, durum wheat; TCL, triticale [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Variance components and entry-mean heritability (H^2) of FHB severity, FDK, DON content, HD and PH across the four crop species in three environments combined

Parameter	FHB (%)	FDK (1–9)	DON (mg/kg)	HD (days)	PH (cm)
σ^2_C	200.61***	1.68*	28.61	61.09***	609.47***
σ^2_{CxE}	10.96	0.51***	22.99**	2.50***	31.89***
Rye					
σ^2_G	1.06***	0.11	1.95	0.85***	93.87***
σ^2_{GxE}	0.00	0.15	1.27	0.08	7.98*
σ^2_e	1.72	0.13	8.24	0.41	16.84
H^2	0.79	0.61	0.52	0.90	0.94
Triticale					
σ^2_G	1.69	0.78*	18.20***	5.75***	43.46***
σ^2_{GxE}	5.41***	0.03	0.13	0.13	11.83***
σ^2_e	3.58	0.44	32.83	0.86	8.18
H^2	0.41	0.90	0.77	0.97	0.89
Durum wheat					
σ^2_G	69.22***	1.55***	405.45***	1.94***	20.75***
σ^2_{GxE}	54.51***	0.98***	53.90	0.25**	1.13
σ^2_e	17.03	0.37	88.78	0.36	6.09
H^2	0.77	0.80	0.93	0.93	0.94
Bread wheat					
σ^2_G	223.22***	3.07***	27.65***	1.13***	37.95***
σ^2_{GxE}	50.95***	0.80***	21.53	0.57**	10.39***
σ^2_e	16.09	0.26	37.11	0.33	5.22
H^2	0.92	0.91	0.67	0.82	0.90

Notes: σ^2_C = crop species variance, σ^2_{CxE} = crop species by environment interaction variance, σ^2_G = genotypic variance, σ^2_{GxE} = genotype by environment interaction variance, σ^2_e = error variance, H^2 = broad sense heritability.

Abbreviations: DON, deoxynivalenol; FHB, Fusarium head blight; FDK, *Fusarium*-damaged kernels; HD, heading date; PH, plant height.

Significantly different from zero at 0.05*, 0.01** and 0.001*** probability level, respectively, according to the likelihood ratio test.

TABLE 3 Phenotypic correlation coefficients among FHB severity, FDK rating and DON concentration for the 12 genotypes per crop

Correlation	Rye	Triticale	Durum wheat	Bread wheat
FHB—FDK	0.29	0.39	0.81***	0.96***
FHB—DON	0.02	0.77***	0.80***	0.82***
FDK—DON	0.34	0.34	0.89***	0.85***

Abbreviations: DON, deoxynivalenol; FDK, *Fusarium*-damaged kernels; FHB, Fusarium head blight.

***Significantly different from zero at 0.001 probability level.

4.1 | Crops are different in their FHB susceptibility and DON accumulation

A methodological challenge with this experiment is that the different crops flower at different times although they have been sown at the same date (Table S2). This could not be changed because in winter crops a different seeding time does not necessarily result in a similar flowering date. We took this into account by inoculating each crop, and each genotype within the crop, at its respective flowering time. This still provides different weather conditions, but the developmental stage should be of higher importance for FHB severity (Siou et al., 2014). The largest difference in flowering time was observed between rye and wheat with 8–11 days difference in the start of the inoculation period, between triticale and wheat with 4–11 days, while rye and triticale and the two wheat species had a maximal difference of 4 and 2 days, respectively. However, under farmer's field conditions the flowering times of the crops vary even more than in our experiment because different sowing dates are usual. So, the different flowering times could also be seen as an inherent characteristic of the crop.

Among the four crop species, rye and triticale consistently exhibited very low mean levels of FHB severity compared to both wheat species. Even the most resistant wheat genotypes were more susceptible than the worst rye or triticale genotypes (Table 1). Accordingly, kernels of rye and triticale were less damaged by Fusarium infection

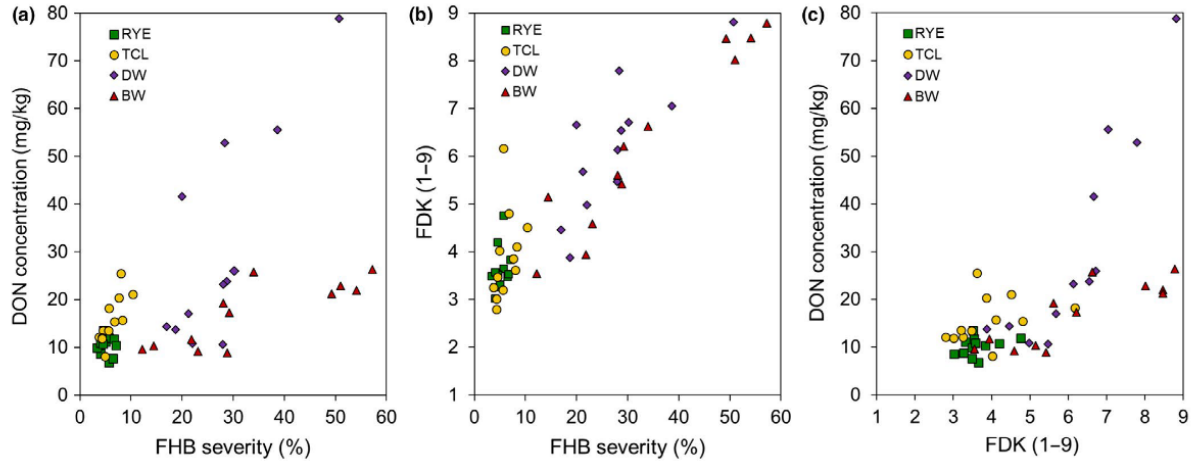


FIGURE 2 Association between (a) Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration, (b) FHB severity and *Fusarium*-damaged kernels (FDK) and (c) FDK and DON concentration in each of 12 cultivars of rye, triticale, durum wheat and bread wheat after inoculation with constant spore concentration

TABLE 4 Means of Fusarium head blight (FHB) severity in rye, triticale, durum wheat and bread wheat under constant and crop-specific spore concentrations across two environments

	FHB (%)	
Crop species	Constant	Crop-specific
Rye	4.24 a	5.56 a
Triticale	4.92 a	6.76 a
Durum wheat	25.46 b	23.01 b
Bread wheat	36.79 c	30.70 c

Notes: Means in the same column or rows sharing the same letters are not significantly different ($p > .05$) according to Tukey's test.

than the kernels of durum and bread wheat cultivars according to the low mean resistance level of the latter to FHB infection. FHB severity was highly correlated with FDK rating only in both wheat species. Correlations were generally not significant for rye, and for triticale only significant for FHB and DON concentration due to the low, although significant, amount of genotypic variation within these crops (Table 2). Because for each correlation only 12 genotypes were available, these data should, however, not be overestimated. Because we used the same time interval between inoculation and rating date for each crop, we might have underestimated the FHB severity of rye and triticale to some extent because in these crops the incubation period is usually longer and a later rating date might have yielded somewhat higher values. However, this should have no large impact because FDK rating after harvest showed exactly the same proportions like FHB severity.

The high susceptibility of durum wheat was reported earlier several times (Langevin et al., 2004; Miedaner, Sieber, et al., 2017). In our study, the most susceptible durum wheat cultivar showed 6.5% less symptoms than the most susceptible bread wheat cultivar because we have chosen the most susceptible bread wheat cultivar to demonstrate the full range of this crop (Table 1).

In previous QTL studies in triticale (Dhariwal et al., 2018; Galiano-Carneiro et al., 2019; Kalih et al., 2015), FHB resistance was attributed to the A and B genomes but also to several rye chromosomes. This might explain the lower susceptibility of hexaploid triticale compared to durum wheat, from which it was derived as female parent. Similarly, Arseniuk et al. (1999) and Miedaner et al. (2001) reported that bread wheat cultivars were less resistant to FHB infection than rye and triticale and always contained a higher amount of DON. Rye and triticale were also found to be more resistant to *F. graminearum* than bread wheat and durum wheat concerning type 2 resistance in the greenhouse (Langevin et al., 2004). Heterogeneity of population and hybrid cultivars in rye (Miedaner & Laidig, 2019) might contribute to the low susceptibility of the crop. In contrast, the other crops include only homogeneous and homozygous cultivars. But still, triticale is only slightly more susceptible for FHB than rye.

For the differences between crop species, passive resistance mechanisms might play a larger role than in assortments within single crops. Among them, plant height, anther extrusion, spike morphology or waxy layer might be of importance. The high basic level of susceptibility of durum and bread wheat to *F. culmorum* head infection could be partly attributed to the presence of reduced height genes (*Rht*), such as *Rht-B1b* and *Rht-D1b* that are widely distributed among Central and Northern European bread wheat cultivars (Miedaner & Voss, 2008) and ubiquitously found in durum wheat cultivars (Miedaner, Sieber, et al., 2017, Table 1). There is strong evidence for the highly significant and negative association between FHB infection and the presence of the semi-dwarfing *Rht-B1b* and *Rht-D1b* alleles (Draeger et al., 2007; Lu et al., 2013; Srinivasachary et al., 2009). In earlier studies, the German bread wheat cultivar 'Toras' known to contain the *Rht-B1b* allele was also found to be quantitatively resistant to FHB (Miedaner & Voss, 2008). Therefore, it was concluded that the presence of FHB resistance QTL in wheat cultivars could counterbalance the negative effects of *Rht* genes and

this has been proved in the meantime (Miedaner, Herter, Ebmeyer, Kollers, & Korzun, 2019). However, this requires a larger population size for selecting short, FHB-resistant cultivars in wheat breeding. In rye, semi-dwarfness plays no role in commercial cultivars and in triticale only a few semi-dwarf cultivars are known (Kalih et al., 2014). Thus, rye and triticale are much taller crops than both wheat species, a fact that might contribute to their higher resistance level, because plant height per se has a positive effect on FHB resistance (Mesterházy, 1995). In wheat, it was shown that genotypes extruding their anthers readily after flowering are less prone to FHB infection than those retaining their anthers inside the florets to a high proportion (Buerstmayr & Buerstmayr, 2015). Moreover, this trait is linked with *Rht* genes in the sense that the semi-dwarf lines have a high proportion of retained anthers and, thus, increased FHB susceptibility (Buerstmayr & Buerstmayr, 2016). In the outcrossing rye, all anthers are fully extruded at flowering. Also, triticale tends to extrude anthers more readily than wheat. In triticale and both wheats, the flowers are very close to the rachis and to each other. Wheat genotypes have, on average, three flowers per rachis node and a denser head than rye and triticale. Genotypes with the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* have an even denser head. In contrast, rye has only one flower per rachis node and a less dense head. Both should give a better aeration than in the other crops and additionally provide a longer distance for the fungus when spreading through the rachis. Also, the waxy layer on the heads is much thicker in rye, and partially in triticale, than in wheat. Caused by the different colours of the ears in triticale, the presence of awns and anthocyanins, and the greyish waxy layer in rye, these crops are more difficult to score visually for FHB severity because the differences between the colour of the ears and the symptoms are less pronounced than in the wheat species. This might also cause the lower correlations among resistance traits in rye and triticale. All these morphological traits may contribute to a lower basic susceptibility of rye and triticale.

Deoxynivalenol accumulation basically reflected the different FHB severities of the crops with rye having the lowest mean values, followed by triticale. Caused by the large variation among genotypes, bread wheat had a similar mean DON concentration to triticale and rye, but a broader variation. This is due to the fact that we have now some rather resistant wheat cultivars on the market. However, durum wheat had, on average, considerably higher (46% more) DON concentrations than bread wheat although exposing a rather similar mean FHB severity. Hence, the mechanism that regulates FHB severity may be at least partially different from the one that regulates DON accumulation, depending on the cereal species (Draeger et al., 2007; Miedaner et al., 2016). The level of DON produced in the kernels of the four cereal crops by isolate FC46 in our study was higher than the DON concentrations reported elsewhere (Arseniuk et al., 1999; Góral & Ochodzki, 2017), but similar to the levels reported by Miedaner et al. (2001). This confirms the high DON-producing ability of isolate FC46. Durum and bread wheat cultivars accumulated, on average, 67% and 39% more DON, respectively, than rye. Furthermore, similar to the observation of Miedaner et al. (2001), triticale cultivars accumulated on average 38% more DON than rye.

Obviously, the different crop species provide a different suitability to serve as substrate for DON production of *F. culmorum*. It is totally unclear whether special ingredients of the crops are responsible for this or whether the differing DON levels just reflect a different fungal colonization of the tissue. There might be compounds suppressing fungal growth in rye and triticale or, alternatively, compounds favouring infection in durum and bread wheat. In previous studies, phenolic acids, such as gallic, chlorogenic and caffeic acids were reported to inhibit *F. graminearum* and *F. culmorum* growth as well as mycotoxin production (Gauthier et al., 2016; Pagnussatt, Medeiros, Ponte, & Garda-Buffon, 2014). Differing breeding efforts could not be the cause because in wheat breeding and research much more attention is given to FHB resistance than in rye or triticale breeding.

Summarizing the results, the cereal crop species with high levels of FDK rating accumulated higher levels of DON (Figure 2) and this was confirmed by the high correlation between FDK rating and DON, especially in the most susceptible crop species, durum and bread wheat. In comparison, rye and triticale showed by far the lowest FHB severity and FDK rating and DON concentrations. Durum wheat displayed higher DON concentrations than bread wheat. There are hints that different regulatory mechanisms are involved in the symptom development and DON accumulation in bread wheat (He, Dreisigacker, Singh, & Singh, 2019).

4.2 | Significant genetic variation occurs within all crops

Significant genetic variation was found between genotypes within each crop species (Table 1). Within bread and durum wheat, a wider genetic variation for FHB resistance and FDK rating was found than within rye and triticale. This explains the higher heritability values detected in bread wheat for these traits compared to the other crop species. For DON concentration, the widest genetic variation was detected in durum wheat with a high heritability. Similar to our findings, Miedaner, Schneider, and Geiger (2003) reported a lower heritability for DON concentration in rye than in bread wheat. G × E interaction variances were important for the majority of the *Fusarium*-related traits as known from literature (Miedaner et al., 2001), and this might be partly due to large differences in average temperature and sum of precipitation in the different environments (location–year combinations, Table S1). The large difference in the wheat species is caused by the selection of FHB-resistant germplasm by breeders. Some cultivars among bread wheat (such as 'Helmond', 'Anapolis' and 'Toras') and durum wheat (such as 'Tempodur', '6134/16' and 'Wintergold', Table 1) had lower FHB severity and may carry several resistance FHB QTL in their genome. On the other hand, there are still highly susceptible cultivars in the market (such as 'Tobak', 'Franz' and 'Inspiration' in bread wheat) greatly enlarging the genetic variation. Accordingly, there is a threefold difference in DON concentrations between the lowest and the highest DON-accumulating cultivar in bread wheat and even a more than sevenfold difference in durum

wheat (Table 1). In triticale, the difference is threefold and in rye twofold only. This shows the large potential of resistance breeding for reducing DON concentration in the most susceptible crops and the relative advantage of rye and triticale in this respect. Given the exclusive use of durum wheat for human consumption as pasta, the high DON concentrations of some released cultivars are alarming. But the best durum wheat 'Wintergold' has almost the same level of FHB resistance as the best bread wheat 'Helmond' (16.93% and 12.16%) with similar DON concentrations (14.35 and 9.57 mg/kg).

4.3 | Inoculum concentration did not change the ranking of crops

According to the considerably different ranking of the crops for FHB severity in the first year, we adjusted in the second year the spore concentrations in an additional experiment in such a way that the more resistant rye and triticale got higher concentrations (1×10^6, 7.5×10^5 spores/ml, respectively) and the more susceptible durum and bread wheat a lower concentration (2×10^5 spores/ml) than initially (4×10^5 spores/ml). The different concentrations, however, did not significantly change ranking of crops for FHB severity (Table 4). Though the crop-specific concentrations caused somewhat higher FHB severity in rye and triticale and somewhat lower FHB severity in the two wheat species, the differences observed in mean values between the two inoculum concentrations were not significantly ($p > .05$) different. Different *F. culmorum* spore concentration levels have been used in different studies to evaluate small-grain cereal crops for FHB resistance and low mycotoxin accumulation (Arseniuk et al., 1999; Boeven et al., 2016; Góral & Ochodzki, 2017; Góral et al., 2018; Miedaner et al., 2016). Our finding, however, demonstrates that a concentration level of 4×10^5 spores/ml is adequate for comparing genotypes of these four crop species against FHB infection without being biased to any of the crop species studied.

5 | CONCLUSIONS

Rye was the most resistant crop to FHB and had the lowest DON content and kernel damage while durum wheat was the crop with the highest kernel damage and DON concentration. The outcome of this study suggests that durum and bread wheat which are among the topmost grown cereal crops worldwide are most susceptible to FHB and may accumulate high levels of DON that are hazardous for human consumption and animal welfare as well. And DON is only one of the *Fusarium* mycotoxins, in the same grain lot additional toxins can be expected. Suchowilska et al. (2010) reported 11 toxic metabolites, including DON, 3-acetyl DON and DON-3-glucoside, isolated from hulled wheat when inoculated with one *F. culmorum* isolate. Hence, breeding for FHB resistance and low DON concentration must have a high priority in these crops. The significant genetic variation within rye, triticale, durum and bread wheat for all FHB-related traits illustrates that genetic progress should be possible. In the meantime, the best durum genotypes nearly reach the FHB level of the most resistant bread wheat. The transfer of resistance QTL from rye to wheat might be a long-term goal. In the past, wheat-rye translocations were a great success in terms of resistances to powdery mildew and rusts and high grain yield, but unfortunately never gave rise to a good baking quality. Future studies to compare FHB resistance levels of rye, triticale, durum wheat and bread wheat should incorporate the effect of different isolates. Further research is required to better understand the regulatory mechanisms of DON accumulation in small-grain cereal crops.

ACKNOWLEDGEMENTS

The authors thank the teams at the respective locations for their excellent technical support.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

DG conceived the manuscript, did the trait recording in 2018 and calculated all data. TM designed and supervised the experiment, BL did the trait recording in 2017 and technically prepared the experiment in both years, and HPM and FL shared the triticale and wheat cultivars, respectively. Further, HPM helped with the statistical analyses. All authors read and edited the manuscript.

ORCID

Thomas Miedaner https://orcid.org/0000-0002-9541-3726

REFERENCES

Ammar, K., Mergoum, M., & Rajaram, S. (2004). The history and evolution of triticale. In M. Mergoum, & H. Gómez-Macpherson (Eds.), *Triticale improvement and production* (pp. 1–9). Rome, Italy: Food and Agriculture Organization of the United Nations.

Arruda, M. P., Brown, P., Brown-Guedira, G., Krill, A. M., Thurber, C., Merrill, K. R., … Kolb, F. L. (2016). Genome-wide association mapping of Fusarium Head Blight resistance in wheat using genotyping-by-sequencing. *The Plant Genome*, *9*(1), https://doi.org/10.3835/plantgenome2015.04.0028

Arseniuk, E., Foremska, E., Góral, T., & Chełkowski, J. (1999). Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye. *Journal of Phytopathology*, *147*(10), 577–590. https://doi.org/10.1046/j.1439-0434.1999.00433.x

Bai, G., Desjardins, A. E., & Plattner, R. D. (2001). Deoxynivalenol-nonproducing Fusarium graminearum causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia*, *153*, 91–98. https://doi.org/10.1023/A:1014419323550

Bai, G., Su, Z., & Cai, J. (2018). Wheat resistance to Fusarium head blight. *Canadian Journal of Plant Pathology*, *40*(3), 1–11. https://doi.org/10.1080/07060661.2018.1476411

Becher, R., Miedaner, T., & Wirsel, S. (2013). Biology, diversity and management of FHB-causing Fusarium species in small-grain cereals. In F. Kempken (Ed.), *Agricultural Applications* (2nd ed., pp. 199–241). Berlin, Heidelberg, Germany: Springer-Verlag. https://doi.org/10.1007/978-3-642-36821-9_8

Boeven, P. H. G., Würschum, T., Weissmann, S., Miedaner, T., & Maurer, H. P. (2016). Prediction of hybrid performance for Fusarium head

blight resistance in triticale (X *Triticosecale* Wittmack). *Euphytica*, *207*(3), 475–490. https://doi.org/10.1007/s10681-015-1498-9

BSL (2017). *Beschreibende Sortenliste für Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben, Zwischenfrüchte* [Descriptive variety list for cereals, maize, oil and fibre plants, pulse crops, beets, catch crops, in German]. Hannover, Germany: Bundessortenamt.

Buerstmayr, M., & Buerstmayr, H. (2015). Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo × Arina. *Theoretical and Applied Genetics*, *128*(8), 1519–1530. https://doi.org/10.1007/s00122-015-2527-8

Buerstmayr, M., & Buerstmayr, H. (2016). The semidwarfing alleles *Rht-D1b* and *Rht-B1b* show marked differences in their associations with anther-retention in wheat heads and with Fusarium head blight susceptibility. *Phytopathology*, *106*(12), 1544–1552. https://doi.org/10.1094/PHYTO-05-16-0200-R

Burlakoti, R. R., Mergoum, M., Kianian, S. F., & Adhikari, T. B. (2010). Combining different resistance components enhances resistance to Fusarium head blight in spring wheat. *Euphytica*, *172*(2), 197–205. https://doi.org/10.1007/s10681-009-0035-0

Butler, D. (2009). *asreml: asreml() fits the linear mixed model*. R package version 3.0. Retrieved from www.vsni.co.uk

Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., & Hohn, T. M. (1996). Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Molecular Plant-Microbe Interactions*, *9*(9), 775–781. https://doi.org/10.1094/MPMI-9-0775

Dhariwal, R., Fedak, G., Dion, Y., Pozniak, C., Laroche, A., Eudes, F., & Randhawa, H. S. (2018). High density single nucleotide polymorphism (SNP) mapping and quantitative trait loci (QTL) analysis in a biparental spring triticale population localized major and minor effect fusarium head blight resistance and associated traits QTL. *Genes*, *9*(1), 1–26. https://doi.org/10.3390/genes9010019

Draeger, R., Gosman, N., Steed, A., Chandler, E., Thomsett, M., Srinivasachary, … Nicholson, P. (2007). Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. *Theoretical and Applied Genetics*, *115*(5), 617–625. https://doi.org/10.1007/s00122-007-0592-3

FAO [Food and Agriculture Organization of the United Nations] (2019). *FAOSTAT Database*. Rome, Italy: FAO [Food and Agriculture Organization of the United Nations].

Galiano-Carneiro, A. L., Boeven, P. H. G., Maurer, H. P., Würschum, T., & Miedaner, T. (2019). Genome-wide association study for an efficient selection of Fusarium head blight resistance in winter triticale. *Euphytica*, *215*(1), 4. https://doi.org/10.1007/s10681-018-2327-8

Gauthier, L., Bonnin-Verdal, M. N., Marchegay, G., Pinson-Gadais, L., Ducos, C., Richard-Forget, F., & Atanasova-Penichon, V. (2016). Fungal biotransformation of chlorogenic and caffeic acids by *Fusarium graminearum*: New insights in the contribution of phenolic acids to resistance to deoxynivalenol accumulation in cereals. *International Journal of Food Microbiology*, *221*(856), 61–68. https://doi.org/10.1016/j.ijfoodmicro.2016.01.005

Góral, T., & Ochodzki, P. (2017). Fusarium head blight resistance and mycotoxin profiles of four *Triticum* species genotypes. *Phytopathologia Mediterranea*, *56*(1), 175–186. https://doi.org/10.14601/Phytopathol_Mediterr-20288

Góral, T., Wiśniewska, H., Ochodzki, P., Nielsen, L. K., Walentyn-Góral, D., & Stępień, Ł. (2018). Relationship between Fusarium head blight, kernel damage, concentration of *Fusarium* biomass, and *Fusarium* toxins in grain of winter wheat inoculated with *Fusarium culmorum*. *Toxins*, *11*(1), 2. https://doi.org/10.3390/toxins11010002

Hallauer, A., Carena, M., & Filho, J. (2010). *Quantitative genetics in maize breeding. Handbook of plant breeding* (3rd ed.). New York, NY: Springer-Verlag. https://doi.org/10.1007/978-1-4419-0766-0

He, X., Dreisigacker, S., Singh, R. P., & Singh, P. K. (2019). Genetics for low correlation between Fusarium head blight disease and deoxynivalenol (DON) content in a bread wheat mapping population. *Theoretical and Applied Genetics*, *132*(8), 2401–2411.

Kalih, R., Maurer, H. P., Hackauf, B., & Miedaner, T. (2014). Effect of a rye dwarfing gene on plant height, heading stage, and Fusarium head blight in triticale (X *Triticosecale* Wittmack). *Theoretical and Applied Genetics*, *127*(7), 1527–1536. https://doi.org/10.1007/s00122-014-2316-9

Kalih, R., Maurer, H. P., & Miedaner, T. (2015). Genetic architecture of Fusarium head blight resistance in four winter triticale populations. *Phytopathology*, *105*(3), 334–341. https://doi.org/10.1094/PHYTO-04-14-0124-R

Langevin, F., Eudes, F., & Comeau, A. (2004). Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium head blight development in six cereal species. *European Journal of Plant Pathology*, *110*(7), 735–746. https://doi.org/10.1023/B:EJPP.0000041568.31778.ad

Lu, Q., Lillemo, M., Skinnes, H., He, X., Shi, J., Ji, F., … Bjørnstad, Å. (2013). Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line "Shanghai-3/Catbird". *Theoretical and Applied Genetics*, *126*(2), 317–334. https://doi.org/10.1007/s00122-012-1981-9

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hack, H., Heß, M., … Weber, E. (2009). The BBCH system to coding the phenological growth stages of plants–history and publications. *Journal Für Kulturpflanzen*, *61*(2), 41–52.

Mesterházy, Á. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding*, *114*, 377–386. https://doi.org/10.1111/j.1439-0523.1995.tb00816.x

Mesterházy, Á., Bartók, T., Kászonyi, G., Varga, M., Tóth, B., & Varga, J. (2005). Common resistance to different *Fusarium* spp. causing Fusarium head blight in wheat. *European Journal of Plant Pathology*, *112*, 267–281. https://doi.org/10.1007/s10658-005-2853-9

Miedaner, T. (1997). Breeding wheat and rye for resistance to Fusarium diseases. *Plant Breeding*, *116*, 201–220. https://doi.org/10.1111/j.1439-0523.1997.tb00985.x

Miedaner, T., Gang, G., & Geiger, H. H. (1996). Quantitative-genetic basis of aggressiveness of 42 isolates of *Fusarium culmorum* for winter rye head blight. *Plant Disease*, *80*, 500–504. https://doi.org/10.1094/PD-80-0500

Miedaner, T., & Geiger, H. H. (1996). Estimates of combining ability for resistance of winter rye to *Fusarium culmorum* head blight. *Euphytica*, *89*, 339–344.

Miedaner, T., Heinrich, N., Schneider, B., Oettler, G., Rohde, S., & Rabenstein, F. (2004). Estimation of deoxynivalenol (DON) content by symptom rating and exoantigen content for resistance selection in wheat and triticale. *Euphytica*, *139*, 123–132. https://doi.org/10.1007/s10681-004-2489-4

Miedaner, T., Herter, C. P., Ebmeyer, E., Kollers, S., & Korzun, V. (2019). Use of non-adapted quantitative trait loci for increasing Fusarium head blight resistance for breeding semi-dwarf wheat. *Plant Breeding*, https://doi.org/10.1111/pbr.12683 (online)

Miedaner, T., Kalih, R., Großmann, M. S., & Maurer, H. P. (2016). Correlation between Fusarium head blight severity and DON content in triticale as revealed by phenotypic and molecular data. *Plant Breeding*, *135*, 31–37. https://doi.org/10.1111/pbr.12327

Miedaner, T., & Laidig, F. (2019). Hybrid breeding in rye (*Secale cereale* L.). In: J. M. Al-Khayri et al. (Eds.), *Advances in Plant Breeding Strategies, Vol 5: Cereals* (pp. 343–372). Cham, Switzerland: Springer International Publishing.

Miedaner, T., & Longin, F. H. C. (2014). Genetic variation for resistance to Fusarium head blight in winter durum material. *Crop & Pasture Science*, *65*, 46–51. https://doi.org/10.1071/CP13170

Miedaner, T., & Perkowski, J. (1996). Correlations among *Fusarium culmorum* head blight resistance, fungal colonization and mycotoxin

contents in winter rye. *Plant Breeding*, *115*, 347–351. https://doi.org/10.1111/j.1439-0523.1996.tb00932.x

Miedaner, T., Reinbrecht, C., Lauber, U., Schollenberger, M., & Geiger, H. H. (2001). Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. *Plant Breeding*, *120*, 97–105. https://doi.org/10.1046/j.1439-0523.2001.00580.x

Miedaner, T., Schneider, B., & Geiger, H. H. (2003). Deoxynivalenol (DON) content and Fusarium head blight resistance in segregating populations of winter rye and winter wheat. *Crop Science*, *43*, 519–526.

Miedaner, T., Schulthess, A. W., Gowda, M., Reif, J. C., & Longin, C. F. H. (2017). High accuracy of predicting hybrid performance of Fusarium head blight resistance by mid-parent values in wheat. *Theoretical and Applied Genetics*, *130*, 461–470. https://doi.org/10.1007/s00122-016-2826-8

Miedaner, T., Sieber, A. N., Desaint, H., Buerstmayr, H., Longin, C. F. H., & Würschum, T. (2017). The potential of genomic-assisted breeding to improve Fusarium head blight resistance in winter durum wheat. *Plant Breeding*, *136*, 610–619. https://doi.org/10.1111/pbr.12515

Miedaner, T., & Voss, H. H. (2008). Effect of dwarfing *Rht* genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. *Crop Science*, *48*, 2115–2122. https://doi.org/10.2135/cropsci2008.02.0107

Pagnussatt, A. F., Medeiros, E., Ponte, D., & Garda-Buffon, J. (2014). Inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* sp. *Pesticide Biochemistry and Physiology*, *108*, 21–26. https://doi.org/10.1016/j.pestbp.2013.11.002

Pasquali, M., Beyer, M., Logrieco, A., Audenaert, K., Balmas, V., Basler, R., … Vogelgsang, S. (2016). A European database of *Fusarium graminearum* and *F. culmorum* trichothecene genotypes. *Frontiers in Microbiology*, *7*, 406. https://doi.org/10.3389/fmicb.2016.00406

Paul, P. A., Lipps, P. E., & Madden, L. V. (2005). Relationship between visual estimates of fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: A meta-analysis. *Phytopathology*, *95*, 1225–1236. https://doi.org/10.1094/PHYTO-95-1225

Pierron, A., Alassane-Kpembi, I., & Oswald, I. P. (2016). Impact of two mycotoxins deoxynivalenol and fumonisin on pig intestinal health. *Porcine Health Management*, *2*, 21. https://doi.org/10.1186/s40813-016-0041-2

Prat, N., Guilbert, C., Prah, U., Wachter, E., Steiner, B., Langin, T., … Buerstmayr, H. (2017). QTL mapping of Fusarium head blight resistance in three related durum wheat populations. *Theoretical and Applied Genetics*, *130*, 13–27.

Proctor, R. H., Hohn, T. M., & McCormick, S. P. (1995). Reduced virulence of *Gibberella zea* caused by disruption of a trichothecene toxin biosynthetic gene. *Molecular Plant-Microbe Interaction*, *8*(4), 593–601.

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ruan, Y., Comeau, A., Langevin, F., Hucl, P., Clarke, J. M., Brule-Babel, A., & Pozniak, C. J. (2012). Identification of novel QTL for resistance to Fusarium head blight in a tetraploid wheat population. *Genome*, *55*, 853–864. https://doi.org/10.1139/gen-2012-0110

Siou, D., Gélisse, S., Laval, V., Repinçay, C., Canalès, R., Suffert, F., & Lannou, C. (2014). Effect of wheat spike infection timing on Fusarium head blight development and mycotoxin accumulation. *Plant Pathology*, *63*, 390–399. https://doi.org/10.1111/ppa.12106

Snijders, C. H. A., & Perkowski, J. (1990). Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. *Phytopathology*, *80*, 566–570. https://doi.org/10.1094/Phyto-80-566

Srinivasachary, Gosman, N., Steed, A., Hollins, T. W., Bayles, R., Jennings, P., & Nicholson, P. (2009). Semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to Fusarium head blight. *Theoretical and Applied Genetics*, *118*, 695–702. https://doi.org/10.1007/s00122-008-0930-0

Suchowilska, E., Kandler, W., Sulyok, M., Wiwart, M., & Krska, R. (2010). Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*. *Journal of the Science of Food and Agriculture*, *90*, 556–565. https://doi.org/10.1002/jsfa.3844

The Commission of the European Communities (2006). Commission Regulation (EC) 1881/2006 Setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, *364*(1074), 5–24.

Zhao, M., Leng, Y., Chao, S., Xu, S. S., & Zhong, S. (2018). Molecular mapping of QTL for Fusarium head blight resistance introgressed into durum wheat. *Theoretical and Applied Genetics*, *131*, 1939–1951. https://doi.org/10.1007/s00122-018-3124-4

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Gaikpa DS, Lieberherr B, Maurer HP, Longin CFH, Miedaner T. Comparison of rye, triticale, durum wheat and bread wheat genotypes for Fusarium head blight resistance and deoxynivalenol contamination. *Plant Breed*. 2020;139:251–262. https://doi.org/10.1111/pbr.12779

SUPPORTING INFORMATION

S1 Average temperature (°C) and sum of precipitation (mm) during inoculation period of rye, triticale, durum and bread wheat in HOH 2017, HOH 2018 and OLI 2018

Crop species	Environment	Temperature (◦C)	Precipitation (mm)
Rye	HOH2017	18.8	0.0
	HOH2018	13.4	15.7
	OLI2018	18.8	0.1
Triticale	HOH2017	21.5	2.9
	HOH2018	14.3	39.8
	OLI2018	17.7	12.3
Durum wheat	HOH2017	16.5	37.9
	HOH2018	18.0	0.0
	OLI2018	18.5	3.8
Bread wheat	HOH2017	14.6	4.3
	HOH2018	20.1	0.0
	OLI2018	18.5	3.8

HOH = Hohenheim, OLI = Oberer Lindenhof

S2 Inoculation and rating periods of rye, triticale, winter durum and bread wheat in HOH 2017, HOH 2018 and OLI 2018

Crop species	Environment	Inoculation period	Rating period
Rye	HOH2017	May 25-27, 2017	June 12-24, 2017
	HOH2018	May 14-16, 2018	June 1-11, 2018
	OLI2018	May 26-28, 2018	June 8-23, 2018
Triticale	HOH2017	May 29-June 2, 2017	June 16-26, 2017
	HOH2018	May 14-22, 2018	June 1-17, 2018
	OLI2018	May 28-June 1, 2018	June 10-28, 2018
Durum wheat	HOH2017	June 2-8, 2017	June 20-30, 2017
	HOH2018	May 25, 2018	June 11-19, 2018
	OLI2018	June 4-7, 2018	June 17-July 2, 2018
Bread wheat	HOH2017	June 4-8, 2017	June 22-July 2, 2017
	HOH2018	May 25-27, 2018	June 11-21, 2018
	OLI2018	June 4-7, 2018	June 17-July 2, 2018

HOH = Hohenheim, OLI = Oberer Lindenhof

S3 Adjusted means and ranges of FHB (%), FDK (1-9), DON (mg kg^{-1}), HD (days) and PH (cm) in rye, triticale, durum and bread wheat across three environments

Crop species	Para-meter	FHB (%)	FDK (1-9)	DON (mg kg^{-1})	HD (days)	PH (cm)
Rye	Mean	5.09 a	3.64 a	10.08 a	131.97 a	144.83 a
	Range	3.32-7.06	3.02-4.76	6.82-13.46	130.67-133.33	132.15-157.67
Triticale	Mean	6.17 a	3.90 a	15.18 ab	140.51 b	110.30 b
	Range	3.75-10.32	2.80-6.17	8.10-25.45	134.83-144.49	99.66-122.80
Durum wheat	Mean	27.72 b	6.17 b	30.68 b	147.93 c	90.73 c
	Range	16.93-50.73	3.88-8.81	10.62-78.81	146.17-151.50	84.50-98.00
Bread wheat	Mean	33.57 b	6.24 b	16.59 ab	148.96 c	92.64 c
	Range	12.16-57.23	3.55-8.78	8.86-26.30	147.56-150.17	84.64-105.67

Means for each trait sharing the same letters are not significantly different at 0.05 probability level according to Tukey's test. FHB = Fusarium head blight, FDK = *Fusarium*-damaged kernel, DON = deoxynivalenol, HD =heading date, PH = plant height

3 Molecular basis of FHB resistance in rye (*Secales cereale* L.)

Received: 24 October 2019 | Revised: 4 February 2020 | Accepted: 5 February 2020
DOI: 10.1111/pbr.12810

ORIGINAL ARTICLE

Plant Breeding WILEY

Genome-wide association mapping and genomic prediction of Fusarium head blight resistance, heading stage and plant height in winter rye (*Secale cereale*)

David Sewordor Gaikpa[1] | Silvia Koch[1] | Franz Joachim Fromme[2] | Dörthe Siekmann[2] | Tobias Würschum[1] | Thomas Miedaner[1]

[1]State Plant Breeding Institute, University of Hohenheim, Stuttgart, Germany
[2]HYBRO Saatzucht GmbH & Co. KG, Schenkenberg, Germany

Correspondence
Thomas Miedaner, State Plant Breeding Institute, University of Hohenheim, Fruwirthstr. 21, 70599 Stuttgart, Germany.
Email: miedaner@uni-hohenheim.de

Funding information
This study was partly financed by the German Academic Exchange Service (DAAD, Bonn, Germany) as a personal grant to David S. Gaikpa, (grant number 91650671), by the company HYBRO Saatzucht GmbH & Co. KG and by a grant of the University of Hohenheim (TG77).

Communicated by: Hermann Buerstmayr

Abstract

Rye is a multi-purpose cereal crop grown in Central and Eastern Europe as well as in Western Canada. Fusarium head blight (FHB) is one of the diseases that have a severe negative impact on rye, but knowledge about FHB resistance at the genomic level is totally missing in rye. The objective of this study was to elucidate the genetic architecture of FHB resistance in winter rye using genome-wide association (GWA) mapping complemented by genomic prediction (GP) in comparison with marker-assisted selection (MAS). Additionally, plant height and heading stage were analysed. A panel of 465 S_1-inbred lines of winter rye was phenotyped in three environments (location–year combinations) for FHB resistance by inoculation with *Fusarium culmorum* and genotyped with a 15k SNP array. Significant genotypic variation and high heritabilities were found for FHB resistance, heading stage and plant height. FHB did not correlate with heading stage, but was moderately correlated with plant height ($r = -.52$, $p < .001$) caused by some susceptible short inbred lines. The GWA scan identified 15 QTL for FHB resistance that jointly explained 74% of the genotypic variance. In addition, we detected 11 QTL for heading stage and 8 QTL for plant height, explaining 26% and 14% of the genotypic variance, respectively. A genome-wide prediction approach resulted in 44% higher prediction abilities than marker-assisted selection for FHB resistance. In conclusion, genomic approaches appear promising to improve and accelerate breeding for complex traits in winter rye.

KEYWORDS
agronomic traits, *Fusarium* resistance, genomic prediction, GWAS, QTL, rye

1 | INTRODUCTION

Winter rye was grown in Northeastern Europe on 3.2 million hectares in 2017 (Food and Agriculture Organization of the United Nations (FAO), 2019). Germany, Poland, Russia, Finno-Scandinavia, Belarus and Ukraine together contribute 74% of the worldwide harvest (FAO, 2019). Rye grain is traditionally used for bread making, but also as home-grown feed and as a substrate for bioethanol and biogas production (Miedaner & Laidig, 2019). Rye is an allogamous and diploid (RR, $2n = 2x = 14$) small-grain cereal crop belonging to the

Triticeae and is reported to have a large genome of approximately 7.9 Gbp (Bartoš et al., 2008). It is the paternal donor for triticale (Ammar, Mergoum, & Rajaram, 2004) and has been used to improve important agronomic traits of wheat (Kim, Johnson, Baenziger, Lukaszewski, & Gaines, 2004; Schlegel & Korzun, 1997; Zhou et al., 2007). Hybrid rye is the most common cultivar type in Central Europe, commercially available in Germany, Austria, Denmark, Sweden, Poland, Belarus and Russia. Hybrid breeding is based on the development of inbred lines from two heterotic pools that are subsequently selected for line per se and testcross performance (Miedaner & Laidig, 2019). Hybrid cultivars cover about 80% of the total rye acreage in Germany and yield 15%–20% more grain than population cultivars, the alternative type of cultivars (Laidig et al., 2017).

Generally, rye has been reported to be more resistant to biotic (Arseniuk, Foremska, Góral, & Chełkowski, 1999; Gaikpa, Lieberherr, Maurer, Longin, & Miedaner, 2019; Miedaner, Reinbrecht, Lauber, Schollenberger, & Geiger, 2001) and abiotic (Bartoš et al., 2008; Myśków, Góralska, Lenarczyk, Czyczyło-Mysza, & Stojałowski, 2018; Villareal, Bañuelos, Mujeeb-Kazi, & Rajaram, 1998) stress factors compared to wheat and triticale. However, rye can be infected with several diseases including Fusarium head blight (FHB), reducing grain size and grain yield and contaminating the grains with mycotoxins, like deoxynivalenol (DON) and zearalenone (ZON) (Miedaner & Geiger, 1996). These mycotoxins pose health threats to humans and animals (Pierron, Alassane-Kpembi, & Oswald, 2016) and are therefore strictly regulated in the European Union. For rye and bread wheat, the same limits apply, being 1.25 and 0.1 mg/kg for DON and ZON, respectively, in unprocessed lots for human consumption (The Commission of the European Communities, 2006). In bread, the maximum allowed levels are 0.5 mg DON/kg and 0.05 mg ZON/kg. For feed, different guidance values are recommended, of which the lowest is for pigs with 0.9 mg DON/kg. In naturally infected rye grains from Denmark, *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* dominated among the *Fusarium* species (Nielsen et al., 2011). Integration of resistant cultivars into other disease management practices such as crop rotation and good soil tillage is an efficient, cost-effective and ecologically safe method of reducing the impact of FHB in cereals. Hence, breeding for FHB resistance in rye is crucial given its use as bread cereal with 22% of the harvest and for feed with 64% of the harvest in Germany (BLE, 2018).

FHB resistance in rye is quantitatively inherited and mainly governed by additive gene action, similar to the other cereal species, with a large genotypic variation in breeding populations (Miedaner, Borchardt, & Geiger, 1993; Miedaner & Geiger, 1996). Highly resistant material, however, can rarely be found in existing nurseries. Genotypic correlation coefficients between FHB symptoms and DON showed a tight association (r = .8–.9), allowing an indirect selection for a reduced DON content by selecting for high FHB resistance (Miedaner, Wortmann, & Geiger, 2003). Genotype-by-environment (G × E) interaction played a major role, illustrating the necessity of selecting in several environments (location × year combinations).

The genetic architecture of FHB resistance has been investigated in bread wheat, durum wheat and to some extent in triticale, but no information is available for rye. In bread wheat (2*n* = 6*x* = 42, genome composition AABBDD), about 550 QTL located on all chromosomes were reported for FHB resistance that could be reduced to 65 meta-QTL (Venske et al., 2019). Some major QTL, that is *Fhb1*, *Fhb5* and *Fhb6* from Chinese wheat, have higher effects on FHB resistance (Bai, Su, & Cai, 2018), and attempts are being made to introduce *Fhb1* into European durum wheat breeding programmes (Prat et al., 2017). For triticale, several minor QTL for FHB resistance were reported on rye chromosomes (Dhariwal et al., 2018; Galiano-Carneiro, Boeven, Maurer, Würschum, & Miedaner, 2019; Kalih, Maurer, & Miedaner, 2015).

In rye, genomics is still lagging behind other small-grain cereals. A few previous studies reported QTL for agronomic traits such as plant height (PH), flowering time, yield-related and quality traits (Falke, Wilde, Wortmann, Geiger, & Miedaner, 2009; Hackauf et al., 2017; Miedaner et al., 2012, 2018), frost tolerance (Li et al., 2011) as well as drought tolerance (Myśków et al., 2018). However, no QTL or genome-wide association study (GWAS) has been reported in rye for FHB resistance. Because FHB resistance is generally caused by many QTL with minor effects, genomic selection (GS) might be more appropriate for improving the trait. GS utilizes genome-wide marker data to predict the genotypic values of individuals to be selected, thus reducing phenotyping once the marker effects have been estimated (Edwards et al., 2019). GS methods were applied in rye for kernel weight and quality traits in two introgression libraries (Mahone et al., 2015) and two bi-parental populations (Schulthess et al., 2016; Wang et al., 2014) as well as diverse breeding material (Bernal-Vasquez et al., 2014). A comprehensive genetic map based on a large SNP array is meanwhile available for rye (Bauer et al., 2017). For FHB resistance, GS yielded cross-validated prediction accuracies of 0.59 to 0.95 in bread wheat (Mirdita et al., 2015; Rutkoski et al., 2012), durum wheat (Miedaner, Herter, Ebmeyer, Kollers, & Korzun, 2019; Miedaner et al., 2017) and triticale (Galiano-Carneiro et al., 2019). Therefore, it is worthwhile to assess the prospects of GS in winter rye as the only out-crossing small-grain cereal.

Our objectives were to (a) assess the genetic variation for FHB resistance and associated traits in rye, (b) identify QTL for FHB resistance by GWA mapping and estimate their effects, (c) investigate their co-localization with QTL for heading stage and plant height, and (iv) compare the potential of marker-assisted selection and genomic prediction (GP) to improve breeding for FHB resistance in winter rye. For this, a large population of 465 rye inbred lines was analysed by inoculation with *F. culmorum*.

2 | MATERIALS AND METHODS

2.1 | Plant materials and field experiments

A panel of 465 rye (*Secale cereale* L.) S_1 lines from the company HYBRO Saatzucht GmbH & Co. KG were used for the study. The lines descended from the 'Carsten' heterotic pool that is used as pollinator pool and were made up of 372 lines that were selected

for FHB resistance in a recurrent selection (RS) programme across five cycles. The RS procedure was a typical S_1-line testing (Lynch & Walsh, 1998) with a three-year cycle: (a) selfing and testcrossing of non-inbred materials, (b) multi-environment selection of FHB rating for inbred line and testcross performance, respectively, with a weighted index of 3:1 and (c) recombination of the superior lines. To widen genetic variation, 93 lines unselected for FHB resistance were added to the last RS cycle that was analysed here. All lines were evaluated in three environments (location × year combinations) at the experimental stations of the University of Hohenheim, Germany, in Hohenheim (HOH) near Stuttgart in 2017 and 2018, and of the company HYBRO Saatzucht GmbH & Co. KG in Wulfsode (WUL) near Wriedel, Lower Saxony in 2017. Entries were mechanically sown in single-row observation plots 0.8–1.2 m long at a sowing density of 270 kernels/m^2. In HOH17 and WUL17, the experimental design used was α-lattice design with two replicates. Each replicate consisted of 54 incomplete blocks and 10 genotypes per block. To fill up the field design, standard lines were used. For the field trial in HOH18, a row–column partially replicated design was used because less seeds were available for some genotypes. The number of rows and columns was 40 and 23, respectively. Eighty-five per cent (85%) of the genotypes were replicated. All genotypes were treated with standard agronomic practices as described by Gaikpa et al. (2019). Genotypes were inoculated with one *Fusarium culmorum* isolate (FC46) at a concentration of 7.5×10^5 spores/ml using a tractor-driven sprayer. The inoculation begun at the onset of flowering of early genotypes and was repeated for 4–5 times at 2–3 days intervals to ensure that all entries were inoculated at least once at mid-anthesis.

The traits recorded included FHB severity, heading stage (HS) and plant height (PH). On plot basis, FHB severity was visually rated using a scale of 0%–100% of infected spikelets per genotype, starting from the onset of FHB symptoms differentiation (Miedaner et al., 2001). Two successive ratings were taken in WUL17, five ratings in HOH17 and four ratings in HOH18. HS was rated on 1–9 scale, where 1 = the ear/head of the crop still remain in the leaf sheath and 9 = ear stalk is at least 10 cm long under the ear or above the leaf sheath. Plant height (cm) was measured from the ground level to the tip of the heads after full flowering using a metre rule.

2.2 | Phenotypic data analysis

Two, four and five FHB ratings from WUL17, HOH18 and HOH17, respectively, were averaged to get mean FHB severity for each genotype per environment and used for all analyses. Adjusted means and variance components of each trait were calculated based on best linear unbiased estimation (BLUE) and best linear unbiased prediction (BLUP), respectively. ASReml package (Butler, 2009) within the statistical software R (R Core Team, 2018) was used for all phenotypic analyses. Because of the different field designs used in 2017 and 2018, a two-step analysis was done to get the adjusted means and variance components. At the first step, means for each trait in WUL17 and HOH17 were estimated separately using the model:

$$Y_{ijk} = \mu + G_i + R_j + B_{jk} + e_{ijk},$$

where Y_{ijk} = the observed phenotypic mean for genotype i in replicate j and block k, μ = general mean, G_i = effect of the ith genotype, R_j = effect of the jth replicate, B_{jk} = effect of the kth block in the jth replicate and e_{ijk} = residual error.

For HOH18, means were estimated using the model:

$$Y_{ijkl} = \mu + G_i + R_j + W_k + c_l + e_{ijkl},$$

where Y_{ijk} = the observed phenotypic mean for genotype i in replicate j, row k and column l, μ = general mean, G_i = effect of the ith genotype, R_j = effect of the jth replicate, W_k = effect of the kth row and C_l = effect of the lth column and e_{ijkl} = residual error.

Row, column and genotype were treated as fixed effects and blocks and replicates considered as random effects. Adjusted entry means and corresponding standard errors of genotypes from each environment were analysed in the second step to obtain genotypic means across environments by using the following mixed model:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij},$$

where Y_{ij} = the observed phenotypic mean for genotype i in environment j, μ = general mean, G_i = effect of the ith genotype, E_j = effect of the jth environment, GE_{ij} = effect of genotype–environment interaction and e_{ij} = residual error.

A weighting factor of one divided by the squared standard error of each mean from the first step was used, so the residual variance was set to one, according to method 3 proposed by Möhring and Piepho (2009). BLUEs were estimated across environments assuming fixed effects for the genotypes and environments. Variance components were determined by the restricted maximum likelihood (REML) method. Genotype, environment and genotype–environment interaction were treated as random.. Significance of variance components was determined using the likelihood ratio test.

Broad sense (entry-mean) heritability (H^2) was estimated based on the generalized method proposed by Cullis, Smith, and Coombes (2006) as follows:

$$H^2 = 1 - \frac{\overline{vBLUP}}{2\sigma_g^2},$$

where $\overline{vBLUP}$ is the squared average standard error of difference of the BLUPs and σ_g^2 = genotypic variance. Phenotypic association between FHB and heading ratings as well as FHB and plant height were estimated by Pearson correlation tests using the "cor.test" function in the R statistical software (R Core Team, 2018).

2.3 | Molecular data analysis

Fresh leaves were collected from the 465 rye lines at four-leaf stage, and genotyping was performed by a commercial laboratory by Illumina Technology (Illumina, San Diego) with a 15 k in-house single nucleotide polymorphism (SNP) chip yielding 8,942 polymorphic SNPs. We checked the quality of the markers using the "check.marker ( )" function in the R package GenABEL (Aulchenko, Ripke, Isaacs, & van Duijn, 2018) and removed SNPs which showed more than 20% missing genotypes or had a minor allele frequency <5% from further analyses. In the end, 7,728 SNPs were available for the genome-wide association mapping across the 465 genotypes. Only 2,719 SNPs in our marker data overlapped with the markers in an already published linkage map (Bauer et al., 2017). To increase the number of mapped markers for our study, we established a consensus map including the 2,719 already mapped SNPs from Bauer et al. (2017) and unpublished maps created from nine bi-parental rye populations in our working group with MergeMap (Wu, Close, & Lonardi, 2008). All markers were used for the analysis. To perform genome-wide prediction, missing values in the marker data were imputed using the software Beagle version 5.0 (Browning, Zhou, & Browning, 2018).

A genome-wide association scan was performed to analyse marker–trait associations for FHB resistance, heading stage and plant height using the R package GenABEL (Aulchenko et al., 2018). Principal component analysis based on the distance matrix of genomic kinship showed two major clusters in the association panel (Figure 1). Therefore, both the genomic kinship matrix (K) and the first principal component were included in the linear mixed model of the "polygenic( )" function to correct for the confounding effects of family and population structure in the data set (Price et al., 2006; Würschum, 2012; Yu et al., 2006). The "ibs( )" function of GenABEL package was used to estimate the kinship matrix based on the SNPs. We conducted the GWA mapping assuming additive effects of markers using the $Q + K$ mixed linear model (Yu et al., 2006):

$$Y = X\beta + S\alpha + Qv + Zu + e,$$

where y = a vector of observed phenotypic means, $X\beta$ = the fixed effects other than the SNP under testing and the population structure, β = a vector of fixed effects other than SNP or population group effects, α = a vector of SNP effects, v = a vector of population effects, $u \sim N\left(0, A\sigma_g^2\right)$ = a vector of random polygenic background effects with A being the genomic relationship matrix of the lines and σ_g^2 the additive genetic variance, e = a vector of residual effects, Q = a matrix from the structure relating y to v, and S, X, Z = incidence matrices of 1s and 0s relating y to β, α and u, respectively.

To control for multiple testing, significant SNP–trait associations were determined using a Bonferroni-corrected threshold of $p < .05$ (0.05/number of hypothesis tested) and in addition by an exploratory significance threshold of $p < .0001$. To identify the likely chromosomal position of unmapped significantly associated SNPs, we assessed the linkage disequilibrium (LD) between these markers and all mapped SNPs. We estimated the LD (r^2) values between the SNPs

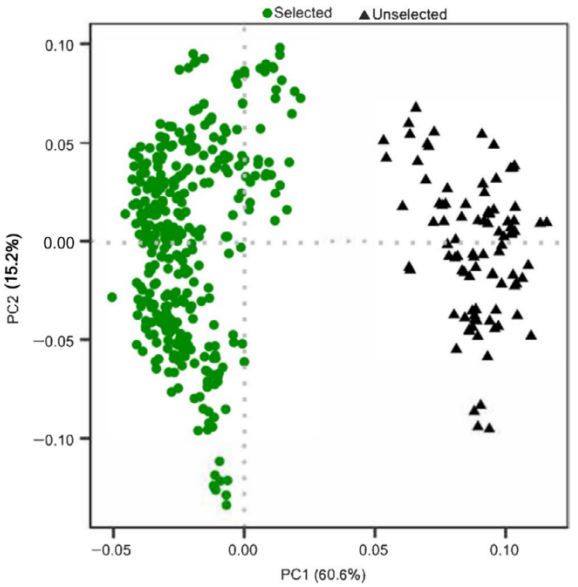


FIGURE 1 Principal component (PC) analysis of 465 S_1 lines of winter rye with the first and second PCs and the percentage of variation explained in brackets. Green filled circles = rye lines selected for Fusarium head blight (FHB) severity (n = 372), black filled rectangles = rye lines unselected for FHB severity (n = 93) [Colour figure can be viewed at wileyonlinelibrary.com]

by applying the function "r2fast( )" which was based on a slightly modified code of Hao, Di, and Cawley (2007). A pair of SNPs having r^2 values >.60 were considered as being in LD. In addition, we used the LD values to correct all significantly associated SNPs for collinearity, that is to determine which of the significant markers likely identify the same putative QTL. The total proportion of genotypic variance (ρ_G) explained by the identified QTL was estimated as:

$$\rho_G = \frac{R^2_{adj}}{H^2},$$

where H^2 is the heritability of the trait, and R^2_{adj} is the adjusted R^2 (Utz, Melchinger, & Schön, 2000). The adjusted R^2 was obtained by fitting all significant SNPs simultaneously in a linear model in a decreasing order of the strength of their association with the trait, that is they were fitted beginning with the SNP that had the lowest P-value (Würschum, Langer, & Longin, 2015). The linear model can be represented as:

$$Y = m_i + m_j + m_k + m_{...} + e,$$

where y is the calculated phenotypic mean, m_i, m_j and m_k are the marker effects where the P-value of $m_i < m_j < m_k < m_{...}$ (i.e. in a decreasing order of the strength of their association with y_{ijk}) and e is the residual error.

The ρ_G of individual QTL was estimated by using the sums of squares obtained from the analysis of variance of the linear model including the significant SNPs (Würschum et al., 2015), that is:

$$\rho_G = \left(SS_m / SS_{total}\right) / h^2 \times 100\%,$$

where SS_m refers to the sums of squares of the individual SNP and SS_{total} refers to the total sums of squares. In addition, we calculated the additive effect (α–effect) of each significant SNP by fitting only one SNP at a time in a linear model.

Furthermore, a genomic prediction (GP) approach was applied to exploit the additive effects of small-effect QTL which cannot be identified in the GWA mapping. The GP was conducted by ridge regression-BLUP (RR-BLUP) with the R package "rrBLUP" (Endelman, 2011; Endelman & Jannink, 2012) using imputed SNPs, including both mapped and unmapped markers. A weighted ridge regression-BLUP (wRR-BLUP) was also performed, where the significant SNPs from the GWA mapping, explaining more than 5% of the genotypic variance, were treated as fixed effect in the GP model (Spindel et al., 2016; Zhao, Mette, Gowda, Longin, & Reif, 2014). Additionally, we compared the predictive ability of MAS and GP. For each trait, the significant SNPs explaining >5% of the genotypic variance in the GWA mapping were used for MAS and all genome-wide SNPs were used for GP (Miedaner et al., 2017). Fivefold cross-validation was done for both MAS and GP by dividing the 465 lines into two sets, (a) estimation set consisting of 80% of the genotypes and (b) prediction set consisting of the remaining 20% of the genotypes (Liu et al., 2013; Würschum, Abel, & Zhao, 2014; Würschum & Kraft, 2014). Resampling of the lines was repeated 1,000 times. The predictive ability was calculated as the correlation coefficient between the predicted and observed trait values of 20% of the genotypes based on the effect estimates from the 80% of the genotypes.

For MAS, we used the model:

$$Y = X\beta + e$$

and for the wRR-BLUP approach the model:

$$Y = X\beta + Zu + e,$$

where Y = the vector of phenotypic observation, β = the vector of fixed marker effects, u = the vector of random marker effects, X and Z = the design matrices coded as −1, 0, 1 relating to β and u, respectively, in Y and e = the residual error. For RR-BLUP, the same model of wRR-BLUP was used by omitting the factor $X\beta$. We assumed additive effects of markers.

3 | RESULTS

3.1 | Phenotypic variation among rye genotypes

The *F. culmorum* isolate FC46 caused FHB infection in all three environments with a slightly higher infection level occurring in HOH18 (Table 1). Heading was earlier in WUL17 compared to HOH17 and HOH18. The highest mean plant height was observed in WUL17. In the combined analysis across environments, wide ranges of mean FHB severity, heading stage and plant height were observed among the rye lines. Both the genotypic variance and the genotype-by-environment interaction variance were significantly different from zero for all recorded traits ($p \leq .001$). Broad sense heritabilities were high throughout, being 0.80 for FHB severity and heading stage and 0.89 for plant height. The correlation between FHB severity and heading stage was low and not significant ($r = -.05$). Between FHB and plant height, a moderate correlation ($r = -.52$, $p \leq .001$) was found that was mainly triggered by some very short susceptible lines (Figure 1). For the selected lines, the correlation was considerably lower, although significant ($r = -.22$, $p \leq .001$). They were, on average, 13.52 cm taller than the non-selected lines, but also 11.9% more FHB resistant (Table S2). Heading stage, by contrast, showed no substantial difference between the selected and the unselected subpopulations (5.37 vs. 4.95, Table S2).

3.2 | Genome-wide association mapping and genomics-assisted selection

Principal coordinate (PC) analysis showed two major population substructures reflecting the genetic background of the lines used in this study (Figure 1). The first and second PC explained 60.6% and 15.2% of the variation, respectively. The larger group comprised of 372 S_1 lines selected for FHB resistance in a recurrent selection breeding programme across five cycles, and the smaller group comprised of 93 S_1 lines not previously selected for FHB resistance. As a result, we used the first PC and the K matrix to correct for population substructure and familial relatedness, respectively.

The GWA scan revealed ten SNP–trait associations for FHB severity on chromosomes 1R, 3R, 5R and 6R that exceeded the Bonferroni-corrected significance threshold with a P-value of 8.13E-06 (Figure 2). At the exploratory threshold ($p < .0001$), significant associations for FHB severity were found on all chromosomes except for chromosome 7R (Table 2, Figure 2). In total, 15 putative QTL were identified with this threshold for FHB severity which jointly explained 74% of the genotypic variance. Each QTL explained between 0.22% and 33.12% of the genotypic variance, five explaining more than 5% ρ_G (Table 2). The SNP Contig1930 significantly associated with the major QTL on chromosome 1R, explaining about 33% of the genotypic variance for FHB severity, was not co-localizing with any of the other significant SNPs at the selected LD threshold ($r^2 > .60$). Additive effects of the FHB QTL ranged from −4.41 to 7.76. The FHB resistance QTL, that explained more than 5% of the genotypic variation, had additive effects except for one locus (isotig15981) that was dominant for the resistant allele and another (isotig14873) that was dominant for the susceptible allele. Generally, the heterozygotes showed intermediate resistance to FHB (Figure 3).

Three SNP–trait associations were identified for heading stage (1R, 2R, 5R) at the Bonferroni-corrected significance threshold (p-value = 8.13E-06, Figure S1). At the exploratory significant threshold, we found significantly associated SNPs on all chromosomes except chromosome 6R (Table 2, Figure S1). Overall, 11 QTL were detected for heading stage and jointly explained 26% of the genotypic variances, with the ρ_G of single QTL ranging between 0.01% and 12.02%. Two SNPs explained more than 5% ρ_G for this trait.

TABLE 1 Means and repeatabilites (in brackets) of 465 rye lines evaluated for Fusarium head blight (FHB) severity, heading stage and plant height in three environments and means, ranges, genotypic variance component (σ^2_g), genotype-by-environment interaction variance ($\sigma^2_{g\times e}$), residual error variance (σ^2) and entry-mean heritability (H^2) of these traits across three environments

Parameter	FHB severity (%)	Heading stage (1–9)	Plant height (cm)
Individual environments			
HOH17	14.33 (0.87)	4.44 (0.78)	114.23 (0.83)
WUL17	12.59 (0.83)	6.64 (0.64)	124.93 (0.80
HOH18	17.82 (0.70)	4.78 (0.69)	107.84 (0.78)
Combined analysis			
Mean	14.97	5.29	115.66
Minimum	5.37	2.14	74.30
Maximum	83.55	8.67	135.98
σ^2_g	48.50***	1.10***	77.00***
$\sigma^2_{g\times e}$	24.92***	0.39***	12.13***
σ^{2a}	1.00	1.00	1.00
H^{2b}	0.80	0.80	0.89

[a]Error term was set to one.
[b]Based on the method described by Cullis et al. (2006).
***Significantly different from zero at $p \leq .001$.

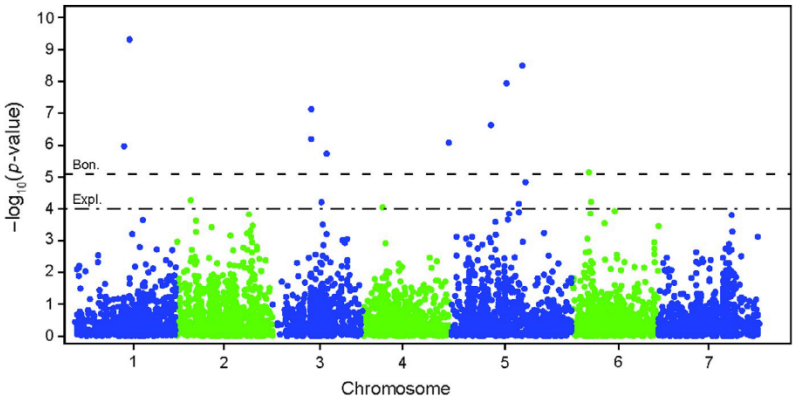


FIGURE 2 Manhattan plot of the genome-wide association scan for Fusarium head blight (FHB) severity (%). Bon. = Bonferroni-corrected significance threshold at $p < .05$ and Expl. = Exploratory significance threshold at $p < .0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

Additive effects of the QTL ranged from −0.62 to 0.44 for heading stage. SNPs isotig12834 and isotig32608 showed an additive and a dominance effect, respectively, for heading time.

For plant height, one significant SNP was found at the Bonferroni-corrected threshold on chromosome 2R. Three significant associations exceeding the exploratory threshold were identified on chromosomes 2R, 3R and 7R, with several significantly associated markers located on chromosome 3R (Table 2, Figure S1). In total, 8 putative QTL were identified for plant height with the exploratory significance threshold. These QTL jointly explained 14% of the genotypic variance and individually between 0.06% and 5.42% (Table 2). Only two SNPs explained slightly more than 5% ρ_G. Additive effects of the QTL for plant height ranged from −2.40 to 3.01. Both loci, isotig24773 and isotig23589, showed dominant allelic effects for mean plant height (Figure 3).

No common QTL were found for FHB severity and heading stage. The significantly associated SNP isotig18865 on chromosome 3R was common to heading stage and plant height. There was a high LD between SNP isotig 15,081 (FHB QTL) and SNP isotig24773 (plant height QTL) on chromosome 3R ($r^2 = 0.84$).

Because of the apparent presence of additively inherited minor-effect QTL contributing to the total genotypic variance of FHB severity, heading stage and plant height, we compared the potential of MAS and GS in a fivefold cross-validation procedure (Figure 4). GS was clearly superior over MAS for all three traits. For FHB severity, the prediction ability of MAS approach was 44% less than the prediction ability of the two genomic prediction approaches. Similarly, genome-wide predictions were 42% and 63% higher than MAS for heading stage and plant height, respectively (Figure 4). A weighted GS approach, incorporating the identified medium- to large-effect QTL as fixed effects, did not yield a higher mean prediction ability than the non-weighted GS approach. Cross-validated prediction ability of the RR-BLUP procedure was 0.86 illustrated by a narrow correlation between observed and predicted FHB severities (Figure S2).

4 | DISCUSSION

Knowledge about the genetic architecture of FHB resistance is vital for genomics-assisted resistance breeding, but to date nothing

TABLE 2 Significant SNP marker detected for Fusarium head blight (FHB) severity, heading stage (HS) and plant height (PH) projected on the map of Bauer et al. (2017) and proportion of explained genotypic variance (ρ_G) and additive (α) effect

Trait	SNP marker	Chromosome	Position (cM)	Bauer et al., 2017 (Chr.; cM)	*p*-value	ρ_G (%)	α-Effect
FHB	Contig00343[b]	1R	264.33		1.07E−06	0.65	5.29
	Contig1930[b]	1R	294.29	1R; 67.47	4.87E−10	33.12	4.79
	isotig19181	2R	80.43		5.31E−05	0.23	0.57
	isotig15081[a,b]	3R	207.56		7.44E−08	5.98	−4.41
	isotig23601[a,b]	3R	263.32		6.11E−05	1.21	4.26
	isotig17662[b]	3R	290.69		1.82E−06	6.99	7.76
	isotig14873	4R	108.76		8.91E−05	7.58	4.49
	isotig10804[b]	5R	0.00		8.30E−07	1.00	2.61
	isotig09091[a]	5R	226.68		2.34E−07	14.17	3.62
	isotig25802[b]	5R	311.06		1.14E−08	3.64	4.35
	C8381_458[b]	5R	377.30	5R; 112.12	6.87E−05	0.58	1.10
	isotig18993[b]	5R	396.33	5R; 116.55	3.21E−09	1.35	0.54
	isotig20329[a]	5R	413.57		1.43E−05	0.22	2.44
	isotig20610[b]	6R	94.99		7.06E−06	2.14	1.82
	isotig25815	6R	105.65	6R; 50.04	6.02E−05	0.85	−1.05
	Total					74.20	
HS	Contig1914[a,b]	1R	184.57		1.71E−05	4.17	−0.32
	isotig22616	2R	255.79		3.02E−05	0.45	0.44
	isotig32608[b]	2R	453.72	2R; 134.37	6.39E−05	6.03	−0.27
	C8904_1380	3R	294.55		7.64E−05	0.01	0.10
	Contig1405	3R	356.51	3R; 106.86	6.41E−05	1.83	−0.14
	isotig18865	3R	379.90	3R; 116.37	5.10E−05	0.67	−0.20
	Contig1056	4R	200.04		2.40E−05	0.97	−0.10
	isotig12834[b]	5R	407.45		2.46E−06	12.02	−0.62
	isotig21263	5R	645.37	5R; 196.14	2.19E−05	1.53	0.19
	isotig21879	5R	641.72	5R; 192.46	2.26E−05	1.14	−0.33
	isotig11542	7R	265.48		1.98E−05	0.89	0.30
	Total					26.10	
PH	Contig808[b]	2R	134.34		1.60E−05	1.96	1.76
	isotig28930	3R	136.25		6.37E−05	0.44	0.08
	isotig30768	3R	157.49		2.05E−05	1.10	0.53
	isotig33248	3R	158.27		8.94E−05	0.19	1.19
	isotig24773	3R	167.77		5.26E−05	5.42	−2.40
	isotig18865	3R	379.90	3R; 116.37	1.60E−05	0.06	0.21
	isotig26122[a]	3R	401.83		5.77E−05	1.71	−0.55
	isotig23589	7R	84.06		1.61E−05	5.34	3.01
	Total					14.09	

[a]Position assigned based on linkage disequilibrium with mapped markers.
[b]Above Bonferroni-corrected significance threshold at $p < .05$.

is known about QTL that confer this resistance in rye. The aim of this study was therefore to (a) perform the first GWA mapping to discover QTL that control FHB resistance in rye and (b) evaluate the potential of genomics-assisted selection for FHB resistance breeding.

4.1 | Construction of the GWA population

For European wheat, many QTL with small effects were reported to govern FHB resistance, as known for other quantitative traits (Lynch & Walsh, 1998). In the cross-pollinating rye, we expect many alleles

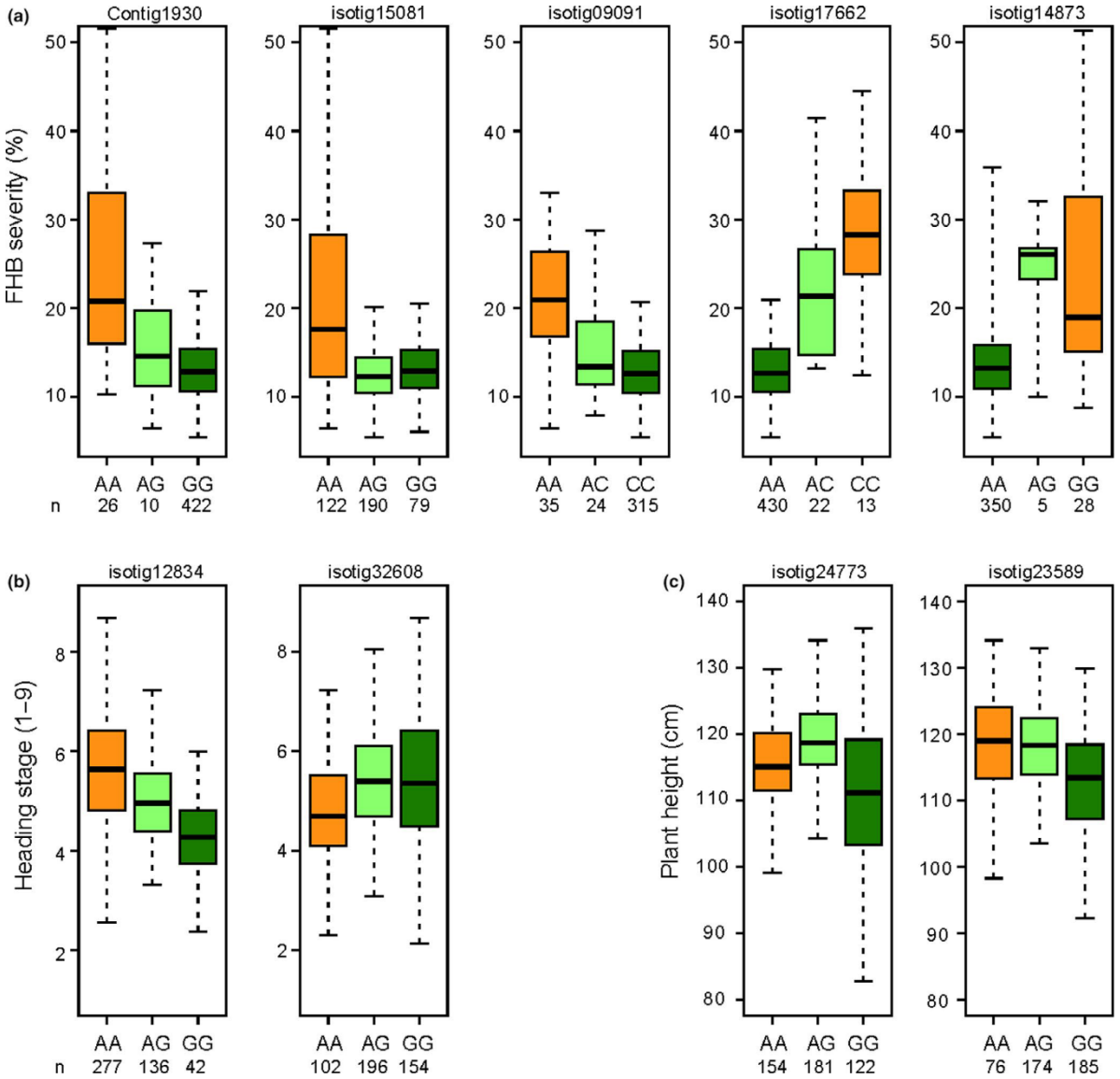


FIGURE 3 Box plots showing the allele effects of significant SNPs explaining >5% of genotypic variation for (a) Fusarium head blight (FHB) severity (b) heading stage, and (c) plant height; n, number of individuals [Colour figure can be viewed at wileyonlinelibrary.com]

per locus (Newell & Butler, 2013). Therefore, a bi-parental mapping population exploring only the effects of the two parental alleles of the population is not adequate to identify QTL for FHB. Moreover, performing a GWAS in unselected populations may not be able to identify effectively QTL present at low frequency. Therefore, we followed here the strategy to enrich our GWA population for FHB resistance by several cycles of recurrent selection. To demonstrate the genetic progress, we added 93 unselected S_1 lines to the 372 selected lines. On average, the selected lines showed an 11.9% higher resistance against FHB than the unselected lines (Table S2). Thus, this strategy can be expected to improve the chances of identifying QTL and superior combinations of resistance QTL alleles. Because all tested lines belonged to the pollinator gene pool, our population can serve as a training population for this heterotic group. Whether it can also be used for the opposite pool requires further research.

4.2 | Phenotypic variation for FHB resistance and agronomic traits

The rye lines analysed in this study showed a high variation for FHB resistance, heading stage and plant height (Table 1). The different genetic background of the genotypes, with selected and unselected lines, might have contributed to the wide range of FHB severity

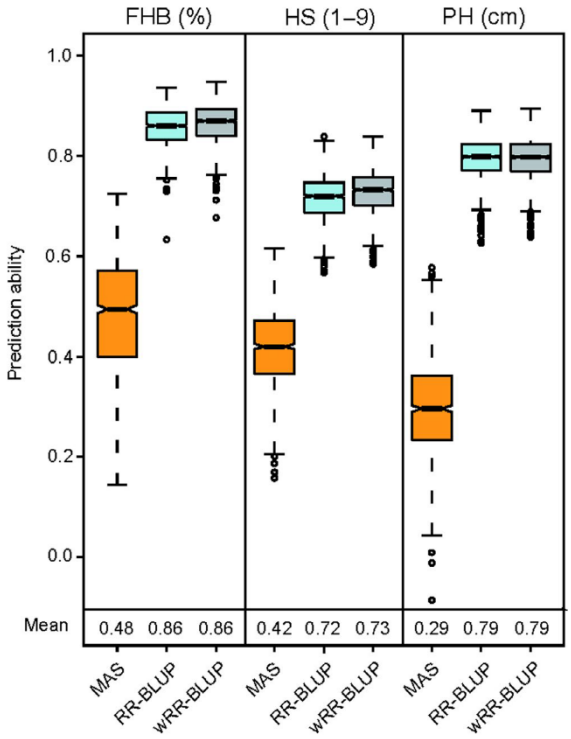


FIGURE 4 Box plots showing the comparison of prediction abilities of marker-assisted selection (MAS), ridge regression-BLUP (RR-BLUP), and weighted ridge regression-BLUP (wRR-BLUP) for Fusarium head blight (FHB) severity, heading stage (HS) and plant height (PH) in winter rye [Colour figure can be viewed at wileyonlinelibrary.com]

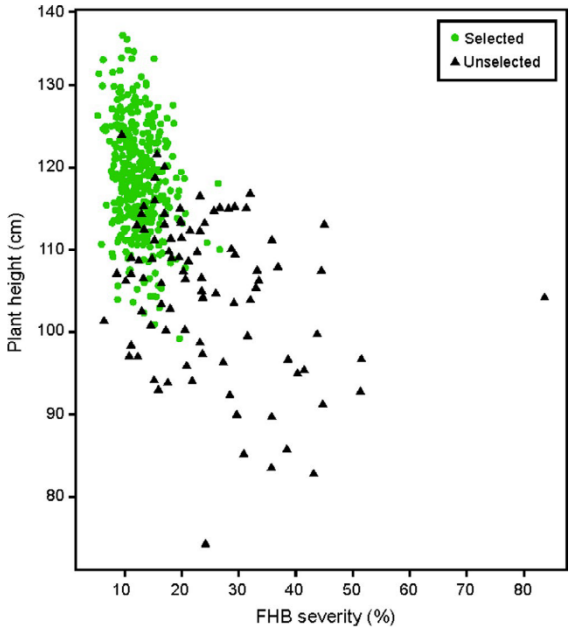


FIGURE 5 Association between Fusarium head blight severity (%) and plant height (cm) for 372 selected (green circles) and 93 unselected (black triangles) S_1 rye lines [Colour figure can be viewed at wileyonlinelibrary.com]

observed in our study. It should be noted, however, that we used only elite lines from a commercial hybrid rye breeding programme without introgression of genetic resources or foreign material. There was a clear molecular distinction between the selected and the unselected lines, although they belong to the same heterotic group (Figure 1), showing the benefits of recurrent selection in combining favourable alleles within breeding populations. Although the main selection trait was FHB resistance, an indirect selection for plant height might have occurred as illustrated by the moderate negative association between plant height and FHB resistance ($r = -0.52$, $p \leq .001$; Figure 5). Several very short and susceptible unselected lines, however, mainly caused this correlation. If all lines <110 cm were omitted, the correlation was much lower ($r = -0.27$, $p \leq .001$). Obviously, in the long-strawed rye the correlation between FHB severity and plant height occurs only when short entries are included. Heading stage varied among the genotypes but did not correlate with FHB severity. This result corresponds to a previous report in triticale (Miedaner, Kalih, Großmann, & Maurer, 2016) and can partly attributed to the synchronization of the plant developmental stage with the date of inoculation, which ensured that each genotype was inoculated at the optimal growth stage (mid flowering), thus reducing the confounding effects of heading stage on disease severity. The finding here indicated that selection for FHB resistance did not affect the maturity period of the rye genotypes, hence simultaneous selection for high FHB resistance and earliness should be possible in this breeding material.

Entry-mean heritabilities were high for all traits, which is in part attributable to the high genotypic variation present in this panel, and similar to the values reported in previous studies in rye (Gaikpa et al., 2019; Hackauf et al., 2017; Miedaner et al., 2012; Wang et al., 2014). Genotype-by-environment interaction variances were significant for all traits, reflecting the importance of phenotyping across several environments for quantitative traits (Fowler, N'Diaye, Laudenci-Chingcuanco, & Pozniak, 2016; Prat et al., 2017; Würschum et al., 2015).

4.3 | GWA mapping identified QTL for FHB resistance in rye

Generally, cross-pollinating, highly heterozygous rye cultivars are more resistant to FHB than self-pollinating homogeneous triticale, durum wheat and bread wheat cultivars (Arseniuk et al., 1999; Gaikpa et al., 2019; Langevin, Eudes, & Comeau, 2004). Therefore, analysis of the genetic mechanisms underlying the increased resistance in rye is of vital importance. For the first time, we performed GWAS to elucidate the genomic basis of FHB resistance in winter rye and identified 15 genomic regions that are associated with FHB resistance (Table 2). These 15 QTL jointly explained a rather high proportion of the genotypic variance (74%), which may in part be due to the accumulation of these QTL in the recurrent selection

programme. Interestingly, we found two major- and several medium- to minor-effect QTL for FHB resistance. For example, SNPs Contig1930 on chromosome 1R and isotig09091 on chromosome 5R explained 33.12% and 14.17% of ρ_G, respectively, and can thus be classified as major-effect QTL. SNPs isotig14873 on chromosome 4R as well as isotig 17,662 and isotig15081 on chromosome 3 were associated with medium-effect QTL and explained 7.58%, 6.99% and 5.98% of ρ_G, respectively. These five SNPs explaining more than 5% of ρ_G of FHB severity are candidates for genomics-assisted breeding for FHB resistance in rye. Their effects on FHB resistance, however, need to be validated in future experiments.

Previous studies conducted in triticale, where rye is the male parent, reported QTL for FHB resistance on chromosomes 3R (Galiano-Carneiro et al., 2019), 4R, 5R, 6R, and 7R (Kalih et al., 2015). The present study identified a significant marker–trait association for FHB resistance on all chromosomes except chromosome 7R (Table 2).The direct comparison of our QTL to previously reported QTL form other small-grain cereals was not possible because of the differences in the genome of rye and triticale as well as the different genetic maps and types of marker used. The several minor and only few medium to major SNP–trait associations demonstrate, however, that the genetic architecture of FHB resistance in rye is complex and mainly controlled by several, additively inherited genes. This agrees well with the findings of other studies involving related *Triticaeae* species such as hexaploid bread wheat, tetraploid durum wheat and triticale (Arruda et al., 2016; Dhariwal et al., 2018; Wang et al., 2017).

4.4 | QTL for heading stage and plant height in rye

Heading stage and plant height are important agronomic traits which might confer passive resistance to FHB in small-grain cereals (Mesterházy, 1995). Therefore, we took these traits into account in our GWAS to investigate their possible co-localization with FHB resistance QTL.

For heading stage, we found 11 QTL, but none of these QTL co-localized with the QTL for FHB severity, which is in line with the lack of phenotypic correlation between these traits. Here, only two of the significantly associated SNPs explained more than 5% of the ρ_G illustrating the high genetic complexity of the trait. We observed both additive and dominance effects of the QTL alleles for heading stage. Recently, Hackauf et al. (2017) reported 7 QTL that jointly explained 85% of ρ_G for heading time across 272 $F_{2:3}$ rye lines derived from a bi-parental population not preselected for FHB resistance. In the present study, by contrast, we used material that was purposefully selected for FHB resistance, but not directly selected for heading stage, which might partly account for the lower ρ_G explained by the 11 QTL for heading stage compared to the QTL identified for FHB resistance.

In triticale, Kalih et al. (2015) reported QTL on chromosomes 4R, 5R, 6R, 7R, explaining 4.55%–39.82% of ρ_G for heading across four populations in triticale. Similarly, we identified significant marker–trait associations for heading stage on all chromosomes except chromosome 6R. Interestingly, the SNP with the highest genotypic effect in our study (isotig12834) is located on chromosome 5R where two large-effect QTL (ρ_G = 15.5, 39.8) were previously reported in triticale (Kalih et al., 2015).

The proportion of genotypic variance jointly explained by the 8 QTL for plant height was lower than the variation explained for FHB resistance and heading stage. Similar to heading stage, two QTL explained more than 5% of the ρ_G for plant height. Non-additive genetic factors, such as QTL × QTL and QTL × environment interactions, might partly account for the remaining unexplained total genotypic variance. Plant height was controlled by dominant alleles for the two most prominent QTL (Figure 3). SNP isotig15081 on chromosome 3, which was significantly associated with FHB resistance, was in LD with the SNP isotig24773 associated with plant height on chromosome 3 (r^2 = .84). Interestingly, these SNPs had medium effects on their respective traits and may partly explain the negative phenotypic correlation observed between FHB severity and plant height. No major dwarfing gene segregated in this population as shown by our GWAS results for plant height, where only minor QTL were found (Table 2). Generally, major genes controlling plant height are not routinely used in rye breeding to date and the height seems to be controlled by a plethora of minor QTL as reported previously in rye (Miedaner et al., 2018; Miedaner, Müller, Piepho, & Falke, 2011) and triticale (Galiano-Carneiro et al., 2019; Kalih et al., 2015).

4.5 | The potential of marker-assisted and genomic prediction in winter rye

For all traits analysed, prediction abilities of both RR-BLUP and wRR-BLUP were by far higher than predictions based on marker-assisted selection (MAS, Figure 4) that considers only QTL with medium to major effects. Overall, the mean prediction abilities of MAS ranged from 29% to 48%, while the mean prediction abilities of the genome-wide approach ranged from 72% to 86% for the three traits (Figure 4). This implies that improvement of FHB resistance, heading stage and plant height by MAS will be slower compared to genomic prediction approaches. This result is in accordance with previous studies reporting higher prediction abilities for genomic prediction than for MAS in triticale (Galiano-Carneiro et al., 2019), bread wheat (Mirdita et al., 2015; Rutkoski et al., 2012) and durum wheat (Miedaner et al., 2019, 2017). However, in the present study, we observed a higher prediction ability for FHB resistance than reported in the earlier studies and even slightly higher than for heading stage and plant height. This is likely due to the continuous selection for FHB resistance, resulting in increased resistance allele frequencies for the QTL underlying this trait (Figure 2). Thus, recurrent selection breeding schemes assisted by genomic prediction appear promising to improve rye resistance against FHB. It is worth to note that our prediction accuracies might be overestimated to some extent, because the training (80% of the lines) and prediction (20%) set were from the same population and have been tested in the same environments.

5 | CONCLUSIONS

Resistance towards FHB might become a trait of increasing importance in hybrid rye breeding. The observed phenotypic variation in elite germplasm is an important and promising prerequisite to gain breeding progress with respect to FHB resistance in rye breeding programmes. There is great potential to improve FHB resistance by genome-based approaches. For the first time, GWA mapping identified several significant marker–trait associations for FHB severity in winter rye of which two can be classified as major QTL. These are candidates for further analyses of FHB resistance to increase our understanding in resistance mechanisms in rye. No co-localization of QTL for FHB and plant height or rather heading stage was observed, which mirrors the moderate correlation between FHB and plant height. Genomic prediction yielded similar high prediction abilities with and without weighted data. Genomics-assisted recurrent selection appears as a promising tool to accelerate breeding for complex disease resistances in rye. These results encourage further research to study FHB resistance in rye hybrids. One opportunity in rye breeding is the reduced level of mycotoxins in the harvest compared to wheat that should facilitate the increase of rye productivity and consumer protection.

ACKNOWLEDGEMENTS

This study was partly financed by the German Academic Exchange Service (DAAD, Bonn, Germany) as a personal grant to David S. Gaikpa (grant number 91650671), by the company HYBRO Saatzucht GmbH & Co. KG and by a grant of the University of Hohenheim (TG77). We thank Ana Carneiro-Galiano and Paul Gruner of the State Plant Breeding Institute, University of Hohenheim, Stuttgart for sharing the R script for the genomic prediction and helping to construct a consensus map, respectively.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

T. Miedaner and F.J. Fromme designed the research and coordinated the experiments, S. Koch and D.S. Gaikpa did the trial organization and phenotyping at Hohenheim in 2017 and 2018, respectively. D.S. Gaikpa performed all statistical analyses and wrote the manuscript. T. Würschum helped with statistical advice, edited and improved the manuscript together with T. Miedaner. D. Siekmann revised the manuscript critically. All authors read and approved the final version of the manuscript.

ORCID

David Sewordor Gaikpa https://orcid.org/0000-0002-1906-0332
Tobias Würschum https://orcid.org/0000-0002-7397-7731
Thomas Miedaner https://orcid.org/0000-0002-9541-3726

REFERENCES

Ammar, K., Mergoum, M., & Rajaram, S. (2004). The history and evolution of triticale. In M. Mergoum, & H. Gómez-Macpherson (Eds.), *Triticale improvement and production* (pp. 1–9). Rome, Italy: Food and Agriculture Organization of the United Nations.

Arruda, M. P., Brown, P., Brown-Guedira, G., Krill, A. M., Thurber, C., Merrill, K. R., … Kolb, F. L. (2016). Genome-wide association mapping of Fusarium head blight resistance in wheat using genotyping-by-sequencing. *The Plant Genome*, *9*(1), 1–14. https://doi.org/10.3835/plantgenome2015.04.0028

Arseniuk, E., Foremska, E., Góral, T., & Chełkowski, J. (1999). Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye. *Journal of Phytopathology*, *147*(10), 577–590. https://doi.org/10.1046/j.1439-0434.1999.00433.x

Aulchenko, Y. S., Ripke, S., Isaacs, A., & van Duijn, C. M. (2018). GenABEL: An R library for genome-wide association analysis. *Bioinformatics*, *23*(10), 1294–1296. https://doi.org/10.1093/bioinformatics/btm108

Bai, G., Su, Z., & Cai, J. (2018). Wheat resistance to Fusarium head blight. *Canadian Journal of Plant Pathology*, *40*(3), 336–346. https://doi.org/10.1080/07060661.2018.1476411

Bartoš, J., Paux, E., Kofler, R., Havránková, M., Kopecký, D., Suchánková, P., … Doležel, J. (2008). A first survey of the rye (*Secale cereale*) genome composition through BAC end sequencing of the short arm of chromosome 1R. *BMC Plant Biology*, *8*, 95. https://doi.org/10.1186/1471-2229-8-95

Bauer, E., Schmutzer, T., Barilar, I., Mascher, M., Gundlach, H., Martis, M. M., … Scholz, U. (2017). Towards a whole-genome sequence for rye (*Secale cereale* L.). *Plant Journal*, *89*(5), 853–869. https://doi.org/10.1111/tpj.13436

Bernal-Vasquez, A. M., Möhring, J., Schmidt, M., Schönleben, M., Schön, C. C., & Piepho, H. P. (2014). The importance of phenotypic data analysis for genomic prediction - a case study comparing different spatial models in rye. *BMC Genomics*, *15*, 646. https://doi.org/10.1186/1471-2164-15-646

BLE (2018). *Bericht zur Markt- und Versorgungslage Getreide 2018* [In German], p. 36. Retrieved from https://www.ble.de/SharedDocs/Downloads/DE/BZL/Daten-Berichte/Getreide_Getreideerzeugnisse/2018BerichtGetreide.pdf?__blob=publicationFile&v=4

Browning, B. L., Zhou, Y., & Browning, S. R. (2018). A one-penny imputed genome from next-generation reference panels. *American Journal of Human Genetics*, *103*(3), 338–348. https://doi.org/10.1016/j.ajhg.2018.07.015

Butler, D. (2009). *asreml: asreml() fits the linear mixed model. R package version 3.0*. Retrieved from https://www.vsni.co.uk

Cullis, B. R., Smith, A. B., & Coombes, N. E. (2006). On the design of early generation variety trials with correlated data. *Journal of Agricultural, Biological, and Environmental Statistics*, *11*(4), 381–393. https://doi.org/10.1198/108571106X154443

Dhariwal, R., Fedak, G., Dion, Y., Pozniak, C., Laroche, A., Eudes, F., & Randhawa, H. S. (2018). High density single nucleotide polymorphism (SNP) mapping and quantitative trait loci (QTL) analysis in a biparental spring triticale population localized major and minor effect Fusarium head blight resistance and associated traits QTL. *Genes*, *9*(1), 19. https://doi.org/10.3390/genes9010019

Edwards, S. M. K., Buntjer, J. B., Jackson, R., Bentley, A. R., Lage, J., Byrne, E., … Hickey, J. M. (2019). The effects of training population design on genomic prediction accuracy in wheat. *Theoretical and Applied Genetics*, *132*(7), 1943–1952. https://doi.org/10.1007/s00122-019-03327-y

Endelman, J. B. (2011). Ridge regression and other kernels for genomic selection with R package rrBLUP. *The Plant Genome Journal*, *4*(3), 250–255. https://doi.org/10.3835/plantgenome2011.08.0024

Endelman, J. B., & Jannink, J.-L. (2012). Shrinkage estimation of the realized relationship matrix. *G3: Genes, Genomes, Genetics*, *2*(11), 1405–1413. https://doi.org/10.1534/g3.112.004259

Falke, K. C., Wilde, P., Wortmann, H., Geiger, H. H., & Miedaner, T. (2009). Identification of genomic regions carrying QTL for agronomic and quality traits in rye (*Secale cereale*) introgression libraries. *Plant Breeding*, *128*(6), 615–623. https://doi.org/10.1111/j.1439-0523.2009.01644.x

Food and Agriculture Organization of the United Nations (FAO) (2019). *FAOSTAT database*. Rome, Italy: FAO. Retrieved from https://www.fao.org/faostat/en/#data/QC

Fowler, D. B., N'Diaye, A., Laudenci-Chingcuanco, D., & Pozniak, C. J. (2016). Quantitative trait loci associated with phenological development, low-temperature tolerance, grain quality, and agronomic characters in wheat (*Triticum aestivum L.*). *PLoS ONE*, *11*(3), e0152185. https://doi.org/10.1371/journal.pone.0152185

Gaikpa, S. D., Lieberherr, B., Maurer, H. P., Longin, C. H. F., & Miedaner, T. (2019). Comparison of rye, triticale, durum wheat, and bread wheat genotypes for Fusarium head blight resistance and deoxynivalenol contamination. *Plant Breeding*, 1–12. https://doi.org/10.1111/pbr.12779

Galiano-Carneiro, A. L., Boeven, P. H. G., Maurer, H. P., Würschum, T., & Miedaner, T. (2019). Genome-wide association study for an efficient selection of Fusarium head blight resistance in winter triticale. *Euphytica*, *215*(1), 4. https://doi.org/10.1007/s10681-018-2327-8

Hackauf, B., Haffke, S., Joachim, F., Steffen, F., Kusterer, B., Musmann, D., … Miedaner, T. (2017). QTL mapping and comparative genome analysis of agronomic traits including grain yield in winter rye. *Theoretical and Applied Genetics*, *130*(9), 1801–1817. https://doi.org/10.1007/s00122-017-2926-0

Hao, K., Di, X., & Cawley, S. (2007). LdCompare: Rapid computation of single- and multiple-marker r2 and genetic coverage. *Bioinformatics*, *23*(2), 252–254. https://doi.org/10.1093/bioinformatics/btl574

Kalih, R., Maurer, H. P., & Miedaner, T. (2015). Genetic architecture of fusarium head blight resistance in four winter triticale populations. *Phytopathology*, *105*(3), 334–341. https://doi.org/10.1094/PHYTO-04-14-0124-R

Kim, W., Johnson, J. W., Baenziger, P. S., Lukaszewski, A. J., & Gaines, C. S. (2004). Agronomic effect of wheat-rye translocation carrying rye chromatin (1R) from different sources. *Crop Science*, *44*(4), 1254–1258. https://doi.org/10.2135/cropsci2004.1254

Laidig, F., Piepho, H. P., Rentel, D., Drobek, T., Meyer, U., & Huesken, A. (2017). Breeding progress, variation, and correlation of grain and quality traits in winter rye hybrid and population varieties and national on-farm progress in Germany over 26 years. *Theoretical and Applied Genetics.*, *130*(5), 981–998. https://doi.org/10.1007/s00122-017-2865-9

Langevin, F., Eudes, F., & Comeau, A. (2004). Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium head blight development in six cereal species. *European Journal of Plant Pathology*, *110*(7), 735–746. https://doi.org/10.1023/B:EJPP.0000041568.31778.ad

Li, Y., Böck, A., Haseneyer, G., Korzun, V., Wilde, P., Schön, C. C., & … Bauer, E., (2011). Association analysis of frost tolerance in rye using candidate genes and phenotypic data from controlled, semi-controlled, and field phenotyping platforms. *BMC Plant Biology*, *11*(1), 146. https://doi.org/10.1186/1471-2229-11-146

Liu, W., Maurer, H. P., Reif, J. C., Melchinger, A. E., Utz, H. F., Tucker, M. R., … Würschum, T. (2013). Optimum design of family structure and allocation of resources in association mapping with lines from multiple crosses. *Heredity*, *110*(1), 71–79. https://doi.org/10.1038/hdy.2012.63

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*, Vol. 1. Sunderland, MA: Sinauer.

Mahone, G. S., Frisch, M., Bauer, E., Haseneyer, G., Miedaner, T., & Falke, K. C. (2015). Detection of donor effects in a rye introgression population with genome-wide prediction. *Plant Breeding*, *134*(4), 406–415. https://doi.org/10.1111/pbr.12283

Mesterházy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding*, *114*, 377–386. https://doi.org/10.1111/j.1439-0523.1995.tb00816.x

Miedaner, T., Borchardt, D. C., & Geiger, H. H. (1993). Genetic analysis of inbred lines and their crosses for resistance to head blight (*Fusarium culmorum*, *F. graminearum*) in winter rye. *Euphytica*, *65*(2), 123–133. https://doi.org/10.1007/BF00022574

Miedaner, T., & Geiger, H. H. (1996). Estimates of combining ability for resistance of winter rye to *Fusarium culmorum* head blight. *Euphytica*, *89*(3), 339–344. https://doi.org/10.1007/BF00022290

Miedaner, T., Haffke, S., Siekmann, D., Fromme, F. J., Roux, S. R., & Hackauf, B. (2018). Dynamic quantitative trait loci (QTL) for plant height predict biomass yield in hybrid rye (*Secale cereale* L.). *Biomass and Bioenergy*, *115*, 10–18. https://doi.org/10.1016/j.biombioe.2018.04.001

Miedaner, T., Herter, C. P., Ebmeyer, E., Kollers, S., & Korzun, V. (2019). Use of non-adapted quantitative trait loci for increasing Fusarium head blight resistance for breeding semi-dwarf wheat. *Plant Breeding*, *138*(2), 140–147. https://doi.org/10.1111/pbr.12683

Miedaner, T., Hübner, M., Korzun, V., Schmiedchen, B., Bauer, E., Haseneyer, G., … Reif, J. C. (2012). Genetic architecture of complex agronomic traits examined in two testcross populations of rye (*Secale cereale* L.). *BMC Genomics*, *13*(1), 706. https://doi.org/10.1186/1471-2164-13-706

Miedaner, T., Kalih, R., Großmann, M. S., & Maurer, H. P. (2016). Correlation between Fusarium head blight severity and DON content in triticale as revealed by phenotypic and molecular data. *Plant Breeding*, *135*(1), 31–37. https://doi.org/10.1111/pbr.12327

Miedaner, T., & Laidig, F. (2019). Hybrid breeding in rye (*Secale cereale* L.). In J. M. Al-Khayri, S. M. Jain, & D. V. Johnson (Eds.), *Advances in plant breeding strategies, Vol 5: Cereals* (pp. 343–372). Cham, Switzerland: Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-030-23108-8_9

Miedaner, T., Müller, B. U., Piepho, H. P., & Falke, K. C. (2011). Genetic architecture of plant height in winter rye introgression libraries. *Plant Breeding*, *130*(2), 209–216. https://10.1111/j.1439-0523.2010.01823.x .

Miedaner, T., Reinbrecht, C., Lauber, U., Schollenberger, M., & Geiger, H. H. (2001). Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. *Plant Breeding*, *120*(2), 97–105. https://doi.org/10.1046/j.1439-0523.2001.00580.x

Miedaner, T., Sieber, A. N., Desaint, H., Buerstmayr, H., Longin, C. F. H., & Würschum, T. (2017). The potential of genomic-assisted breeding to improve Fusarium head blight resistance in winter durum wheat. *Plant Breeding*, *136*(5), 610–619. https://doi.org/10.1111/pbr.12515

Miedaner, T., Wortmann, H., & Geiger, H. H. (2003). Genetics of deoxynivalenol (DON) contamination caused by Fusarium head blight in hybrid rye. *Plant Breeding and Seed Science*, *48*(2), 69–78.

Mirdita, V., Liu, G., Zhao, Y., Miedaner, T., Longin, C. F. H., Gowda, M., … Reif, J. C. (2015). Genetic architecture is more complex for resistance to Septoria tritici blotch than to Fusarium head blight in Central European winter wheat. *BMC Genomics*, *16*(1), 430. https://doi.org/10.1186/s12864-015-1628-8

Möhring, J., & Piepho, H. P. (2009). Comparison of weighting in two-stage analysis of plant breeding trials. *Crop Science*, *49*(6), 1977–1988. https://doi.org/10.2135/cropsci2009.02.0083

Myśków, B., Góralska, M., Lenarczyk, N., Czyczyło-Mysza, I., & Stojałowski, S. (2018). Putative candidate genes responsible for leaf rolling in rye (*Secale cereale* L.). *BMC Genetics*, *19*(1), 57. https://doi.org/10.1186/s12863-018-0665-0

Newell, M. A., & Butler, T. J. (2013). Forage rye improvement in the southern United States: A review. *Crop Science*, *53*(1), 38–47. https://doi.org/10.2135/cropsci2012.05.0319

Nielsen, L. K., Jensen, J. D., Nielsen, G. C., Jensen, J. E., Spliid, N. H., Thomsen, I. K., … Jørgensen, L. N. (2011). Fusarium head blight of cereals in Denmark: Species complex and related mycotoxins. *Phytopathology*, *101*(8), 960–969. https://doi.org/10.1094/phyto-07-10-0188

Pierron, A., Alassane-Kpembi, I., & Oswald, I. P. (2016). Impact of two mycotoxins deoxynivalenol and fumonisin on pig intestinal health. *Porcine Health Management*, *2*(1), 21. https://doi.org/10.1186/s40813-016-0041-2

Prat, N., Guilbert, C., Prah, U., Wachter, E., Steiner, B., Langin, T., … Buerstmayr, H. (2017). QTL mapping of Fusarium head blight resistance in three related durum wheat populations. *Theoretical and Applied Genetics*, *130*(1), 13–27. https://doi.org/10.1007/s00122-016-2785-0

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics*, *38*(8), 904–909. https://doi.org/10.1038/ng1847

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org

Rutkoski, J., Benson, J., Jia, Y., Brown-Guedira, G., Jannink, J.-L., & Sorrells, M. (2012). Evaluation of genomic prediction methods for fusarium head blight resistance in wheat. *The Plant Genome Journal*, *5*(2), 51. https://doi.org/10.3835/plantgenome2012.02.0001

Schlegel, R., & Korzun, V. (1997). About the origin of IRS.IBL wheat-rye chromosome translocations from. *Plant Breeding*, *116*(6), 537–540. https://doi.org/10.1111/j.1439-0523.1997.tb02186.x

Schulthess, A. W., Wang, Y., Miedaner, T., Wilde, P., Reif, J. C., & Zhao, Y. (2016). Multiple-trait- and selection indices-genomic predictions for grain yield and protein content in rye for feeding purposes. *Theoretical and Applied Genetics*, *129*(2), 273–287. https://doi.org/10.1007/s00122-015-2626-6

Spindel, J. E., Begum, H., Akdemir, D., Collard, B., Redoña, E., Jannink, J. L., & McCouch, S. (2016). Genome-wide prediction models that incorporate *de novo* GWAS are a powerful new tool for tropical rice improvement. *Heredity*, *116*(4), 395–408. https://doi.org/10.1038/hdy.2015.113

The Commission of the European Communities (2006). Commission Regulation (EC) 1881/2006 Setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, *364*(1074), 5–24. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20140701&from=EN

Utz, H. F., Melchinger, A. E., & Schön, C. C. (2000). Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. *Genetics*, *154*(4), 1839–1849. https://doi.org/10.3389/fpls.2019.00727

Venske, E., Dos Santos, R. S., Farias, D. D. R., Rother, V., Maia, L. C. D., Pegoraro, C., & Costa De Oliveira, A. (2019). Meta-analysis of the QTLome of Fusarium head blight resistance in bread wheat: Refining the current puzzle. *Frontiers in Plant Science*, *10*, 727. https://doi.org/10.3389/fpls.2019.00727

Villareal, R. L., Bañuelos, O., Mujeeb-Kazi, A., & Rajaram, S. (1998). Agronomic performance of chromosomes 1B and T1BL.1RS near-isolines in the spring bread wheat Seri M82. *Euphytica*, *103*(2), 195–202. https://doi.org/10.1023/A:1018392002909

Wang, R., Chen, J., Anderson, J. A., Zhang, J., Zhao, W., Wheeler, J., … Dong, Y. (2017). Genome-wide association mapping of Fusarium head blight resistance in spring wheat lines developed in the Pacific Northwest and CIMMYT. *Phytopathology*, *107*(12), 1486–1495. https://doi.org/10.1094/phyto-02-17-0073-r

Wang, Y., Mette, M. F., Miedaner, T., Gottwald, M., Wilde, P., Reif, J. C., & Zhao, Y. (2014). The accuracy of prediction of genomic selection in elite hybrid rye populations surpasses the accuracy of marker-assisted selection and is equally augmented by multiple field evaluation locations and test years. *BMC Genomics*, *15*, 556. https://doi.org/10.1186/1471-2164-15-556

Wu, Y., Close, T. J., & Lonardi, S. (2008). On the accurate construction of consensus genetic maps. *Computational Systems Bioinformatics*, *7*, 285–296.

Würschum, T. (2012). Mapping QTL for agronomic traits in breeding populations. *Theoretical and Applied Genetics*, *125*(2), 201–210. https://doi.org/10.1007/s00122-012-1887-6

Würschum, T., Abel, S., & Zhao, Y. (2014). Potential of genomic selection in rapeseed (*Brassica napus* L.) breeding. *Plant Breeding*, *133*(1), 45–51. https://doi.org/10.1111/pbr.12137

Würschum, T., & Kraft, T. (2014). Cross-validation in association mapping and its relevance for the estimation of QTL parameters of complex traits. *Heredity*, *112*, 463–468. https://doi.org/10.1038/hdy.2013.126

Würschum, T., Langer, S. M., & Longin, C. F. H. (2015). Genetic control of plant height in European winter wheat cultivars. *Theoretical and Applied Genetics*, *128*(5), 865–874. https://doi.org/10.1007/s00122-015-2476-2

Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., … Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*(2), 203–208. https://doi.org/10.1038/ng1702

Zhao, Y., Mette, M. F., Gowda, M., Longin, C. F. H., & Reif, J. C. (2014). Bridging the gap between marker-assisted and genomic selection of heading time and plant height in hybrid wheat. *Heredity*, *112*(6), 638–645. https://doi.org/10.1038/hdy.2014.1

Zhou, Y., He, Z. H., Sui, X. X., Xia, X. C., Zhang, X. K., & Zhang, G. S. (2007). Genetic improvement of grain yield and associated traits in the Northern China Winter Wheat Region from 1960 to 2000. *Crop Science*, *47*(1), 245–253. https://doi.org/10.2135/cropsci2006.03.0175

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Gaikpa DS, Koch S, Fromme FJ, Siekmann D, Würschum T, Miedaner T. Genome-wide association mapping and genomic prediction of Fusarium head blight resistance, heading stage and plant height in winter rye (*Secale cereale*). *Plant Breed.* 2020;139:508–520. https://doi.org/10.1111/pbr.12810

SUPPORTING INFORMATION

Table S1. Consensus map of rye (*Secale cereale* L.) compared to the map of Bauer et al. (2017)

See separate EXCEL file (available online at https://doi.org/10.1111/pbr.12810)

Table S2. Mean, minimum and maximum values of Fusarium head blight (FHB), heading stage and plant height for selected and unselected rye lines

Parameter	FHB (%)		Heading (1-9)		Plant height (cm)	
	Selected	Unselected	Selected	Unselected	Selected	Unselected
Mean	12.58	24.51	5.37	4.95	118.36	104.84
Minimum	5.37	6.46	3.00	2.14	99.17	74.30
Maximum	26.69	83.55	8.67	8.10	135.98	123.89

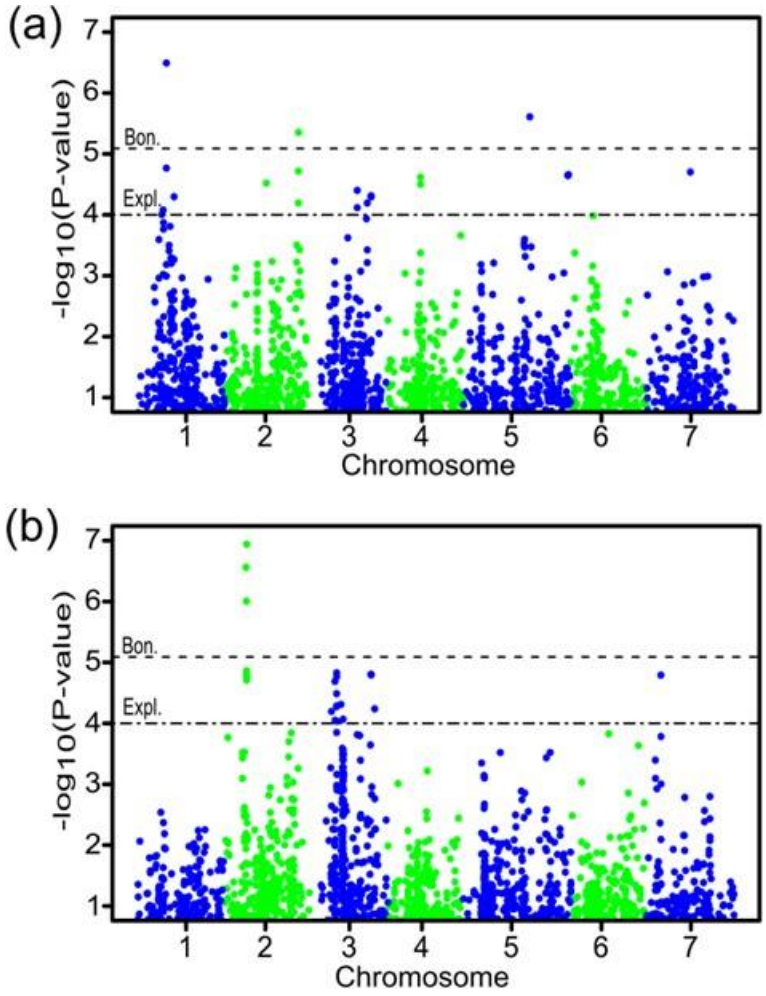


Figure S1. Manhattan plot of the genome-wide association scan for (a) heading stage (1-9), and (b) plant height (cm). Bon. =Bonferroni corrected significance threshold at P<0.05 and Expl. = Exploratory significance threshold at P<0.0001

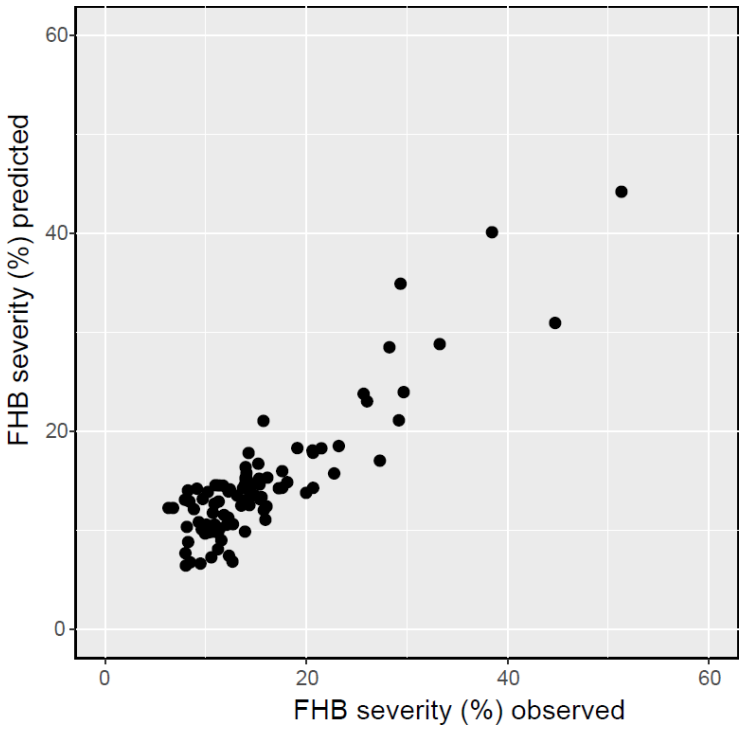


Figure S2. Association between observed and predicted FHB severities based on 20% of inbred lines (=validation set)

4 Genomics-assisted breeding for ear rot resistances and reduced mycotoxin contamination in maize

Genomics-assisted breeding for ear rot resistances and reduced mycotoxin contamination in maize: methods, advances and prospects

David Sewordor Gaikpa[1], Thomas Miedaner[1*]

[1]State Plant Breeding Institute, University of Hohenheim, Fruwirthstr. 21, 70599 Stuttgart, Germany

*Correspondence should be addressed to Thomas Miedaner (miedaner@uni-hohenheim.de)

Published in *Theoretical and Applied Genetics* (2019), 132(10):2721-2739.

https://doi.org/10.1007/s00122-019-03412-2

Received: 13 December 2018 | Accepted: 13 August 2019 | Published online: 22 August 2019

Abstract

Globally, maize is a very important crop for humans and animals. However, fungal diseases such as Gibberella, Fusarium and Aspergillus ear rots (ERs) can result in about 30% yield loss in most maize-growing regions of the world. These diseases do not only reduce yield, but also contaminate the grains with mycotoxins such as deoxynivalenol, zearalenone, fumonisins and aflatoxins, respectively. These mycotoxins pose serious health concerns in both humans and livestock. Over the past decades, several studies have been conducted to dissect the genetic architecture of resistance to these three toxigenic ear rots. The review provides spotlight on studies carried out to identify quantitative trait loci (QTL) and candidate genes (CG) as well as the application of genome-wide selection in maize for resistance to *Fusarium graminearum*, *Fusarium verticillioides* and *Aspergillus flavus*. Genetic mapping (linkage mapping and genome-wide association studies), genomic profiling (proteomics, transcriptomics and metabolomics) and bioinformatic approaches are used in current studies to propose resistance genes against maize ear rot fungi. Though a multitude of QTLs and CGs are reported, only a few specific genes have been cloned and validated to directly confer resistance to ear rots. The way forward is to combine available gene identification methods. Genome-wide selection might speed up ER resistance breeding, but this area is not adequately exploited yet. Tapping resistance alleles from genetic resources may improve resistance in elite maize materials.

5 Genome-wide association studies and genomic prediction for Gibberella ear rot resistance in two European maize landraces

Theoretical and Applied Genetics
https://doi.org/10.1007/s00122-020-03731-9

ORIGINAL ARTICLE

Exploiting genetic diversity in two European maize landraces for improving Gibberella ear rot resistance using genomic tools

David Sewordor Gaikpa[1] · Bettina Kessel[2] · Thomas Presterl[2] · Milena Ouzunova[2] · Ana L. Galiano-Carneiro[1] · Manfred Mayer[4] · Albrecht E. Melchinger[3] · Chris-Carolin Schön[4] · Thomas Miedaner[1]

Received: 29 September 2020 / Accepted: 13 November 2020

Abstract

Key message **High genetic variation in two European maize landraces can be harnessed to improve Gibberella ear rot resistance by integrated genomic tools.**

Abstract *Fusarium graminearum* (Fg) causes Gibberella ear rot (GER) in maize leading to yield reduction and contamination of grains with several mycotoxins. This study aimed to elucidate the molecular basis of GER resistance among 500 doubled haploid lines derived from two European maize landraces, "Kemater Landmais Gelb" (KE) and "Petkuser Ferdinand Rot" (PE). The two landraces were analyzed individually using genome-wide association studies and genomic selection (GS). The lines were genotyped with a 600-k maize array and phenotyped for GER severity, days to silking, plant height, and seed-set in four environments using artificial infection with a highly aggressive Fg isolate. High genotypic variances and broad-sense heritabilities were found for all traits. Genotype-environment interaction was important throughout. The phenotypic (r) and genotypic (r_g) correlations between GER severity and three agronomic traits were low ($r = -0.27$ to 0.20; $r_g = -0.32$ to 0.22). For GER severity, eight QTLs were detected in KE jointly explaining 34% of the genetic variance. In PE, no significant QTLs for GER severity were detected. No common QTLs were found between GER severity and the three agronomic traits. The mean prediction accuracies (ρ) of weighted GS (wRR-BLUP) were higher than ρ of marker-assisted selection (MAS) and unweighted GS (RR-BLUP) for GER severity. Using KE as the training set and PE as the validation set resulted in very low ρ that could be improved by using fixed marker effects in the GS model.

Introduction

Ear rot infections caused by *Fusarium graminearum, F. verticillioides, Aspergillus flavus*, and/or *Stenocarpella maydis* are global threats to maize production. In Germany, a recent survey on the prevalence of *Fusarium* species showed that *F. graminearum* (Fg) and *F. verticillioides* (Fv) were dominant, their relative occurrence depending on temperature and humidity (Pfordt et al. 2020). *F. graminearum* (sexual stage: *Gibberella zeae*) causes Gibberella ear rot (GER) which reduces the quantity and quality of maize kernels and more importantly, contaminates the grains with mycotoxins such as deoxynivalenol (DON) and zearalenone (ZON) (Trail 2009; Ding et al. 2011; Martin et al. 2012a; Mesterházy et al. 2012). These mycotoxins are associated with serious health problems such as kidney diseases, poor growth, and disorders of reproduction in animals and humans (Pinton and Oswald 2014; Zhou et al. 2018). Empirical studies revealed high correlations between GER severity and DON as well as ZON contents in European maize by artificial infection

Communicated by Thomas Lubberstedt.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00122-020-03731-9) contains supplementary material, which is available to authorized users.

✉ Thomas Miedaner
miedaner@uni-hohenheim.de

[1] State Plant Breeding Institute, University of Hohenheim, Stuttgart, Germany

[2] Kleinwanzlebener Saatzucht (KWS) KWS SAAT SE & Co. KGaA, Einbeck, Germany

[3] Institute of Plant Breeding, Population Genetics and Seed Science, University of Hohenheim, Stuttgart, Germany

[4] Plant Breeding, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany

Published online: 03 December 2020

with individual isolates (Bolduan et al. 2009; Martin et al. 2012a; Mesterházy et al. 2020). Because of the adverse health and economic effects of mycotoxins, regulatory bodies in most parts of the world have set recommended limits in maize kernels and products (FAO 2003; The Commission of the European Communities 2006; Foroud et al. 2019). An integrated disease management strategy can support existing efforts to reduce ear rots and associated mycotoxin contaminations in maize with GER resistant cultivars being an essential prerequisite.

In maize, GER resistance is inherited quantitatively with mostly small-effect quantitative trait loci (QTLs) (Xiang et al. 2010; Martin et al. 2012a). In the past years, a genome-wide association study (GWAS) was performed to identify QTLs for GER resistance using single-locus models (Han et al. 2018). However, multi-locus models such as fixed and random model circulating probability unification (FarmCPU, Liu et al. 2016) have been found to be more powerful in detecting SNP-trait associations with a lower rate of false positives and false negatives than single SNP-based models, especially for traits with complex genetic architecture (Abed and Belzile 2019; Kaler et al. 2020; Malik et al. 2019; Miao et al. 2019; Odilbekov et al. 2019; Wei et al. 2017; Xu et al. 2018; Zhang et al. 2019; Zhu et al. 2018).

For complex polygenic traits, genomic selection (GS) offers an attractive alternative to conventional or marker-assisted selection (Meuwissen et al. 2001). The potential of GS for improving quantitative resistances has been analyzed for several pathosystems, e.g., for resistance to lethal necrosis (Gowda et al. 2015), Diplodia ear rot (dos Santos et al. 2016) and Northern corn leaf blight (Technow et al. 2013). Two studies (Han et al. 2018; Riedelsheimer et al. 2013) investigated the prospects of GS for GER resistance in European elite maize lines.

Landraces serve as repositories of diverse alleles of agronomic importance and have great potential for broadening the genetic diversity of elite maize germplasm as illustrated for several agronomical traits (Yao et al. 2007; Strigens et al. 2013; Bedoya et al. 2017). European maize landraces have experienced several hundred years of adaptation to European growing conditions and can have a higher chance of successful allele transfer to elite backgrounds compared to non-adapted lines. The molecular diversity of 35 European maize landraces was investigated by Mayer et al. (2017) using high-density genotypic data and landraces, "Kemater Landmais Gelb" (KE, originating from Austria) and "Petkuser Ferdinand Rot" (PE, originating from Germany) represented a high proportion of the total molecular diversity (Mayer et al. 2017; Hölker et al. 2019). Thus, they were chosen for large-scale production of doubled haploid (DH) lines, which were extensively genotyped and phenotyped for numerous agronomic traits but not for Fusarium diseases (Hölker et al. 2019).

Our objective was to investigate the genetic architecture of Fg resistance in two DH libraries derived from landraces KE and PE and the potential of genetic improvement by marker-assisted (MAS) and genomic selection (GS). Specifically, we aimed to (1) estimate variances and covariances for GER severity and the agronomic traits, days to silking, plant height, and seed-set, (2) map QTLs using a multi-SNP GWAS model based on the markers from a 600 k SNP array, and (3) compare the prediction accuracies of MAS and two GS approaches for GER severity. Therefore, 250 DH lines from each landrace were artificially infected by *F. graminearum* in four environments.

Materials and methods

Plant materials, experimental design and data collected

Plant materials consisted of a panel of 500 DH lines produced from two European flint landrace populations, KE and PE by KWS SAAT SE & Co. KGaA, Einbeck, Germany. We phenotyped 250 DH lines per population plus 10 checks (including the two original source populations) in 2018 and 2019 at Hohenheim (HOH) near Stuttgart, Germany, and at Gondelsheim (GON) near Karlsruhe, Germany. The DH lines represent a random sample of the DH lines described by Hölker et al. (2019). The experimental design was a 51×10 alpha lattice design (10 genotypes per 51 incomplete blocks) with 2 replicates in both locations and years. Sowing was done mechanically. Each plot was a single row of 3 m length and consisted of 20 plants at intra-row spacing of 15 cm. Inter-row spacing was 75 cm. Eight to ten maize ears per plot, leaving out border plants, were inoculated with inoculum prepared from a highly aggressive *F. graminearum* (Fg) isolate, FG 163 (= IFA 66, Martin et al. 2012a, b) at a concentration of 1.5×10^4 spores mL^{-1}. The isolate was shared by Prof. Marc Lemmens, BOKU, Vienna, Austria. Each upper ear was inoculated by a one-needle vaccinator on the silk channel of the maize cobs with approximately 2 ml of the inoculum at 4–6 days after 50% silk emergence (Reid et al. 1996). Though significant genotype-isolate interaction for ear rot severity and DON content was reported in previous studies, Miedaner et al. (2010) found no rank reversals for GER resistance in early maize inbred lines inoculated with eight *F. graminearum* isolates where our isolate used in this study was one of them. Therefore, inoculation with one highly aggressive isolate should be adequate to discriminate resistant and susceptible lines. Days to silking (DS), plant height (PHT, cm), seed-set (SS, %), and GER severity (%) were recorded in all 4 environments (= location × year combinations). Briefly, days to silking were recorded as the number of days taken to achieve ≥ 50% female flowering per

plot. PHT was measured plotwise from ground level to the first tassel branch using a meter rule in cm. At physiological maturity (about 18–20% grain moisture), we manually dehusked each ear and assessed visually seed-set as the proportion of kernels per cob (%), where 0% = no kernels on the cob and 100% = cob fully covered with kernels. GER severity was visually assessed on the same ears on a quantitative scale from 0 to 100%, where 0% = no Fg mold visible and 100% = entire ear covered with Fg mold.

Data analysis

Phenotypic analysis

ASReml R package version 3.0 (Butler 2009) was used to estimate means and variance components for all four traits. Trait values from each environment were used to calculate best linear unbiased estimates (BLUEs), regarding genotypes as fixed effects. Estimates of variance components and best linear unbiased predictors (BLUPs) were calculated by the following model, regarding genotypes within each population and the other factors as random:

$$Y_{ijklm} = \mu + P_i + G_{j(i)} + E_k + R_{l(k)} + B_{m(kl)} + PE_{ik} + GE_{jk} + e_{ijklm}$$

where Y_{ijklm} = the observed phenotypic value for genotype j from population i in replicate l and block m at environment k, μ = general mean, P_i = effect of the ith population, $G_{j(i)}$ = effect of the jth genotype nested in the ith population, E_k = effect of the kth environment, $R_{l(k)}$ = effect of the lth replicate nested in the kth environment, $B_{m(kl)}$ = effect of the mth block nested in the lth replicate and the kth environment, PE_{ik} = interaction effect between the ith population and the kth environment, GE_{Jk} = interaction effect between the jth genotype and the kth environment, and e_{ijklm} = residual error. We assumed heterogeneous variances of residual effects in different environments. Dummy variables were used to separate the genotypes into checks and the two landraces (KE and PE) in the random statement to obtain the variance components for each population (Piepho et al. 2006). The likelihood ratio test based on full and reduced models was used to determine the significance of variance components. The same model was used for calculations in individual environments by omitting the environment factor. Repeatabilities and broad-sense heritabilities (H^2) were estimated by standard procedures described by Hallauer et al. (1988). Pearson correlation coefficient (r) between BLUEs of traits were estimated using the function "cor.test" in R programming language (R Core Team 2018). Genotypic correlations (r_g) between traits and their P-values were calculated using bivariate models described in details by Wilson et al. (2010), in Asreml-R 3.0 (Butler 2009).

Molecular analysis

The 500 DH lines (250 derived from PE and KE, respectively) were previously genotyped using a high-density Affymetrix® Axiom® Maize Genotyping Array optimized for temperate maize (Unterseer et al. 2014, Mayer et al. 2020). SNP markers having call rate < 90%, minor allele frequency < 5%, and too high heterozygosity (false discovery rate < 1%) were excluded from the marker data. The remaining heterozygous loci were replaced with missing values, and the new data set without heterozygous loci was filtered again as described above. Remaining missing values were imputed using Beagle 5.0 software (Browning et al. 2018). A total of 388,999 SNPs and 462 DH lines (KE = 236, PE = 226) were left for further statistical analyses after quality check. Physical positions of all markers are available on the public maize reference genome, B73 RefGen_v4, AGPv4 (Jiao et al. 2017).

Principal component analysis and genomic kinship

Principal component analysis (PCA) was carried out by the default method in the R package Genome Association and Integrated Prediction Tool (GAPIT) 3.0 (Lipka et al. 2012). In addition, a kinship plot was created from the genomic relationship matrix of the high-density SNP marker data using the default kinship.algorithm, VanRaden (VanRaden 2008) in GAPIT 3.0 (Lipka et al. 2012).

Genome-wide association studies (GWAS)

The BLUEs and the high density filtered SNPs were used to perform GWAS for GER severity (%), DS (days), PHT (cm), and SS (%), employing the multi-locus-based method, FarmCPU (Liu et al. 2016) implemented in the R package GAPIT 3.0 (Lipka et al. 2012). The GWAS was conducted with the filtered DH lines from each population (KE = 236 and PE = 226) separately. In FarmCPU, false positives are controlled by using a special kinship (K) matrix created from pseudo-quantitative traits nucleotides (pseudo-QTNs) as random effect (Liu et al. 2016). The parameter, "method.bin" was set to "optimum" for the optimization process, using the default bin.size = c(5e5,5e6,5e7) and bin.selection = seq(10,100,10). The "bin.size" function refers to the division of the whole genome into bins in kilo base pairs and represents the window size used to select a probable QTN. The "bin.selection" indicates the number of possible QTNs that can be selected into the model as covariates in loops. After the optimization process in a random effect model, the marker having the most significant P-value in a particular bin is used to represent that bin (Liu et al. 2016).

The two steps of FarmCPU model, which are run iteratively are described in detail by Liu et al. (2016) and can be represented as.

Step 1. Fixed effect model (FEM): $y_j = M_{j1}T_1 + M_{j2}T_2 + ... + M_{jt}T_t + S_{jn}e_n + \varepsilon_j$
Step 2. Random Effect Model (REM): $y_j = u_j + \varepsilon_j$

In both FEM and REM, y_j=the trait value (i.e., BLUE across environments) of the jth maize DH line and ε_j= residual ~ $N(0,\sigma_\varepsilon^2)$. In FEM, $M_{j1}, M_{j2}, ..., M_{jt}$= the genotypes of t pseudo-QTNs, initiated as an empty set (Liu et al. 2016), $T_1, T_2, ..., T_t$= the corresponding effects of the pseudo-QTNs; S_{jn} = the genotype score of the jth DH line at the nth SNP marker and e_n = the corresponding effect of the nth SNP marker. In REM, u_j= the total genetic effect of the jth DH line, where the variance and covariance matrix is represented by $G = 2K\sigma_g^2$, K= the kinship matrix constructed based on the pseudo-QTNs and σ_g^2 = the genetic variance pertaining to the REM (Liu et al. 2016).

In order to identify which SNPs were most likely associated with each trait, we adopted an exploratory significant threshold of P-value ≤ 0.0001 (–log10 (P-value) ≤ 4) and Bonferroni-corrected threshold of (–log10 (P-value) = 6.89). The total proportion of genotypic variance (p_G) explained by the QTLs detected were calculated using the formula.

$$p_G = \frac{R^2_{adj}}{H^2}$$

where H^2 is the broad-sense heritability of the trait, and R^2_{adj} is the adjusted R^2 from a linear model (Utz et al. 2000). Calculation of R^2_{adj} and p_G for (a) a simultaneous fit of all significant QTL and (b) individual QTL followed the procedure described by Würschum et al. (2015).

Candidate gene identification for GER resistance

We searched for possible genes for GER resistance using the publicly available B73 reference genome version 4 (Zm-B73-REFERENCE-GRAMENE-4.0, Jiao et al. 2017) from MaizeGDB (https://www.maizegdb.org/gene_center/gene) based on the positions of two most important SNPs explaining > 5% of genotypic variance for GER resistance in KE (i.e., ZmSYNBREED_24070_673 on chromosome (chr.) 2 and ZmSYNBREED_53695_527 on chr. 6). Descriptions and ontology terms of genes located within ≤ 1 cM (approx. ≤ 250 kb) around the SNPs (Coan et al. 2018) were obtained from the Gramene Annotations (http://www.gramene.org/).

Marker-assisted and genomic selection for GER severity

We evaluated the potential of GS for GER resistance using two models, ridge regression-BLUP (RR-BLUP) and weighted ridge regression-BLUP (wRR-BLUP) using the R package "rrBLUP" (Endelman 2011; Endelman and Jannink 2012). In wRR-BLUP, the significant SNPs from the GWAS explaining > 5% p_G for GER severity were fitted in the GS model as a fixed effect, and all other SNPs fitted as a random effect (Zhao et al. 2014; Spindel et al. 2016). In addition, we compared the prediction accuracies of marker-assisted selection (MAS, i.e., by using the significant SNPs from the GWAS explaining > 5% p_G) and the two genome-wide prediction models (RR-BLUP and wRR-BLUP) for GER resistance.

The quality of prediction of these models was evaluated by cross-validation using 80% of the data as training set (TS) and the remaining 20% as validation set (VS) (Liu et al. 2013; Würschum et al. 2014; Würschum and Kraft 2014). Sampling was stratified by landrace population and repeated 1000 times. To reduce computation time, we did not perform a de novo QTL detection for each calibration set for the MAS and wRR-BLUP. Instead, we predicted based on QTL positions and effects detected in the whole dataset. For RR-BLUP and wRR-BLUP, we also investigated the prediction accuracy of GS for GER resistance across the two landraces. Here, KE was exclusively used as the TS and PE as the VS, and vice versa. The prediction accuracy (ρ) was determined by expressing the predictive ability (i.e., correlation coefficient between the observed BLUEs and the predicted values) as a fraction of the square root of the broad-sense heritability of the trait. The model used to estimate marker effects in the TS is given by the following:

$$Y = X\beta + Zu + e$$

where = Y the vector of BLUEs for GER; β = vector of fixed effects; $u \sim N(0, A\sigma_u^2)$ = the vector of random marker effects, A is a relationship matrix and the residuals are normal with constant variance; X and Z = the design matrices; e = the residual error (Endelman 2011). We calculated the genomic estimated breeding values (GEBV) of the individuals of the VS by using the relation.

$$Y_0 = X_0\beta + Z_0u$$

where Y_0= the vector of GEBV of the VS; X_0 and Z_0 = design matrixes of individuals in the VS. The predictions were based on additive effects of markers.

Results

Phenotypic and genetic variation for GER resistance and agronomic traits

GER symptoms were observed among maize lines in all four environments with the highest mean severity in HOH 2019 and the lowest in GON 2018 (Fig. 1). Repeatability values per environment were moderate to high, ranging from 0.61 to 0.96, depending on the trait (Supplementary Table 1). Across the four environments, KE source population was slightly more resistant than PE source population (Fig. 2a). Accordingly, KE DH lines had a lower mean GER severity than PE lines (Fig. 1 and Fig. 2a). Variation within each population was high, GER severity ranging from 1 to 87% for KE and 7% to 97% for PE. On average, KE lines were about 25 cm taller than PE lines while DS was similar for both landraces. The average SS was slightly higher for KE than PE lines. Accordingly, the KE source population had a slightly higher seed set than the PE population (80% *vs.* 75%) (Table 1, Fig. 2b). We found significant ($P \leq 0.0001$) genotypic and genotype–environment interaction variances and high H^2 estimates for all traits (Table 1). H^2 was higher for KE than PE for most traits. Phenotypic and genotypic correlations between GER severity and DS and SS were significant ($P \leq 0.01$) in most cases but very low and similar for KE and PE (Table 2). DS was significantly and moderately correlated with SS. No significant correlations were found between GER severity and plant height.

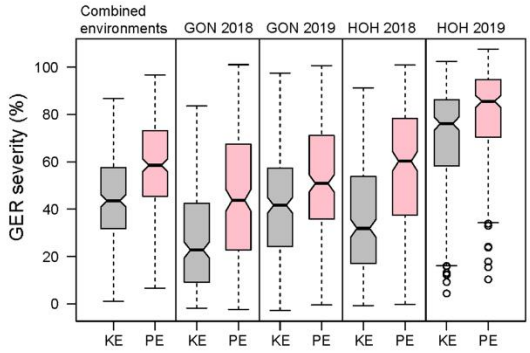


Fig. 1 Box plots of adjusted means for Gibberella ear rot (GER) infection among Kemater (KE) and Petkuser (PE) DH lines at Gondelsheim (GON) and Hohenheim (HOH) in 2018 and 2019 plus the four environments combined. Horizontal thick lines in boxes indicate the median

Principal component analysis and genomic relationship

The PCA grouped the 462 DH lines used for the molecular analyses into two major clusters corresponding to the two maize landrace populations, KE and PE (Supplementary Fig. 1). The first, second, and third PCs explained 16.75%, 3.36%, and 3.25% of the molecular variation, respectively. Within KE, the percentage of variation explained by the first three PCs were 7.27%, 4.41%, and 4.16%, respectively. Similarly, among PE lines, the first three PCs explained 8.56%,

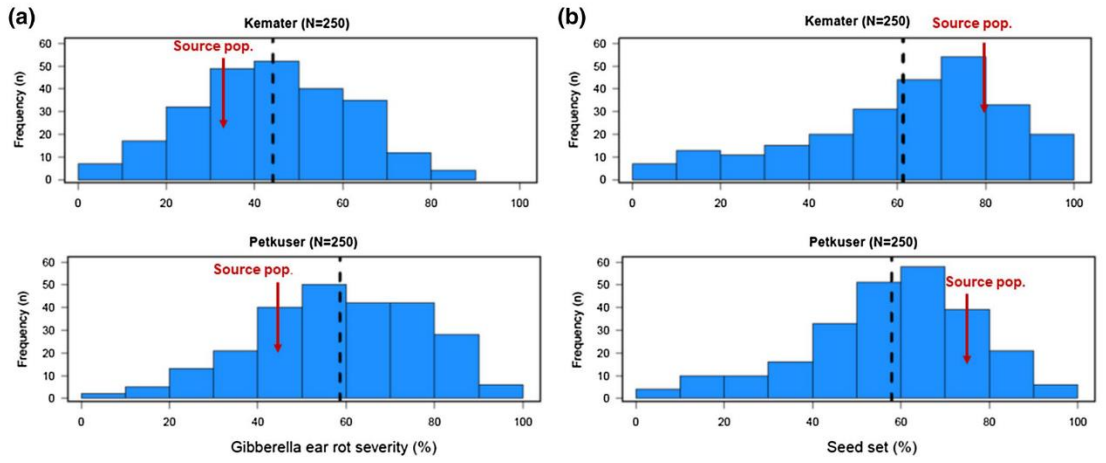


Fig. 2 Histograms showing the distribution of **a** Gibberella ear rot (GER) severity and **b** seed-set among 250 DH lines within each landrace, across four environments. The red arrows indicate the mean value of GER severity and seed-set for the respective source populations (replicated 4-fold). Vertical dashed lines represent the mean disease severity and seed-set of DH lines

Table 1 Means, genotypic variance (σ^2_G), genotype-environment interaction variance ($\sigma^2_{G\times E}$) and residual variance (σ^2_ε) components and broad-sense heritabilities (H^2) of Gibberella ear rot (GER) severity, days to silking (DS), plant height (PHT), and seed-set (SS) within landraces

Parameter	GER (%)	DS (days)	PHT (cm)	SS (%)
Kemater (KE)				
Mean	44.12	80.44	133.61	61.34
σ^2_G	251.30	14.65	377.49	473.83
$\sigma^2_{G\times E}$	94.64	2.00	53.35	123.45
σ^2_ε	305.01	2.53	85.10	182.04
H^2	0.80	0.95	0.94	0.90
Petkuser (PE)				
Mean	58.57	79.86	108.70	57.88
σ^2_G	255.60	14.75	324.03	302.01
$\sigma^2_{G\times E}$	146.95	3.57	44.57	143.16
σ^2_ε	305.01	2.53	85.10	182.04
H^2	0.77	0.92	0.94	0.84

σ^2_G and $\sigma^2_{G\times E}$ for all traits and populations were significantly different from zero at $P < 0.0001$

Table 2 Phenotypic and genotypic (in brackets) correlations between Gibberella ear rot (GER) severity and days to silking (DS), plant height (PHT), and seed-set (SS) within landraces across four environments

Traits	DS	PHT	SS
Kemater DH			
GER severity	−0.25 (−0.32)***	−0.03 (−0.04)	0.20 (0.22)**
DS			−0.52 (−0.56)***
Petkuser DH			
GER severity	−0.27 (−0.31)***	−0.05 (−0.06)	0.18 (0.22)**
DS			−0.54 (−0.59)***

,*Significantly different from zero at $P \le 0.01$ and $P \le 0.0001$, respectively (for both the phenotypic and genotypic correlations)

5.22%, and 3.47% of the molecular variation, respectively. The genomic relationship plot also showed two major groups corresponding to KE and PE landraces, with smaller subclusters within each landrace (Supplementary Fig. 2).

QTLs for GER severity

Among KE DH lines ($N = 236$), at $P = 0.0001$, 8 QTLs collectively explaining 34% of p_G for GER severity were found (Fig. 3a, Table 3). One SNP on chr. 2 (ZmSYNBREED_29737_831) exceeded the Bonferroni threshold (Fig. 3, Table 3). We detected 13, 11, and 1 QTL(s) for DS, PHT, and SS, respectively. None of the QTLs identified for GER severity colocalized with the QTLs detected for the agronomic traits in KE (Supplementary Table 2).

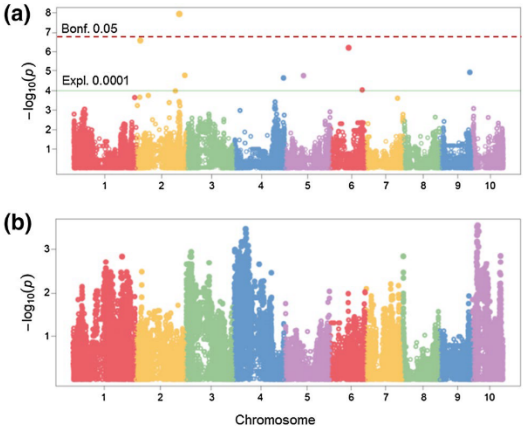


Fig. 3 Manhattan plot of the GWAS scan for Gibberella ear rot (GER) severity in **a** "Kemater Landmais gelb" (N=236), and **b** "Petkuser Ferdinand rot" (N=226). ***Ex pl.*** Exploratory threshold at $P \le 0.0001$; ***Bonf.*** Bonferroni-corrected threshold at $P \le 0.05$

For PE ($N = 226$), no QTL were detected for GER severity at $P = 0.0001$ (Fig. 3b). SNP-GER resistance associations among PE lines were found at or near some of the loci identified in KE only with a lower significance level (e.g., $P \le 0.01$). Ten QTLs were detected for DS and PHT while one QTL was detected for SS in PE ($P = 0.0001$, Supplementary Table 3).

The two most important SNPs with the largest p_G for GER severity in KE (i.e., ZmSYNBREED_24070_673 and ZmSYNBREED_53695_527) were placed in 25 protein-coding genes/gene models in the chosen interval, which could be placed into 10 functional categories (Supplementary Table 4).

Genomic prediction *versus* marker-assisted selection for GER resistance

We evaluated the prospects of MAS and GS for GER resistance. For KE, we used the two SNPs explaining > 5% p_G from the GWAS for MAS (Zhang et al. 2005) and all 388,999 markers for GS by adopting two models, RR-BLUP and wRR-BLUP. In wRR-BLUP, we used the medium-to-large-effect SNPs associated with GER QTLs as fixed effects as described in the Material and Methods. For KE ($N_{TS} = 189$, $N_{VS} = 47$), MAS and RR-BLUP yielded similar ρ for GER severity (~0.40) while wRR-BLUP yielded the highest ρ (0.51, Fig. 4). In PE

Table 3 Significant SNPs detected for Gibberella ear rot (GER) severity, their chromosomal position, *P*-value, frequency of the favorable allele (FAF), additive effects and proportion of explained genotypic variance (p_G) in "Kemater Landmais gelb" (KE)

Marker	Chr[a]	Coordinate (cM)	*P*-value	FAF	Additive effect	p_G (%)
ZmSYNBREED_24070_673	2	49.00	2.70E–07	0.42	5.00	15.04
ZmSYNBREED_29737_831	2	119.54	1.17E–08	0.26	4.56	1.28
ZmSYNBREED_30537_486	2	162.00	1.70E–05	0.41	−3.33	2.84
ZmSYNBREED_44869_210	4	162.93	2.33E–05	0.36	3.27	4.35
ZmSYNBREED_47633_944	5	78.30	1.75E–05	0.47	3.41	3.27
ZmSYNBREED_53695_527	6	31.15	6.36E–07	0.50	−3.52	6.04
ZmSYNBREED_55609_889	6	91.78	9.50E–05	0.67	−3.14	0.46
ZmSYNBREED_70955_321	9	110.30	1.18E–05	0.19	−4.11	3.53
Total						33.69

[a]Chromosome

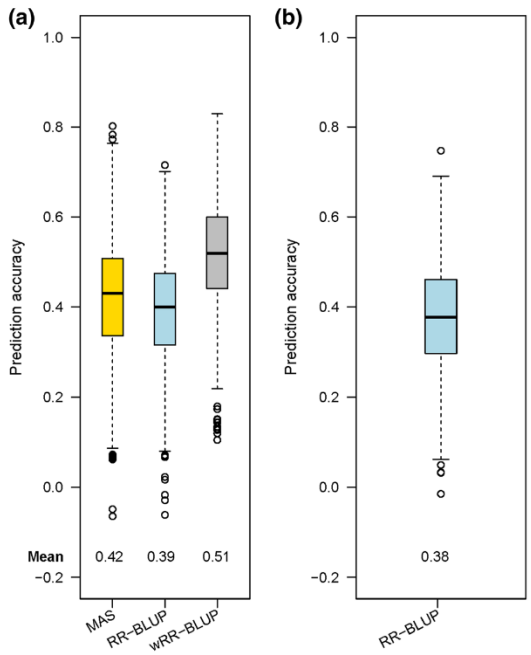


Fig. 4 Box plots showing the prediction accuracies (%) of marker-assisted selection (MAS), ridge regression-BLUP (RR-BLUP) and weighted RR-BLUP (wRR-BLUP) models for Gibberella ear rot severity (%) in **a** "Kemater Landmais Gelb", **b** "Petkuser Ferdinand Rot"

($N_{VS} = 181$, $N_{VS} = 45$), the mean ρ of RR-BLUP was 0.38 (Fig. 4b). For GS across the two landraces, when only DH lines from KE were used as TS and PE as VS in RR-BLUP, ρ for GER severity was 0.03 and vice versa ρ was -0.01. When the two SNPs explaining > 5% p_G for GER severity in KE were used as fixed effects in the GS model (wRR-BLUP), ρ increased to 0.22 when KE lines constituted the TS and PE lines the VS.

Discussion

Over the past decade, only few phenotypic and molecular studies have been conducted on GER resistance in elite maize germplasm compared to wheat (Gaikpa and Miedaner 2019) and exploitation of the genetic diversity among European flint maize landraces for GER resistance using genomic tools has not been pursued hitherto. In this study, we conducted GWAS and GS for GER resistance in two European flint maize landraces (KE from Austria and PE from Germany). To analyze potential covariations, we additionally evaluated days to silking (DS), plant height (PHT), and seed-set (SS).

Variation for GER severity and agronomic traits in European landraces

Inoculation with the highly aggressive *F. graminearum* isolate FG163 resulted in GER infection in all locations and years. The environment and its interaction with the DH lines influenced GER severity in both landraces. Although the genotypic variances were quite similar for both landraces, PE showed higher genotype × environment variance resulting in a slightly higher H^2 for KE (Table 1). A large effect of the environmental conditions on ear rot resistance has been reported several times in the literature (Giomi et al. 2016; Han et al. 2018; Galić et al. 2019; Morales et al. 2019). Therefore, it is important to phenotype lines for GER resistance in multi-environmental trials. The broad-sense heritability values were similar to previous reports (Martin et al. 2012a, b; Giomi et al. 2016; Han et al. 2016; Kebede et al. 2016).

We analyzed additionally agronomic traits such as DS, PHT, and SS, because they may lead to physiological escape or could have pleiotropic effects on GER resistance. All three traits had high H^2 estimates ranging from 0.84 to 0.95 (Table 1). Within each population, low correlations ($r = -0.27$ to 0.20; $r_g = -0.32$ to 0.22) were found between

GER severity and the three agronomic traits analyzed, though significant in most instances (Table 2). Similarly to our findings, Han et al. (2018) and Martin et al. (2012c) reported low negative correlations between GER severity and days to silking illustrating that late materials tend to get less infected.

In wheat, PHT can highly affect severity of Fusarium head blight infection (Mesterházy 1995; Gaikpa et al. 2020), but this was not the case in our study with maize as judged from the non-significant correlations between PHT and GER severity (Table 2). This might be explained by the direct inoculation of the maize ears by hand while in wheat spray inoculation from above is commonly practiced.

Seed-set is an important fertility and yield-related trait in maize and also highly affected by inbreeding depression. DH lines from landraces are known to suffer more from inbreeding depression (Böhm et al. 2017; Strigens et al. 2013) because they have not experienced several cycles of inbreeding like elite material. *Fusarium* species are notorious in benefitting from host stress and the proportion of kernels on maize cobs itself might influence GER severity because many missing kernels reduce the nutrient ability of the fungus. In both cases, a close correlation between GER severity and SS should occur. The respective correlation coefficients between the two traits were significant, but low (Table 2). This implies that those DH lines that were heavily affected by inbreeding depression and consequently showing a low SS did not systematically suffer more from GER. The large differences in SS (Fig. 2b) within our populations might have been caused also by variation in flowering date as indicated by moderate negative correlations between both traits ($r = -0.52$ to -0.54, $r_g = -0.59$ to -0.56, $P < 0.0001$). Genotypes flowering late had reduced seed-set, but this might have been caused by unusually low rainfall and higher temperatures towards the end of the silking period in 2018 and partially also in 2019 rather than by inbreeding depression. PHT is also affected by inbreeding depression, but the association between PHT and SS was not significant ($r_g = 0.02$ in KE and -0.06 in PE). Hence, we conclude that in our study GER was not strongly affected by inbreeding depression among lines. This is supported by the fact that the mean of the DH lines is not drastically higher than the mean of the source populations (Fig. 2a).

Our findings corroborate earlier studies reporting a high amount of genetic variation among landraces for Fusarium ear rot caused by *F. verticillioides* (Böhm et al. 2017). In the latter study, some DH lines from landraces were even less susceptible to FER than elite maize lines. The high phenotypic variation observed in this study can be exploited for GER resistance breeding and can be used for genomic-based approaches, like GWAS and GS. GER resistance was not much affected by the three agronomic traits and can, thus, be selected without undesirable correlated response.

Marker-trait associations for GER severity among maize landraces

Although genotypic variation for GER severity found within PE was similar to KE (Table 1), no significant QTLs could be detected in PE, whereas in KE, we detected eight QTLs. This is astonishing as both landraces were evaluated in the same environments, with about the same number of genotypes ($N \sim 230$) and a high-density marker array ($N = 388{,}999$). As the complexity of a trait highly affects the outcome of the molecular analyses (Schön et al. 2004), many small-effect QTLs with rare alleles might control GER resistance in PE that could not be detected by GWAS at the chosen significant threshold. Though we did not find QTLs for GER severity within PE, QTLs were detected for the three other agronomic traits evaluated ($P = 0.0001$, Supplementary Table 3). In GWAS, strong linkage disequilibrium (LD) between a marker and a QTL allele is required to detect minor-effect QTLs (van Inghelandt et al. 2011). As LD decay was faster in PE than in KE (Mayer et al. 2017, 2020), this might partly explain the difference in QTL detection between both populations. Additionally, the presence of rare alleles in a population can result in low QTL detection power (Korte and Farlow 2013). At most of the QTL positions, minor alleles improved GER resistance in KE (Table 3). Also, the study of Han et al. (2018) found no QTLs for GER severity by GWAS in a line sortiment, but several QTLs for DON content, some of which were located in the same bin (2.02) where GER QTLs were identified in this present study (Table 3). Similar to the present outcome, QTLs have been reported on chromosome bins 2.03, 5.04, 6.07, and 9.05 for GER resistance in previous linkage mapping studies (Giomi et al. 2016; Han et al. 2016; Martin et al. 2011, 2012b). Although we found several QTLs for DS, PHT, and SS, none colocalized with QTLs for GER severity in KE. This accords to the low r and r_g found between GER and the agronomic traits.

Our molecular results agree with the assumption that GER resistance is controlled by many loci each contributing a small effect to the total genetic variation. Most intermediate and small-effect QTLs remain undetected in QTL mapping with small population size and lead to overestimation of the genotypic variance explained by the few detected QTLs (Beavis 1998; Melchinger et al. 1998; Schön et al. 2004; Xu 2003). Thus, larger population sizes are required to obtain an unbiased estimate of the proportion of explained genotypic variance of detected QTLs. The unexplained genetic variance by the QTLs detected in KE might be explained by QTLs with small additive effects that were below the significant threshold and QTLs with non-additive genetic effects on GER severity.

Increase in population size and precision of disease ratings as well as exploration of GWAS models that can account for non-additive QTL effects are recommended. In an analysis combining both landraces KE and PE, however, we could not detect more QTL than in KE alone when including population (KE and PE) as a fixed effect in the model.

Candidate genes associated with GER resistance

The two prioritized SNPs, ZmSYNBREED_24070_673 (chr. 2), and ZmSYNBREED_53695_527 (chr. 6) detected for GER resistance in KE, were associated with candidate genes which code for proteins belonging to families like cytochrome P450, mitogen-activated protein kinase kinase kinase (MAP3Ks), serine/threonine kinase, tetratricopeptide repeat (TPR)-like superfamily protein, leucine-rich repeat (LRR) family protein and armadillo (ARM) repeat superfamily protein. They are associated with functional groups such as binding activities, kinase activity, response to stress/stimulation, signal transduction, catalytic activity, metabolic and biosynthetic processes (Supplementary Table 4). Similar functional categories were reported for differentially expressed genes for *F. graminearum* (Yuan et al. 2020) and *F. verticillioides* (Fv, Yao et al. 2020) resistances in maize.

In previous studies, cytochrome P450 metabolism was found to be involved in Fv resistance in maize (Yao et al. 2020) because it regulates lipid metabolism and influences the production and activity of jasmonic acid as well as synthesis of secondary metabolites such as flavonoid and plant hormones (Koo et al. 2011). Mitogen-activated protein kinases (MAPKs) are highly conserved and transduce signals from the environment into cellular response in plants (Sopeña-Torres et al. 2018). MAP3Ks YODA found in the present study was previously reported to confer broad-spectrum resistance to fungi, bacteria, and oomycetes in *Arabidopsis* (Sopeña-Torres et al. 2018). Additionally, a combined linkage mapping or GWAS and transcriptomic data identified kinase genes for Fv resistance in maize (Maschietto et al. 2017; Yao et al. 2020). Han et al. (2018) also found a protein serine/threonine kinase annotated gene on chr. 2 associated with DON accumulation in maize. The significant roles of TPR-like superfamily protein, LRR family protein and ARM repeats in biotic and abiotic stress regulations have been extensively documented (Shanmugam 2005; Rosado et al. 2006; Padmanabhan et al. 2009; Sharma and Pandey 2016) and LLR family protein has been validated to control *A. flavus* resistance in maize (Dhakal et al. 2017).

Weighted genomic selection outperformed marker-assisted selection for GER resistance

In practice, independent TS and VS are used for GS. However, in this study, we simulated the prospect of MAS and GS in the same experimental material using a fivefold cross-validation procedure (Liu et al. 2013; Würschum et al. 2014; Würschum and Kraft 2014). Additionally, the prospect of using each landrace population exclusively as TS or VS was evaluated. Within KE, the average prediction accuracy of MAS and unweighted GS (RR-BLUP) were similar implying that the QTLs detected by the multilocus GWAS model (FarmCPU) were able to capture most of the important additive variance controlling GER severity. MAS is expected to yield better predictions only when major QTLs are underlying a trait, *e.g., Fhb1*, *Fhb2, Fhb4*, *Fhb5* for Fusarium head blight resistance in wheat (Buerstmayr et al. 2002; Ma et al. 2020). The ρ estimated by RR-BLUP was similar for both, KE and PE DH libraries (39% and 38%, respectively). In RR-BLUP, the effects of many QTLs with small effects are estimated simultaneously and can result in underestimation of the effects of major genes in a population (Bernardo 2014). In contrast, the weighted GS (wRR-BLUP) approach outperformed MAS and RR-BLUP (Fig. 4). Therefore, we hypothesize that different information is captured by the fixed compared to the random effects (Spindel et al. 2016). The superiority of wRR-BLUP agrees with the findings for Fusarium head blight and Septoria tritici blotch resistance in small-grain cereals (Galiano-Carneiro et al. 2019; Herter et al. 2019; Odilbekov et al. 2019). However, estimates of MAS and wRR-BLUP are likely to be somewhat inflated in our study, because we based predictions in the VS on QTLs detected from GWAS in the entire data set. An alternative for getting an unbiased estimate would be the cross-validation procedure suggested by Utz et al. (2000). However, this procedure would be computationally very demanding for our study with about 390,000 markers as it requires in each of the n runs (1) performing GWAS for QTL detection and (2) establishing the GS model with 80% of the population in the training set, and application of the model to the remaining 20% of the population. The unweighted GS approach is a possibility when most of the low-effect QTLs underlying a trait cannot be detected by a GWAS model like in PE.

A close relationship between training set and validation set is positively influencing GS (Albrecht et al. 2011; Riedelsheimer et al. 2013; Kadam et al. 2016; Brauner et al. 2018, 2020; Herter et al. 2019). Prediction across different maize heterotic pools or highly unrelated individuals can even lead to a negative mean ρ (Riedelsheimer et al. 2013; Han et al. 2018). It should be noted that the materials used

for our present work were DH lines derived from two landraces both belonging to the same flint pool, but are not as closely interrelated as lines from bi-parental or interconnected families. Therefore, GS may yield higher ρ for GER severity in breeding programs incorporating pre-selected lines (Albrecht et al. 2011; Brauner et al. 2018).

Across landraces, prediction accuracies close to zero were expected. Differences among landraces in the linkage phases between QTL and markers might account for this result (Brauner et al. 2018; Han et al. 2018), because GS basically utilizes the LD between SNPs and QTLs. When the TS contained only lines from PE even negative ρ was found. KE yielded somewhat higher ρ than PE when used as TS, especially when the two SNPs with intermediate to major effects in KE were used as fixed effect in the GS model. This reflects the results found for each population in GWAS, i.e., the landrace having no major QTL (i.e., PE) was a poorer predictor of GER resistance in the landrace where GER QTLs could be detected (i.e., KE) while the latter was a slightly better predictor of GER resistance in PE.

In an analysis combining both landraces KE and PE for GS, the ρ obtained for GER severity were reduced compared to the results obtained for individual landraces when accounting for differences in mean GER severity between populations (KE and PE) ($\rho = -0.03$ for RR-BLUP and 0.36 for wRR-BLUP).

Conclusions

This study presents phenotypic and molecular analyses of GER resistance among DH lines originating from two European maize landraces, KE and PE. The present findings suggest that favorable alleles in the two landraces can be harnessed for improving GER resistance of elite germplasm with genomic tools. Beneficial QTL alleles from KE need to be validated and then marker-assisted backcrossed (BC) into elite flint lines to increase GER resistance in this heterotic group. The BC lines should be subjected to testcrossing for selecting maturity, further adaptation traits, and finally grain yield. A subsequent selection for GER resistance on testcross basis could be beneficial, because the correlation between line and testcross performance for this resistance trait has been shown to be only moderate (Löffler et al. 2011; Martin et al. 2012c). Although no GER QTLs could be detected within PE, ρ estimated by RR-BLUP was of similar magnitude than within KE, indicating that beneficial effects can be expected also from PE. In future, it might be useful to cross selected DH lines from KE and PE to accumulate their respective resistance alleles in the flint heterotic group.

Acknowledgements We express our profound gratitude to the technical teams of KWS (Einbeck and Gondelsheim) and University of Hohenheim (Institute of Plant Breeding, Seed Science, and Population Genetics, Chair of Applied Genetics and Plant Breeding, State Plant Breeding Institute) for their immense support in conducting the trials. We highly appreciate the help of Maria Belén Kistner, INTA (National Institute of Agricultural Technology), Pergamino, Argentina, in disease rating in 2019. We also thank Dr. Claude Urbany (KWS SAAT SE & Co. KGaA -Einbeck) and Dr. Hans Peter Maurer (State Plant Breeding Institute, University of Hohenheim, Germany) for providing statistical advice.

Author contribution statement TM, TP, BK, and MO conceived the study, supervised the project, and discussed the outcome; DSG collected phenotypic data, investigated genotypic data, conducted all statistical analyses and drafted the manuscript. ALGC helped with phenotypic data collection on all four environments, MM generated and curated the genotypic data, MO, AEM and CCS acquired funding and supervised the genotyping part, TM, AEM, and CCS edited the manuscript. All authors read the final version for publication.

Funding Open Access funding enabled and organized by Projekt DEAL. This research was partly funded by the German Academic Exchange Service, Bonn, Germany as a doctoral study grant to David S. Gaikpa (grant no. 91650671). KWS SAAT SE and Co. KGaA, Einbeck, Germany, co-financed the project by conducting the maize experiments in Gondelsheim. MO, AEM, CCS received funding through MAZE ("Accessing the genomic and functional diversity of maize to improve quantitative traits", BMBF, Funding ID 031B0195) for genotyping the lines.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors declare that the experiments comply with the current laws of Germany.

Human and animal rights This research does not contain any studies on humans or animals.

References

Abed A, Belzile F (2019) Comparing Single-SNP, Multi-SNP, and haplotype-based approaches in association studies for major traits in barley. Plant Genome 12:190036. https://doi.org/10.3835/plantgenome2019.05.0036

Albrecht T, Wimmer V, Auinger H et al (2011) Genome-based prediction of testcross values in maize. Theor Appl Genet 123:339. https://doi.org/10.1007/s00122-011-1587-7

Arruda MP, Brown P, Brown-Guedira G et al (2016) Genome-wide association mapping of Fusarium head blight resistance in wheat using genotyping-by-sequencing. Plant Genome. https://doi.org/10.3835/plantgenome2015.04.0028

Bedoya CA, Dreisigacker S, Hearne S et al (2017) Genetic diversity and population structure of native maize populations in Latin America and the Caribbean. PLoS ONE 12:1–21. https://doi.org/10.1371/journal.pone.0173488

Beavis WD (1998) QTL analyses: power, precision, and accuracy. In: Paterson A (ed) Molecular dissection of complex traits. CRC Press, New York, pp 145–162

Bernardo R (2014) Genome wide selection when major genes are known. Crop Sci 54:68–75

Böhm J, Schipprack W, Utz HF, Melchinger AE (2017) Tapping the genetic diversity of landraces in allogamous crops with doubled haploid lines: a case study from European flint maize. Theor Appl Genet 130:861–873. https://doi.org/10.1007/s00122-017-2856-x

Bolduan C, Miedaner T, Schipprack W et al (2009) Genetic variation for resistance to ear rots and mycotoxins contamination in early European maize inbred lines. Crop Sci 49:2019–2028. https://doi.org/10.2135/cropsci2008.12.0701

Brauner PC, Müller D, Molenaar WS, Melchinger AE (2020) Genomic prediction with multiple biparental families. Theor Appl Genet 133:133–147. https://doi.org/10.1007/s00122-019-03445-7

Brauner PC, Müller D, Schopp P et al (2018) Genomic prediction within and among doubled-haploid libraries from maize landraces. Genetics 210:1185–1196. https://doi.org/10.1534/genetics.118.301286

Browning BL, Zhou Y, Browning SR (2018) A one-penny imputed genome from next-generation reference panels. Am J Hum Genet 103:338–348. https://doi.org/10.1016/j.ajhg.2018.07.015

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Genet 104:84–91. https://doi.org/10.1007/s001220200009

Butler D (2009) asreml: asreml() fits the linear mixed model. R package version 3.0. https://www.vsni.co.uk

Coan MMD, Senhorinho HJC, Pinto RJB et al (2018) Genome-wide association study of resistance to ear rot by *F. verticillioides* in a tropical field maize and popcorn core collection. Crop Sci 58:564–578. https://doi.org/10.2135/cropsci2017.05.0322

Dhakal R, Chai C, Karan R et al (2017) Expression profiling coupled with in-silico mapping identifies candidate genes for reducing aflatoxin accumulation in maize. Front Plant Sci 8:503. https://doi.org/10.3389/fpls.2017.00503

Ding L, Xu H, Yi H et al (2011) Resistance to hemi-biotrophic *F. graminearum* infection is associated with coordinated and ordered expression of diverse defense signaling pathways. PLoS One. https://doi.org/10.1371/journal.pone.0019008

dos Santos JPR, Pires LPM, de Castro Vasconcellos RC et al (2016) Genomic selection to resistance to *Stenocarpella maydis* in maize lines using DArTseq markers. BMC Genet 17:1–10. https://doi.org/10.1186/s12863-016-0392-3

Endelman JB (2011) Ridge regression and other kernels for genomic selection with R package rrBLUP. Plant Genome J 4:250. https://doi.org/10.3835/plantgenome2011.08.0024

Endelman JB, Jannink J-L (2012) Shrinkage estimation of the realized relationship matrix. G3 Genes. Genomes, Genetics 2:1405–1413. https://doi.org/10.1534/g3.112.004259

FAO (2003) Worldwide regulations for mycotoxins in food and feed 2003. Food Nutr Pap 81:1–165

Foroud NA, Baines D, Gagkaeva TY et al (2019) Trichothecenes in cereal grains—An update. Toxins 11:634. https://doi.org/10.3390/toxins11110634

Gaikpa DS, Koch S, Fromme FJ et al (2020) Genome-wide association mapping and genomic prediction of Fusarium head blight resistance, heading stage and plant height in winter rye (*Secale cereale*). Plant Breed 139:508–520. https://doi.org/10.1111/pbr.12810

Gaikpa DS, Miedaner T (2019) Genomics-assisted breeding for ear rot resistances and reduced mycotoxin contamination in maize: methods, advances and prospects. Theor Appl Genet 132:2721–2739. https://doi.org/10.1007/s00122-019-03412-2

Galiano-Carneiro AL, Boeven PHG, Maurer HP et al (2019) Genome-wide association study for an efficient selection of Fusarium head blight resistance in winter triticale. Euphytica 215:4. https://doi.org/10.1007/s10681-018-2327-8

Galić V, Šimić D, Franić M et al (2019) Analysis of Fusarium ear rot and fumonisin contamination in testcrosses of a maize biparental population. Crop Breed Appl Biotechnol 19:40–46. https://doi.org/10.1590/1984

Gao J, Wang S, Zhou Z et al (2019) Linkage mapping and genome-wide association reveal candidate genes conferring thermotolerance of seed-set in maize. J Exp Bot 70:4849–4863. https://doi.org/10.1093/jxb/erz171

Giomi GM, Kreff ED, Iglesias J et al (2016) Quantitative trait loci for Fusarium and Gibberella ear rot resistance in Argentinian maize germplasm. Euphytica 211:287–294. https://doi.org/10.1007/s10681-016-1725-z

Gowda M, Das B, Makumbi D et al (2015) Genome-wide association and genomic prediction of resistance to maize lethal necrosis disease in tropical maize germplasm. Theor Appl Genet 128:1957–1968. https://doi.org/10.1007/s00122-015-2559-0

Hallauer A, Carena M, Filho J (1988) Quantitative genetics in maize breeding. Handbook of plant breeding, 2nd edn. Springer-Verlag, New York

Han S, Miedaner T, Utz HF et al (2018) Genomic prediction and GWAS of Gibberella ear rot resistance traits in dent and flint lines of a public maize breeding program. Euphytica 214:1–20. https://doi.org/10.1007/s10681-017-2090-2

Han S, Utz HF, Liu W et al (2016) Choice of models for QTL mapping with multiple families and design of the training set for prediction of Fusarium resistance traits in maize. Theor Appl Genet 129:431–444. https://doi.org/10.1007/s00122-015-2637-3

Herter CP, Ebmeyer E, Kollers S et al (2019) Accuracy of within- and among-family genomic prediction for Fusarium head blight and Septoria tritici blotch in winter wheat. Theor Appl Genet 132:1121–1135. https://doi.org/10.1007/s00122-018-3264-6

Hölker AC, Mayer M, Presterl T et al (2019) European maize landraces made accessible for plant breeding and genome-based studies. Theor Appl Genet 132:3333–3345. https://doi.org/10.1007/s00122-019-03428-8

Hu G, Li Z, Lu Y et al (2017) Genome-wide association study identified multiple genetic loci on chilling resistance during germination in maize. Sci Rep 7:10840. https://doi.org/10.1038/s41598-017-11318-6

Jiao Y, Peluso P, Shi J et al (2017) Improved maize reference genome with single-molecule technologies. Nature 546:524–527. https://doi.org/10.1038/nature22971

Kadam DC, Potts SM, Bohn MO et al (2016) Genomic prediction of single crosses in the early stages of a maize hybrid breeding pipeline. G3 Genes. Genomes, Genet 6:3443–3453. https://doi.org/10.1534/g3.116.031286

Kaler AS, Gillman JD, Beissinger T, Purcell LC (2020) Comparing different statistical models and multiple testing corrections for association mapping in soybean and maize. Front Plant Sci 10:1794. https://doi.org/10.3389/fpls.2019.01794

Kebede AZ, Woldemariam T, Reid LM, Harris LJ (2016) Quantitative trait loci mapping for Gibberella ear rot resistance and associated agronomic traits using genotyping-by-sequencing in maize. Theor Appl Genet 129:17–29. https://doi.org/10.1007/s00122-015-2600-3

Koo AJK, Cooke TF, Howe GA (2011) Cytochrome P450 CYP94B3 mediates catabolism and inactivation of the plant hormone jasmonoyl-L-isoleucine. Proc Natl Acad Sci U S A 108:9298–9303. https://doi.org/10.1073/pnas.1103542108

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:1–9. https://doi.org/10.1186/1746-4811-9-29

Li C, Fu Y, Sun R et al (2018) Single-locus and multi-locus genome-wide association studies in the genetic dissection of fiber quality traits in upland cotton (*Gossypium hirsutum* L.). Front Plant Sci 9:1–16. https://doi.org/10.3389/fpls.2018.01083

Lipka AE, Tian F, Wang Q et al (2012) GAPIT: Genome association and prediction integrated tool. Bioinformatics 28:2397–2399. https://doi.org/10.1093/bioinformatics/bts444

Liu X, Huang M, Fan B et al (2016) Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet 12:1–24. https://doi.org/10.1371/journal.pgen.1005767

Liu W, Maurer HP, Reif JC et al (2013) Optimum design of family structure and allocation of resources in association mapping with lines from multiple crosses. Heredity 110:71–79. https://doi.org/10.1038/hdy.2012.63

Löffler M, Kessel B, Ouzunova M, Miedaner T (2011) Covariation between line and testcross performance for reduced mycotoxin concentrations in European maize after silk channel inoculation of two *Fusarium* species. Theor Appl Genet 122:925–934. https://doi.org/10.1007/s00122-010-1499-y

Ma Z, Xie Q, Li G, Jia H, Zhou J, Kong Z, Li N, Yuan Y (2020) Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. Theor Appl Genet 133:1541–1568. https://doi.org/10.1007/s00122-019-03525-8

Malik PL, Janss L, Nielsen LK et al (2019) Breeding for dual-purpose wheat varieties using marker–trait associations for biomass yield and quality traits. Theor Appl Genet 132:3375–3398. https://doi.org/10.1007/s00122-019-03431-z

Martin GB, Bogdanove AJ, Sessa G (2003) Understanding the functions of plant disease resistance proteins. Annu Rev Plant Biol 54:23–61. https://doi.org/10.1146/annurev.arplant.54.031902.135035

Martin M, Dhillon BS, Miedaner T, Melchinger AE (2012) Inheritance of resistance to Gibberella ear rot and deoxynivalenol contamination in five flint maize crosses. Plant Breed 131:28–32. https://doi.org/10.1111/j.1439-0523.2011.01908.x

Martin M, Miedaner T, Dhillon BS, Ufermann U, Kessel B, Ouzunova M, Schipprack W, Melchinger AE (2011) Colocalization of QTL for Gibberella ear rot resistance and low mycotoxin contamination in early European maize. Crop Sci 51:1935–1945. https://doi.org/10.2135/cropsci2010.11.0664

Martin M, Miedaner T, Schwegler DD et al (2012) Comparative quantitative trait loci mapping for Gibberella ear rot resistance and reduced deoxynivalenol contamination across connected maize populations. Crop Sci 52:32–43. https://doi.org/10.2135/cropsci2011.04.0214

Martin M, Schipprack W, Miedaner T et al (2012) Variation and covariation for Gibberella ear rot resistance and agronomic traits in testcrosses of doubled haploid maize lines. Euphytica 185:441–451. https://doi.org/10.1007/s10681-012-0623-2

Maschietto V, Colombi C, Pirona R et al (2017) QTL mapping and candidate genes for resistance to Fusarium ear rot and fumonisin contamination in maize. BMC Plant Biol 17:1–21. https://doi.org/10.1186/s12870-017-0970-1

Mayer M, Hölker AC, González-Segovia E, Presterl T, Ouzunova M, Melchinger AE, Schön C-C (2020) Discovery of beneficial haplotypes for complex traits in maize landraces. Nat Commun 11:4954. https://doi.org/10.1038/s41467-020-18683-3

Mayer M, Unterseer S, Bauer E et al (2017) Is there an optimum level of diversity in utilization of genetic resources? Theor Appl Genet 130:2283–2295. https://doi.org/10.1007/s00122-017-2959-4

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Melchinger AE, Utz HF, Schön CC (1998) Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149:383–403. https://doi.org/10.1016/1369-5266(88)80015-3

Mesterházy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breed 114:377–386. https://doi.org/10.1111/j.1439-0523.1995.tb00816.x

Mesterházy Á, Lemmens M, Reid LM (2012) Breeding for resistance to ear rots caused by *Fusarium* spp. in maize—A review. Plant Breed 131:1–19. https://doi.org/10.1111/j.1439-0523.2011.01936.x

Mesterházy Á, Toldine Toth E, Szel S, Varga M, Toth B (2020) Resistance of maize hybrids to *Fusarium graminearum, F. culmorum*, and *F. verticillioides* ear rots with toothpick and silk channel inoculation, as well as their toxin production. Agronomy. https://doi.org/10.3390/agronomy10091283

Miao C, Yang J, Schnable JC (2019) Optimising the identification of causal variants across varying genetic architectures in crops. Plant Biotechnol J 17:893–905. https://doi.org/10.1111/pbi.13023

Miedaner T, Bolduan C, Melchinger AE (2010) Aggressiveness and mycotoxin production of eight isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for ear rot on susceptible and resistant early maize inbred lines. Eur J Plant Pathol 127:113–123. https://doi.org/10.1007/s10658-009-9576-2

Morales L, Zila CT, Mejía DEM et al (2019) Diverse components of resistance to *Fusarium verticillioides* infection and fumonisin contamination in four maize recombinant inbred families. Toxins (Basel). https://doi.org/10.3390/toxins11020086

Odilbekov F, Armonienė R, Koc A et al (2019) GWAS-assisted genomic prediction to predict resistance to Septoria tritici blotch in Nordic winter wheat at seedling stage. Front Genet 10:1–10. https://doi.org/10.3389/fgene.2019.01224

Padmanabhan M, Cournoyer P, Dinesh-Kumar S (2009) The leucine-rich repeat domain in plant innate immunity: a wealth of possibilities. Cell Microbiol 11:191–198. https://doi.org/10.1111/j.1462-5822.2008.01260.x

Pfordt A, Ramos Romero L, Schiwek S et al (2020) Impact of environmental conditions and agronomic practices on the prevalence of Fusarium species associated with ear- and stalk rot in maize. Pathogens 9:236. https://doi.org/10.3390/pathogens9030236

Piepho HP, Williams ER, Fleck M (2006) A note on the analysis of designed experiments with complex treatment structure. HortScience 41:446–452

Pinton P, Oswald I (2014) Effect of deoxynivalenol and other type b trichothecenes on the intestine: a review. Toxins (Basel) 6:1615–1643. https://doi.org/10.3390/toxins6051615

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org

Reid L, Hamilton RI, Mather DE (1996) Screening maize for resistance to Gibberella ear rot. Agric Agri-Food Canada Res. Branch, Eastern Cereal Oilseed Res. Cent. Tech. Bull. 1996–5E

Riedelsheimer C, Endelman JB, Stange M et al (2013) Genomic predictability of interconnected biparental maize populations. Genetics 194:493–503. https://doi.org/10.1534/genetics.113.150227

Rosado A, Schapire AL, Bressan RA et al (2006) The Arabidopsis tetratricopeptide repeat-containing protein TTL1 is required for osmotic stress responses and abscisic acid sensitivity. Plant Physiol 142:1113–1126. https://doi.org/10.1104/pp.106.085191

Schön CC, Utz HF, Groh S, Truberg B, Openshaw S, Melchinger AE (2004) Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. Genetics 167:485–498

Shanmugam V (2005) Role of extracytoplasmic leucine rich repeat proteins in plant defence mechanisms. Microbiol Res 160:83–94. https://doi.org/10.1016/j.micres.2004.09.014

Sharma M, Pandey GK (2016) Expansion and function of repeat domain proteins during stress and development in plants. Front Plant Sci 6:1–15. https://doi.org/10.3389/fpls.2015.01218

Sopeña-Torres S, Jordá L, Sánchez-Rodríguez C et al (2018) YODA MAP3K kinase regulates plant immune responses conferring broad-spectrum disease resistance. New Phytol 218:661–680. https://doi.org/10.1111/nph.15007

Spindel JE, Begum H, Akdemir D et al (2016) Genome-wide prediction models that incorporate de novo GWAS are a powerful new tool for tropical rice improvement. Heredity (Edinb) 116:395–408. https://doi.org/10.1038/hdy.2015.113

Strigens A, Schipprack W, Reif JC, Melchinger AE (2013) Unlocking the genetic diversity of maize landraces with doubled haploids opens new avenues for breeding. PLoS ONE 8:7–9. https://doi.org/10.1371/journal.pone.0057234

Technow F, Bürger A, Melchinger AE (2013) Genomic prediction of northern corn leaf blight resistance in maize with combined or separated training sets for heterotic groups. Genes, Genomes, Genetics 3:197–203. https://doi.org/10.1534/g3.112.004630

The Commission of the European Communities (2006) Commission regulation (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union 364:5–24

Trail F (2009) For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiol 149:103–110. https://doi.org/10.1104/pp.108.129684

Unterseer S, Bauer E, Haberer G et al (2014) A powerful tool for genome analysis in maize: development and evaluation of the high density600 k SNP genotyping array. BMC Genomics 15:823. https://doi.org/10.1186/1471-2164-15-823

Utz HF, Melchinger AE, Schön CC (2000) Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849

van Inghelandt D, Reif JC, Dhillon BS et al (2011) Extent and genome-wide distribution of linkage disequilibrium in commercial maize germplasm. Theor Appl Genet 123:11–20. https://doi.org/10.1007/s00122-011-1562-3

VanRaden PM (2008) Efficient methods to compute genomic predictions. J Dairy Sci 91:4414–4423. https://doi.org/10.3168/jds.2007-0980

Warburton ML, Tang JD, Windham GL et al (2015) Genome-wide association mapping of aspergillus flavus and aflatoxin accumulation resistance in maize. Crop Sci 55:1857–1867. https://doi.org/10.2135/cropsci2014.06.0424

Wei W, Mesquita ACO, de Figueiró A, A, et al (2017) Genome-wide association mapping of resistance to a Brazilian isolate of *Sclerotinia sclerotiorum* in soybean genotypes mostly from Brazil. BMC Genomics 18:1–16. https://doi.org/10.1186/s12864-017-4160-1

Wen YJ, Zhang H, Ni YL et al (2018) Methodological implementation of mixed linear models in multi-locus genome-wide association studies. Brief Bioinform 19:700–712. https://doi.org/10.1093/bib/bbw145

Wilson AJ, Réale D, Clements MN et al (2010) An ecologist's guide to the animal model. J Anim Ecol 79:13–26. https://doi.org/10.1111/j.1365-2656.2009.01639.x

Würschum T, Abel S, Zhao Y (2014) Potential of genomic selection in rapeseed (*Brassica napus* L.) breeding. Plant Breed 133:45–51. https://doi.org/10.1111/pbr.12137

Würschum T, Kraft T (2014) Cross-validation in association mapping and its relevance for the estimation of QTL parameters of complex traits. Heredity 112:463–468. https://doi.org/10.1038/hdy.2013.126

Würschum T, Langer SM, Longin CFH (2015) Genetic control of plant height in European winter wheat cultivars. Theor Appl Genet 128:865–874. https://doi.org/10.1007/s00122-015-2476-2

Xiang K, Reid LM, Zhang Z et al (2010) A meta-analysis of QTL associated with ear rot resistance in maize. Maydica 55:281–290

Xu S (2003) Theoretical basis of the Beavis effect. Genetics 165:2259–2268

Xu Y, Yang T, Zhou Y et al (2018) Genome-wide association mapping of starch pasting properties in maize using single-locus and multi-locus models. Front Plant Sci 9:1–10. https://doi.org/10.3389/fpls.2018.01311

Yao L, Li Y, Ma C et al (2020) Combined genome-wide association study and transcriptome analysis reveal candidate genes for resistance to Fusarium ear rot in maize. J Integr Plant Biol. https://doi.org/10.1111/jipb.12911

Yao Q, Yang K, Pan G, Rong T (2007) Genetic diversity of maize (*Zea mays* L.) landraces from Southwest China based on SSR data. J Genet Genomics 34:851–860. https://doi.org/10.1016/S1673-8527(07)60096-4

Yuan G, He X, Li H et al (2020) Transcriptomic responses in resistant and susceptible maize infected with *Fusarium graminearum*. Crop J 8:153–163. https://doi.org/10.1016/j.cj.2019.05.008

Zhang YM, Jia Z, Dunwell JM (2019) Editorial: The applications of new multi-locus gwas methodologies in the genetic dissection of complex traits. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00100

Zhang YM, Mao Y, Xie C et al (2005) Mapping quantitative trait loci using naturally occurring genetic variance among commercial inbred lines of maize (Zea mays L.). Genetics 169:2267–2275. https://doi.org/10.1534/genetics.104.033217

Zhao Y, Mette MF, Gowda M et al (2014) Bridging the gap between marker-assisted and genomic selection of heading time and plant height in hybrid wheat. Heredity (Edinb) 112:638–645. https://doi.org/10.1038/hdy.2014.1

Zhou M, Yang L, Shao M et al (2018) Effects of zearalenone exposure on the TGF-β1/Smad3 signaling pathway and the expression of proliferation or apoptosis related genes of post-weaning gilts. Toxins (Basel) 10:1–13. https://doi.org/10.3390/toxins10020049

Zhu XM, Shao XY, Pei YH et al (2018) Genetic diversity and genome-wide association study of major ear quantitative traits using high-density SNPs in maize. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00966

Supplementary material

Supplementary Tables

Supplementary Table 1 Repeatability values for Gibberella ear rot (GER) severity and agronomic traits in individual environments

Trait	GON 2018	GON 2019	HOH 2018	HOH 2019
Kemater (N=250)				
GER (%)	0.71	0.61	0.70	0.75
Silking (days)	0.93	0.93	0.95	0.90
Plant height (cm)	0.90	0.92	0.91	0.91
Seedset (%)	0.88	0.85	0.90	0.87
Petkuser (N=250)				
GER (%)	0.81	0.62	0.76	0.61
Silking (days)	0.93	0.93	0.96	0.90
Plant height (cm)	0.88	0.90	0.91	0.90
Seed set (%)	0.85	0.82	0.88	0.80

GON=Gondelsheim, HOH=Hohenheim

Supplementary Table 2 Significant SNPs detected for days to silking (DS), plant height (PHT) and seed-set (SS) and the proportion of explained genotypic variance (PG, %) within "Kemater Landmais Gelb" population (N=236)

Trait	Marker	Chr[a]	Coordinate (cM)	P-value	MAF	Effect	PG(%)
Silking	ZmSYNBREED_13571_319	1	109.74	2.29E-05	0.35	0.76	7.04
	PZE-101177457	1	173.40	6.95E-07	0.37	-0.72	4.22
	ZmSYNBREED_24171_409	2	54.70	2.42E-08	0.37	0.92	0.46
	PZE-102057292	2	68.05	6.88E-06	0.09	-1.16	4.06
	ZmSYNBREED_25614_200	2	85.63	1.24E-07	0.40	-0.93	12.02
	ZmSYNBREED_30702_382	2	179.08	6.63E-05	0.29	0.92	1.30
	PZE-103020403	3	36.33	8.61E-05	0.49	0.62	3.99
	ZmSYNBREED_36594_421	3	136.27	8.38E-05	0.26	0.60	3.54
	ZmSYNBREED_43496_660	4	135.18	3.07E-05	0.30	0.73	2.58
	ZmSYNBREED_60539_814	7	89.00	5.06E-06	0.42	-0.75	6.38
	ZmSYNBREED_61375_398	7	138.60	1.31E-05	0.28	0.78	2.06
	PZE-108043501	8	53.20	4.55E-07	0.30	0.88	4.54
	ZmSYNBREED_66468_531	8	134.55	3.06E-07	0.08	1.65	12.57
	Total						62.21
PHT	SYN25732	1	98.12	2.8E-08	0.05	8.96	6.56
	ZmSYNBREED_26883_932	2	87.30	2.67E-13	0.26	6.84	14.53
	ZmSYNBREED_30884_970	3	18.40	1.04E-06	0.14	-4.61	3.99
	ZmSYNBREED_31089_919	3	36.33	9.43E-05	0.32	2.85	1.50
	ZmSYNBREED_36058_252	3	114.24	1.56E-11	0.47	5.17	10.58
	ZmSYNBREED_45996_103	5	61.00	6.55E-05	0.50	-2.68	2.27
	ZmSYNBREED_48515_186	5	79.07	5.54E-05	0.34	-4.05	14.60
	ZmSYNBREED_56241_197	7	10.95	1.22E-06	0.38	3.35	2.74
	ZmSYNBREED_64964_415	8	80.15	1.96E-09	0.31	4.64	7.36
	ZmSYNBREED_65956_413	8	98.42	9.48E-08	0.22	4.79	8.51
	ZmSYNBREED_67307_966	9	47.00	5.32E-08	0.29	-3.75	0.72
	Total						71.71
SS	SYN34979	5	99.08	4.01E-05	0.07	-12.25	9.84

[a]Chromosome; MAF, minor allele frequency

Supplementary Table 3 Significant SNPs detected for days to silking, plant height and seed-set and the proportion of explained genotypic variance (PG, %) within "Petkuser Ferdinand Rot" population (N=226)

Trait	Marker	Chr[a]	Coordinate (cM)	P-value	MAF	Effect	PG(%)
Silking	ZmSYNBREED_18736_535	1	252.70	1.32E-09	0.46	1.04	3.90
	ZmSYNBREED_24440_109	2	66.95	2.06E-11	0.49	-1.36	12.33
	ZmSYNBREED_30876_878	3	17.00	2.99E-07	0.08	1.51	8.38
	ZmSYNBREED_44949_124	4	173.20	6.05E-09	0.02	3.11	6.65
	ZmSYNBREED_45112_401	5	13.20	3.55E-05	0.38	0.62	2.64
	ZmSYNBREED_53883_603	6	37.25	3.2E-06	0.13	-1.03	0.66
	ZmSYNBREED_55424_201	6	79.40	1.8E-05	0.44	0.68	0.30
	ZmSYNBREED_21984_457	10	61.53	5.52E-06	0.28	0.97	15.42
	ZmSYNBREED_23095_276	10	86.50	1.51E-05	0.23	-0.97	5.07
	ZmSYNBREED_23313_200	10	102.40	7.33E-08	0.14	1.31	0.33
	Total						53.04
PHT	ZmSYNBREED_16431_599	1	164.77	1.04E-05	0.14	-3.73	1.93
	PZE-101171667	1	168.80	9.4E-08	0.12	-5.33	7.23
	PUT-163a-16927623-1182	2	62.87	3.66E-05	0.47	-2.90	4.28
	SYN4699	2	134.28	1.11E-06	0.04	-6.90	2.41
	ZmSYNBREED_42399_177	4	90.51	6.45E-07	0.18	3.97	13.28
	ZmSYNBREED_53359_839	6	27.00	2.16E-11	0.35	-6.40	22.81
	ZmSYNBREED_55722_432	6	99.00	4.86E-06	0.29	-4.67	7.13
	SYN14712	6	135.80	1.32E-05	0.48	-2.39	1.58
	ZmSYNBREED_60462_165	7	86.50	4.73E-09	0.50	-3.86	0.02
	ZmSYNBREED_66119_558	8	103.30	1.91E-05	0.04	7.52	3.90
	Total						62.62
SS	ZmSYNBREED_24191_259	2	55.37	1.9E-05	0.29	7.59	3.60

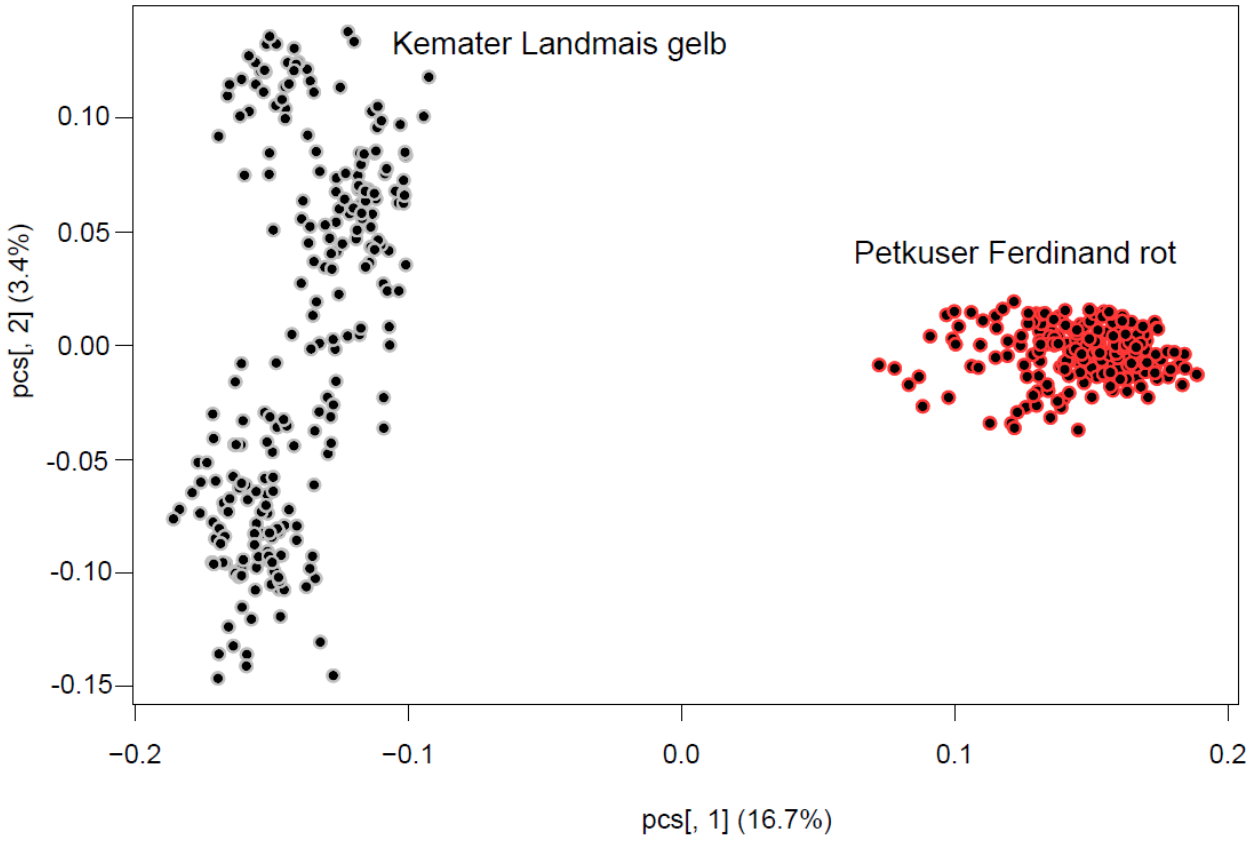
[a]Chromosome; MAF, minor allele frequency

Supplementary Table 4 Number of candidate genes associated with ontological terminologies for Gibberella ear rot severity

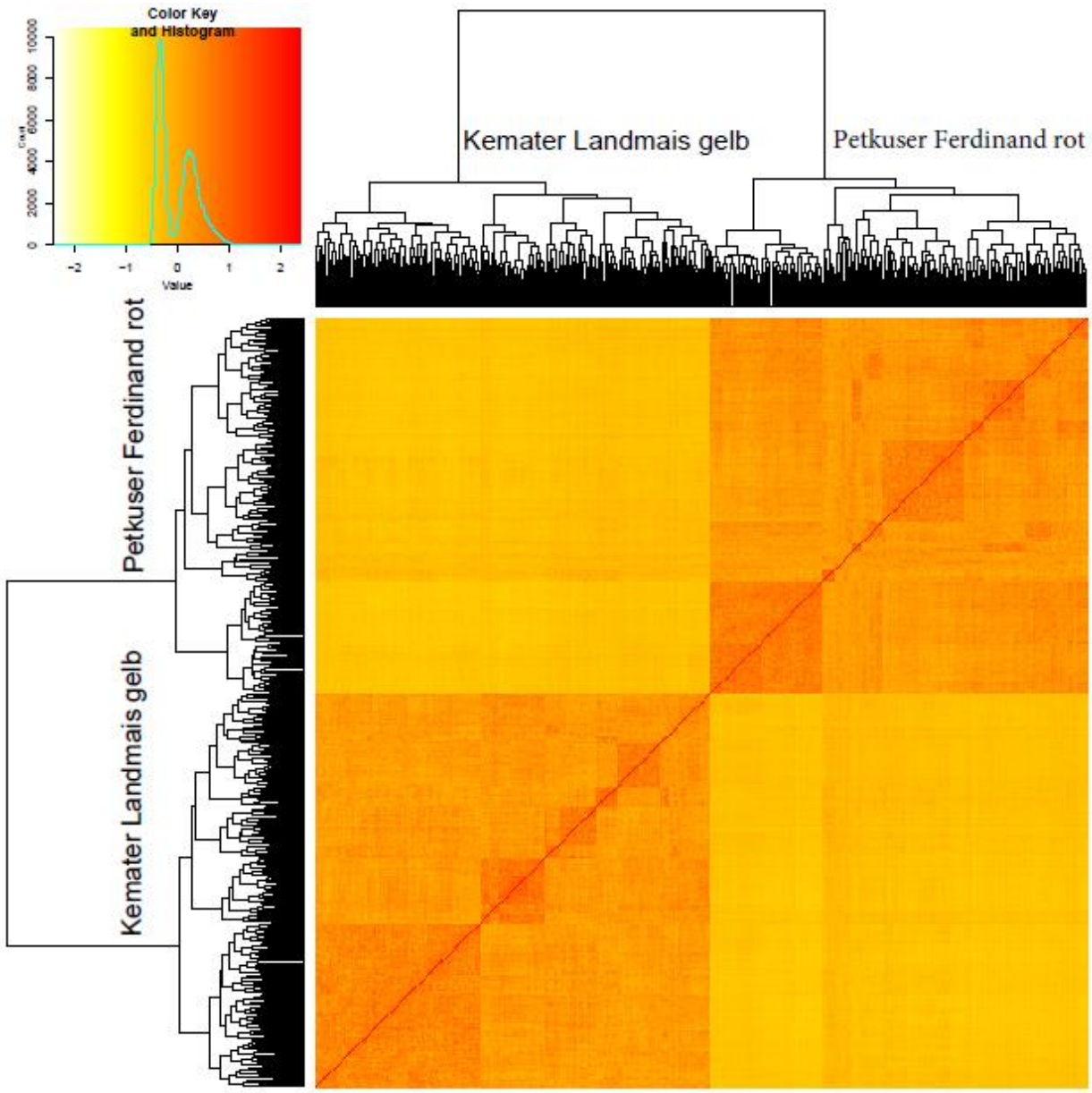
Functional group/gene ontology term	Number of genes*
(ATP, DNA, Protein, Ion) binding activity	9
(Protein) kinase activity	4
Molecule/membrane modification/repair	2
Defense/response to stress or stimuli	4
Catalytic/transferase activity	5
Structural component of cell wall/membrane/ribosome	3
Compound biosynthesis/metabolism	5
Protein phosphorylation/signal transduction	3
Regulation of DNA replication/transcription	2
Oxidation-reduction process	3

*Some of the 25 protein-coding genes/gene models associated with the two most important SNPs of GER severity had multiple functions

Supplementary Figures



Supplementary Figure 1 Principal component (PC) analysis of the 462 DH lines originating from two landraces based on the marker data. Percentages of variation explained by the first and second PCs are shown in the brackets



Supplementary Figure 2 A heat map of dendrogram and the genomic relationship matrix constructed using VanRaden algorithm in R package GAPIT.

6 General discussion

Fusarium spp. are ubiquitous in nature, infecting maize and small-grain cereals such as rye, triticale, durum wheat, bread wheat and many other crops. Quantitative host plant resistance is an effective way of reducing the negative impacts of Fusarium ear diseases and mycotoxin contaminations in maize and small-grain cereals. In this thesis, Fusarium head blight (FHB) resistance and deoxynivalenol accumulation in four small-grain winter cereal crops were compared, and the molecular mechanism of FHB resistance examined in rye for the first time. The thesis also covers the genomics of ear rots (ER) and mycotoxin resistances in maize, and empirical phenotypic and genomic analyses of Gibberella ear rot (GER) resistance among doubled-haploid (DH) lines derived from two European flint maize landraces.

FHB resistance and deoxynivalenol accumulation in four winter small-grain cereals

Systematic comparison of 12 cultivars or advanced genotypes each of rye, triticale, durum wheat and bread wheat under artificial infection using constant and crop-specific inoculum concentrations showed differential response to *F. culmorum* head infection, kernel damage and DON accumulation (Chapter 2). Interestingly, although FHB severity in durum wheat was lower than in bread wheat, durum wheat accumulated more DON than bread wheat on the overage. The higher mean of FHB severity for bread wheat than durum wheat can be attributed to the presence of two highly susceptible genotypes, Franz and Tobak (Chapter 2). In addition, the regulatory mechanisms of FHB severity and DON accumulation in these crop species may be partially different. He et al. (2019) identified two major quantitative trait loci (QTLs) for DON accumulation among 197 recombinant bread wheat inbred lines by spray-inoculation of a mixture of five DON producing *F. graminearum* isolates. The QTL located on chromosome 3BL had only minor impact on FHB resistance while the other QTL on chromosome 3DL had no impact on FHB. Other factors such as moisture content and phenolic compounds might

influence symptom development and DON synthesis in small-grain cereals (Gauthier et al., 2016; Pagnussatt et al., 2014). The strength of association between traits, FHB severity, FDK and DON depended on the crop species, being high in both wheat species and generally low in rye and triticale. Hence, it is important to determine mycotoxin contents of cereal crops at the later stages of variety development.

The ranking of the four winter crop species for FHB severity was not influenced by change in inoculum concentration (i.e., constant *vs*. crop-specific concentrations, Chapter 2). Other authors also found rye to be more resistant to FHB and DON accumulation than triticale and wheat genotypes (Arseniuk et al., 1999; Langevin et al., 2004; Miedaner et al., 2001). Arseniuk et al. (1999) found both spring and winter wheat varieties to be more susceptible to FHB than rye and triticale varieties under artificial infection with a composite isolate of four *Fusarium* spp. Triticale might have inherited the high level of resistance from rye. Previous QTL analyses in hexaploid triticale showed that several rye chromosomes contained QTLs for FHB resistance, though some QTLs were also found on the A and B genomes (Dhariwal et al., 2018; Galiano-Carneiro et al., 2019; Kalih et al., 2015). The differential response of these four winter *Triticeae* species to Fusarium infection can also be attributed to passive resistance mechanisms such as variation in anther extrusion, spike morphology, waxy layer and plant height (Chapter 2; Buerstmayr & Buerstmayr, 2015; Mesterházy, 1995). In addition, the reduced height (*Rht*) genes such as *Rht-B1b* and *Rht-D1b* in durum wheat and bread wheat highly influence FHB severity among cultivars of these crops (Miedaner & Voss, 2008; Miedaner et al., 2017).

In future studies, the regulatory mechanisms of mycotoxin accumulation in harvested small-grain cereal crops should be investigated into more detail. Studies aimed at elucidating the molecular mechanism of FHB resistance in rye will be worthwhile to understand to which extent its high resistance level is governed by a high frequency of strong QTL alleles that could be transferred in future to wheat and triticale.

Molecular basis of FHB resistance in rye (*Secales cereale* L.)

Though advances have been made in rye genomics in the last decades, rye still lagged behind most small-grain winter cereals in terms of genomics (Miedaner et al., 2019b). Few genomic analyses have been done to identify QTLs for agronomic and quality traits (Falke et al., 2009; Hackauf et al., 2017; Miedaner et al., 2012, 2018) as well as abiotic stress tolerance (Myşków et al., 2018) in rye. However, QTLs that regulate FHB resistance in rye, the only cross-pollinating winter small-grain cereal, were unknown until now. Therefore, as part of this thesis, a premier GWAS and genomic prediction was conducted to unravel the genomic mechanisms of FHB resistance in rye (Chapter 3).

The high total impact of the QTLs detected for FHB resistance (Chapter 3) can partly be attributed to the accumulation of FHB resistance alleles in the recurrent selection program where the material was derived from. According to Beavis (1998), ρ_G of detected QTLs can greatly be estimated upward when population size, $n \leq 100$ because most small effect QTLs are difficult to detect in small population sizes. Given that close to 500 lines were analyzed (Chapter 3), the proportion of genotypic variance explained by the detected QTLs might only be slightly overestimated (Beavis, 1998; Xu, 2003). The two SNPs, Contig1930 located on chromosome 1R and isotig09091 on chromosome 5R, which explained 33 % and 14 % of ρ_G, respectively, can be investigated further and used as candidates for genomic-assisted breeding against FHB in rye (Chapter 3). The outcome of this study shows that the genetic architecture of FHB resistance in rye is complex, controlled by several additive alleles. Similar genetic architecture was found for FHB resistance traits in other small-grain winter crops (Arruda et al., 2016b; Dhariwal et al., 2018; Wang et al., 2017) and Gibberella ear rot resistance in maize (Chapter 4; Chapter 5). No common QTL was found between FHB severity and HS, which was in consonance with the observed low phenotypic relationship between these traits (Chapter 3). However, one medium-effect FHB QTL (isotig15081, 3R) was in LD ($r^2 = 0.84$) with the

PHT QTL (isotig24773, 3R) and might represent a common QTL between the two traits, partially explaining the moderate phenotypic correlation found between the two traits (Chapter 3). None of the QTLs for PHT had a large effect, indicating the absence of major dwarfing genes in the material analyzed. Therefore, resistant lines can be selected without having large negative impacts on earliness and PHT in hybrid rye breeding programs (Chapter 3).

Because of the presence of several additive genes each with minor effects on the traits analyzed in rye, MAS (i.e., using marker effects of QTLs explaining >5 % of ρ_G) was compared to GS using unweighted and weighted GP approaches (Chapter 3). The two GP approaches outperformed MAS for all three traits, FHB severity, HS and PHT. For example, for FHB severity, the prediction accuracy, ρ (i.e., prediction ability divided by the square root of H^2) of MAS was 0.54 while that of both GP approaches was 0.96. This trend corroborates other reports in triticale (Galiano-Carneiro et al., 2019), bread wheat (Herter et al., 2019; Mirdita et al., 2015; Odilbekov et al., 2019; Rutkoski et al., 2012) and durum wheat (Miedaner et al., 2017). The high ρ can be due to the presence of increased resistance alleles for FHB resistance and the close relatedness of lines from the elite breeding germplasm. Weighted and unweighted GP yielded similar prediction abilities. Thus, inclusion of most important QTLs in the GP model did not result in further improvement of ρ, because the alleles associated with QTLs for FHB resistance were already high in the material analyzed GP (Chapter 3). In breeding materials where few QTLs with small to moderate cumulative effects are present, the inclusion of detected QTLs as fixed effects may result in higher prediction accuracy (Galiano-Carneiro et al., 2018; Odilbekov et al., 2019; Herter et al., 2019). In addition, the magnitude of the power of weighted GP over unweighted GP and MAS is dependent on the trait and the genetic material evaluated (Chapter 3 and 4). Galiano-Carneiro et al. (2018) detected six QTLs jointly explaining 56.64 % ρ_G for FHB resistance in triticale by GWAS. They used the four QTLs explaining >5 % ρ_G as weight in the GP model, which led to about 20 % increase in the ρ for

FHB resistance. Hence, breeding programs can target accumulation of QTLs in populations prior to GP for improved predictability. The findings in Chapter 3 imply that genomic-assisted recurrent selection scheme can catalyze improvement of rye genotypes against toxigenic Fusarium head infection. It should be noted that the prediction accuracies reported (Chapter 3) and other cross-validation studies (Galiano-Carneiro et al., 2018; Herter et al., 2019) might be over calculated since individuals were evaluated in the same trials and randomly sampled to constitute the training set, TS (80 %) and the validation set, VS (20 %). However, in real-world breeding programs, individuals of the TS and VS are evaluated in different trials or environments, and sometimes, disease symptoms are scored by different people. In this case, prediction accuracies may be lower than what has been reported in literature. Efforts should be made to optimize GS in applied breeding by constantly updating the training set.

Breeding for ear rot and mycotoxin resistances in maize (*Zea mays* L.)

In the past decades, conventional breeding techniques like backcrossing, single seed descent, recurrent and multistage or mass selection have been extensively used to breed maize against toxigenic ear rots (ERs) such as Gibberella ear rot (GER), Fusarium ear rot (FER) and Aspergillus ear rots (Mesterházy et al., 2012). However, these traditional methods are time-consuming and labor intensive. Meanwhile, there is the need to expedite breeding cycles to increase selection gain to produce safe and more food, to feed the ever-increasing human and animal populations. The advancement and availability of molecular markers, high-throughput sequencing technologies and internet-based “omics” data have led to the use of genomic tools such as linkage mapping, GWAS, gene expression analyses and genomic prediction in ER resistance breeding programs (Chapter 4). Chitinase gene 2 and geranyl geranyl transferase-like protein found to contribute to resistance to *Fusarium spp*. in maize have been cloned (Dowd et al., 2018a; Dowd et al., 2018b). However, there is slow progress in using the several QTLs and candidate genes (CGs) detected in real-world resistance breeding due to many

factors. Major constraints to the power and usefulness of genomic analyses are precision phenotyping and the highly polygenic nature of ER resistance and mycotoxin accumulation (Chapter 4; Cobb et al., 2013). Application of automated high-throughput phenotyping platforms such as 3D scanning to improve transferability and repeatability of assessing ER symptoms (Kuska & Mahlein, 2018; Mutka & Bart, 2015) might be a long-term goal. A possible solution to optimize results from genomic studies is to combine different analytical methods in order to overcome the inherent weaknesses of each individual method. In addition, the use of landraces to increase the genetic variation for ER and mycotoxin resistances was proposed (Chapter 4).

Genome-wide association studies and genomic prediction for harnessing GER resistance from two European maize landraces

Phenotypic and molecular analyses of Gibberella ear rot (GER) resistance in elite maize materials have been conducted (Han et al., 2016; Martin et al., 2012; Martin et al., 2011) but until now, the genetic diversity among landraces of European flint maize is not exploited for GER resistance breeding using integrated genomic tools. Therefore, 500 doubled-haploid (DH) lines originating from two flint maize landraces, “Kemater Landmais Gelb” (KE) from Austria and “Petkuser Ferdinand Rot” (PE) from Germany, were phenotyped and genotyped for *F. graminearum* ear rot resistance using silk channel inoculation method (Figure 2a, b).

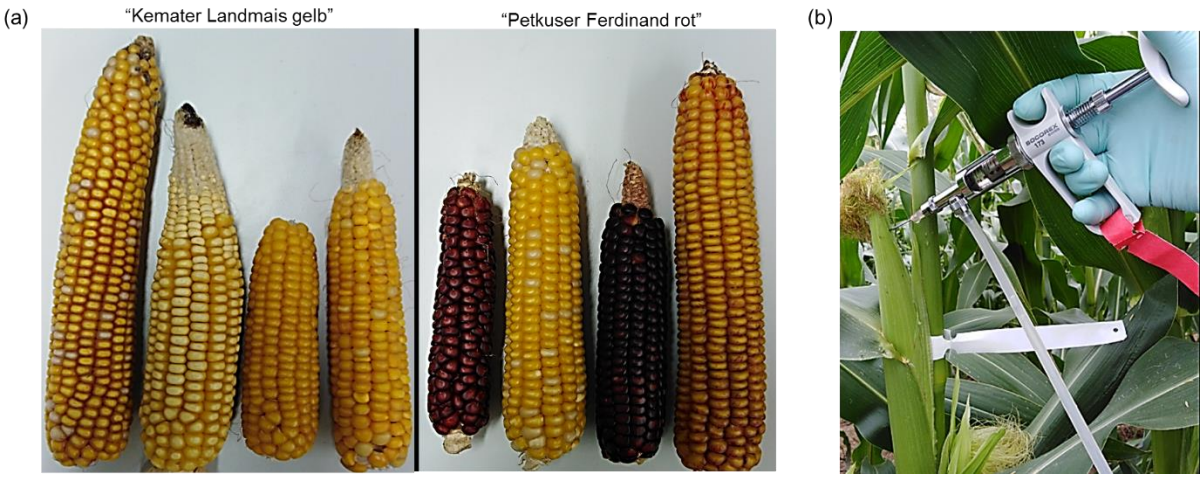


Figure 2 (a) The source landrace populations, "Kemater Landmais Gelb" and "Petkuser Ferdinand Rot", (b) Silk channel inoculation of maize ear, 4-6 days after silk emergence

Maximum phenotypic variation was found for GER severity in both combined DH libraries (COMB) and within the two landraces evaluated, ranging from approximately 1 % to 87 % in KE and 7 % to 97 % in PE (Figure 3). This shows that highly resistant and susceptible lines can be found in maize landraces (Chapter 5; Böhm et al., 2017). Similarly, previous studies reported high phenotypic and molecular variation for agronomic and quality traits among DH lines originating from other European flint maize landraces (Böhm et al., 2017; Mayer et al., 2017; Stringens et al., 2013). Böhm et al. (2017) evaluated 389 DH lines from six European flint landraces together with 53 elite flint lines and reported higher phenotypic variation and broad-sense heritability for landraces than the elite lines for *F. verticilliodes* ear rot (FER) severity. They also found improved resistance for FER within landraces than elite lines. The significance of G x E interaction and the influence of the environment on GER resistance has been reported several times in literature (Chapter 4 and Chapter 5; Martin et al., 2012; Han et al. 2018). The importance of G x E in GER resistance makes evaluation of lines in multi-environments highly necessary, to improve heritability values. Phenotypic and genotypic correlations between GER severity and the three agronomic traits were weak ($P \leq 0.01$). Similarly, previous research revealed negative but weak to moderate relationships between

GER severity and days to female flowering (Martin et al., 2011, Han et al., 2016, 2018). This phenomenon allows simultaneous selection for GER resistance, and the three agronomic traits, plant height, earliness, and seed-set (Chapter 5).

Because of the high level of genetic diversity found among the landraces (Chapter 5), novel alleles present can be harnessed to broaden the narrow genetic background of elite breeding materials. It is important to note that remnant genetic load among DH lines obtained from landraces can lead to undesirable agronomic traits like poor emergence rate, poor growth, lodging, low seed-set, and poor grain yield (Chapter 5; Böhm et al. 2017; Strigens et al. 2013). Besides, inbreeding depression among DH libraries may results in unwanted phenotypes like leaf chlorosis, tillering, extreme susceptibility to diseases such as maize ear rots, common smut (*Ustilago maydis*) and common rust (*Puccinia sorghi*) (Figure 4; Strigens et al. 2013). Therefore, introgression of resistance alleles from landraces such as KE and PE into elite materials may require further selection for superior agronomically adapted traits, to reduce the effect of detrimental alleles. In a previous study, about 70 % of DH lines derived from European flint landraces were recommended to be excluded from subsequent breeding program because of the impact of inbreeding depression (Böhm et al. 2017).

Figure 3 A sample of the most resistant line (from Kemater) and susceptible line (from Petkuser) found in our field trial in 2019

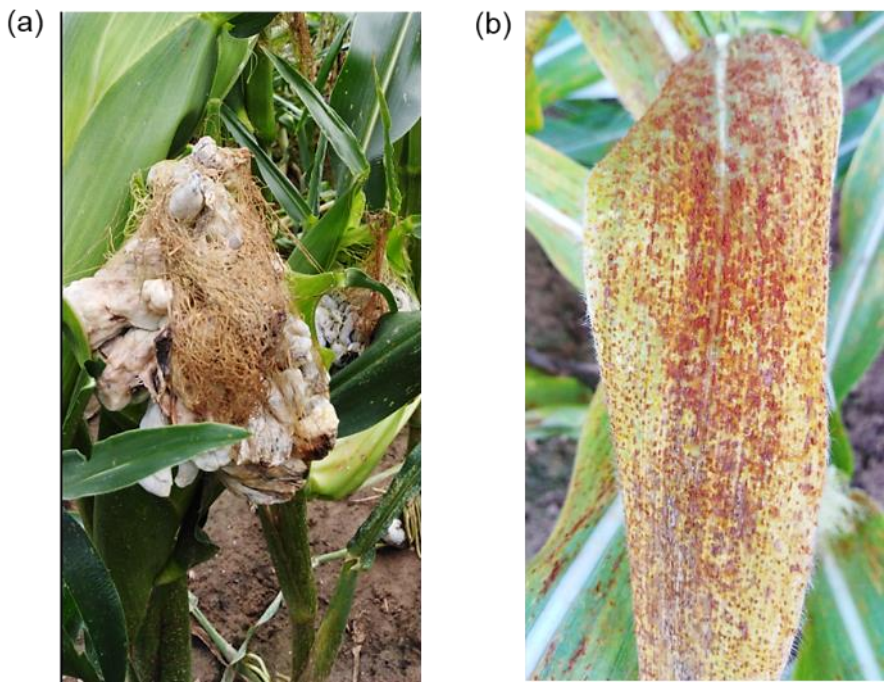


Figure 4 Maize plants showing symptoms of (a) common smut and (b) common rust, under natural infection on the field in 2019

The premier single-SNP based GWAS performed for *F. graminearum* ear rot resistance in elite European flint maize detected six QTLs for DON accumulation but none for GER severity (Han et al., 2018). However, the present GWAS conducted using a multi-locus method, fixed and random model circulating probability unification (FarmCPU, Liu et al., 2016) detected 14 QTLs for GER severity among DH lines from KE and PE (COMB), when first three principal components (PCs) were fitted as fixed effects in the model. These SNPs jointly explained about 52 % of ρ_G . Similar to the findings for rye (Chapter 3), though the cumulative effect of QTLs detected by FarmCPU was large, majority of the single QTLs (10 out of 14) had small effects for GER severity (i.e., contributed <5 % ρ_G). By classical QTL mapping approaches, other authors reported several minor-effect and a few medium- to major-effect QTLs for GER resistance, with cumulative QTL effects ranging from about 20 % to 60 % (Chapter 4). Generally, compared to most other crops, the linkage disequilibrium (LD) decay in maize is faster (Miao et al., 2019) which makes QTL detection for complex traits like GER resistance very difficult (Han et al., 2018). Studies showed that LD decreased even faster in landraces

than elite lines because of long historical recombination events (Strigens et al., 2013). Among the landraces analyzed in the present study, LD decreases more rapidly within PE than KE and the decay pattern is less rapid among lines from COMB (Chapter 5; Mayer et al., 2017). The findings in Chapter 5 illustrate that large population sizes and increased number of alleles associated with GER severity at a particular genomic region largely influence the power to detect QTLs at stringent significant threshold such as the Bonferroni corrected threshold at *P* = 0.05. GWAS allows for QTL detection at high resolution but population structure in association-mapping panels can lead to spurious marker-trait associations (Yu et al. 2006). However, over the years, GWAS methods such as compressed mixed linear model, CMLM (Zhang et al., 2010) and FarmCPU (Liu et al., 2016), have been developed to control false positives and false negatives by the inclusion of kinship matrix and principal components in the model as covariates. Large population sizes and appropriate GWAS methods help to overcome "the Beavis effect" (Beavis, 1998). Therefore, since the number of DH lines analyzed across landraces (n=462, Chapter 5) was similar to the number analyzed in rye (Chapter 3), the proportion of genotypic variance explained by the QTLs might be close to the expected value (King and Long, 2017). The remaining unexplained genotypic variance in the GWAS can partly be attributed to the presence of QTLs having non-additive effects, and QTLs with rare alleles that could not be detected at the significant threshold applied. In our study, none of the QTLs detected for DS, PHT and SS colocalized with the QTLs for GER severity, confirming the low correlations found between these traits in both COMB and within populations (Chapter 5). Similar trend was found between FHB severity and earliness in rye (Chapter 3). This outcome implies that there is no strong genetic linkage between the resistance alleles and the alleles for earliness, plant height and poor seed-set, making the introgression of GER QTLs into commercial flint germplasms less cumbersome.

Several candidate genes (CGs) have been reported for ear rots resistance in past studies (Chapter 4). In this thesis, 25 CGs were associated with two most important SNPs for GER severity. These CGs encoded for proteins which fall into functionary categories such as response to stress, molecule binding activities, molecule modification, kinase activity, catalytic activity, signal transduction, oxidation-reduction process, cellular process , etc., similar to earlier reports for Fusarium resistance in maize (Han et al., 2018; Lanubile et al., 2017; Yao et al., 2020; Yuan et al., 2020). The current study (Chapter 5) confirms that GER resistance is governed by multiple loci containing several genes. For both COMB library and within DH libraries, multi-SNP GWAS algorithm (FarmCPU) was more powerful than single-SNP based GWAS (CMLM) for GER QTL detection at stringent significance thresholds (P = 0.0001 and Bonferroni corrected threshold at P = 0.05, e.g. Table 1, Figure 5). Within KE, FarmCPU detected eight significant SNPs jointly explaining 34% of genotypic variance for GER severity wihile CMLM detected only two SNPs on chromosome 2, which jointly explained 15% genotypic variance for GER severity. Both models failed to detect significant SNPs in PE for GER severity: However, at less stringent significance thresholds (-log (0.001) = 3), CMLM detected SNP-trait associations at genomic regions similar to FarmCPU (Figure 5). This corroborates previous reports illustrating the power of FarmCPU over conventional MLM for other complex traits (Kaler et al., 2020; Malik et al., 2019; Miao et al., 2019; Wen et al., 2018; Zhang et al., 2019a). The advantage of detecting more MTAs and CGs become even higher when both single- and multi-locus GWAS methods are used for the same data set because the inherent weaknesses of each method is overcome (Abed and Bezile, 2019; Li et al., 2018; Wei et al., 2017; Zhang et al., 2019a). Single-locus based GWAS methods such as CMLM have lower power of quantitative trait nucleotides (QTNs) detection for complex traits and requires correction for multiple testing to control false positives (Zhang et al., 2018; Zhang et al. 2019a). When the number of SNPs is large, some important QTL may not be detected under the

stringent screening criterion such as the Bonferroni correction for multiple testing for significance, $P = 0.05$/number of markers (Zhang et al., 2018, Figure 5). This can results in many false negatives (Liu et al., 2016; Miao et al., 2019). An individual SNP may not be able to capture existing allelic diversity for complex traits in a given population (Abed and Bezile, 2019). An advantage of single-locus GWAS is that peaks can be localized precisely because significant markers in LD are not removed (Figure 5). This makes it more beneficial for CG identification and comparison of QTLs between populations. However, according to Kaler et al. (2020), single-locus GWAS models can fail to identify other important loci that may have slightly lower *P*-value than SNPs in the peaks that are in strong LD with the most significant SNP. Multi-locus GWAS methods have higher QTL detection power and accuracy than single-locus GWAS methods (Abed and Bezile, 2019; Kaler et al., 2020; Miao et al., 2019; Malik et al., 2019). This is because associated markers are fitted as covariates and multiple markers are tested simultaneously, which reduces the background noise by other loci that may be associated to the trait (Segura et al., 2012; Liu et al., 2016). Treating SNP effect as random in the multi-locus GWAS model results in shrinkage estimate of QTL effects which is more stable than the least square estimate (Wang et al. 2016; Liu et al., 2016). Multi-locus GWAS does not require correction for multiple testing (Zhang et al., 2019a). On the other hand, multi-SNP GWAS such as FarmCPU removes significant SNPs that are in LD with the SNPs at detected peaks (Figure 5), but these SNPs in LD at the peaks might provide additional information for MTA validation purposes and CG identifications. Hence, some information may be reduced in FarmCPU GWAS compared to CMLM at detected peaks (Wei et al., 2017). The comparison of available GWAS methods for QTL identification have been reviewed recently (Zhang et al., 2019a).

Table 1 Number of QTLs detected by CMLM and FarmCPU and the total proportion of explained genotypic variance (ρ_G) for Gibberella ear rot (GER) severity, days to silking (DS), plant height (PHT) and seed-set (SS) in combined DH libraries, across four environments

Trait	GWAS method	Number of QTLs	ρ_G (%)
GER severity	CMLM	5	28.30
	FarmCPU	14	52.20
DS	CMLM	16	26.45
	FarmCPU	23	56.37
PHT	CMLM	13	57.78
	FarmCPU	17	53.21
SS	CMLM	13	31.12
	FarmCPU	13	43.90

GWAS, genome-wide association studies; CMLM, compressed mixed linear model; FarmCPU, fixed and random model circulating probability unification

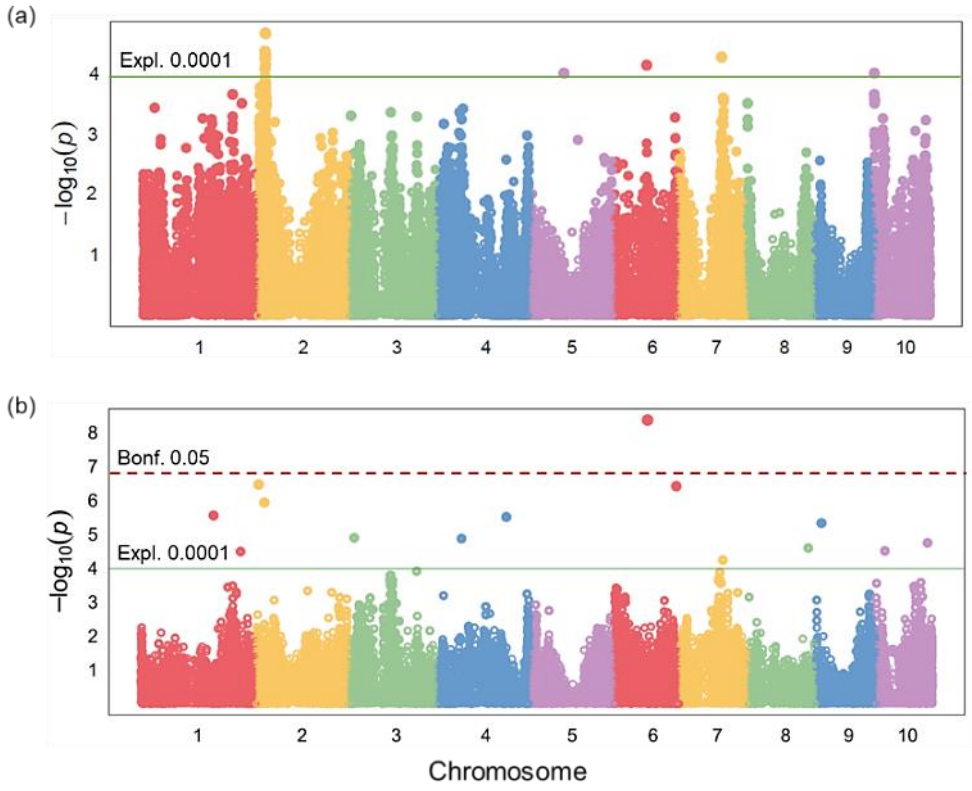


Figure 5 Manhattan plots of (a) compressed mixed linear model (CMLM), and (b) fixed and random model circulating probability unification (FarmCPU) GWAS methods for GER severity among 462 DH lines (combined library). ***Expl***. Exploratory threshold; ***Bonf***. Bonferroni-corrected threshold

In marker-assisted selection (MAS), marker effects of detected QTLs are estimated and used to predict breeding values of lines in prediction models such as the best linear unbiased prediction, BLUP (Zhang et al., 2005). However, GWAS may not be able to fully capture several rare additive alleles that control quantitative traits such as FHB and ER resistances and agronomic traits (Miedaner and Korzun, 2019; Chapter 3, 4). To account for the effects of undetected QTL alleles in genetic mapping, estimated genome-wide marker effects can be used to predict superior lines for selection in GP models, such as ridge regression-BLUP (RR-BLUP) (Chapter 3; Endelman, 2011; Endelman & Jannink, 2012). To further improve prediction accuracies, significant SNPs (QTLs) having intermediate to large effects can be fixed in the RR-BLUP model as fixed effect in a GP approach termed weighted RR-BLUP, wRR-BLUP (Chapter 3; Bernado, 2014; Spindel et al., 2016; Zhao et al., 2014). MAS based on medium effect GER QTLs detected from the multi-locus GWAS algorithm (i.e., FarmCPU) performed similar to the unweighted GP approach (RR-BLUP). However, the weighted GP approach (i.e., wRR-BLUP) outperformed MAS and RR-BLUP both in the combined DH libraries and within landraces (Chapter 5). The higher performance of wRR-BLUP over RR-BLUP and MAS (Chapter 5) is in consonance with what has been reported earlier for Fusarium resistance in small-grain cereals (Galiano-Carneiro et al., 2019; Herter et al., 2019; Odilbekov et al., 2019). Using the medium- to major-effect QTLs (i.e., QTLs explaining >5 % ρ_G) as a weighting factor in the GP model might have reduced the background noise, which improved the predictability for GER resistance further. Unweighted GP approach seems promising for populations (e.g., PE) where it is difficult to detect QTLs for MAS. When members of a training set are unrelated to members of the validation set, ρ may be very low, even negative in some materials (Chapter 5; Brauner et al., 2018; Han et al. 2018). Using only DH lines from one landrace population to predict GER resistance in another population was not promising at all (Chapter 5). However, fitting the two significant SNPs explaining >5 % ρ_G in KE as fixed

effect in the GP model where KE library was used exclusively as TS and PE as the VS increased the ρ from 3 % to 22 % (Chapter 5). In another study involving six European maize landraces, GP between pairs of DH libraries resulted in approximately zero ρ for all landraces and six agronomic traits analyzed. However ρ improved when the TS and VS contained lines from both landraces (Chapter 5; Brauner et al., 2018). Additionally, an increase in the size of TS resulted in improved ρ (Chapter 5, Brauner et al., 2018; Schopp et al., 2017). The pattern of QTL detection and ρ in GWAS and GP, respectively, in both COMB and within landraces as well as the GP between landraces (Chapter 5) showed that the population having no or only few QTLs underlying GER resistance cannot serve as a good TS in GS. Hence, accumulation of resistance QTLs in a breeding material prior to GP might improve the ρ for Fusarium resistance traits considerably (Chapter 3; Galiano-Carneiro et al., 2018). Composition of the genetic materials, differences in allele frequencies of important QTLs underlying a particular trait in the TS and VS, population size, GP approach used, etc., largely affect the predictive ability of GP and have been well document elsewhere (Lozada & Carter, 2019; Robertsen et al., 2019; Schopp et al. 2017; Zhang et al., 2019b). These factors must be critically considered and addressed before using results of GP for practical breeding against GER.

This part of the thesis demonstrates that there is maximum genetic variation in KE and PE landraces and can be exploited using combined genome-based analyses. The QTLs with medium impacts can be employed in marker-assisted backcrossing (MABC) after validation. To reduce or eliminate the effect of many negative loci segregating in these landraces (Chapter 5), implementation of GS can be more beneficial after the introgression of best QTLs into elite materials and selection for agronomically adapted traits. Both phenotypic and molecular analyses showed that selection gains may be higher when using lines from KE than PE landrace (Chapter 5).

Implications for practical breeding against Fusarium ear infections in small-grain cereals and maize

In small-grain cereals, breeding against mycotoxigenic fungi in durum wheat and bread wheat should be prioritized. It can be facilitated by the introduction of resistance alleles from rye or triticale, which will however require several cycles of backcrossing to remove unfavorable alleles that may be linked to poor flour quality and yield (Chapter 2). Furthermore, genomics-assisted recurrent selection strategy can be adopted in breeding programs aiming at reducing FHB and mycotoxins in small-grain cereals.

The wide genetic variation for GER resistance both across and within KE and PE can be harnessed to improve elite European flint maize against toxigenic Fusarium ear infection. The DH lines from the landraces (Chapter 5) can be crossed with susceptible elite lines and their off-springs (F_1) backcrossed to the elite parents (F_1BC) for better agronomic adaptation, i.e., to reduce or eliminate the impact of deleterious alleles associated with traits like early development, lodging, shortness, poor fertility and yield. Application of GS to select resistant lines from the backcross population is expected to produce higher returns (Hölker et al., 2019) because close genetic relationship between the training set and validation set can improve the prediction accuracy (Brauner et al., 2018, 2020; Herter et al., 2019; Kadam et al., 2016; Riedelsheimer et al., 2013). Brauner et al. (2018) found higher prediction accuracy for six agronomic traits in elite flint lines than within six landraces. GP exploits LD between markers and the QTLs underlying a trait to predict the genomic estimated breeding values of individuals, but LD decreases more rapidly among landraces than elite populations (Strigen et al., 2013). To reduce the problem of unrelatedness of individuals of the TS and the VS in GS, the TS must be periodically updated by phenotyping about 10 % to 20 % of the population, which represent parents of used crosses, in subsequent cycles (Chapter 4). Besides, when the medium- to major-effect GER QTLs are successfully validated and molecular markers such as

Kompetitive allele specific PCR markers (KASPs) developed, MABC can be employed to assist in the transfer of resistance alleles from the landraces into susceptible elite materials.

7 Summary

The world's human and livestock population is increasing and there is the need to increase quality food production to achieve the global sustainable development goal 3, zero hunger by year 2030 (United Nations, 2015). However, biotic stresses such as Fusarium ear infections pose serious threat to cereal crop production. Breeding for host plant resistance against toxigenic *Fusarium* spp. is a sustainable way to produce more and safer cereal crops such as maize and small-grain winter cereals. Many efforts have been made to improve maize and small-grain cereals for ear rot (ER) and Fusarium head blight (FHB) resistances, using conventional and genomic techniques.

Among small-grain cereals, rye had the shortest maturity period followed by the descendant, hexaploid triticale while both wheat species had the longest maturity period. In addition, rye and triticale were more robust to Fusarium infection and deoxynivalenol accumulation, making them safer grain sources for human and animal consumption. However, a few resistant cultivars have been produced by prolonged conventional breeding efforts in durum wheat and bread wheat. High genetic variation was present within each crop species and can be exploited for resistance breeding. In this thesis, the genetic architecture of FHB resistance in rye was investigated for the first time, by means of genome-wide association study (GWAS) and genomic prediction (GP). GWAS detected 15 QTLs for *Fusarium culmorum* head blight severity, of which two had major effects. Both weighted and unweighted GP approaches yielded higher prediction abilities than marker-assisted selection (MAS) for FHB severity, heading stage and plant height. Genomics-assisted breeding can shorten the duration of breeding rye for FHB resistance.

In the past decade, genetic mapping and omics were used to identify a multitude of QTLs and candidate genes for ear rot resistances and mycotoxin accumulation in maize. The polygenic nature of resistance traits, high genotype x environment interaction, and large-scale

phenotyping remain major bottlenecks to increasing genetic gains for ear rots resistance in maize. Phenotypic and molecular analyses of DH lines originating from two European flint landraces ("Kemater Landmais Gelb", KE, and "Petkuser Ferdinand Rot", PE) revealed high variation for Gibberella ear rot (GER) severity and three agronomic traits *viz*. number of days to female flowering, plant height and proportion of kernels per cob. By employing multi-SNP GWAS method, we found four medium-effect QTLs and many small-effect (10) QTLs for GER severity in combined DH libraries (when PCs used as fixed effects), none co-localized with the QTLs detected for the three agronomic traits analyzed. However, one major QTL was detected within KE DH library for GER severity. Two prioritized SNPs detected for GER resistance were associated with 25 protein-coding genes placed in various functional categories, which further enhances scientific knowledge on the molecular mechanisms of GER resistance. Genome-based approaches seems promising for tapping GER resistance alleles from European maize landraces for applied breeding. After several cycles of backcrossing and sufficient selection for agronomic adaptation traits, the resistant lines identified in this thesis can be incorporated into existing maize breeding programs to improve immunity against *F. graminearum* ear infection. Breeding progress can be faster using KE landrace than PE.

A successful validation of QTLs identified in this thesis can pave way for MAS in rye and marker-assisted backcrossing in maize. Effective implementation of genomic selection requires proper design of the training and validation sets, which should include part of the current breeding population.

8 Zusammenfassung

Um das Ziel 3 für nachhaltige Entwicklung, das Ende des Hungers bis 2030 (United Nations, 2015) zu erreichen, muss durch den Anstieg der Weltbevölkerung die Nahrungsmittelproduktion deutlich erhöht werden. Gleichzeitig aber bedrohen Pflanzenkrankheiten wie Fusariosen die Getreideproduktion. Die Züchtung von Sorten mit Resistenzen gegen die (für Mensch und Tier) giftigen Pilze der Gattung *Fusarium* ist ein nachhaltiger Weg, um größere Mengen und weniger toxin-belastetes Getreide zu produzieren. Viele Versuche wurden unternommen, um die Resistenz gegen Kolbenfäule in Mais und gegen Ährenfusariosen (Fusarium head blight, FHB) in kleinkörnigem Getreide mit konventionellen und genomischen Züchtungsmethoden zu verbessern.

In unseren Untersuchungen waren Roggen und Triticale am widerstandsfähigsten gegen Fusarium-Infektionen und hatten die geringste Deoxynivalenol-Kontamination, was sie zu weniger toxischen Nahrungs- und Futtermitteln macht. Aber auch für Hart- und Weichweizen gibt es durch langjährige konventionelle Züchtung einzelne resistente Sorten. Eine hohe genetische Variation konnte bei allen Getreidearten beobachtet werden und kann damit für zukünftige Resistenzzüchtung verwendet werden. In dieser Arbeit wurde zum ersten Mal mit Hilfe einer genomweiten Assoziationsstudie (genome-wide association study, GWAS) und genomischer Vorhersage (genomic prediction, GP) die genetische Architektur der *Fusarium*-Resistenz in Roggen untersucht. GWAS konnten 15 Loci (quantitative trait loci, QTL) für die Resistenz gegen *Fusarium culmorum* gefunden werden, zwei davon mit Haupt-Effekten (major effects). Sowohl die gewichtete als auch die ungewichtete genomische Vorhersage erzielten für Fusariumbefall, Ährenschieben und Wuchshöhe höhere Genauigkeiten als die markergestützte Selektion (marker-assisted selection, MAS). Genomische Daten können damit die Züchtung von *Fusarium*-resistentem Roggen beschleunigen.

In den letzten zehn Jahren wurden genetische Kartierungen und Omics verwendet, um eine Vielzahl von QTLs und Kandidatengenen für Kolbenfäule-Resistenzen und Mykotoxin-Akkumulation in Mais zu identifizieren. Die komplexe Vererbung der Resistenzen, die hohen Genotyp x Umwelt-Wechselwirkungen und der Bedarf großer Versuche zur Phänotypisierung den genetischen Zuchtfortschritt für die Resistenz gegen Kolbenfäule bei Mais. Die phänotypische und genotypische Analyse von doppelt-haploiden Maislinien, die aus zwei europäischen Flint-Landrassen ("Kemater Landmais Gelb", KE, and "Petkuser Ferdinand Rot", PE) erstellt wurden, zeigte eine hohe genetische Variation für Kolbenfäule (Giberella ear rot, GER) und die drei weiteren agronomischen Merkmale Tage bis zur weiblichen Blüte, Wuchshöhe und Kornansatz. Durch Verwendung einer GWAS-Methode, die mehrere Markerloci gleichzeitig berücksichtigt (multi-SNP), konnten vier QTL mit mittleren Effekten und 10 QTL mit kleinen Effekten für die GER-Befallsstärke in kombinierten DH-Bibliotheken gefunden werden; keine davon war co-lokalisiert mit QTL für die drei analysierten agronomischen Merkmale. Innerhalb der KE DH-Bibliothek wurde jedoch ein Haupt-QTL für die GER-Befallsstärke festgestellt. Zwei ausgewählte SNP-Marker für die GER-Befallstärke waren mit 25 proteincodierenden Sequenzen assoziiert, die unterschiedlichen Funktionen zugeordnet werden konnten und damit das Wissen über die molekularen Mechanismen zur GER-Resistenz erweiterten. Eine genom-basierte Züchtungsmethode erscheint vielversprechend, um die GER-Resistenz in europäischen Mais-Landrassen für die angewandte Züchtung zu erschließen. Nach mehreren Zyklen von Rückkreuzung und Selektion auf agronomische Merkmale, können die resistenten Linien in einem bestehenden Mais-Zuchtprogramm verwendet werden, um die Resistenz gegen Kolbenfusariosen zu erhöhen. Der Zuchtfortschritt dürfte bei Verwendung der Landrasse KE höher sein als bei PE.

Eine erfolgreiche Validierung der QTL, die in dieser Arbeit gefunden wurden, kann den Weg für eine markergestützte Selektion bei Roggen und Mais zur Erhöhung der Fusarium-Resistenz

ebnen. Die effiziente Anwendung genomischer Selektionsmethoden bedarf der laufenden Erstellung von aktuellen Trainings- und Validierungssets, die jeweils einen Teil der aktuellen Zuchtpopulationen umfassen sollten.

9 General references

Abed, A., & Belzile, F. (2019). Comparing single-SNP, multi-SNP, and haplotype-based approaches in association studies for major traits in barley. Plant Genome, 12(3):190036. https://doi.org/10.3835/plantgenome2019.05.0036

Ammar, K., Mergoum, M., & Rajaram, S. (2004). The history and evolution of triticale. In M. Mergoum & H. Gómez-Macpherson (Eds.), Triticale improvement and production (pp. 1–9). Plant production and protection paper 179. Food and Agriculture Organization of the United Nations.

Anderson, N. R., Romero Luna, M. P., Ravellette, J. D., & Wise, K. A. (2017). Impact of foliar fungicides on Gibberella ear rot and deoxynivalenol levels in Indiana corn. Plant Health Progress, 18(3), 186–191. https://doi.org/10.1094/PHP-01-17-0010-RS

Arruda, M. P., Lipka, A. E., Brown, P. J., Krill, A. M., Thurber, C., Brown-Guedira, G., Dong, Y., Foresman, B. J., & Kolb, F. L. (2016). Comparing genomic selection and marker-assisted selection for Fusarium head blight resistance in wheat (*Triticum aestivum* L.). Molecular Breeding, 36(7), 1–11. https://doi.org/10.1007/s11032-016-0508-5

Arruda, M. P., Brown, P., Brown-Guedira, G., Krill, A. M., Thurber, C., Merrill, K. R., Foresman, B. J., & Kolb, F. L. (2016a). Genome-wide association mapping of Fusarium head blight resistance in wheat using genotyping-by-sequencing. The Plant Genome, 9(1), 1-14. https://doi.org/10.3835/plantgenome2015.04.0028

Arseniuk, E., Foremska, E., Góral, T., & Chełkowski, J. (1999). Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye. Journal of Phytopathology, 147(10), 577–590. https://doi.org/10.1046/j.1439-0434.1999.00433.x

Ayalew, H., Kumssa, T.T., Butler, T.J., & Ma, X-F. (2018). Triticale improvement for forage and cover crop uses in the Southern Great Plains of the United States. Frontiers in Plant Science, 9:1130. https://doi.org/10.3389/fpls.2018.01130

Baenziger, P.S. (2016). Wheat breeding and genetics. Reference Module in Food Science, Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.03001-8

Bai, G., Su, Z., & Cai, J. (2018). Wheat resistance to Fusarium head blight. Canadian Journal of Plant Pathology, 40(3), 1–11. https://doi.org/10.1080/07060661.2018.1476411

Bartoš, J., Paux, E., Kofler, R., Havránková, M., Kopecký, D., Suchánková, P., Šafář, J., Šimková, H., Town, C. D., Lelley, T., Feuillet, C., & Doležel, J. (2008). A first survey of the rye (*Secale cereale*) genome composition through BAC end sequencing of the short arm of chromosome 1R. BMC Plant Biology, 8, 1–12. https://doi.org/10.1186/1471-2229-8-95

Becher, R., Miedaner, T., & Wirsel, S. (2013). Biology, diversity and management of FHB-causing *Fusarium* species in small-grain cereals. In F. Kempken (Ed.), Agricultural Applications (2nd ed., pp. 199–241). Springer-Verlag. https://doi.org/10.1007/978-3-642-36821-9_8

Bernardo, R. (2014). Genomewide selection when major genes are known. Crop Science, 54:68–75

Böhm, J., Schipprack, W., Utz, H. F., & Melchinger, A. E. (2017). Tapping the genetic diversity of landraces in allogamous crops with doubled haploid lines: a case study from European flint maize. Theoretical and Applied Genetics, 130(5), 861–873. https://doi.org/10.1007/s00122-017-2856-x

Bolduan, C., Miedaner, T., Schipprack, W., Dhillon, B., & Melchinger, A. (2009). Genetic variation for resistance to ear rots and mycotoxins contamination in early European maize inbred lines. Crop Science, 49, 2019–2028. https://doi.org/10.2135/cropsci2008.12.0701

Boyacioglu, H. (2017, October 23). Global durum wheat use trending upward. https://www.world-grain.com/articles/8777-global-durum-wheat-use-trending-upward

Brauner, P.C., Müller, D., Molenaar, W. S., & Melchinger, A. E. (2020). Genomic prediction with multiple biparental families. Theoretical and Applied Genetics, 133(1), 133–147. https://doi.org/10.1007/s00122-019-03445-7

Brauner, P.C., Müller, D., Schopp, P., Böhm, J., Bauer, E., Schön, C. C., & Melchinger, A. E. (2018). Genomic prediction within and among doubled-haploid libraries from maize landraces. Genetics, 210(4), 1185–1196. https://doi.org/10.1534/genetics.118.301286

Brauner, P.C., Melchinger, A. E., Schrag, T. A., Utz, H. F., Schipprack, W., Kessel, B., Ouzunova, M., & Miedaner, T. (2017). Low validation rate of quantitative trait loci for Gibberella ear rot resistance in European maize. Theoretical and Applied Genetics, 130(1), 175–186. https://doi.org/10.1007/s00122-016-2802-3

Buerstmayr, H., Steiner, B., Lemmens, M., & Rucjenbauer, P. (2000). Heritability and Trait Associations. Crop Science, 40, 662–664.

Buerstmayr, M., & Buerstmayr, H. (2015). Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo × Arina. Theoretical and Applied Genetics, 128(8), 1519–1530. https://doi.org/10.1007/s00122-015-2527-8

Buerstmayr, M., Steiner, B., & Buerstmayr, H. (2019). Breeding for Fusarium head blight resistance in wheat—Progress and challenges. Plant Breeding, 139 (3), 429-454. https://doi.org/10.1111/pbr.12797

Butrón, A., Reid, L. M., Santiago, R., Cao, A., & Malvar, R. A. (2015). Inheritance of maize resistance to Gibberella and Fusarium ear rots and kernel contamination with deoxynivalenol and fumonisins. Plant Pathology, 64(5), 1053-1060. https://doi.org/10.1111/ppa.12351

Castiblanco, V., Castillo, H. E., & Miedaner, T. (2020). Be flexible and adapt easily—The great role of plasticity relative to genetic variation for aggressiveness of *Fusarium culmorum* isolates. Journal of Phytopathology, 168(3), 162–174. https://doi.org/10.1111/jph.12878

Chaudhary, H. K., Kaila, V., & Rather, S. A. (2014). Maize. In A. Pratap & J. Kumar (Eds.), Alien Gene Transfer in Crop Plants: Achievemnets and Impacts, 2, pp. 27–51. Springer New York. https://doi.org/10.1007/978-1-4614-9572-7

Chungu, C., Mather, D., Reid, L., & Hamilton, R. (1996). Inheritance of kernel resistance to *Fusarium graminearum* in maize. Journal of Heredity, 87(5), 382–385. https://doi.org/10.1093/oxfordjournals.jhered.a023019

Cobb, J. N., DeClerck, G., Greenberg, A., Clark, R., & McCouch, S. (2013). Next-generation phenotyping: Requirements and strategies for enhancing our understanding of

genotype-phenotype relationships and its relevance to crop improvement. Theoretical and Applied Genetics, 126(4), 867–887. https://doi.org/10.1007/s00122-013-2066-0

Crespo-Herrera, L. A., Garkava-Gustavsson, L., & Åhman, I. (2017). A systematic review of rye (*Secale cereale* L.) as a source of resistance to pathogens and pests in wheat (*Triticum aestivum* L.). Hereditas, 154(1), 14. https://doi.org/10.1186/s41065-017-0033-5

Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., & Hohn, T. M. (1996). Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. In Molecular Plant-Microbe Interactions (Vol. 9, Issue 9, pp. 775–781). https://doi.org/10.1094/MPMI-9-0775

Dhariwal, R., Fedak, G., Dion, Y., Pozniak, C., Laroche, A., Eudes, F., & Randhawa, H. S. (2018). High density single nucleotide polymorphism (SNP) mapping and quantitative trait loci (QTL) analysis in a biparental spring triticale population localized major and minor effect Fusarium head blight resistance and associated traits QTL. Genes, 9(1), 1–26. https://doi.org/10.3390/genes9010019

Díez, C. M., Gaut, B. S., Meca, E., Scheinvar, E., Montes-Hernandez, S., Eguiarte, L. E., & Tenaillon, M. I. (2013). Genome size variation in wild and cultivated maize along altitudinal gradients. New Phytologist, 199(1), 264–276. https://doi.org/10.1111/nph.12247

dos Santos, J. P. R., Pires, L. P. M., de Castro Vasconcellos, R. C., Pereira, G. S., Von Pinho, R. G., & Balestre, M. (2016). Genomic selection to resistance to *Stenocarpella maydis* in maize lines using DArTseq markers. BMC Genetics, 17(1), 1–10. https://doi.org/10.1186/s12863-016-0392-3

Dowd, P. F., Naumann, T. A., Price, N. P. J., & Johnson, E. T. (2018). Identification of a maize (Zea mays) chitinase allele sequence suitable for a role in ear rot fungal resistance. Agri Gene, 7(March 2018), 15–22. https://doi.org/10.1016/j.aggene.2017.10.001

Dowd, P. F., Zilkowski, B. W., Johnson, E. T., Berhow, M. A., & Muturi, E. J. (2018). Transgenic expression of a maize geranyl geranyl transferase gene sequence in maize callus increases resistance to ear rot pathogens. Agri Gene, 7(March 2018), 52–58. https://doi.org/10.1016/j.aggene.2018.01.001

Edwards, S. M. K., Buntjer, J. B., Jackson, R., Bentley, A. R., Lage, J., Byrne, E., Burt, C., Jack, P., Berry, S., Flatman, E., Poupard, B., Smith, S., Hayes, C., Gaynor, R. C., Gorjanc, G., Howell, P., Ober, E., Mackay, I. J., & Hickey, J. M. (2019). The effects of training population design on genomic prediction accuracy in wheat. Theoretical and Applied Genetics, 0123456789. https://doi.org/10.1007/s00122-019-03327-y

Endelman, J. B. (2011). Ridge Regression and Other Kernels for Genomic Selection with R Package rrBLUP. The Plant Genome Journal, 4(3), 250. https://doi.org/10.3835/plantgenome2011.08.0024

Endelman, J. B., & Jannink, J.L. (2012). Shrinkage Estimation of the Realized Relationship Matrix. G3: Genes,Genomes,Genetics, 2(11), 1405–1413. https://doi.org/10.1534/g3.112.004259

Fakhfakh, M. M., Yahyaoui, A., Rezgui, S., Elias, E. M., & Daaloul, A. (2011). Inheritances of Fusarium head blight resistance in a cross involving local and exotic durum wheat cultivars. Crop Science, 51(6), 2517–2524. https://doi.org/10.2135/cropsci2011.03.0140

Falke, K. C., Wilde, P., Wortmann, H., Geiger, H. H., & Miedaner, T. (2009). Identification of genomic regions carrying QTL for agronomic and quality traits in rye (*Secale cereale*) introgression libraries. Plant Breeding, 128(6), 615–623. https://doi.org/10.1111/j.1439-0523.2009.01644.x

FAO. (2020). FAOSTAT database. http://www.fao.org/faostat/en/#data/QC%0A

FAO. (2003). Worldwide Regulations for mycotoxins in food and feed 2003. In Food and Nutrition Paper 81 (pp. 1–165). FAO. https://www.fooddiagnostics.dk/seekings/uploads/Worldwide_mycotoxin_regulations_in_food_and_feed_2003.pdf

Federeal Ministry of Food and Agriculture. (2019). Understanding farming, facts and figures about German farming. 1–36. https://www.bmel.de/SharedDocs/Downloads/EN/Publications/UnderstandingFarming.html

Fingstag, M. D., Casa, R. T., Bogo, A., Kuhnem, P., Sangoi, L., Valente, J. B., Bevilaqua, D., Fiorentin, O. A., & Gonçalves, M. J. (2019). Fungicide performance on *Fusarium*

meridionale control, grain yield and grain damage in maize. Summa Phytopathologica, 45(3), 265–271. https://doi.org/10.1590/0100-5405/193255

Foroud, N. A., Baines, D., Gagkaeva, T. Y., Thakor, N., Badea, A., Steiner, B., Bürstmayr, M., & Bürstmayr, H. (2019). Trichothecenes in cereal grains - An update. Toxins, 11(11). https://doi.org/10.3390/toxins11110634

Galiano-Carneiro, A. L., Boeven, P. H. G., Maurer, H. P., Würschum, T., & Miedaner, T. (2019). Genome-wide association study for an efficient selection of Fusarium head blight resistance in winter triticale. Euphytica, 215(4), 1-12. https://doi.org/10.1007/s10681-018-2327-8

Gauthier, L., Bonnin-Verdal, M. N., Marchegay, G., Pinson-Gadais, L., Ducos, C., Richard-Forget, F., & Atanasova-Penichon, V. (2016). Fungal biotransformation of chlorogenic and caffeic acids by *Fusarium graminearum*: New insights in the contribution of phenolic acids to resistance to deoxynivalenol accumulation in cereals. International Journal of Food Microbiology, 221(856), 61–68. https://doi.org/10.1016/j.ijfoodmicro.2016.01.005

Goddard, M. E., & Hayes, B. J. (2007). Genomic selection. Journal of Animal Breeding and Genetics , 124(6), 323–330. https://doi.org/10.1111/j.1439-0388.2007.00702.x

Gowda, M., Das, B., Makumbi, D., Babu, R., Semagn, K., Mahuku, G., Olsen, M. S., Bright, J. M., Beyene, Y., & Prasanna, B. M. (2015). Genome-wide association and genomic prediction of resistance to maize lethal necrosis disease in tropical maize germplasm. Theoretical and Applied Genetics, 128(10), 1957–1968. https://doi.org/10.1007/s00122-015-2559-0

Gunupuru, L. R., Perochon, A., & Doohan, F. M. (2017). Deoxynivalenol resistance as a component of FHB resistance. Tropical Plant Pathology, 42(3), 175–183. https://doi.org/10.1007/s40858-017-0147-3

Hackauf, B., Haffke, S., Joachim, F., Steffen, F., Kusterer, B., Musmann, D., Kilian, A., & Miedaner, T. (2017). QTL mapping and comparative genome analysis of agronomic traits including grain yield in winter rye. Theoretical and Applied Genetics, 130(9), 1801–1817. https://doi.org/10.1007/s00122-017-2926-0

Han, S., Miedaner, T., Utz, H. F., Schipprack, W., Schrag, T. A., & Melchinger, A. E. (2018). Genomic prediction and GWAS of Gibberella ear rot resistance traits in dent and flint lines of a public maize breeding program. Euphytica, 214(1), 1–20. https://doi.org/10.1007/s10681-017-2090-2

Han, S., Utz, H. F., Liu, W., Schrag, T. A., Stange, M., Würschum, T., Miedaner, T., Bauer, E., Schön, C. C., & Melchinger, A. E. (2016). Choice of models for QTL mapping with multiple families and design of the training set for prediction of Fusarium resistance traits in maize. Theoretical and Applied Genetics, 129(2), 431–444. https://doi.org/10.1007/s00122-015-2637-3

Harris, L. J., Desjardins, A. E., Plattner, R. D., Nicholson, P., Butler, G., Young, J. C., Weston, G., Proctor, R. H., & Hohn, T. M. (1999). Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Disease, 83(10), 954–960. https://doi.org/10.1094/PDIS.1999.83.10.954

He, X., Dreisigacker, S., Singh, R. P., & Singh, P. K. (2019). Genetics for low correlation between Fusarium head blight disease and deoxynivalenol (DON) content in a bread wheat mapping population. Theoretical and Applied Genetics, 132(8), 2401–2411. https://doi.org/10.1007/s00122-019-03362-9

Herter, C. P., Ebmeyer, E., Kollers, S., Korzun, V., Würschum, T., & Miedaner, T. (2019). Accuracy of within- and among-family genomic prediction for Fusarium head blight and *Septoria tritici* blotch in winter wheat. Theoretical and Applied Genetics, 132(4), 1121–1135. https://doi.org/10.1007/s00122-018-3264-6

Hölker, A. C., Mayer, M., Presterl, T., Bolduan, T., Bauer, E., Ordas, B., Brauner, P. C., Ouzunova, M., Melchinger, A. E., & Schön, C. C. (2019). European maize landraces made accessible for plant breeding and genome-based studies. Theoretical and Applied Genetics, 132(12), 3333–3345. https://doi.org/10.1007/s00122-019-03428-8

Jiao, Y., Peluso, P., Shi, J., Liang, T., Stitzer, M. C., Wang, B., Campbell, M. S., Stein, J. C., Wei, X., Chin, C. S., Guill, K., Regulski, M., Kumari, S., Olson, A., Gent, J., Schneider, K. L., Wolfgruber, T. K., May, M. R., Springer, N. M., … Ware, D. (2017). Improved maize reference genome with single-molecule technologies. Nature, 546(7659), 524–527. https://doi.org/10.1038/nature22971

Kadam, D. C., Potts, S. M., Bohn, M. O., Lipka, A. E., & Lorenz, A. J. (2016). Genomic prediction of single crosses in the early stages of a maize hybrid breeding pipeline. Genes, Genomes, Genetics, 6(11), 3443–3453. https://doi.org/10.1534/g3.116.031286

Kaler, A. S., Gillman, J. D., Beissinger, T., & Purcell, L. C. (2020). Comparing different statistical models and multiple testing corrections for association mapping in soybean and maize. Frontiers in Plant Science, 10:1794. https://doi.org/10.3389/fpls.2019.01794

Kalih, R., Maurer, H. P., & Miedaner, T. (2015). Genetic architecture of Fusarium head blight resistance in four winter triticale populations. Phytopathology, 105(3), 334–341. https://doi.org/10.1094/PHYTO-04-14-0124-R

Kazan, K., & Gardiner, D. M. (2018). Transcriptomics of cereal–*Fusarium graminearum* interactions: what we have learned so far. Molecular Plant Pathology, 19(3), 764–778. https://doi.org/10.1111/mpp.12561

Kebede, A. Z., Johnston, A., Schneiderman, D., Bosnich, W., & Harris, L. J. (2018). Transcriptome profiling of two maize inbreds with distinct responses to Gibberella ear rot disease to identify candidate resistance genes. BMC Genomics, 19, 131. https://doi.org/10.1186/s12864-018-4513-4

King, E. G., & Long, A. D. (2017). The Beavis Effect in Next-Generation Mapping Panels in Drosophila melanogaster. G3 (Bethesda, Md.), 7(6), 1643–1652. https://doi.org/10.1534/g3.117.041426

Kim, W., Johnson, J. W., Baenziger, P. S., Lukaszewski, A. J., & Gaines, C. S. (2004). Agronomic Effect of Wheat-Rye Translocation Carrying Rye Chromatin (1R) From Different Sources. Crop Science, 44, 1254–1258.

Kuska, M. T., & Mahlein, A. K. (2018). Aiming at decision making in plant disease protection and phenotyping by the use of optical sensors. European Journal of Plant Pathology, 152(4), 987–992. https://doi.org/10.1007/s10658-018-1464-1

Langevin, F., Eudes, F., & Comeau, A. (2004). Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium head blight development in six cereal species. European Journal of Plant Pathology, 110(7), 735–746. https://doi.org/10.1023/B:EJPP.0000041568.31778.ad

Lanubile, A., Maschietto, V., Borrelli, V. M., Stagnati, L., Logrieco, A. F., & Marocco, A. (2017). Molecular basis of resistance to Fusarium ear rot in maize. Frontiers in Plant Science. https://doi.org/10.3389/fpls.2017.01774

Leng, P., Lübberstedt, T., & Xu, M. (2017). Genomics-assisted breeding – A revolutionary strategy for crop improvement. Journal of Integrative Agriculture, 16(12), 2674–2685. https://doi.org/10.1016/S2095-3119(17)61813-6

Li, C., Fu, Y., Sun, R., Wang, Y., & Wang, Q. (2018). Single-locus and multi-locus genome-wide association studies in the genetic dissection of fiber quality traits in upland cotton (*Gossypium hirsutum* L.). Frontiers in Plant Science, 9(1083), 1–16. https://doi.org/10.3389/fpls.2018.01083

Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. PLoS Genetics, 12(2). https://doi.org/10.1371/journal.pgen.1005767

Lofgren, L. A., LeBlanc, N. R., Certano, A. K., Nachtigall, J., LaBine, K. M., Riddle, J., Broz, K., Dong, Y., Bethan, B., Kafer, C. W., & Kistler, H. C. (2018). *Fusarium graminearum*: pathogen or endophyte of North American grasses? New Phytologist, 217(3), 1203–1212. https://doi.org/10.1111/nph.14894

Lozada, D. N., & Carter, A. H. (2019). Accuracy of single and multi-trait genomic prediction models for grain yield in US Pacific Northwest winter wheat. Crop Breeding, Genetics and Genomics, 1(e190012), 1–23. https://doi.org/10.20900/cbgg20190012

Ma, H. X., Bai, G. H., Zhang, X., & Lu, W. Z. (2006). Main effects, epistasis, and environmental interactions of quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population. Phytopathology, 96(5), 534–541. https://doi.org/10.1094/PHYTO-96-0534

Ma, Z., Xie, Q., Li, G., Jia, H., Zhou, J., Kong, Z., Li, N., & Yuan, Y. (2020). Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. Theoretical and Applied Genetics, 133, 1541–1568. https://doi.org/10.1007/s00122-019-03525-8

Malik, P. L., Janss, L., Nielsen, L. K., Borum, F., Jørgensen, H., Eriksen, B., Schjoerring, J. K., & Rasmussen, S. K. (2019). Breeding for dual-purpose wheat varieties using

marker–trait associations for biomass yield and quality traits. Theoretical and Applied Genetics, 132(12), 3375–3398. https://doi.org/10.1007/s00122-019-03431-z

Martin, M., Miedaner, T., Schwegler, D. D., Kessel, B., Ouzunova, M., Dhillon, B. S., Schipprack, W., Utz, H. F., & Melchinger, A. E. (2012). Comparative quantitative trait loci mapping for Gibberella ear rot resistance and reduced deoxynivalenol contamination across connected maize populations. Crop Science, 52(1), 32–43. https://doi.org/10.2135/cropsci2011.04.0214

Martin, M, Miedaner, T., Dhillon, B., Ufermann, U., Kessel, B., Ouzunova, M., Schipprack, W., & Melchinger, A. E. (2011). Colocalization of QTL for Gibberella ear rot resistance and low mycotoxin contamination in early European maize. Crop Science, 51(5), 1935–1945. https://doi.org/10.2135/cropsci2010.11.0664

Martin, Matthias, Dhillon, B. S., Miedaner, T., & Melchinger, A. E. (2012). Inheritance of resistance to Gibberella ear rot and deoxynivalenol contamination in five flint maize crosses. Plant Breeding, 131(1), 28–32. https://doi.org/10.1111/j.1439-0523.2011.01908.x

Massart, F., Meucci, V., Saggese, G., & Soldani, G. (2008). High Growth Rate of Girls with Precocious Puberty Exposed to Estrogenic Mycotoxins. Journal of Pediatrics, 152(5), 690-695.e1. https://doi.org/10.1016/j.jpeds.2007.10.020

Mayer, M., Unterseer, S., Bauer, E., de Leon, N., Ordas, B., & Schön, C. C. (2017). Is there an optimum level of diversity in utilization of genetic resources? Theoretical and Applied Genetics, 130(11), 2283–2295. https://doi.org/10.1007/s00122-017-2959-4

Mesterházy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. Plant Breeding, 114(5), 377–386. https://doi.org/10.1111/j.1439-0523.1995.tb00816.x

Mesterházy, Á., Lemmens, M., & Reid, L. M. (2012). Breeding for resistance to ear rots caused by *Fusarium* spp. in maize - A review. Plant Breeding, 131(1), 1–19. https://doi.org/10.1111/j.1439-0523.2011.01936.x

Miao, C., Yang, J., & Schnable, J. C. (2019). Optimising the identification of causal variants across varying genetic architectures in crops. Plant Biotechnology Journal, 17(5), 893–905. https://doi.org/10.1111/pbi.13023

Miedaner, T., Bolduan, C., & Melchinger, A. E. (2010). Aggressiveness and mycotoxin production of eight isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for ear rot on susceptible and resistant early maize inbred lines. European Journal of Plant Pathology, 127(1), 113–123. https://doi.org/10.1007/s10658-009-9576-2

Miedaner, T., & Geiger, H. H. (1996). Estimates of combining ability for resistance of winter rye to Fusarium culmorum head blight. Euphytica, 89(3), 339–344. http://www.scopus.com/inward/record.url?eid=2-s2.0-0029961931&partnerID=40

Miedaner, T., Haffke, S., Siekmann, D., Fromme, F. J., Roux, S. R., & Hackauf, B. (2018). Dynamic quantitative trait loci (QTL) for plant height predict biomass yield in hybrid rye (*Secale cereale* L.). Biomass and Bioenergy, 115, 10–18. https://doi.org/10.1016/j.biombioe.2018.04.001

Miedaner, T., Heinrich, N., Schneider, B., Oettler, G., Rohde, S., & Rabenstein, F. (2004). Estimation of deoxynivalenol (DON) content by symptom rating and exoantigen content for resistance selection in wheat and triticale. Euphytica, 139(2), 123–132. https://doi.org/10.1007/s10681-004-2489-4

Miedaner, T., Herter, C. P., Ebmeyer, E., Kollers, S., Korzun, V. (2019a). Use of non-adapted quantitative trait loci for increasing Fusarium head blight resistance for breeding semi-dwarf wheat. Plant Breeding, 138:140–147. https://doi.org/10.1111/pbr.12683

Miedaner, T., Hübner, M., Korzun, V., Schmiedchen, B., Bauer, E., Haseneyer, G., Wilde, P., & Reif, J. C. (2012). Genetic architecture of complex agronomic traits examined in two testcross populations of rye (Secale cereale L.). BMC Genomics, 13, 706. https://doi.org/10.1186/1471-2164-13-706

Miedaner, T., & Korzun, V. (2019). Editorial: genetics became to be genomics. In. T. Miedaner & V. Korzun (eds.). Applications of genetic and genomic research in cereals. Elsevier Ltd. pp xvii-xxii. https://doi.org/https://doi.org/10.1016/C2016-0-04192-X

Miedaner, T., Korzun, V., & Bauer, E. (2019b). Genomics-based hybrid rye breeding. In. T. Miedaner & V. Korzun (eds.). Applications of genetic and genomic research in cereals. Elsevier Ltd. pp 329-348. https://doi.org/10.1016/B978-0-08-102163-7.00015-6

Miedaner, T., & Laidig, F. (2019). Hybrid breeding in rye (*Secale cereal* L.). In J. M. Al-Khayri, S. M. Jain, & D. V. Johnson (Eds.), Advances in plant breeding strategies, Vol 5: Cereals (pp. 343-372). Cham, Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-030-23108 -8_9

Miedaner, T., Reinbrecht, C., Lauber, U., Schollenberger, M., & Geiger, H.H. (2001). Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. Plant Breeding, 120(2), 97–105. https://doi.org/10.1046/j.1439-0523.2001.00580.x

Miedaner, T., Sieber, A. N., Desaint, H., Buerstmayr, H., Longin, C. F. H., & Würschum, T. (2017). The potential of genomic-assisted breeding to improve Fusarium head blight resistance in winter durum wheat. Plant Breeding, 136(5), 610–619. https://doi.org/10.1111/pbr.12515

Miedaner, T., & Voss, H. H. (2008). Effect of dwarfing *Rht* genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Science, 48(6), 2115–2122. https://doi.org/10.2135/cropsci2008.02.0107

Mielniczuk, E., & Skwaryło-Bednarz, B. (2020). Fusarium head blight, mycotoxins and strategies for their reduction. Agronomy, 10(4), 11–15. https://doi.org/10.3390/agronomy10040509

Mirdita, V., Liu, G., Zhao, Y., Miedaner, T., Longin, C. F. H., Gowda, M., Mette, M. F., & Reif, J. C. (2015). Genetic architecture is more complex for resistance to *Septoria tritici* blotch than to Fusarium head blight in Central European winter wheat. BMC Genomics, 16(1), 1–8. https://doi.org/10.1186/s12864-015-1628-8

Mohammadi, M., Anoop, V., Gleddie, S., & Harris, L. J. (2011). Proteomic profiling of two maize inbreds during early Gibberella ear rot infection. Proteomics, 11(18), 3675–3684. https://doi.org/10.1002/pmic.201100177

Moreno-Amores, J., Michel, S., Miedaner, T., Longin, C. F. H., & Buerstmayr, H. (2020). Genomic predictions for Fusarium head blight resistance in a diverse durum wheat panel: an effective incorporation of plant height and heading date as covariates. Euphytica, 216(2), 1–19. https://doi.org/10.1007/s10681-019-2551-x

Mouton, M. (2014). Resistance in South African maize inbred lines to the major ear rot diseases and associated mycotoxin contamination. March, 60–76.

Mutka, A. M., & Bart, R. S. (2015). Image-based phenotyping of plant disease symptoms. Frontiers in Plant Science, 5, 734. https://doi.org/10.3389/fpls.2014.00734

Myşków, B., Góralska, M., Lenarczyk, N., Czyczyło-Mysza, I., & Stojałowski, S. (2018). Putative candidate genes responsible for leaf rolling in rye (*Secale cereale* L.). BMC Genetics, 19(1), 1–11. https://doi.org/10.1186/s12863-018-0665-0

Newell, M., & Jannink, J. (2014). Genomic selection in plant breeding. In D. Fleury & R. Whitford (Eds.), Crop Breeding. Methods in Molecular Biology (Methods and Protocols) (1145th ed., pp. 117–130). Humana Press. https://doi.org/10.1007/978-1-4939-0446-4_10

Odilbekov, F., Armoniené, R., Koc, A., Svensson, J., & Chawade, A. (2019). GWAS-assisted genomic prediction to predict resistance to *Septoria tritici* blotch in nordic winter wheat at seedling stage. Frontiers in Genetics, 10(1224), 1–10. https://doi.org/10.3389/fgene.2019.01224

OECD/FAO. (2019). OECD-FAO agricultural outlook 2019-2028 chapter 3: Cereals. http://www.agri-outlook.org/commodities/Cereals.pdf

Ollier, M., Talle, V., Brisset, A. L., Le Bihan, Z., Duerr, S., Lemmens, M., Goudemand, E., Robert, O., Hilbert, J. L., & Buerstmayr, H. (2020). QTL mapping and successful introgression of the spring wheat-derived QTL Fhb1 for Fusarium head blight resistance in three European triticale populations. Theoretical and Applied Genetics, 133(2), 457–477. https://doi.org/10.1007/s00122-019-03476-0

Pagnussatt, A. F., Medeiros, E., Ponte, D., & Garda-buffon, J. (2014). Inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* spp. Pesticide Biochemistry and Physiology, 108, 21–26. https://doi.org/10.1016/j.pestbp.2013.11.002

Pariona, A. (2019, June 7). What Are the World's Most Important Staple Foods?. WorldAtlas. https://www.worldatlas.com/articles/most-important-staple-foods-in-the-world.html

Pasquali, M., Beyer, M., Logrieco, A., Audenaert, K., Balmas, V., Basler, R., Boutigny, A. L., Chrpová, J., Czembor, E., Gagkaeva, T., González-Jaén, M. T., Hofgaard, I. S., Köycü, N. D., Hoffmann, L., Levic, J., Marin, P., Miedaner, T., Migheli, Q., Moretti, A., … Vogelgsang, S. (2016). A European database of Fusarium graminearum and *F. culmorum* trichothecene genotypes. Frontiers in Microbiology, 7, 406. https://doi.org/10.3389/fmicb.2016.00406

Pereira, G. S., Pinho, R. G. V., Pinho, E. V. R. V., Pires, L. P. M., Junior, L. A. Y. B., Pereira, J. L. A., & Melo, M. P. (2017). Selection of maize inbred lines and gene expression for resistance to ear rot. Genetics and Molecular Research, 16(3), 1–21. https://doi.org/10.4238/gmr16039415

Pfordt, A., Ramos Romero, L., Schiwek, S., Karlovsky, P., & von Tiedemann, A. (2020). Impact of environmental conditions and agronomic practices on the prevalence of *Fusarium* species associated with ear- and stalk rot in maize. Pathogens, 9(3), 236. https://doi.org/10.3390/pathogens9030236

Pinton, P., & Oswald, I. (2014). Effect of deoxynivalenol and other type B trichothecenes on the intestine: A Review. Toxins, 6(5), 1615–1643. https://doi.org/10.3390/toxins6051615

Prat, N., Guilbert, C., Prah, U., Wachter, E., Steiner, B., Langin, T., Robert, O., & Buerstmayr, H. (2017). QTL mapping of Fusarium head blight resistance in three related durum wheat populations. Theoretical and Applied Genetics, 130(1), 13–27. https://doi.org/10.1007/s00122-016-2785-0

Rebourg, C., Chastanet, M., Gouesnard, B., Welcker, C., Dubreuil, P., & Charcosset, A. (2003). Maize introduction into Europe: the history reviewed in the light of molecular data. Theoretical and Applied Genetics, 106, 895–903. https://doi.org/10.1007/s00122-002-1140-9

Riedelsheimer, C., Endelman, J. B., Stange, M., Sorrells, M. E., Jannink, J. L., & Melchinger, A. E. (2013). Genomic predictability of interconnected biparental maize populations. Genetics, 194(2), 493–503. https://doi.org/10.1534/genetics.113.150227

Robertsen, C. D., Hjortshøj, R. L., & Janss, L. L. (2019). Genomic selection in cereal breeding. Agronomy, 9(95): 1-16. https://doi.org/10.3390/agronomy9020095

Rutkoski, J., Benson, J., Jia, Y., Brown-Guedira, G., Jannink, J.-L., & Sorrells, M. (2012). Evaluation of genomic prediction methods for Fusarium head blight resistance in wheat. The Plant Genome Journal, 5(2), 51-61. https://doi.org/10.3835/plantgenome2012.02.0001

Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T. A., Minx, P., Reily, A. D., Courtney, L., Kruchowski, S. S., Tomlinson, C., Strong, C., Delehaunty, K., Fronick, C., Courtney, B., Rock, S. M., … Braidotti, M. (2009). The B73 Maize genome: complexity, diversity, and dynamics. Science, 326(5956), 1112–1115. https://doi.org/10.1126/science.1178534

Schopp, P., Müller, D., Technow, F., & Melchinger, A.E. (2017) Accuracy of genomic prediction in synthetic populations depending on the number of parents, relatedness, and ancestral linkage disequilibrium. Genetics, 205(1):1-14. https://doi.org/10.1534/genetics.116.193243

Segura, V., Vilhjalmsson, B. J., Platt, A., Korte, A., Seren, U., Long, Q., & Nordborg, M. (2012). An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nature Genetics, 44(7):825–30

Sperschneider, J., Gardiner, D. M., Thatcher, L. F., Lyons, R., Singh, K. B., Manners, J. M., & Taylor, J. M. (2015). Genome-wide analysis in three Fusarium pathogens identifies rapidly evolving chromosomes and genes associated with pathogenicity. Genome Biology and Evolution, 7(6), 1613–1627. https://doi.org/10.1093/gbe/evv092

Spindel, J. E., Begum, H., Akdemir, D., Collard, B., Redoña, E., Jannink, J. L., & McCouch, S. (2016). Genome-wide prediction models that incorporate de novo GWAS are a powerful new tool for tropical rice improvement. Heredity, 116(4), 395–408. https://doi.org/10.1038/hdy.2015.113

Strigens, A., Schipprack, W., Reif, J.C., & Melchinger, A.E. (2013). Unlocking the genetic diversity of maize landraces with doubled haploids opens new avenues for breeding. PLoS One, 8 (2): e57234. https://doi.org/10.1371/journal.pone.0057234

Suchowilska, E., Kandler, W., Sulyok, M., Wiwart, M., & Krska, R. (2010). Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*. Journal of the Science of Food and Agriculture, 90(4), 556–565. https://doi.org/10.1002/jsfa.3844

Technow, F., Bürger, A., & Melchinger, A. E. (2013). Genomic prediction of Northern Corn leaf blight resistance in maize with combined or separated training sets for heterotic groups. Genes, Genomes, Genetics, 3(2), 197–203. https://doi.org/10.1534/g3.112.004630

Tenaillon, M. I., & Charcosset, A. (2011). A European perspective on maize history. Comptes Rendus Biologies, 334(3), 221-228. https://doi.org/10.1016/j.crvi.2010.12.015

The Commission of the European Communities. (2006). Commission Regulation (EC) 1881/2006 Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 364(1074), 5–24. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20140701&from=EN

The International Wheat Genome Sequencing Consortium. (2014). A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science, 345(6194), 1251788. https://doi.org/http://dx.doi.org/10.1126/science.1251788

Trail, F. (2009). For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiology, 149(1), 103–110. https://doi.org/10.1104/pp.108.129684

Tsen, C., Hoover, W. ., & Farrell, E. (1971). Baking quality of triticale flours. 56th Annual Meeting, Dallas, Manhattan. pp. 17–26.

United Nations (2015). Transforming our world: the 2030 Agenda for sustainable development. In Resolution adopted by the General Assembly on 25 September 2015. United Nations. https://sustainabledevelopment.un.org/post2015/transformingourworld/publication

Venske, E., dos Santos, R. S., Farias, D. da R., Rother, V., da Maia, L. C., Pegoraro, C., & Costa de Oliveira, A. (2019). Meta-analysis of the QTLome of Fusarium head blight resistance in bread wheat: Refining the current puzzle. Frontiers in Plant Science, 10, 727. https://doi.org/10.3389/fpls.2019.00727

Villareal, R. L., Bañuelos, O., Mujeeb-Kazi, A., & Rajaram, S. (1998). Agronomic performance of chromosomes 1B and T1BL.1RS near-isolines in the spring bread wheat Seri M82. Euphytica, 103(2), 195–202. https://doi.org/10.1023/A:1018392002909

Wallace, J. G., Larsson, S. J., & Buckler, E. S. (2014). Entering the second century of maize quantitative genetics. Heredity, 112(1), 30–38. https://doi.org/10.1038/hdy.2013.6

Wang, R., Chen, J., Anderson, J. A., Zhang, J., Zhao, W., Wheeler, J., Klassen, N., See, D. R., & Dong, Y. (2017). Genome-wide association mapping of Fusarium head blight resistance in spring wheat lines developed in the Pacific Northwest and CIMMYT. Phytopathology, 107(12), 1486–1495. https://doi.org/10.1094/phyto-02-17-0073-r

Wang, S., Feng, J., Ren, W., Huang, B., Zhou, L., Wen, Y., Zhang, J., Dunwell, J. M., Xu, S., & Zhang, Y. (2016). Improving power and accuracy of genome-wide association studies via a multi-locus mixed linear model methodology. Scientific Report 6, 19444. https://doi.org/10.1038/srep19444

Wen, Y. J., Zhang, H., Ni, Y. L., Huang, B., Zhang, J., Feng, J. Y., Wang, S. B., Dunwell, J. M., Zhang, Y. M., & Wu, R. (2018). Methodological implementation of mixed linear models in multi-locus genome-wide association studies. Briefings in Bioinformatics, 19(4), 700–712. https://doi.org/10.1093/bib/bbw145

Wei, W., Mesquita, A.C.O., Figueiró, A. d. A., Wu, X., Manjunatha, S., Wickland, D. P., Hudson, M. E., Juliatti, F. C., & Clough, S. J. (2017). Genome-wide association mapping of resistance to a Brazilian isolate of *Sclerotinia sclerotiorum* in soybean genotypes mostly from Brazil. BMC Genomics, 18, 849. https://doi.org/10.1186/s12864-017-4160-1

Wise, K., Allen, T., Chilvers, M., Faske, T., Freije, A., Isakeit, T., Mueller, D., Price, T., Sisson, A., Smith, D., Tenuta, A., & Woloshuk, C. (2016). Corn disease management: ear rots. Crop Protection Network. https://cropprotectionnetwork.org/download/2553/

Xiang, K., Reid, L. M., Zhang, Z., Zhu, X., & Pan, G. (2010). A meta-analysis of QTL associated with ear rot resistance in maize. Maydica, 55(3), 281–290.

Yao, L., Li, Y., Ma, C., Feili Du, L. T., & Xu, M. (2020). Combined genome-wide association study and transcriptome analysis reveal candidate genes for resistance to Fusarium ear rot in maize. Journal of Integrative Plant Biology, 00(00). https://doi.org/10.1111/jipb.12911

Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., …Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for

multiple levels of relatedness. Nature Genetics, 38(2), 203–208. https://doi.org/10.1038/ng1702

Yuan, G., He, X., Li, H., Xiang, K., Liu, L., Zou, C., Lin, H., Wu, J., Zhang, Z., & Pan, G. (2020). Transcriptomic responses in resistant and susceptible maize infected with *Fusarium graminearum*. Crop Journal, 8(1), 153–163. https://doi.org/10.1016/j.cj.2019.05.008

Zhang, H., Yin, L., Wang, M., Yuan, X., & Liu, X. (2019b). Factors affecting the accuracy of genomic selection for agricultural economic traits in maize, cattle, and pig populations. Frontiers in Genetics, 10, 189. https://doi.org/10.3389/fgene.2019.00189

Zhang, K., Liu, S., Li, W., Liu, S., Li, X., Fang, Y., Zhang, J., Wang, Y., Xu, S., Zhang, J., Song, J., Qi, Z., Tian, X., Tian, Z., Li, W-X., & Ning, H. (2018). Identification of QTNs controlling seed protein content in soybean using multi-locus genome-wide association studies. Front. Plant Sci., 9, 1690. https://doi.org/10.3389/fpls.2018.01690

Zhang, Y. M., Jia, Z., & Dunwell, J. M. (2019a). Editorial: The applications of new multi-locus GWAS methodologies in the genetic dissection of complex traits. Frontiers in Plant Science, 10, 100. https://doi.org/10.3389/fpls.2019.00100

Zhang, Y. M., Mao, Y., Xie, C., Smith, H., Luo, L., & Xu, S. (2005). Mapping quantitative trait loci using naturally occurring genetic variance among commercial inbred lines of maize (*Zea mays* L.). Genetics, 169(4), 2267–2275. https://doi.org/10.1534/genetics.104.033217

Zhang, Z., Ersoz, E., Lai, C. Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., Bradbury, P. J., Yu, J., Arnett, D. K., Ordovas, J. M., & Buckler, E. S. (2010). Mixed linear model approach adapted for genome-wide association studies. Nature Genetics, 42(4), 355–360. https://doi.org/10.1038/ng.546

Zhao, Y., Mette, M. F., Gowda, M., Longin, C. F. H., & Reif, J. C. (2014). Bridging the gap between marker-assisted and genomic selection of heading time and plant height in hybrid wheat. Heredity, 112(6), 638–645. https://doi.org/10.1038/hdy.2014.1

Zhou, M., Yang, L., Shao, M., Wang, Y., Yang, W., Huang, L., Zhou, X., Jiang, S., & Yang, Z. (2018). Effects of zearalenone exposure on the TGF-β1/Smad3 signaling pathway

and the expression of proliferation or apoptosis related genes of post-weaning gilts. Toxins, 10(2), 1–13. https://doi.org/10.3390/toxins10020049

10 Curriculum vitae

Name: David Sewordor Gaikpa

Date and Place of Birth: 12th May, 1984; Donkorkrom, Ghana

EDUCATION

2017 – 2021 **PhD (Plant Genetics and Breeding)**

State Plant Breeding Institute, University of Hohenheim, Stuttgart, Germany under apl. Prof. Dr. Thomas Miedaner

Thesis: Breeding for Fusarium resistance in maize and small-grain cereals using genomic tools

2012 – 2015 **Master of Philosophy in Agronomy (Plant Breeding), Grade "A"**

Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Thesis: Molecular and phenotypic screening of groundnut varieties for Cercospora leaf spots resistance

2007 - 2011 **Bachelor of Education in Agriculture (1st Class Honours)**

University of Education, Winneba, Ghana

2002-2005 **3-year Post Secondary Teacher's Certificate "A" (Associate Degree)**

College of Education, Akropong-Akuapem, University of Cape-Coast, Ghana

1999-2001 **Senior Secondary School Certificate**

West African Examinations Council

Donkorkrom Agriculture Senior High School, Donkorkrom, Ghana

ADDITIONAL STUDIES

May 4 – June 7, 2020 **Certificate in Digital Agriculture**

Online course, World Bank Group

WORK/RESEARCH EXPERIENCE

2017-2021 **Doctoral Researcher at State Plant Breeding Institute (720), University of Hohenheim, Stuttgart, Germany**

- Planned and conducted multi-environment trials for maize and four small-grain cereals in different teams
- Prepared inoculum for silk-channel and spray inoculations
- Collected data on multiple cereal crops for fungal resistance and agronomic traits
- Used mixed models to estimate quantitative genetic parameters from phenotypic data and performed genome-wide association studies and genomic prediction with R packages
- Wrote reports and published four scientific articles as main author plus other two as a co-author.
- Made oral presentations at seminars and conferences

2013 - 2014 **Graduate Researcher at Crop Research Institute, Fumesua-Kumasi, Ghana**

- Prepared inoculum for spray inoculation
- Did DNA extraction and ran PCR
- Conducted field and greenhouse trials
- Analysed phenotypic and molecular data and published the results
- Helped in pollinations

2011 – 2017;	**Principal Teacher at the Ghana Education Service, Ministry of**
2005 - 2007	**Education, Ghana**

- Planned taught, and evaluated lessons in General Science, Agriculture and Biology for improved students' performance
- Planned and implemented extra-curricular activities
- Coordinated special programs such as National Science and Mathematics Quiz programs (H'Mount Sinai Senior High School)

GRANTS/SCHOLARSHIPS

- Research Grants - Doctoral Programmes in Germany (57299294) by German Academic Exchange Service (DAAD) (2017 - Date)
- Doctoral Studies Scholarship ("Ideelle Förderung") in Germany by Catholic Academic Exchange Service (KAAD), Germany (2017 - Date)
- In-country Master Studies Scholarship by KAAD, Germany (2013 - 2014)

PUBLICATIONS

Nine (9)

David Sewordor Gaikpa

Stuttgart,

Dedication

I dedicate this doctoral thesis to my lovely mother, Veronica Adorshi; my late uncle, Jacob W. Awudja; my wife, Stella Kposu; children, Manel and Johann Sewordor; and the German Academic Exchange Service (DAAD).

Acknowledgements

I am most grateful to God almighty for his care and blessings. My profound gratitude to my mother, Veronica Adorshi and my late uncle, Jacob W. Awudja for raising me and taking the cost of my initial education. Special thanks to you, my wife, Stella S. Kposu and children, Manel and Johann for your understanding, spiritual and emotional support. To the bigger family, sisters and brothers, thank you so much.

I express my heartfelt appreciation to my greatest mentor and the main supervisor of this PhD work, apl. Prof. Dr. Thomas Miedaner for accepting me to study in his group and playing instrumental role in getting the PhD funding and projects. Thank you so much, Thomas!. I am thankful to Prof. Dr. Ralf T. Vögele for his annual reviews and letters of support for scholarship extension. I thank Drs. T. Würschum, H. Maurer, Willmar Leiser and H. Piepho for their statistical advice. My special gratitude to all the technical persons at the Rye and Biotic stress group of the State Plant Breeding Institute and at the Chair of Applied Genetics and Plant Breeding, University of Hohenheim as well as KWS-SE breeding station in Gondelsheim for their great help in all experiments. Silvia, Bärbel, Heike, Mark, Dorethee, and others, I am grateful ☺!. I thank Maria Belén Kistner, INTA (National Institute of Agricultural Technology), Pergamino, Argentina, for her immense help in the field experiments of 2019. Thank you, Paul Gruner, Anna Kodisch and Jana Liedtke for your support. I thank Ana Galiano-Carneiro for sharing office and ideas with me and being supportive in my experiments. I recognize the special contributions of all the staff and PhD students at the State Plant Breeding Institute (720) and the Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany. Your emotional, social and academic supports were very useful. All friends and persons, who helped me directly or indirectly through my journey of Ph.D studies, you are highly acknowledged. I am highly indebted to the German

Academic Exchange Service (DAAD), Bonn, for the great financial support throughout my doctoral studies. To my contact persons at DAAD, I am most grateful to you. I thank the Catholic Academic Exchange Service (KAAD) for providing me with the mutual and networking support funding, Marko and your team, Thank you.

Affidavit

Declaration in lieu of an oath on independent work

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

"Breeding for resistance to Fusarium ear diseases in maize and small-grain cereals using genomic tools"

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

_____ _____

Place, Date Signature

Instructions on the importance and criminal legal consequences of the declaration in lieu of an oath

according to Sec. 18(3) sentence 6 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

The University of Hohenheim requires a declaration in lieu of oath on the independence of the scientific work done in order to ensure that the doctoral candidates have done the scientific work independently.

Because the legislators place a particular importance on declarations in lieu of oath and these declarations can have serious consequences, the legislators have placed criminal penalties on false declarations in lieu of oath. If a person willfully (that means knowingly) submits a false declaration, the punishment can be imprisonment for up to three years or a fine.

If a person negligently submits a false declaration (that is, it is submitted even though the person should have realized that the declaration was not correct), then the punishment can be imprisonment for up to one year or a fine.

The criminal provisions can be found in Sec. 156 of the Criminal Code (StGB, false declaration in lieu of oath) and in Sec. 161 StGB (negligent false oath, negligent false declaration in lieu of oath).

Sec. 156 StGB: False Declaration in Lieu of Oath

Persons who make a false declaration in lieu of oath to an institution responsible for accepting such declarations or persons who make false statements on such a declaration are subject to imprisonment of up to three years or a fine.

Sec. 161 StGB: Negligent False Oath, Negligent False Declaration in Lieu of Oath

161(1): If an action described in Secs. 154 and 156 are done negligently, the punishment is imprisonment of up to one year or a fine.

161(2): There is impunity if the perpetrator corrects the false declaration in a timely manner. The provisions in Sec. 158(2 and 3) apply mutatis mutandis.

I acknowledge the instructions on declarations in lieu of oath.

_____ _____

Place, Date Signature