Genomics-assisted breeding strategies for quantitative resistances to Northern corn leaf blight in maize (*Zea mays* L.) and Fusarium diseases in maize and in triticale (×*Triticosecale* Wittm.)

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

Faculty of Agricultural Sciences
University of Hohenheim
State Plant Breeding Institute

Submitted by
Master of Science
Ana Luísa Galiano Carneiro

from Sorocaba, SP-Brazil

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Dedicated to Fernando Galiano

Contents

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Abbreviations

BC	Backcross
Ddw1	Dominant dwarfing gene
DH	Double haploid
DON	Deoxynivalenol
Dpi	Days post infection
ELISA	Enzyme-linked immunosorbent assay
FHB	Fusarium head blight
Fhb1	FHB resistance gene
GBLUP	Genomic best linear unbiased prediction
GEBVs	Genomic estimated breeding values
GER	Gibberella ear rot
(w)GS	(weighted)Genomic selection
GWA(S)	Genome-wide association (study)
Ht	Resistance gene against *Setosphaeria turcica*
Lr34	Rust resistance gene
MAS	Marker-assisted selection
NCLB	Northern corn leaf blight
NIRS	Near-infrared spectroscopy
Pan1	Receptor-like kinase
QTL	*Quantitative trait loci*
Remorin	Group of plant-specific proteins
(w)RR-BLUP	(weighted)Ridge regression best linear unbiased prediction
WAKs	Wall-associated receptor-like kinase
ZON	Zearalenone

1 General introduction

Estimates attribute more than 20% of global pre-harvest yield losses in major field crops to plant diseases and pests (Savary et al. 2019). At the same time, agricultural production should double by 2050 to meet the demands for food, feed and fuel by a growing world population (Ray et al. 2013). Resistant crops can assist farmers to exploit higher crop yield potential in a sustainable way. However, pathogen populations evolve quickly and new resistance alleles are continuously needed to restore effective host-resistance (Nelson et al. 2018). Exotic germplasm is a potential source of novel disease resistances, but is mostly untapped by breeding programs due to insufficient knowledge of its merits or lack of adaption traits (Kraja et al. 2000; Hallauer et al. 2010; Sood et al. 2014; Mayer et al. 2017). The advent of high-throughput molecular markers and decreasing genotyping costs enable genomics-assisted breeding strategies to harness the disease resistance potential of elite and exotic germplasm.

Genomic tools to assist resistance breeding in crops: A brief overview

The genetic architecture underlying quantitative resistances can be dissected by *quantitative trait loci* (QTL) mapping (Nelson et al. 2018). The first method was based on linkage mapping in biparental populations (Edwards

et al. 1987). This is a powerful method to identify rare alleles as controlled crosses artificially increase their frequency (Myles et al. 2009; Würschum 2012). However, linkage mapping with biparental populations is limited by low allelic diversity and often low mapping resolution that can hinder the transferability of QTL to other genetic backgrounds (Würschum 2012). Therefore, Rebai and Goffinet (1993) and Muranty (1996) proposed the multi-parent QTL analysis. This method is based on multiple crosses between biparental populations carrying one or more common parents. Furthermore, it can potentially increase power for QTL identification in the same ancestral locus and increase QTL transferability success (Li et al. 2005; Blanc et al. 2006; Würschum 2012; Garin et al. 2017). Another approach aims at the identification of QTL in a diverse panel of populations and is commonly known as genome-wide association (GWA) mapping (Würschum 2012). Genome-wide association studies (GWAS) investigate more alleles at a time and evaluate a greater sample of the available germplasm compared to biparental mapping. GWA has higher mapping resolution and potentially leads to higher QTL transferability success but has lower power to identify favorable alleles at low frequency (Würschum 2012). Regardless of the method of choice, high phenotyping quality and intensity, large QTL mapping populations, and high marker density evenly distributed through the genome are common features for a successful QTL discovery and application in marker assisted selection (MAS).

MAS is a powerful and well established approach to transfer target genes or QTL from a donor to a recipient germplasm to increase the diversity of breeding programs (Frisch and Melchinger 2001; Cobb et al. 2019). However, MAS is not an efficient method to select superior genotypes for diseases with complex genetic architecture (Poland and Rutkoski 2016). On the other hand, genomic selection (GS) exploits whole-genome molecular profiles to predict genomic estimated breeding values (GEBVs) of phenotyped and un-phenotyped genotypes (Heffner et al. 2009). The potential of GS to correctly classify genotypes is often measured by the prediction accuracy between the training and prediction sets, and confirmed by cross validation.

Prediction accuracy is usually defined as the Pearson correlation between the GEBVs and the observed values divided by the square root of the heritability. Standard ridge regression best linear unbiased prediction (RR-BLUP) or genomic best linear unbiased prediction (GBLUP) models give equal weight to markers and do not distinguish between major- and minor-effect QTL. As an extension, weighted genomic selection, where few assumptive major QTL are considered as fixed effects, has been suggested (Bernardo 2014). Such an approach gives special weight to major QTL and often outperforms standard GS models for different traits and crops (Herter et al. 2019; Moreno-Amores et al. 2020; Gaikpa et al. 2020).

Fusarium spp.: A crop non-specific pathogen

Fusarium spp. is one of the most important crop pathogens worldwide. It affects many plant species such as rye (*Secale cereale*), triticale (×*Triticosecale* Wittmack), durum and bread wheat (*Triticum* spp.), and maize (*Zea mays* L.). *Fusarium* spp. is a hemibiotrophic pathogen which causes, among other diseases, Fusarium head blight (FHB), one of the most destructive and health-threatening diseases in small grain cereals (Goswami and Kistler 2004; Scherm et al. 2013). In addition, it is the causal pathogen of Gibberella ear rot (GER), a serious threat for maize production (Han et al. 2018). *F. graminearum* Schw. [teleomorph: *Gibberella zeae* (Schw.) Petch] and *F. culmorum* (W.G. Sm.) Sacc. are the main causes for FHB in Europe (Gaikpa et al. 2019). In Germany, *F. graminearum*, *F. verticillioides* (Sacc.) Nirenberg and *F. temperatum* (new species separated from *F. subglutinans*) (Scauflaire et al. 2011) are the main species causing ear rot in maize (Pfordt et al. 2020). *F. graminearum* and *F. culmorum* produce mainly deoxynivalenol (DON), DON derivatives, and zearalenone (ZON) (Castiblanco et al. 2020). *F. verticillioides* and *F. temperatum* produce mainly fumonisins (Scauflaire and Gourgue 2012; Alshannaq and Yu 2017). DON is a secondary methabolite from the group trichothecene

which damages the cell membrane, reduces protein synthesis and accelerates host cell death (Rocha et al. 2005; Kebede et al. 2018). DON reduces voluntary feed intake, ZON is an estrogen-like substance that causes fertility disorders including abortions in farm animals (Döll and Dänicke 2011) and fumonisins have carcinogenic properties (Gelderblom et al. 1993; Gaikpa and Miedaner 2019). Among farm animals, swine are the most sensitive to DON and ZON (Pierron et al. 2016). Triticale, wheat and maize are mainly vulnerable to Fusarium infections and often present high levels of DON and ZON (Döll and Dänicke 2011). As pigs consume a high percentage of these cereals, the European Union established an orientation limit of 0.9 mg DON kg^{-1} in pig feed (European Commission (EC) 2006; Pierron et al. 2016).

Fusarium head blight: A major challenge in triticale production

Triticale is an amphiploid man-made hybrid between durum wheat (× *Triticum turgidum* var. *durum*) as seed-parent and rye (*Secale cereale* L.) as pollen-donor parent composed by the wheat genomes A, B, and the rye genome R leading to $2n = 6x = 42$ chromosomes (Oettler 2005). In 2018, 3.8 million hectares and 12.8 million tons of triticale were produced globally (FAOSTAT 2020). About 90% of this production is concentrated in Europe where Poland, Germany and Belarus are the greatest producers (FAOSTAT 2020). However, this production can be potentially decreased due to damages caused by FHB and farm animals can be endangered by FHB mycotoxins (Becher et al. 2013). Among small grain cereals affected by FHB, triticale warrants special attention to the risk of mycotoxin contamination as this crop is mainly employed as a homegrown component for swine feed formulations and is usually not tested for DON content (Oettler 2005; Boeven et al. 2016; Pierron et al. 2016). Among crop protection measures, host-genetic resistance is one of the most efficient methods to increase FHB resistance levels.

FHB resistance in triticale is quantitatively inherited and few linkage mapping studies in biparental populations have been conducted to dissect its genetic architecture (Kalih et al. 2014, 2015; Miedaner et al. 2016; Dhariwal et al. 2018). On the other hand, GWAS can describe resistances in elite diverse panels for the identification of superior genotypes for direct application in breeding programs, but has not been conducted for FHB in triticale to date. In addition, FHB assessment in triticale is time consuming as it requires several scorings from flowering to full maturity stage, and the presence of strip rust on ears can hamper visual scoring in specific years (Miedaner et al. 2004; Losert et al. 2017). Therefore, selecting superior genotypes with complex traits by means of genomic selection is one of the most appropriate methods to efficiently increase selection gain (Meuwissen et al. 2001; Heffner et al. 2010; Jannink et al. 2010). However, few studies have investigated the potential of GS to accumulate favorable alleles for FHB resistance in triticale (Würschum et al. 2017). Hence, we conducted a GWAS and assessed the potential of different GS models to efficiently select superior triticale genotypes for FHB resistance.

Maize: A genetically diverse crop sowed worldwide

Maize is the world largest crop in production, a main source of feed, food and fuel, and the raw material of a number of products (Hallauer et al. 2010; USDA/IPAD 2020). In 2018, the world maize production amounted to 1147.62 and 11.52 million tons of grain maize and silage maize, respectively. Grain maize production corresponds to 38.7% of the total cereal production (FAOSTAT 2020). Europe is the third largest producer of grain maize with 128.6 million tons, just behind the U.S. and China, and the second producer of green maize, just behind the U.S. (3.3 million tons) with 2.8 million tons (FAOSTAT 2020). The high average yield of 7.5 tons ha^{-1} for grain maize and 23.9 tons ha^{-1} for green maize in Europe, world average is 5.9 and 9.8 tons

ha^{-1}, respectively, is partially attributed to the successful exploitation of the European Flint and U.S. Dent heterotic patterns, that leads to high yielding hybrids (Hallauer et al. 2010). As maize is grown worldwide, natural and artificial selections led to distinct varieties of maize that can be exchanged among geographical locations to increase genetic gains and safeguard maize against disease outbreaks (Kraja et al. 2000; Hallauer et al. 2010; Hallauer and Carena 2014).

Exotic germplasm: Source of novel resistance alleles to reduce vulnerability of plant cultivars to diseases

Dommestication and plant breeding have led to the reduction of allelic diversity increasing the vulnerability of plant cultivars to diseases (Hawbaker et al. 1997; Tanksley and McCouch 1997; Šimić 1999). For instance, since the devastating South corn leaf blight (incided by *Bipolaris maydis*) maize disease outbreak in the 1970's in the U.S. due to crop uniformity, identifying new sources of disease resistance became an important task to broaden resistance genetic base in many breeding programs (Kraja et al. 2000). Exotic germplasm is a potential and promising source of disease resistances (Kraja et al. 2000). In applied breeding, exotic germplasm can be defined as genotypes that are not adapted to the target environment and cannot be directly employed in elite single crosses (Carena and Hallauer 2001; Hallauer e al. 2010). Thus, exotic material is mainly untapped by breeding programs due to the absence of phenotypic and genotypic information of its merits, and lesser adaptability (Hallauer et al. 2010; Sood et al. 2014; Mayer et al. 2017). Whereas lower adaptability can limit the direct usefulness of exotic germplasm, while the introgression of few major QTL from the exotic to the adapted germplasm can accelerate trait improvement (Hallauer et al. 2010). Moreover, sampling selected exotic germplasm can optimize the

genetic diversity of a trait and increase the efficiency of new quantitative traits incorporation to elite background (Mayer et al. 2017). In the context of resistance breeding, Brazilian germplasm represents a promissing source of resistance alleles as frequent disease epidemics occur in the maize production areas. For instance, the Northern corn leaf blight (NCLB) epidemics in the Southwest of Brazil enabled selection for resistant genotypes (Poland et al. 2011; Romero 2016). The goal of this thesis was to assess genetic variation for GER and NCLB, two of the most important maize diseases in European temperate regions (Butrón et al. 2015; Romero 2016), in multiple environments in Brazil and in European countries (Austria, France, Germany and Italy).

Gibberella ear rot: Main cause of mycotoxin contamination in maize grown in Europe

Central Europe has a severe risk of mycotoxin contamination where 82% of the sampled maize finished feed and raw commodities were contaminated and presented an average of 903 ppb of DON (mycotoxin monitoring in Europe from January to March 2020, Biomin 2020). DON is mainly synthesized by *F. graminearum*, specie causing GER in maize. GER symptoms are white to pinkish mold starting on the tip of the cobs that may cover the entire cob in a susceptible genotype. As no fungicides are released to control Fusarium diseases in maize in Europe, host resistance is one of the most promising methods to control disease spread and mycotoxin accumulation (Bolduan et al. 2009; Pfordt et al. 2020). However, little effort in breeding resistant varieties against ear rot was made in the past and nowadays most of commercial hybrids have a lower ear rot resistance than desirable (Bush et al. 2004; Zila et al. 2013). Therefore, introduction of GER resistances from exotic germplasm can be highly beneficial to reduce GER damages and mycotoxin contamination levels in European maize, but few studies have been conducted to date.

Northern corn leaf blight: The most devastating maize leaf disease in Europe

NCLB is the most destructive maize leaf pathogen in Europe. The disease is caused by the hemibiotrophic heterothallic ascomycete *Setosphaeria turcica* (Luttrell) Leonard Suggs (anamorph form, *Exserohilum turcicum*). *S. turcica* originated from tropical regions and spread through Europe together with maize expansion (Vidal-Villarejo et al., 2021). First NCLB symptoms in Europe were observed in Italy in 1876 (Drechsler 1923; Jordan 1983), and in the Southwest of France in 1903, with epidemics every 10 years and yield losses between 20 and 25% (Cassini 1973; Welz 1998). In Austria and in Switzerland, first epidemics were observed in the 1990s on seed production and later on commercial fields (Zwatz 1988; Welz et al. 1996). In Germany, NCLB was first observed in 1995 in seed production fields in the upper Rhine valley (Welz 1998) and is nowadays the most important disease in the entire country. In Europe, clonal pathogen lineages were identified and assigned to four genetically distinct groups: French, Diverse, Small clonal and Big clonal. They differ in their genetic diversity (Diverse represents 82% of the total genetic diversity in Europe), split times within the groups (French had the latest split, in 1999) and the number of pathogenic races (Big clonal and Small clonal presented four different races) (Vidal-Villarejo et al., 2021). *S. turcica* can possess the virulence genes 0, 1, 2, 3, N, being the most complex race 123N already identified in Heilongjiang region in China (Ma et al. 2020). Hanekamp (2016) conducted a race monitoring in Europe in 2011 and 2012, and identified different race compositions where the complex races 12 and 23N were also present. In his study, race 0 was the most abundant race in the south of Germany/Austria, while races 3N and 3 in Southwest of France and north of Italy, race 1 in Austria/Hungary and Upper Rhine region were the most abundant in Germany. Another monitoring was conducted from 2017 to 2019 in Europe with samples from Germany, Southwest of France, north of Italy and Austria. Race 3 was predominant in the overall monitoring while a remarkable switch from race 0 to race 1 between 2017 and 2018 was

observed (Navarro, personal communication). Although this dynamic race composition can be related to sampling effects, it can also be attributed to the high evolution potential of *S. turcica* (McDonald and Linde 2002; Vidal-Villarejo et al. 2020). Therefore, regular race monitoring is essential to assist breeding decisions on the employement of host resistances.

Host resistance to NCLB can be both qualitatively or quantitatively inherited (Welz and Geiger 2000). The *Ht1* gene, first detected in 1961 (Hooker 1961), was effective for about 10 years, when the race 1, virulent to the gene *Ht1*, was identified in seed production fields in Hawaii (Bergquist and Masias 1974; Welz 1998). Other *Ht* genes such as *Ht2*, *Ht3* and *HtN* have a much shorter history, where in few years the disease was overcome by virulent *S. turcica* races. Gene pyramiding, stacking more than one *Ht* gene in one genotype, can increase the durability of the resistance genes (Sánchez-Martín and Keller 2019). However, more complex races (Hanekamp 2016) and the strong directional selection for pathogen virulence may render gene pyramiding ineffective (Pilet-Nayel et al. 2017). Employing the same QTL in large acreages may gradually increase pathogen aggressiveness levels and lead to resistance erosion (McDonald and Linde 2002). Therefore, quantitative resistance is a promising strategy to breed for NCLB resistance durably (Welz and Geiger 2000). The identification and employment of novel quantitative resistances are essential to reduce maize plants vulnerability to NCLB epidemics. In this context, Brazilian germplasm is a potential exotic source to increase NCLB resistance levels in European materials. However, resistance levels of Brazilian exotic germplasm in European field conditions for GER and NCLB have not been described to date.

Objectives

The goal of this thesis was to exploit the potential of genomics-assisted breeding strategies to increase the resistance levels against three important pathosystems: Triticale/*Fusarium culmorum*, maize/*Fusarium graminearum* and maize/*Setosphaeria turcica*.

In particular, the objectives were to:

1. Dissect the genetic architecture underlying FHB resistance in triticale and investigate the potential of genomic selection in applied triticale breeding programs
2. Review the current status of the maize/*Setosphaeria turcica* pathosystem to identify sustainable strategies for host-resistance management in breeding programs
3. Characterize Brazilian germplasm for GER and NCLB resistances, applying phenotypic and molecular approaches
4. Evaluate the potential of genomics-assisted breeding strategies for the introgression of GER and NCLB resistances from exotic to adapted backgrounds

2 Genome-wide association study for an efficient selection of Fusarium head blight resistance in winter triticale

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Abstract Fusarium head blight (FHB) is one of the most serious diseases in small-grain cereals including triticale (× Triticosecale Wittmack). The disease reduces yield and accumulates mycotoxins which are harmful to human and animal health. Triticale grain is almost exclusively used on-farm in feed

formulations for swine and other animals, and swine is the most susceptible farm animal to Fusarium mycotoxins. In order to evaluate the potential of genomics-assisted breeding to FHB, we performed the first genome-wide association study for FHB resistance in triticale. QTL for FHB resistance were identified on chromosomes 2A, 2B, 5B and 3R with an explained genotypic variance ranging from 0.28 to 30.23% and a total explained genetic variance of 56.64%. A QTL on chromosome 3R that explained 15.38% of the genotypic variance was identified for the first time. Association mapping was complemented by genome-wide prediction, which yielded a high prediction accuracy of 0.78 for FHB resistance when weighted genomic selection was performed. Collectively our findings highlight the potential of genomics-assisted approaches to improve Fusarium resistance in triticale in early generations.

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Multi-parent QTL mapping reveals stable QTL conferring resistance to Gibberella ear rot in maize

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Abstract Maize production is on risk by Gibberella ear rot (GER) caused by *Fusarium graminearum*. This is one of the most important ear rot diseases in temperate zones as it leads to yield losses and production of harmful mycotoxins. We investigated, for the first time, the potential use of Brazilian tropical maize to increase resistance levels to GER in temperate European flint germplasm by analyzing six interconnected biparental populations. We assessed GER symptoms in Brazil and in Europe in up to six environments (= location × year combinations) during the growing seasons of 2018 and 2019. We conducted multi-parent QTL and biparental QTL mapping, and identified four QTLs with additive gene action, each explaining 5.4 to 21.8% of the total genotypic variance for GER resistance. Among them, QTL q1 was stable across test environments, populations, and between inbred lines and testcrosses. The accuracies of genomic prediction ranged from 0.50 to 0.59 depending on the resistance donor and prediction model. Jointly, our study reveals the potential use of Brazilian resistance sources to increase GER resistance levels by genomics-assisted breeding.

Keywords Gibberella ear rot (GER) · *Fusarium graminearum* · Stable resistance · Genetic resources · QTL mapping · Genomic selection

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Introduction

Fusarium spp. is one of the most important crop pathogens in maize (*Zea mays* L.) reducing yield and threatening human and animal health by mycotoxins. These hemibiotrophic fungi can cause diseases during all growth stages of the plant including stalk and ear rots (Munkvold et al. 1997; Pfordt et al. 2020). *F. graminearum*, *F. verticillioides*, and *F. temperatum*, a new species separated from *F. subglutinans*, are the main species causing ear rot in temperate zones (Pfordt et al. 2020). The composition of species in each environment is mainly associated with weather conditions during silking. At this developmental stage,

F. graminearum is favored by high precipitation and moderate temperatures whereas *F. verticillioides* is favored by low humidity and high temperatures (Bottalico 1998; Munkvold 2003; Pfordt et al. 2020).

In NW Europe, the main use of maize is for animal feed as silage or corn-cob-mix (Deutsches Maiskomitee 2020). GER symptoms appear as white to pinkish mold starting on the tip of the cobs and may cover the entire cob in a susceptible genotype. *F. graminearum* produces mainly deoxynivalenol (DON), a mycotoxin causing reduction of voluntary feed intake and even vomiting, and zearalenone (ZEA), an estrogen-like mycotoxin that causes fertility disorders including abortions (Döll and Dänicke 2011). Among livestock, pigs are the main consumers of corn-cob-mix (Deutsches Maiskomitee 2020) and they are the most sensitive animals to these mycotoxins (Pierron et al. 2016). For this reason, the European Union established an orientation value of maximum 0.9 mg DON kg^{-1} for pig feed (European Commission 2006). However, this limit can be easily surpassed when the environmental conditions are favorable for the sporulation and spread of this pathogen (LSV Bayern 2019). For instance, Europe has a high to severe risk of mycotoxin contamination in animal feed where 83% of the maize samples were tested positive for DON in recent years (Biomin 2020). Little effort in breeding resistant varieties against ear rot was made in the past and nowadays most of commercial hybrids have a lower ear rot resistance than desirable (Bush et al. 2004; Mesterházy et al. 2012; Zila et al. 2013).

In the European Union, no fungicides are released to control Fusarium diseases in maize and, thus, agronomical practices such as ploughing and host resistance are the most promising methods to control disease spread and mycotoxin accumulation (Bolduan et al. 2009; Pfordt et al. 2020). Reduction of DON accumulation through resistance breeding has been observed for all maize maturity groups (LSV Bayern 2019; Löffler et al. 2009). Furthermore, genotypes with less DON accumulation do not negatively affect the expression of agronomical traits (Martin et al. 2012c), thus allowing breeding of high yielding cultivars (Vigier et al. 2001; Eller et al. 2008; Martin et al. 2012c).

For GER, uniquely quantitative resistance has been identified to date (Martin 2012; Gaikpa and Miedaner 2019). Several studies based on adapted germplasm have been conducted to dissect the genetic architecture of this trait. They identified QTLs explaining 21 to 59% of the total genotypic variance (Martin et al. 2011, 2012b; Giomi et al. 2016; Han et al. 2016, 2018; Kebede et al. 2016; Gaikpa and Miedaner 2019). Employing exotic germplasm may introduce new sources of resistance alleles to adapted European germplasm (Gaikpa and Miedaner 2019). Tropical and subtropical maize as well as popcorn populations are possible sources of resistant alleles for Fusarium ear rot for temperate maize breeding pools (Zila et al. 2013) and should be explored to achieve higher resistance levels. However, only few studies exploiting genetic resources to increase ear rot resistance have been conducted (Mesterházy et al. 2012; Zila et al. 2013; Butrón et al. 2015).

With the aim to identify QTL with a high environmental stability we evaluated six biparental populations originating from crosses between Brazilian resistant genotypes and European susceptible germplasm. In Brazil, two biparental populations comprising 273 double haploid (DH) lines were tested while four bi-parental populations comprising 486 hybrids were tested in Europe with one common resistance donor being the same. In particular, our objectives were to: (1) validate the use of Brazilian genetic resources in Europe; (2) dissect the genetic architecture of GER resistance in these sources; (3) verify the usefulness of genomics-assisted breeding to boost breeding progress for this complex quantitative trait.

Materials and methods

Plant material and field trials

Our experiments comprised six biparental populations: T3 × A6, T3 × A7, T3 × A8, T3 × A12, T4 × A4 and T4 × A5, with 99, 174, 155, 71, 110, and 150 individuals, respectively, each resulting from a cross between a GER resistance donor (Brazilian tropical DH line, "T") and a GER susceptible recipient (European adapted DH line, "A"). Recipients "A6", "A7", "A8" and "A12" belong to the stiff stalk synthetic (SSS) while recipients "A4" and "A5" belong to the non-stiff stalk (NSSS) heterotic groups, respectively. Populations T3 × A6 and T3 × A7 comprised 99 and 174 double haploid (DH) lines, respectively, and were assessed in Brazil as line per se in Campo Largo in 2018 and in Ponta Grossa in 2019,

both cities in Paraná state located in the southern region of Brazil. Jointly, 486 testcrosses from the other four biparental populations were evaluated in Europe in three locations: Monselice, Italy, and Gondelsheim and Bernburg, Germany, during the growing seasons of 2018 and 2019 (except by donor T4 which was assessed uniquely in 2019) leading to up to six testing environments, (= combination of location × year). All progenies intended to be tested in Europe were crossed with the same susceptible early flint tester aiming to establish chilling tolerance and an earlier maturity for the European testing locations. For simplification, we will refer to T3 × A6 and T3 × A7 as T3 donor, tested in Brazil as per se populations, to T3 × A8 and T3 × A12 as T3 donor, and T4 × A4 and T4 × A5 as T4 donor, both tested in Europe as testcross populations.

Our experiments were allocated in an alpha design with two replications where each experimental unit comprised a four-meter row with approximately 20 plants. Standard agricultural practices including insecticides and fungicides not being effective against Fusarium were applied at the Brazilian locations.

Inoculation and trait assessment

In Brazil, inoculum was kindly provided by Dr. Lygia Vitória Galli Terasawa (Federal University of Paraná, Curitiba, Brazil). The inoculum was obtained by isolating three sources of *Fusarium graminearum* from contaminated maize cobs collected at three different locations in the state of Paraná, Brazil, in the growing seasons 2015 and 2016. An inoculum suspension with a concentration of 50,000 conidia ml^{-1} containing these three inoculum sources was produced and 1 ml was inoculated into the maize silk channel. In Europe, the highly aggressive *Fusarium graminearum* strain IFA 66 was kindly provided by Prof. Dr. Marc Lemmens (University of Natural Resources and Life Sciences, Vienna, Austria) and used to prepare our inoculum suspension following the protocol of Reid et al. (1996). Two ml of the inoculum suspension containing 1.5×10^4 spores ml^{-1} were applied with a one-needle vaccinator on the silk channel of the maize cobs in the European locations. Both in Brazil and in Europe, ten plants of each experimental unit were inoculated, excluding the first plant of the row due to possible border effect, three to 6 days after the experimental unit was flowering. Rows were declared as flowering when at least 50% of the plants on the row presented extruded silks. Female flowering (FF) dates were collected for each row in a two-day interval.

Approximately 50 days after inoculation, cobs were dehusked and all plants were visually assessed for GER symptoms by estimating the percentage of the ear covered by mycelium (Fig. 1). The 10 non-inoculated plants were used as a control of the proportions of naturally infected cobs. The arithmetic means of the 10 assessed inoculated and the 10 control plants (= naturally infected), respectively, were employed for further statistical analyses.

Phenotypic data analysis

Phenotypic analyses for single environments were performed using linear mixed models and outlier detection procedures as proposed by Bernal-Vasquez et al. (2016). All GER phenotypic data were arcsine square root transformed to attend the normality assumption and reduce the heterogeneity of variances. Combined analysis without critical outliers (not more than 15% of the complete data were removed) were conducted according to the following mixed model:

$$y_{ijklm} = \mu + G_i + Y_j + L_k + LY_{kj} + LYR_{kjl} + LYRB_{kjlm} + e_{ijklm}$$

where y_{ijklm} was the phenotypic observation of the ith genotype, jth year, kth location, lth replication and mth incomplete block. The symbol μ represents the overall mean, G_i the effect of the ith genotype, Y_j is the effect of the jth year, L_k the effect of the kth location, and its interaction terms, R_l is the effect of the lth replication, B_m the effect of the mth incomplete block, and e_{ijklm} is

Fig. 1 Assessment scale of damaged maize cobs by GER. 0% represents healthy and 100% completely diseased cobs. The percentage is assigned depending on the percentage of the cob with GER symptoms

the heterogeneous error variance. The same model excluding the year effects was used for the single location analysis.

G_i and Y_j effects were included in the fixed statement of the model to obtain the best linear unbiased estimators (BLUEs). The variance components were obtained through the restricted maximum likelihood method (REML) by including only the Y_j effects in the fixed statement of the model above. The significance of the variance components was obtained by likelihood ratio test between the full and incomplete model (Stram and Lee 1994). Binary dummy variables were used to separate the effects of each population, checks and replicates as proposed by Piepho et al. (2006). For the sake of simplicity, they were not shown in the described model.

The broad sense heritability (H^2) was estimated following the formula:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_L^2}{L} + \frac{\sigma_Y^2}{Y} + \frac{\sigma_{LY}^2}{LY} + \frac{\sigma_e^2}{LYR}}$$

where σ_G^2, σ_L^2, σ_Y^2, σ_{LY}^2 and σ_e^2 are the genotypic, location, year, location $\times$ year and error variances, respectively. L, Y and R correspond to the number of locations, years, and replicates, respectively. Phenotypic correlations based on BLUEs were calculated with Pearson product moment correlation coefficients.

In the inbred populations tested in Brazil, the correlation coefficient between GER and FF was significant ($r = -0.49$ and $p < 0.001$) (data not shown). Therefore, GER was adjusted for FF by including FF as fixed effect in the mixed model to estimate the best linear unbiased estimators (BLUEs) as described by Emrich et al. (2008). After the corrections, the correlation between GER and FF was reduced to $r = -0.30$ ($p < 0.001$). This GER rating adjusted for FF (GER_FF) was used for all further analysis. In the testcross populations tested in Europa, the correlation coefficient between GER and FF was low and not significant ($r = -0.033$ and $p > 0.05$), therefore no corrections for FF were necessary.

All analyses were conducted in R environment (R Development Core Team 2018, version 3.5.1). Mixed-model computations were performed by using ASReml-R 3.0 (Gilmour et al. 2009).

Molecular data

DH lines were genotyped at KWS molecular laboratory with an Illumina 15 k SNP chip based on the public Illumina MaizeSNP50 BeadChip. The ten maize chromosomes were partitioned into bins of 0.5 cM (genetic map IBM, physical map AGPv02, Ganal et al. 2011) to construct the genetic map. Regions adjacent to centromers were especially markedly enriched to account for the low recombination rates in this chromosome area.

The number of polymorphic markers in each population ranged from 5832 to 7039. Quality control was conducted by removing monomorphic or missing alleles for both parents, genotypes with more than 25% missing values, markers with more than 10% missing data and markers with minor allele frequency (MAF) lower than 5% in each population. After the quality check, 4603, 5585 and 2784 SNP markers were available for the Brazilian crosses with T3, the European crosses with T3 and T4, respectively.

QTL mapping analysis

Multi-parent QTL mapping analysis was conducted with the R package mppR version 1.2.1 (Garin et al. 2018). By employing this package, interconnected biparental populations from each continent were analyzed jointly by the method of composite interval mapping (CIM) (Zeng 1993, 1994). We obtained the allele-substitution effect of the identified QTL through a bi-allelic model where alleles from different populations are considered to be identical by state (IBS), same SNP score transmitted the same allele for all individuals with common parents (e.g., model B in Würschum et al. 2012; Garin et al. 2017). For this model, population structure was accounted by the k-model proposed by Yu et al. (2006).

Permutation tests were conducted by performing 1000 iterations and the significance thresholds were obtained from the 90th percentile of the maximum LOD score distribution of all iterations (Broman and Sen 2009). QTL mapping for each model was conducted in a first step by a simple interval mapping (SIM) and the significant QTL from this analysis were applied as cofactors for the CIM. The confidence interval of each QTL was obtained by $-\log10\ (p)$ value drop off interval. The contribution of each QTL to the phenotypic variance was computed by

comparing the full model, containing all the QTL, and the incomplete model, excluding only the detected QTL of interest. Individual explained genotypic variance (p_G) were obtained following the equation proposed by Utz et al. (2000):

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

where R^2_{adj} corresponds to the adjusted R^2 from the linear model containing all identified QTL and H^2 to the average heritability of heritability estimates for individual populations with a common donor.

Biparental QTL mapping for population T3 × A8 was also evaluated individually with the software for meta-QTL analysis (PlabMQTL) (Utz 2011) by the CIM method, as population T3 × A12 was not included in the QTL analysis due to the low genetic variance. Additive and additive by additive epistatic models were investigated. Empirical thresholds for LOD scores were determined using 1000 permutation tests and assuming an experiment-wise error of 0.10. The selection of cofactors was done according to the modified BIC (mBIC) model (Baierl et al. 2006). The identified QTL were assumed as co-located when their confidence intervals overlapped.

Marker-assisted, genomic and weighted genomic predictions

Marker-assisted predictions were conducted for breeding values of testcrosses with all QTLs. Genomic prediction was carried out by ridge-regression BLUP (RR-BLUP; Whittaker et al. 2000) with the R package ''rrBLUP'' (Endelman 2011; Endelman and Jannink 2012) within each donor group. Missing SNP marker information was imputed for each donor group with the software LinkImpute (Money et al. 2015) and resulted in high imputation accuracies (> 90%). In addition, we performed a weighted ridge-regression BLUP (wRR-BLUP) where the same significant markers applied for marker-assisted predictions were included in the fixed statement of the genomic prediction model (Zhao et al. 2014; Spindel et al. 2016). The prediction accuracy was defined as the Pearson's product-moment correlation coefficient between observed and predicted trait values divided by the square root of the broad-sense heritability.

Results

Adjusted means for GER severity ranged, on average, from 4.9 to 10.0 for per se populations and 24.4 to 28.9% for the testcross populations (Table 1). Entry-mean heritabilities were moderate to high ranging from 0.68 to 0.72 for per se populations and 0.44 to 0.72 for testcross populations except for population T3 × A12 where the heritability was only 0.24 due to the non-significant genetic variation (Table 1). For this reason, the population T3 × A12 was not included in the QTL mapping analysis. Both GER_FF and GER showed a quantitative distribution with T3 being more resistant than the adapted parental lines (Fig. 2). Within Europe, most of the locations showed higher GER severity in 2019 compared to 2018 and this tendency was observed for all biparental populations (Fig. 3).

Jointly, we identified four QTL that explained 5.4 to 21.8% of the genetic variance, most of them had minor effects (< 15% p_G) only. They were located on chromosome bins 1.02, 3.08, 5.06, and 8.05. No dominance or additive × additive QTL were identified indicating uniquely additive QTL for GER_FF and GER in our study. QTL q1 was identified across all QTL analyses performed including different biparental populations, and line and testcross populations across both continents. QTL q1 explained between 10.2 and 21.8% of the genotypic variance where the highest variance was observed for population T3 × A8 (Table 2). Moreover, none of the identified QTL for GER were overlapping with the identified QTL for FF (data not shown).

Prediction accuracy by weighted genomic prediction (wRR-BLUP) was slightly higher compared to marker-assisted selection for both donors (MAS, Fig. 4). Prediction accuracy for GER in testcrosses with donor T3 were of 0.53, 0.50 and 0.59 estimated for MAS, RR-BLUP and wRR-BLUP, respectively. Lower prediction accuracy for MAS (0.47), wRR-BLUP (0. 57) and RR-BLUP (0.55) was obtained for testcrosses with donor T4. For both donors, the wRR-BLUP method led to the highest prediction accuracies. For population T3 × A8 only wRR-BLUP led to slightly improved predictions compared to MAS (data not shown).

Table 1 Statistics summary and variance components for GER_FF (arcsin transformed Gibberella ear rot adjusted for female flowering date, original data without transformation in parentheses) of two populations of DH inbreds evaluated in Brazil and GER (arcsin transformed Gibberella ear rot) of testcrosses of four DH inbred populations evaluated in Europe

Trait	Brazil			Europe		
Pop	T3xA6	T3xA7	T3xA8	T3xA12	T4xA4	T4xA5
No. env.	*2*		*6*		*3*	
Phenotypic data						
Mean	4.94 (7.66)	10.01 (10.99)	27.81 (31.20)	28.86 (33.45)	28.61 (31.99)	24.33 (27.63)
Median	3.25 (2.64)	8.00 (8.30)	27.96 (31.50)	27.80 (31.52)	28.23 (30.57)	23.65 (26.60)
Min	0.16 (0.00)	0.00 (0.00)	5.23 (6.83)	10.70 (11.49)	4.12 (5.05)	5.89 (6.25)
Max	47.56 (51.13)	76.24 (77.50)	59.26 (55.00)	79.30 (77.99)	62.41 (63.49)	62.96 (61.75)
$LSD_{5\ \%}$	2.38	2.38	4.34	4.34	4.34	4.34
n	99	174	155	71	110	150
Variance components						
σ^2_G	0.01***	0.04***	0.010***	0.002	0.007***	0.007***
$\sigma^2_{G\times L}$	0.01***	0.01***	0.001	0	0.012***	0.003*
$\sigma^2_{G\times Y}$	–	–	0	0	–	–
$\sigma^2_{G\times Y\times L}$	–	–	0.008**	0.018***	–	–
σ^2_e	0.01	0.01	0.060	0.060	0.03	0.03
H^2	0.72	0.68	0.61	0.24	0.44	0.54

Gibberella ear rot was estimated as the percentage of ear affected. Minimum (Min), median, mean, and maximum (Max.) scores are shown for the backtransformed phenotypic data. Number of genotypes (n) and least square of a difference (LSD5%) are also indicated. The variance components include the genetic (σ^2_G), genotype-location ($\sigma^2_{G\times L}$), genotype-year ($\sigma^2_{G\times Y}$), genotype-year-location interactions ($\sigma^2_{G\times Y\times L}$), and residuals ($\sigma^2_e$) variances. Entry mean heritability (H^2) for each population are also assigned

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

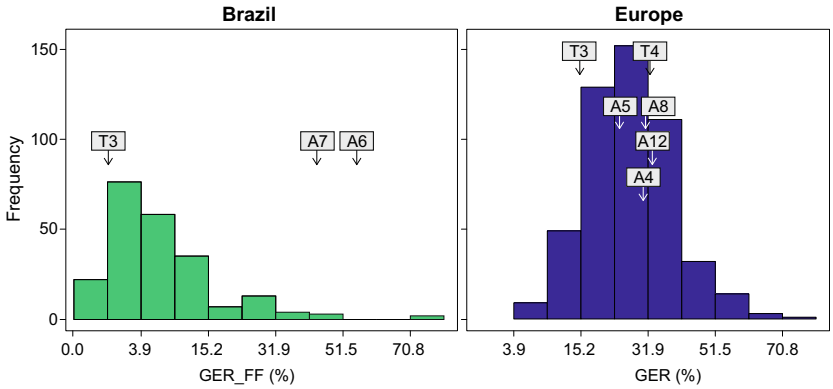

Fig. 2 Phenotypic distribution of the backtransformed Gibberella ear rot (GER) data assessed in Brazil adjusted for female flowering date (FF) and in Europe; pointing the respective tropical (T) and adapted (A) parental lines by arrows

Discussion

The extension of maize acreage to attend the increasing demand in combination with short crop rotations including the Fusarium susceptible wheat will increase the risk of ear rots by *Fusarium* spp. and subsequent mycotoxin contamination in the near future (Ray et al. 2013; Pfordt et al. 2020). To keep

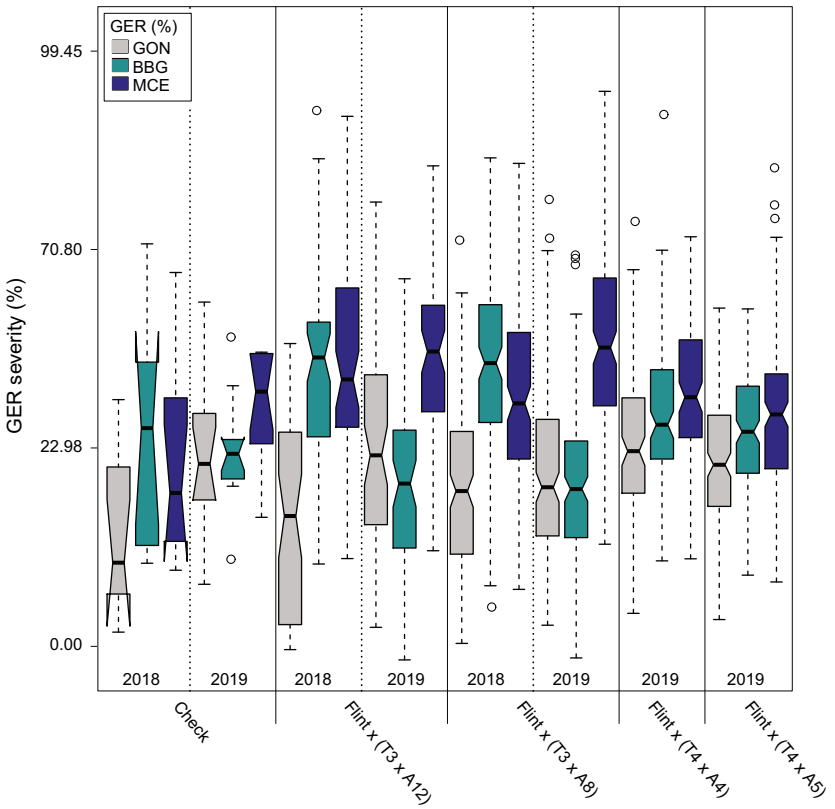

Fig. 3 Box plots for Gibberella ear rot (GER) severity (backtransformed values) of different biparental populations evaluated in Europe in six environments (year-location combinations, environments: GON = Gondelsheim/DE, BBG = Bernburg/DE, MCE = Monselice/IT; in 2018 and 2019). Horizontal lines within boxes indicate the median, black squares refer to outliers. The checks comprised parental lines and commercial resistant and susceptible hybrids

Table 2 QTL for Gibberella ear rot resistance identified across populations sharing the same inbred donor and inbreeding level (multi-parent QTL mapping

Population	n_G	n_M	Type	QTL	Bin	QTL (cM)	Range (cM)	LOD score	p_G (%)	α-effect
Brazil										
T3 × A6_A7	266	4603	PS	q1	1.02	60.54	58.89–62.92	5.57	10.17	− 0.96
T3 × A6_A7	266	4603	PS	q2	3.08	196.72	194.99–197.03	4.63	14.86	− 1.33
T3 × A6_A7	266	4603	PS	q3	5.06	162.53	161.56–162.71	4.65	5.37	− 0.43
Europe										
T4 × A4_A5	229	2784	TC	q1	1.02	58.64	50.40–85.62	3.74	10.92	0.35
T4 × A4_A5	229	2784	TC	q4	8.05	120.04	119.75–120.56	3.78	11.67	0.35
T3 × A8	145	5585	TC	q1	1.02	60.00	59.93–61.04	6.56	21.84	− 0.34

Populations T3 × A6 and T3 × A7 were written as T3 × A6_A7 for simplification, as well as populations T4 × A4 and T4 × A5, T4 × A4_A5) or T3 × A8 (with PLABMQTL), number of genotypes (n_G), number of markers used (n_M), type of population assessed (PS for per se and TC for testcrosses), QTL location (cM), QTL confidence interval range (cM), explained genotypic variance (*pG*) and the backtransformed allele substitution effect (α-effect) of the tropical parent for GER_FF assessed in Brazil and GER assessed in Europe. Bolded name indicates co-located QTL

resistance levels high in the long-term, it is essential to employ diversified resistance sources (Nelson et al. 2018). Tropical maize, including Brazilian germplasm, could be valuable sources of resistance alleles

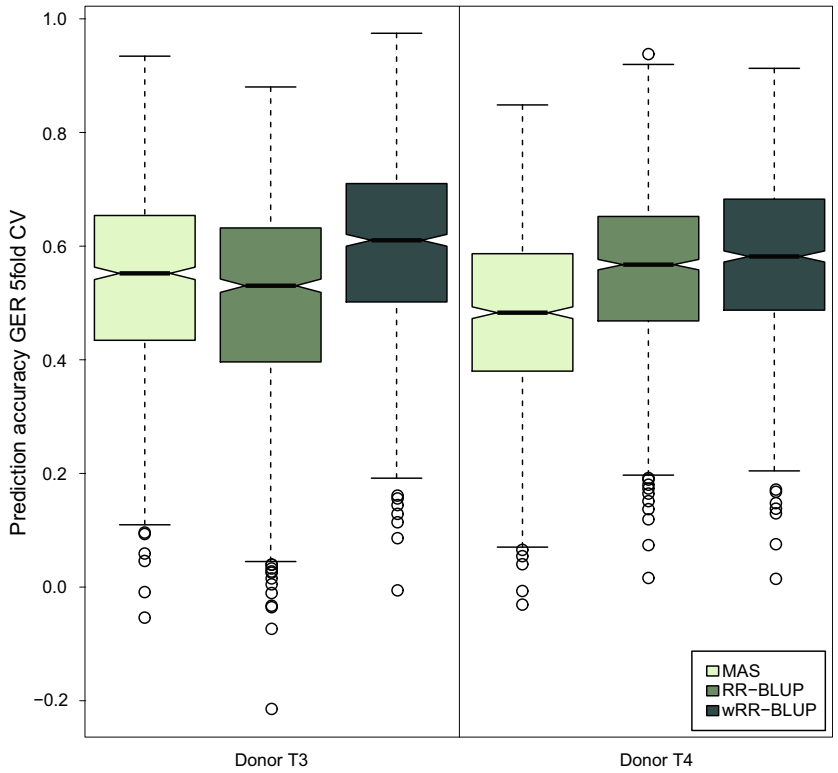

Fig. 4 Prediction accuracies obtained from marker assisted selection (MAS), genomic selection (RR-BLUP) and weighted genomic selection (wRR-BLUP) for each donor group and continent for testcrosses

for temperate germplasm (Hallauer et al. 2010; Poland et al. 2011), but are not yet fully exploited. Therefore, we investigated the potential use of Brazilian sources as GER resistance donors for European flint maize. Aiming for environmentally stable resistances we tested a total of six interconnected biparental populations both in Brazil and in Europe.

Assessing GER in contrasting environments

In Brazil, tropical parent "T3" showed higher resistance levels compared to the European adapted parents "A6" and "A7" as expected. However, the population mean for GER damage was low in both environments. This could be explained by the concentration of spores. We applied 50,000 spores ml^{-1} in each maize ear in the experiments located in Brazil, but an even higher concentration might be necessary to increase disease severity. Still, genetic variance was significant with moderate to high heritabilities. Conversely, in Europe, the genetic variance was lower than in Brazil and only the tropical parent T3 was more resistant than the adapted lines. The tropical parent T4 and adapted European parent inbred lines, however, were similarly susceptible.

Our phenotypic data was assessed after inoculation of maize cobs through the silk channel. This is the most important infection pathway for *F. graminearum* in the absence of insect injury and the most common in the northern maize growing regions (Reid et al. 1992, 1996; Munkvold et al. 1997; Bolduan et al. 2009). However, this method has the disadvantage to be unstable across different weather conditions (Reid et al. 1996; Mesterházy et al. 2012; Butrón et al. 2015). This can be one of the reasons why the GER severity was lower in 2018 compared to 2019 for most of the European locations.

The genotype × environment interactions were high and significant both in Brazil and in Europe. This is in accordance with other studies where

resistance was found to be variable when assessing GER resistance in several contrasting environments (Bolduan et al. 2009; Löffler et al. 2009). Independent selection for each geographic region was recommended (Butrón et al. 2015) and is practiced in Europe according to the different breeding programs assigned to each maturity group. In our study, we assessed phenotypic data in up to six contrasting environments as the main objective of this research work was to identify stable resistance QTL that are effective even in the current global warming conditions.

QTL mapping reveals stable QTL across continents, environments, and populations

We identified four QTL explaining between 5.37 and 21.84% of the GER genotypic variance where most of them had minor effects ($< 15\% p_G$). This is in accordance with other studies that identified many QTL with small effects and a global explained genotypic variance varying between 21% and 59% for GER resistance (Martin et al. 2011, 2012b; Kebede et al. 2016; Gaikpa and Miedaner 2019). Martin et al. (2012a) identified QTL explaining between 21 and 49% of the global genotypic variance in three biparental European populations with no common QTL identified across populations. QTL q1 was identified across populations. In addition, this QTL had a major effect on population T3 × A8 and could alone explain 21.8% of the genotypic variance. The favorable allele originated from the tropical parent T3 indicating that this Brazilian donor can be a great source of stable QTL for GER resistance. However, we identified only a low number of QTL. This might indicate that possibly each family was segregating for a different set of QTLs and/or other genomic regions conferring resistance to GER could not be identified due to the highly quantitative nature of this trait (Blanc et al. 2006; Ogut et al. 2015; Han et al. 2016). However, we also conducted a QTL mapping for each family separately (data not shown) and did not identify a larger number of QTL. Another main reason might be that only few QTL are stable across six European environments including two very contrasting years and field locations (northern Italy and Germany). This conclusion is supported by the high genotype × environment interaction variances.

QTL conferring GER resistance were identified on chromosome bins 1.02, 3.08, 5.06 and 8.05 in our study, namely QTL q1, q2, q3 and q4, respectively (Table 1). The QTL q1 identified across environments and populations is located in a genomic region known to confer resistance to ear rot caused by multiple pathogens (Wisser et al. 2006). QTL q2 was identified in the same bin position previously reported to significantly contribute to GER resistance and reduced DON contamination, while the QTL on chromosome bin 5.06 was in the same bin as a QTL previously reported to be associated to DON contamination (Martin et al. 2012; Martin et al. 2012b). Kebede et al. (2016) identified one QTL for GER resistance near the QTL identified on chromosome bin 8.05. Overlapping QTL between GER and DON are expected as both traits are highly correlated ($r > 0.86$; Butrón et al. 2015; Miedaner et al. 2015). This was confirmed by co-located QTL for GER resistance and reduced DON contamination in QTL mapping studies suggesting that both traits are likely to be controlled by a set of the same genes (Martin et al. 2012b; Han et al. 2016). Additionally, different genes might also play a role in GER resistance and reduced DON accumulation (Gaikpa and Miedaner 2019).

Our germplasm included families belonging to the SSS and NSSS heterotic groups, comprising populations of donors T3 and T4, respectively. We identified a larger number of QTL within the SSS group compared to the NSSS, but this is probably due to the unbalanced number of families per heterotic group in our study with four families from SSS and two from NSSS, and the GER severity discrepancy between the parental components of each heterotic group. Conversely, other studies identified that the flint germplasm was more susceptible to ear rot and showed higher DON and ZEA concentrations compared to the dent pool. These differences were assigned to the few founding populations composing the flint pool compared to the dent pool which had a constant influx from germplasms from other regions (Reif et al. 2005; Löffler et al. 2010).

The major infection pathway of *F. graminearum* is via the silks, but some species such as *F. verticillioides* can infect cobs after silking additionally via insect injuries on the cobs (Reid et al. 1992, 1996; Pfordt et al. 2020). Kebede et al. (2016) investigated infection by *F. graminearum* both through silk and kernels and identified only three QTLs overlapping for both infection pathways. These co-located QTL were

identified on chromosomes 1, 2, and 8, where the QTL on chromosome 8 was identified in a close location to our QTL q4 (Kebede et al. 2016). With rising temperatures due to global climatic change damage by insects might increase in frequency and severity, especially in the tropics and subtropics (Juroszek and von Tiedemann 2013). For this reason, the identification of QTL that are common among different infection pathways can lead to a broader resistance.

In summary, the QTL identified in our study showed mainly additive effects and no additive × additive epistasis. This is in accordance with other studies where GER was found to be controlled by several additive QTL (Martin et al. 2012a) and epistatic gene effects were of little importance in most of the testing environments (Butrón et al. 2015). Therefore, mainly additive and dominance effects should be considered in a breeding program aiming to increase ear rot resistances and decrease mycotoxin accumulation (Butrón et al. 2015). In a study of GER resistance in maize, mid-parent heterosis was observed indicating partial dominance (Martin et al. 2012c). This is in accordance to results of Gendloff et al. (1986) and Chungu et al. (1996) who identified dominance and dominance × dominance gene action although additive effects were more important.

Genomics-assisted breeding can successfully select superior resistant genotypes for GER

QTL q1 alone explained 21.8% of genetic variation for GER in testcrosses of the mapping population T3 × A8, 10.2% across per se populations derived from T3 × A6 and T3 × A7, and 10.9% across testcrosses of the mapping populations derived from T4 × A4 and T4 × A5. Therefore, genomic selection did not lead to a significantly higher prediction accuracy compared to the marker assisted selection approach (Fig. 4). It is important to notice that our prediction accuracies might be overestimated as the same germplasm was composing both the training and prediction sets. In addition, the phenotypic data of all genotypes were collected in the same environments which may not illustrate the reality of commercial breeding programs. Moreover, before the application of the identified QTLs in MAS a QTL validation is necessary. Brauner et al. (2016) conducted the first validation study for QTLs on GER resistance. They tested six QTL identified in a previous mapping study and introgressed them into two different genetic backgrounds. Resistance alleles at three QTLs significantly increased resistance to GER, but the effects were significant only for a small subset of lines due to linkage drag and/or epistasis with residual loci in non-target regions.

To date, only two studies conducted a genomic selection for GER resistance in maize (Gaikpa and Miedaner 2019). Riedelsheimer et al. (2013) investigated the influence of the training set (TS) composition on the prediction accuracy of agronomic traits and GER on five interconnected biparental DH populations. They identified a decline on prediction accuracy when full-sibs were replaced by half-sibs in the TS. In our analysis, the prediction accuracy of genomic selection was slightly higher for donor T3, for which the TS was composed by the same families of the validation set. The TS of donor T4 was composed by two biparental populations with one common tropical line and had slightly lower predictions than donor T3 (0.50 for T4 and 0.55 for T3). Han et al. (2018) reported that increasing the TS set size with genetically distant individuals, in this case of the opposite heterotic group, did not improve the genomic prediction of GER resistance.

Conclusions

In this research project we tested two Brazilian lines as resistance donors of GER. The tropical parent T3 was resistant even in northern Italian and German locations illustrating the independence of this resistance source from environment. QTL q1 was proven to be stable across populations and continents explaining 10.2 to 21.8% of the genotypic variance of GER resistance depending on the situation. An independent validation of this QTL would be very valuable. In addition, genomics-assisted breeding can boost selection for GER resistance by wRR-BLUP. Given the different maturity groups and other adaptation problems of tropical germplasm, however, marker-assisted backcrossing of q1 might be recommendable to integrate this prominent QTL into adapted European germplasm.

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Authors' contributions TM, TP and BK planned the experiments and supervised the project. BK, DSG and MBK supported data collection. AG collected phenotypic data, conducted all statistical analyses, and wrote the manuscript, TM edited it. All authors read the final version for publication.

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Compliance with ethical standards

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

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REVIEW
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Genetics of Resistance and Pathogenicity in the Maize/*Setosphaeria turcica* Pathosystem and Implications for Breeding

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Northern corn leaf blight (NCLB), the most devastating leaf pathogen in maize (*Zea mays* L.), is caused by the heterothallic ascomycete *Setosphaeria turcica*. The pathogen population shows an extremely high genetic diversity in tropical and subtropical regions. Varietal resistance is the most efficient technique to control NCLB. Host resistance can be qualitative based on race-specific *Ht* genes or quantitative controlled by many genes with small effects. Quantitative resistance is moderately to highly effective and should be more durable combatting all races of the pathogen. Quantitative resistance must, however, be analyzed in many environments (= location × year combinations) to select stable resistances. In the tropical and subtropical environments, quantitative resistance is the preferred option to manage NCLB epidemics. Resistance level can be increased in practical breeding programs by several recurrent selection cycles based on disease severity rating and/or by genomic selection. This review aims to address two important aspects of the NCLB pathosystem: the genetics of the fungus *S. turcica* and the modes of inheritance of the host plant maize, including successful breeding strategies regarding NCLB resistance. Both drivers of this pathosystem, pathogen, and host, must be taken into account to result in more durable resistance.

Keywords: *Exserohilum turcicum*, genomic selection (GS), *Ht* genes, marker-assisted selection (MAS), northern corn leaf blight (NCLB), recurrent selection (RS), resistance breeding

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INTRODUCTION

Setosphaeria turcica (Luttrell) Leonard and Suggs (syn. *Helminthosporium turcicum,* teleomorph *Exserohilum turcicum* [Pass.] Leonard and Suggs), subclass Loculoascomycetidae, order Pleosporales, is a heterothallic ascomycete overwintering on host plant debris as dormant mycelium or as chlamydospores in the soil (Leach et al., 1977). Primary infections result from

Abbreviations: AUDPC, area under the disease progress curve; BLUP, best linear unbiased predictor; CMS, cytoplasmic-male sterility; DAMP, danger-associated molecular patterns; ETI, effector-triggered immunity; GBLUP, genomic best linear unbiased predictor; GCA, general combining ability; GEBV, genomic estimated breeding value; GS, genomic selection; GWAS, genome wide association study; MAS, marker-assisted selection; NBS-LRR, nucleotide-binding site-leucine-rich repeat; NCLB, northern corn leaf blight; PAMP, pathogen associated molecular patterns; PRR, plasma membrane-anchored pattern recognition receptors; QTL, quantitative trait loci; RS, recurrent selection; SCAR, sequence characterized amplified region; SCLB, southern corn leaf blight; SNP, single nucleotide polymorphism; SSR, single sequence repeat.

airborne conidia produced on maize debris which are transported by wind, rain, and seed borne inoculum (De Rossi and Reis, 2014). Infections are favored by temperatures between 15 and 25°C, dew periods of at least 4 h and 90–100% relative humidity (Levy and Cohen, 1983; Bentolila et al., 1991; Ogliari et al., 2005). The fungal mycelium penetrates directly the leaf cuticle and epidermis (Setyawan et al., 2016). Hyphae grow intracellularly into the mesophyll, proceed to vascular bundles, penetrate the xylem (Jennings and Ullstrup, 1957) and secrete HT (from *Helminthosporium turcicum*) toxin. HT toxin is composed of water soluble low molecular weight compounds inhibiting chlorophyll synthesis and are, therefore, phytotoxic (Bashan et al., 1995; Li et al., 2016). HT toxin is an important factor affecting pathogenicity, the pathogen's ability to infect a resistant host, and virulence, which is the possibility to overcome non-specific host resistance genes (Vanderplank, 1984; Wathaneeyawech et al., 2015b). Moreover, the toxin induces disease symptoms and is associated with fungal aggressiveness (Bashan and Levy, 1992), the quantitative ability of a fungus to cause infection in the host (Vanderplank, 1984; Becher et al., 2013). This qualitative interaction between the resistance (*R*) gene of the host, and the Avirulence (*Avr*) gene of the pathogen directly affects conidial germination and ramification, and increases lesion size when the phytotoxin concentration is >250 ppm (Bashan et al., 1996). Hence, HT toxin is non-host specific (Yoka and Albertini, 1975; Petitprez et al., 1984; Bashan et al., 1995) and can affect many host plants (Mitchell, 1984).

About 14 days after infection, depending on host, pathogen, and environment, the first symptoms appear and expand further to a 2–30 cm long elliptical lesion of gray-green color which turns tan brown parallel to leaf margins (Welz, 1998). When no host resistance is available and optimal infection conditions persist, these lesions can coalesce and the entire leaf becomes blighted (**Figure 1**; Mengesha, 2013).

In the field, maize lesions grow 1.6–3.9 times faster at night than at the day, thus a day length shorter than 12 h enhances lesion growth. This is one factor making NCLB so severe in tropical and subtropical regions (Leach et al., 1977). Highly aggressive *S. turcica* isolates, however, can compensate suboptimal weather conditions resulting in severe epidemics also in temperate zones (Welz, 1998). In dead leaf tissue, sporulation commences with cloudy sky and 12 h day length as well as an extended period of high humidity (>90%) and a minimum of 14 h of dew period, resulting in higher spore production (Leach et al., 1977; Welz, 1998). This secondary inoculum spreads to other maize leaves, thus continuing the infection cycle.

Yield losses caused by NCLB depend on (i) host growth stage when the infection occurs, (ii) disease severity governed by the epidemic situation (Perkins and Pedersen, 1987), (iii) leaf insertion, (iv) level of host plant resistance, and (v) pathogen aggressiveness. Generally, yield losses are highest, when infection occurs before silking (Fajemisin and Hooker, 1974; Raymundo and Hooker, 1981; Ding et al., 2015) and the cob leaf is damaged (Welz, 1998). The percentage of yield loss due to the reduction in photosynthesis of the injured leaves under NCLB infection was around 63, 43, and 17% for an early maturing susceptible hybrid, a hybrid with quantitative resistance and intermediate maturity, and a hybrid with quantitative and qualitative resistances combined and late maturing, respectively (Raymundo and Hooker, 1981; Levy and Pataky, 1992). Additionally, NCLB may cause a reduction of feeding value and increases pre-disposition of maize to stalk rot (Hooker et al., 1965; Fajemisin and Hooker, 1974). To reduce these negative effects, fungicides, biological control, improved management practices, and resistant cultivars can be used.

Some carboxamides (Iprodione), phenylpyrroles (Fludioxonil), and sulfur compounds (Thiram) are the most efficient fungicides against *S. turcica* mycelium growth, the latter two are used in maize seed treatment (Rossi et al., 2015). Wathaneeyawech et al. (2015a) found that spraying contact fungicides (Chlorothalonil, Mancozeb) or azoles (Difenoconazole) 7 days before inoculation was the best timing with Difenoconazole being the most effective fungicide. Robertson and Pecinovsky (2016) demonstrated that the application of fungicides at five-leaf stage and at visible silk stage results in reduction of 50% in NCLB severity compared to the non-treated control or to application in five-leaf stage only. However, application of fungicides in maize is costly and can represent a risk to the farmer and to the environment when not handled properly.

Some *Bacillus* and *Enterococcus* species reduce *S. turcica* growth effectively (Sartori et al., 2015) and can be used as biological control agents. Moreover, chaetoglobosin A and chaetoglobosin C, metabolites produced by *Chaetomium globosum* N°05 strain (Ascomycota), have been reported to prevent symptom development on detached maize leaves (Zhang et al., 2013). Further research is necessary to identify the effect of these agents under field conditions and optimize their efficiency, their stability, and to address security issues (Zhang et al., 2013).

Among the management practices, tillage is the most important. In the last decades, reduced tillage or even no-tillage systems were largely exploited by farmers to prevent soil from erosion and to save time and costs. Consequently, the plant debris remains on the soil and enable the viable propagules of many fungi including *S. turcica* to survive the period where no host plant is grown. Tillage practices, therefore, indirectly reduce NCLB incidence and severity in the following crop (Sumner et al., 1981). Given this complex situation, only an integrated management system with improved cultural practices (crop rotation, burial or removal of crop residues) and resistant cultivars as the most important components should effectively control NCLB and avoid significant economic damage (Welz, 1998).

Resistant cultivars are important to control NCLB since they do not present additional costs for the farmer, do not harm the environment and reduce costs of seed production. Varietal resistance occurs in two forms in this pathosystem: (i) qualitative resistance governed by single, race-specific genes called *Ht* genes, and (ii) quantitative resistance, controlled by several to many genes each of which has only a small impact on disease resistance. In commercial cultivars both forms of resistance can be present.

Epidemiological aspects and management practices have been recently reviewed in detail by Hooda et al. (2017). This review,

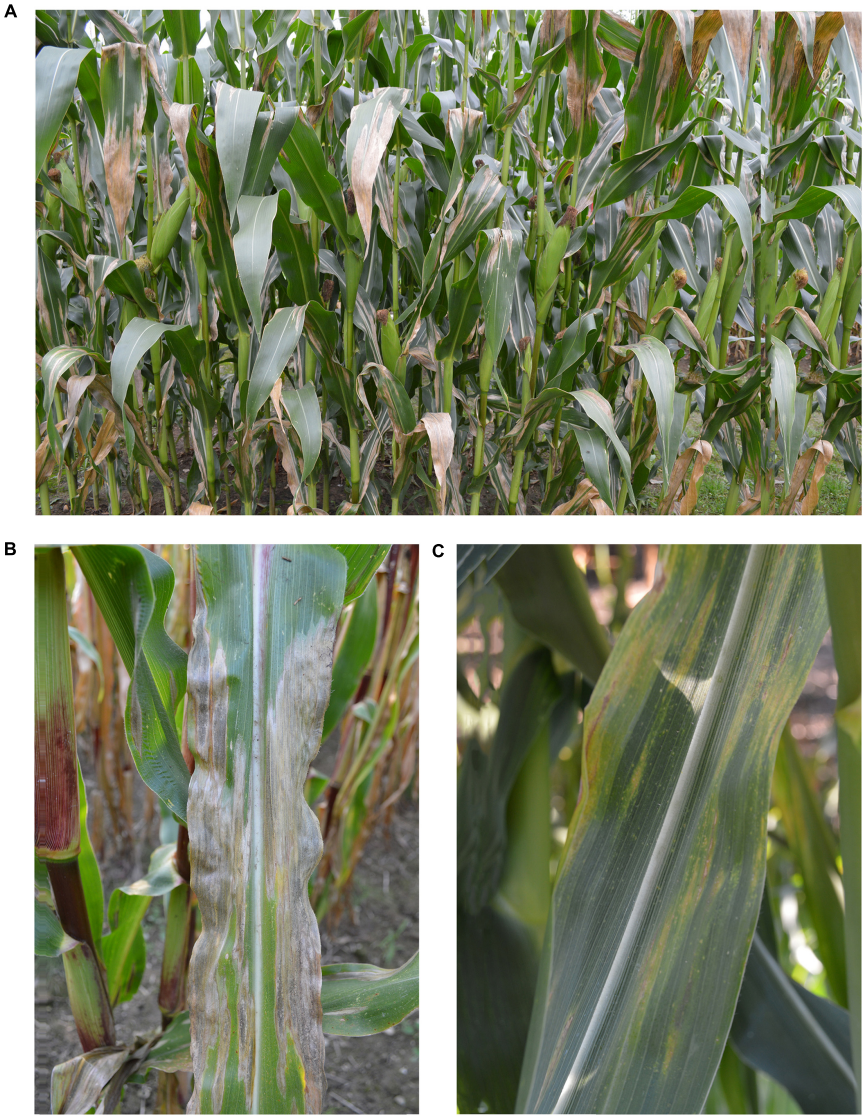

FIGURE 1 | Northern corn leaf blight symptoms. **(A)** Symptoms of *Setosphaeria turcica* on a maize field with a susceptible cultivar. **(B)** Single leaf of a susceptible cultivar with symptoms of *S. turcica,* and **(C)** Single leaf with resistance reaction (Photos: Dr. Lucia Ramos-Romero, University of Göttingen, Germany).

therefore, concentrates on population genetics of the fungus and resistance of the host including consequences for breeding. A high genetic variation in pathogenicity is indicative for a high evolutionary potential of a pathogen providing the basis for adaptation to fungicides and single resistance genes (McDonald and Linde, 2002). This often leads to low durability of resistances and, therefore, both drivers of this pathosystem must be analyzed to result in a sustainable management of resistance.

GENETIC VARIATION OF *Setosphaeria turcica* POPULATIONS

Setosphaeria turcica populations are distinct among continents. In Mexico, the highest molecular diversity was found compared to *S. turcica* samples from Kenya, southern and northern China, Germany, Switzerland, France, and Austria. Mexico, therefore, is most likely the center of origin (Borchardt et al., 1998a).

Tropical populations from Kenya, Mexico, and southern China are, in general, more genotypically diverse, have a lower gametic phase disequilibrium and a more even distribution of mating types when compared to temperate populations from Europe and northern China (Borchardt et al., 1998a). In addition, in the tropics, no clonal lineages were identified while in Europe, one third of the isolates had the same haplotype (Borchardt et al., 1998b).

Natural occurrence of the sexual stage, *Exserohilum turcicum*, was first reported in Thailand in 2013 (Bunkoed et al., 2014). Sexual hybridization enhances pathogen virulence by combining diverse virulences and generating new races (Bunkoed et al., 2014), thus playing a key role for pathogenic variation. The mating type is controlled by a single locus with two alleles (MAT-1 and MAT-2; Nelson, 1959). In tropical environments, an equal proportion of MAT-1 and MAT-2 was observed suggesting a frequent sexual hybridization that leads to a higher adaptation potential compared to temperate areas (Borchardt et al., 1998a). The reason why sexual hybridization occurs mainly in the tropics is still unknown.

Ferguson and Carson (2004) evaluated the diversity of *S. turcica* in the United States. by analyzing 251 maize isolates collected in the fields of 19 Eastern United States. A high pathogenic diversity was observed indicating the existence of sexual reproduction and a long-distance migration between states (Ferguson and Carson, 2004). The presence of nearly equal proportions of MAT-1 and MAT-2 alleles in some and dominance of MAT-1 or MAT-2 in other United States indicates the presence of both sexually and asexually reproducing populations depending on the region. Sexual reproduction tends to occur in the Southern United States, where the average annual temperature is higher, rather than in the Corn Belt (Ferguson and Carson, 2004).

Since *S. turcica* populations behave in large parts panmictic, it is impossible to identify diagnostic markers of virulence since recombination rapidly breaks down associations between the markers and the genomic region of interest. These markers would work, therefore, only with strictly asexual multiplication (Welz, 1998) or when directly placed within the avirulence gene.

The potential of a pathogen to adapt to quantitative disease resistances should be proportional to the level of genetic variation present in the fungal population (Fisher, 1930). According to McDonald and Linde (2002) pathogens with a mixed, i.e., sexual and asexual, reproductive system, high potential of genetic flow, and large population sizes are more likely to overcome host resistance and are, therefore, considered as "high-risk" pathogens. All these evolution forces apply for tropical *S. turcica* populations (Bergquist and Masias, 1974; Thakur et al., 1989; Borchardt et al., 1998a) resulting in highly diverse populations with a high probability of adapting to single-site fungicides or monogenic *Ht* genes.

QUALITATIVE RESISTANCE TO NCLB

The first element of plant defense against pathogens is based on PRR. PRR monitors the extracellular presence of PAMPs or DAMPs. When PAMPs or DAMPs are recognized by PRR, a signaling cascade response starts (Hurni et al., 2015). The pathogen has specific effectors that are injected into the host cytoplasm and suppress this plant response. When host proteins from the NBS-LRR family, like those coded by the *Ht* genes, recognize these effectors intracellularly, a second signaling cascade response starts (McHale et al., 2006; Dangl et al., 2013; Hurni et al., 2015) usually resulting in the death of the infected cell due to a hypersensitivity reaction. This reaction turns out to be qualitative and leads to "vertical" or race-specific resistance. The pathogens' mutation from avirulence to virulence leads to a modification or suppression of these specific effectors. Consequently, the host plant cannot recognize the presence of the pathogen anymore leading to infection and subsequent pathogen reproduction. Due to its selective advantage the fungal subpopulation containing the virulence mutation can rapidly increase. When an *Ht* gene is not effective anymore due to a high frequency of the virulence mutation the resistance is colloquially called "broken" (McDonald and Linde, 2002), but indeed it is only ineffective due to a change in the pathogen population.

TABLE 1 | Gene-by-gene interaction between the pathogen and host plant (Welz, 1998).

Pathogen races	*Ht* gene reaction				
	Ht0	***Ht1***	***Ht2***	***Ht3***	***HtN***
0	+	−	−	−	−
1	+	+	−	−	−
2	+	−	+	−	−
3	+	−	−	+	−
N	+	−	−	−	+
12	+	+	+	−	−
2N	+	−	+	−	+
23	+	−	+	+	−
23N	+	−	+	+	+
123N	+	+	+	+	+

− Incompatible reaction between Avirulence (Avr) gene and Ht gene, infection do not occur (= host resistance).
+ Compatible reaction between the Avr and Ht genes (= host susceptibility).

In the presence of qualitative *Ht* genes, the leaf presents chlorotic lesions with different levels of necrosis, wilting does not occur and sporulation is greatly reduced or even prohibited (**Figure 1**; Hilu and Hooker, 1963). The pathogen races are designated according to their virulence to the corresponding *Ht* gene (**Table 1**). Race 0 can infect only susceptible cultivars (*Ht0*) showing an incompatible host-pathogen interaction with all cultivars possessing an *Ht* gene. In contrast, race 1 is able to infect cultivars with *Ht1* gene due to a mutated avirulence (*Avr*) gene that turns the reaction into virulence. A single gene in *S. turcica* conditions the inheritance of virulence to *Ht1* gene and a gene-for-gene interaction occurs between the respective *Avr* gene and *Ht1* (Flor, 1956). The race with the highest virulence complexity known yet, race 123N, can infect all cultivars with the corresponding *Ht* genes. The expression of virulence to *Ht* genes depends on light and temperature conditions (Welz, 1998).

Sixteen races of the pathogen could be, theoretically, identified by four *Ht* genes. Among them, 13 races have already been detected in northern China (Dong et al., 2008; Hooda et al., 2017) indicating a high race diversity of *S. turcica*.

Worldwide, race 0 showed the highest abundancy with a frequency of 55% (Welz, 1998). In Europe, race 0 represented 88% of the pathogen population while races N and 23N represented about 14 and 7%, respectively, in the 1990s (Welz, 1998). A monitoring from 2014 showed that in Central Europe, on average, race 0 occurred with 50.2% frequency among 255 isolates, 23.1% of them were identified as race 1 and 11% as race 3, and the races 3N, 123, 23, 2, 13, 23N, and 12 occurred, together, with a frequency of 15.7% (Hanekamp et al., 2014). There were, however, large regional differences. In the warmer areas of Central Europe, where maize growing is more abundant, race 0 represented only 25% of the described isolates and the remaining races were mainly virulent against *Ht1* and *Ht3* (Hanekamp et al., 2014).

Also, in the Eastern states of the United States race 0 declined from 83% in 1974 to 50% in 1990s most likely because of the use of *Ht1* gene in commercial maize hybrids as reported in a study with 242 isolates (Ferguson and Carson, 2007). Races 23 and 23N were only present in low levels. Accordingly, in the United States Corn Belt race 1 is nowadays more frequent than race 0 (Perkins, 2005; Pataky and Ledencan, 2006). Nine *Ht* genes have already been described in more detail (**Table 2**).

In genotypes possessing the *Ht1* gene, sporulation is greatly suppressed in chlorotic lesions (Hilu and Hooker, 1963, 1964; Welz and Geiger, 2000) and lesion expansion is reduced since the hyphae spread only slowly from the xylem to the mesophyll of necrotic cells (Welz, 1998). This gene is partially dominant (Hooker, 1963; Dunn and Namm, 1970) and the degree of resistance depends on the genetic background where it occurs (Hooker, 1963; Calub et al., 1973; Leath and Pedersen, 1986). *Ht1* has been mapped on the long arm of chromosome 2 on bin 2.08, close to the RFLP markers *sgcr506* (Gupta et al., 1989; Welz, 1998) and *umc150B* (Bentolila et al., 1991; Welz, 1998).

Ht2 presents similar chlorotic lesions but less necrosis than genotypes with *Ht1* (Welz, 1998). It is partially dominant (Hooker, 1977; Ceballos and Gracen, 1989). The gene *Ht2* has been mapped on the long arm of chromosome 8 in the *umc48-umc89* interval (Zaitlin et al., 1992; Welz and Geiger, 2000) on bin 8.06 (Zaitlin et al., 1992; Ding et al., 2015). A single dominant suppressor gene of *Ht2* was found in lines related to inbred 'B14' hampering backcross programs aiming to transfer *Ht2* gene (Ceballos and Gracen, 1989) into elite germplasm.

The gene *Ht3* was introgressed from *Tripsacum floridanum* into maize (Van Inghelandt et al., 2012) and it was mapped on bin 7.04 (Zhang et al., 2014). Another gene that confers race-specific resistance is the recessive gene *ht4* located on the short arm of the chromosome 1 near the centromere. In the presence of this gene the plant presents circular chlorotic halos of about 1 cm diameter (Carson, 1995a; Wang et al., 2012). Gene *HtM* was identified in inbred line 'H102' from the cross 'C123' × 'PI 209135' ('Mayorbela') (Robbins and Warren, 1993; Welz and Geiger, 2000). *HtP* was mapped on the long arm of chromosome 2 on bin 2.08 (Ogliari et al., 2005), *HtNB* gene, located on bin 8.07 was identified in the Indonesian line Bramadi and confers non-lesion resistance to *S. turcica* (Wang et al., 2012).

Gene *Htn1*, located on bin 8.05, tracing back to the Mexican landrace Pepitilla, confers partial resistance to NCLB (Welz and Geiger, 2000; Hurni et al., 2015). Differently from the genes *Ht1, Ht2,* and *Ht3, Htn1* delays lesion development up to 4 weeks after infection, reduces the number of lesions and delays the sporulation (Raymundo et al., 1981; Welz and Geiger, 2000). *Htn1* is effective to most NCLB races (Welz and Geiger, 2000), however, its level of resistance depends on environment and genetic background (Thakur et al., 1989). This gene has been mapped on the long arm of chromosome 8 (bin 8.05), while *Ht2* was mapped on bin 8.06 (Zaitlin et al., 1992; Simcox and Bennetzen, 1993; Ding et al., 2015). Hurni et al. (2015) associated a wall-associated receptor-like kinase (WAKs) with the *Htn1*. The WAKs attach the cell wall to the plasma membrane allowing these proteins to notify changes on cell wall structure (Brutus et al., 2010; Kohorn and Kohorn, 2012; Hurni et al., 2015). WAKs confer a new recognition pattern of the host defense immunity system since they can serve as DAMP receptors that recognize changes on cell wall during pathogen penetration in leaf tissue (Hurni et al., 2015). The recessive gene *rt* was identified by Ogliari et al. (2005) in the elite Brazilian line L40 and mapped on bin 3.06 (Ding et al., 2015).

Qualitative resistance usually leads to a high level of resistance when avirulent races dominate the fungal population. On the other hand, some *Ht* genes can quickly get ineffective in case of the occurrence of a virulent strain. Therefore, their use in breeding programs should be accompanied by regular analyses of race abundancy to select those genes that are still effective in the target region. In temperate environments, where the disease pressure is not as high as in the tropics, breeders readily introgress *Ht* genes since it is a faster strategy than improving NCLB resistance by means of quantitative resistance. Durability is hoped to be prolonged by pyramiding several *Ht* genes in the same cultivars. In tropical environments with high disease severity, high pathogen abundancy, and highly diverse *S. turcica* populations, *Ht* genes, however, provide only partial resistance (Welz and Geiger, 2000; Hakiza et al., 2004). The environment, mainly temperature and light intensity, may modify the expression of *Ht* genes and/or the corresponding avirulence genes in *S. turcica.* Maize breeders working in those regions are more reluctant to exploit monogenic resistances due to the higher risk of major gene resistance breakdown (Welz and Geiger, 2000; McDonald and Linde, 2002).

QUANTITATIVE RESISTANCE TO NCLB

In environments, where the disease pressure and the genetic diversity of *S. turcica* populations are high, broad-based quantitative resistance to NCLB is essential. Maize cultivars with quantitative, "horizontal" or non-race specific resistance show a significant reduction of disease severity, but may still produce conidiophores and conidia (Hilu and Hooker, 1964). Typically, fewer and smaller lesions and a prolonged incubation period are

TABLE 2 | Origin of qualitative resistance genes against *Setosphaeria turcica* and its defense reactions.

Genes	Location (bin)	Origin	Defense reaction	Reference
Ht1	2.08	Breeding material from the United States, Australia, Peru	Chloroses	Hooker, 1963, 1977; Ullstrup, 1963; Bentolila et al., 1991
Ht2	8.06	Breeding material from Australia	Chloroses	Hooker, 1977; Zaitlin et al., 1992
Ht3	7.04	*Tripsacum floridanum*	Chloroses	Hooker, 1981
ht4	1[a]	Breeding material from the United States	Chlorotic ring (ca. 1 cm)	Carson, 1995a
HtM	NA[b]	Variety from Puerto Rico	Full resistance	Robbins and Warren, 1993
HtP	2.08	Breeding material from Brazil	Full resistance or chloroses	Ogliari et al., 2005
HtNB	8.07	Landrace from Indonesia	Fewer lesions	Wang et al., 2012
Htn1	8.05	Landrace from Mexico	Fewer and delayed lesions	Gevers, 1975; Simcox and Bennetzen, 1993
rt	3.06	Breeding material from Brazil	Full resistance or chloroses	Ogliari et al., 2005

[a]*Short arm of chromosome 1 near the centromere.*
[b]*Not applied.*

observed in resistant hosts when compared to susceptible hosts (Ullstrup, 1970; Brewster et al., 1992; Smith and Kinsey, 1993; Carson, 1995b; Welz and Geiger, 2000).

Quantitative NCLB resistance is governed by many genes (polygenic). Most of the QTL have minor (0.5–5%) and only a few have major phenotypic effects (>20%). Entry-mean heritability (h^2) of resistance is usually moderate to high: 0.53–0.95 as shown in a review by Welz (1998). Gene action varies with plant age, being purely additive in juvenile plants (Carson, 1995b) and dominance becomes gradually more important over the course of an epidemic (Schechert et al., 1997). Maternal and cytoplasmic effects are not important in this pathosystem (Geiger and Heun, 1989; Welz and Geiger, 2000), which differs from SCLB caused by *Cochliobolus heterostrophus* (Drechs.) Drechs. [anamorph: *Bipolaris maydis* (Nisikado and Miyake) Shoemaker]. Here, genotypes with CMS induced by the T cytoplasm are highly susceptible (Levings and Siedow, 1992).

Schechert et al. (1997) estimated genetic parameters for incubation period and AUDPC. These are important trait components for quantitative NCLB resistance being tightly correlated ($r = \sim 0.8$) and highly heritable ($h^2 = \sim 0.8$) (Welz and Geiger, 2000). The incubation period revealed mainly additive effects in crosses of susceptible by resistant lines while dominance effects were observed only in some crosses of resistant by resistant lines. For both resistance parameters, epistatic gene effects were not important (Schechert et al., 1997).

Quantitative trait loci for resistance were found on all chromosomes (Welz et al., 1999b; Wang et al., 2012; Chen et al., 2016) (**Table 3**). In the meanwhile, multiple-resistant loci including NCLB resistance loci, have been detected. McMullen and Simcox (1995), Wisser et al. (2006), and Jamann et al. (2014) identified clusters of multiple disease resistance factors in bins 3.04, 6.01, and 1.06, respectively. Wisser et al. (2006) revealed strong evidences of association between resistance loci for NCLB, head smut, and common rust resistance. In a fine mapping study, a QTL was found on chromosome 1 conferring resistance to NCLB, Stewart's wilt (caused by *Pantoea stewartii*) and common rust (caused by *Puccinia sorghi*) (Jamann et al., 2016).

Van Inghelandt et al. (2012) demonstrated that 15.95% of genotypic variance was explained by four QTL on chromosome bins 2.08, 5.03, 6.05, and 7.02 in a GWAS of 1487 inbred lines. A SNP marker on bin 5.03 was identified in a region of unknown function, while a SNP with minor effect was located in the *GPC4* gene (bin 5.05), involved in sugar metabolism and showing expression differences upon anaerobiosis as well as heat shock (Russell and Sachs, 1992; Van Inghelandt et al., 2012). The SNP on bin 7.02 is located in a *DBF1* gene, which is a member of the Apetala 2/Ethylene transcription factor family (Kizis and Pagès, 2002; Van Inghelandt et al., 2012) and has a role in abiotic stress responses. Plants that are sensitive to drought stress have a tendency to show early senescence symptoms. Since *S. turcica* is a necrotrophic pathogen, NCLB tends to progress quicker in senescing tissue (Rupe et al., 1982; Van Inghelandt et al., 2012), mainly after anthesis (Rupe et al., 1982).

Another GWAS study was conducted by Ding et al. (2015) where 999 inbred lines were analyzed using 56,110 SNPs. They significantly associated 12, 14, and 19 markers to the traits AUDPC, mean disease rating, and final disease rating, respectively. Genes associated to two or three of the traits simultaneously were identified on chromosomes 4, 7, and 10 and the functional annotation of three of these genes correspond to biotic stress resistance, such as the SANT domain-associated protein and the DNA-binding gene WRKY.

POTENTIAL CANDIDATE GENES

Besides candidate genes derived from GWAS, other genes have been suggested earlier. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), an antimicrobial substance in maize, affects *S. turcica*, European Corn Borer, and *Fusarium* spp. Plants homozygous for the mutant gene *bx1* (benzoxazinless 1) do not produce DIMBOA and the host becomes extremely susceptible

TABLE 3 | Synthesis of some QTL mapping studies using composite interval mapping (CIM).

Parents		Test site[a]	Population size	% of phenotypic variance		Reference
Resistant	**Susceptible**			**Range[b]**	**Total**	
Incubation period						
Mo17[c]	B52	Tr	121	9.8–38.0	40.9	Dingerdissen et al., 1996
CML202	Lo951	Tr	194	7.0–11.8	52.2	Schechert et al., 1999
B73	Mo17	Te	302	4.1–6.9	51.6	Balint-Kurti et al., 2010
DK888	S11	Te	96	NA[d]	61.0	Chung et al., 2011
Disease severity						
Mo17	B52	Te	150	7.5–13.4	51.5	Freymark et al., 1994
D32; D145[c]		Te	220	5.2–20.9	61.5	Welz et al., 1999b
CML202	Lo951	Tr	194	7.2–24.8	55.4	Welz et al., 1999a
IL731a; W6786		Te	157	4.6–10.7	49.4	Brown et al., 2001
K22	By815	Te	207	6.7–15.5	56.3	Chen et al., 2016
AUDPC						
Mo17	B52	Tr	121	9.8–18.3	47.8	Dingerdissen et al., 1996
CML202	Lo951	Tr	194	6.9–18.3	55.8	Schechert et al., 1999
CML52	B73	Te	98	NA[d]	12.0	Chung et al., 2011
Historical minnesota inbreds		Te	284	NA[d]	55.0	Schaefer and Bernardo, 2013

[a]*Tr: tropical, countries below the equator, all locations in Kenya were assumed to be tropical. Te: temperate.*
[b]*Phenotypic variance explained by the smallest and largest QTL effect, respectively.*
[c]*Moderate resistance.* [d]*Not given.*
Three traits related to NCLB resistance are shown: incubation period, disease severity (affected leaf area or lesion width), and AUDPC.

to NCLB. The genes *bx1* to *bx5* are located on the short arm of chromosome 4S (McMullen and Simcox, 1995). In another study, Frey et al. (1997) assigned a different bin position from *bx1* to *bx5* on chromosome 4. The hypothesis that variation at the *bx1* locus is responsible for DIMBOA production is less probable to be validated. An *in vitro* experiment confirmed the significant inhibition of *S. turcica* mycelium growth by DIMBOA (Rostás, 2007). With 0 μg ml^{-1} DIMBOA, the mean growth size of the mycelium was about 2 cm^2, while with 250 and 750 μg ml^{-1} the mean size was 1.5 and 1.0 cm^2, respectively (Rostás, 2007). Besides inhibition of mycelium growth, DIMBOA can also affect spore germination of *S. turcica* (Couture et al., 1971; Long et al., 1975).

Lesion-mimic mutant (*Les/les*) is one of the most common stress phenotypes in plants (Johal, 2007). Some of these lesion-mimic mutants can induce similar symptoms like NCLB (Hoisington et al., 1982). *Les1*, a lesion-mimic dominant mutant gene located on the short arm of chromosome 2 in maize, induces lesion formation with specific size, shape, and coloration (Hoisington et al., 1982). *Les 1*, therefore, could be involved in induction of NCLB necrosis. In total, more than 50 *Les/les* mutants have been identified in maize (Walbot et al., 1983; Johal et al., 1995; Buckner et al., 2000; Johal, 2007) and it is assumed that more than 200 *Les/les* mutants may exist (Walbot et al., 1983; Johal, 2007). Further research is necessary to explore this topic in relation to NCLB.

Micro RNAs (*miRNAs*) are gene expression regulators that are related to many stress responses. Wu et al. (2014) demonstrated that *miR811* and *miR829* confer a high degree of resistance to NCLB. The relationship between *S. turcica* and *miRNAs* remains to be explored (Wu et al., 2014).

IMPLICATIONS FOR BREEDING OF NCLB RESISTANCE

Successful resistance-breeding programs need effective resistance sources, testing systems to reliably assess genetic differences in resistance, and adequate selection and breeding methods.

Resistance sources can be identified especially in areas where the disease pressure is high. Eastern and Southern Africa, Latin America, China, and India are hot spots for the development of NCLB preferrently in the mid-altitude regions, 900–1600 m above the see level, where long dew periods, moderate temperatures, and short day length lead to a high disease pressure (Renfro and Ullstrup, 1976; CIMMYT, 1988; Welz and Geiger, 2000). Materials from Kenya (Muiru et al., 2007) and Uganda (Adipala et al., 1993), for example, have been demonstrated to be highly resistant to NCLB. More resistance sources and their origins are listed, for example, in Welz and Geiger (2000), Ding et al. (2015), and Hooda et al. (2017).

Northern corn leaf blight phenotypic evaluations are usually assessed in the field in adult-plant stage. Artificial inoculation ensures high NCLB pressure and uniform disease distribution in the nursery. This maximizes genetic differentiation and, thus, ensures high heritability and large potential selection gains (Welz, 1998). An advisable inoculation technique for large populations is to collect infected leaves, ideally 4–6 weeks after anthesis in order to avoid a mix of *S. turcica* and other leaf pathogens (Hooker, 1973). The leaf samples must be kept dry and cool to avoid the loss of *S. turcica* pathogenicity and the ability to sporulate. Crushed infected leaves are placed about 10 days before flowering in the maize whorl, ideally in the same field locations where the infected leaves were collected (Hooker, 1973;

TABLE 4 | Scoring method of NCLB incidence on the field useful for assessing large maize populations (Hurni et al., 2015).

Score	Phenotype
1	Plants do not show disease symptoms
2	First small lesions appear on few plants per row and occupies less than 5% of leaf surface
3	Many plants per row present in one leaf level lesions occupying 5–10% of the leaf
4	Many plants per row present in several leaf level lesions occupying 10–20% of the leaf
5	Lesions occupying 20–40% of the leaf and start to merge
6	Lesions occupying 40–60%
7	Lesions occupying 60–80%. Half of the leaf is dry due to disease infection
8	Lesions occupying 80–90%. More than half of the leaf is dry due to disease infection
9	Lesions occupying 90–100%. Nearly the whole plant is dry due to disease infection

Hurni et al., 2015). The infection tends to be higher when the inoculum is added during or just after light rain or prior to irrigation (Hooker, 1973). When the weather is dry and hot, the secondary spread of inoculum may happen naturally, in unfavorable weather conditions a second spread of inoculum and/or sowing spreader rows of susceptible genotypes may be necessary (Hooker, 1973). Craven and Fourie (2016) visually assessed NCLB lesions in the field at the growth stages of visible silks (R1), kernels start to fill (R2), milk stage (R3), top part of kernel filled with starch (R4), and dent stage (R5), respectively (Anonymous, n.d.). Based on these multiple disease ratings the incubation period and AUDPC can be estimated (Welz and Geiger, 2000). In routine breeding programs, field evaluation is realized one to three times, depending on the development of disease symptoms. Scoring is based on disease severity (**Table 4**) in the field. Ratings are performed plotwise with scores ranging from 1 to 9 or 1 to 5 where the lowest number represents a plot without NCLB symptoms and the highest number is a plot with severest disease symptoms. NCLB symptoms can be confounded by other diseases such as Stewart's wilt caused by *Pantoea stewartii* in locations where both diseases occur. A microscopic examination of leaf tissue can easily differentiate both disease symptoms (Pataky, 2004).

Evaluation of NCLB resistance in line *per se* performance is tightly correlated ($r = 0.94–0.98$) to its GCA (Schechert et al., 1997). The high correlation for *per se* evaluation corroborates to the fact that gene expression of NCLB resistance is mainly additive (Abera et al., 2016). Maize resistance-breeding programs should allocate their resources in early selection stages, therefore, for *per se* evaluation of NCLB resistance rather than for testcross performance (Schechert et al., 1997). However, the disease shows some heterosis for resistance (18–27%) and consequently experimental hybrids should be also tested for NCLB resistance in a later selection stage in order to exploit this heterosis (Schechert et al., 1997).

Some studies reveal low (Balint-Kurti et al., 2010) to moderate correlations (Van Inghelandt et al., 2012; Bernardo and Thompson, 2016) between flowering date and NCLB severity with early flowering lines being more susceptible. However, none of the studies shows a clear correlation pattern between flowering date and disease development.

The choice of the most adequate resistance type in a breeding program depends on the population structure and the evolutionary capacity of a pathogen (McDonald and Linde, 2002). In environments where the pathogen population is highly diverse and the gene or genotype flow is high quantitative resistance or exploiting qualitative resistances by using multilines or cultivar mixture are recommended. Producing complex hybrids, such as three-way and double-cross hybrids, with inbred lines differing in resistance gene(s) can be another strategy to retard gene erosion (McDonald and Linde, 2002), since these complex hybrids are heterogeneous and, therefore, present a large genetic variation within the cultivar (Welz, 1998). They are routinely produced in some breeding programs due to the lower costs of hybrid seed production compared to single-cross hybrids. In environments, where the pathogen diversity is lower, the use of qualitative resistances is recommended since it is easier to identify diseased plants and can be employed in a breeding program more easily (McDonald and Linde, 2002).

While *Ht* genes can be easily introgressed by multiple backcrosses with or without molecular markers, improving quantitative resistances can be accomplished by RS procedures. The main objective is to improve the frequency of favorable alleles and maintain a sufficient genetic variation in order to increase the population performance in the subsequent cycles (Falconer and Mackay, 1996). This method includes the development of progenies from a population with some resistance level, evaluation of progenies and selection of the best progenies for recombination of selected individuals for the next selection cycle. The selection response to this breeding method depends, among others, on the square root of the heritability. In NCLB resistance tests, the heritability is usually moderate to high; therefore, it is expected to achieve rapid improvement progress by RS, considering a large genetic variance within the source population (Schipprack, personal communication). It is important, however, that the presence of effective *Ht* genes mask the selection of quantitative resistance and, thus, should be avoided when breeding for quantitative resistance (Welz, 1998).

RS has been successfully used for improving NCLB resistance by several groups. Ceballos et al. (1991) used RS for NCLB resistance improvement and observed with 19% per cycle a high selection gain. Carson (2006) studied the response to selection of two traits related to partial resistance to NCLB: latent period and lesion length. Selection gain per cycle for latent period was higher than for lesion length, 20–27% and 14–18%, respectively, after three cycles of RS with a selection intensity of 10% per cycle. Ayiga-Aluba et al. (2015) studied the efficiency for selection of NCLB traits through a S1 RS program across two cycles. Among other traits the measurement of AUDPC provided a reduction of 26% per cycle indicating that the S1 RS was efficient. Ribeiro et al. (2016) applied seven cycles of RS among 200 half-sib popcorn families and also concluded that selection was effective. Brewbaker (2009) released a synthetic population after 10 cycles of RS to NCLB resistance without giving disease scoring data. The

selection was conducted in Hawaii in a location where the disease incidence was high and the known *Ht* genes were not effective anymore.

When the NCLB resistance level in a population is already high enough, a multi-stage selection integrated in the commercial breeding program can be routinely applied. With this method, selection is realized through successive screenings of different sets of traits per generation. In each screening step, different information and selection intensity are used for selection (Cunningham, 1975). NCLB resistance can be selected in early stages of inbred line development because heritability is high. Other qualitative and qualitative traits, including agronomic traits and other disease resistances, can be simultaneously selected.

Marker-assisted selection is an important breeding tool when selecting for resistant material, especially when introgressing *Ht* genes or major QTL via backcrossing. With molecular markers, it is possible to identify in the early stages of plant development plants containing the gene or QTL of interest (foreground selection), increase the proportion of recurrent parent genome (background selection), and reduce linkage drag (Miedaner, 2016). Codominant SSR markers linked to the known *Ht* genes *Ht1*, *Ht2*, and *Htn1* have already been identified, such as *bnlg1721* and *umc1042* being closely linked to the resistance gene *Ht1* (R^2 = 0.2948 and 0.2626, respectively, $p < 0.0001$, Puttarach et al., 2016). These SSR markers can also be used to select for absence of *Ht* genes during selection for quantitative resistances, thus avoiding results biased by the presence of race-specific genes.

For using QTL, it is necessary to validate them prior to the backcross steps in independent populations or materials derived from the original crossing, like near-isogenic lines. Asea et al. (2009) validated a QTL on bin 3.06 while Chung et al. (2010) validated the QTLs qNLB1.02B73 and qNLB1.06Tx303, identified in bin 1.02 in genotype B73, and bin 1.06 in line Tx303, respectively. The identification of molecular markers closely linked to the gene or QTL of interest is also crucial for a successful MAS. Asea et al. (2012) demonstrated that the use of markers linked to the target QTL is highly efficient and a cost-effective tool to improve foliar disease resistances in maize. Some dominant SCAR markers such as *SCA07496*, *SCA16420*, *SCB09464*, and *SCE20429* were identified and can be successfully used to identify NCLB resistant genotypes (Khampila et al., 2008) although it is not possible to discriminate homozygous from heterozygous resistant plants. In maize, large SNP marker chips are available such as SNP50 Beadchip (Illumina, Inc.) containing 56,110 SNPs (Ganal et al., 2011) that have been used in quantitative resistance studies to NCLB (e.g., Schaefer and Bernardo, 2013; Ding et al., 2015; Chen et al., 2016).

Improving quantitative NCLB resistance by combining several QTL is nowadays considered as less effective (Bernardo, 2008; Xu and Crouch, 2008; Jannink et al., 2010). In MAS, firstly QTL are identified and later on estimates of their effects are computed. This leads to a long procedure and a biased estimation, especially when only QTL with small effects are detected (Lande and Thompson, 1990; Jannink et al., 2010). GS seems to be more promising than MAS since it enables the simultaneous estimation of all marker effects of a genotype and, thus, can be effectively used in selecting quantitative traits, even when only small-effect QTL are available (Jannink et al., 2010). Prerequisites for GS are (i) large training populations segregating for NCLB resistance that are intensively phenotyped across locations and years and genotyped by high-density markers, (ii) adequate GS models, and (iii) genotyped test populations that are selected by using the most appropriate GS model. Thus, the most resistant genotypes to NCLB are predicted on the basis of their GEBV (Jannink et al., 2010). Thus, greatly reduces the amount of necessary test units in the field because only the most resistant predicted progenies are field tested. Thus, resources can be reallocated in order to increase selection gain per breeding generation by testing larger populations. Technow et al. (2013) demonstrated a high prediction accuracy for NCLB resistance of 0.71 (dent gene pool) and 0.69 (flint gene pool) when using the GBLUP model, thus encouraging the application of GS.

CONCLUSION

Northern corn leaf blight resistance can be monogenically or polygenically inherited. The most adequate resistance type used in a breeding program depends on the population structure and the evolutionary capacity of the pathogen. In environments with lower disease pressure and low diversity of *S. turcica* populations, like in the temperate regions, introgression of *Ht* genes by recurrent backcrossing might be favored, because it is easy to accomplish for the breeder. Durability, however, might also here be restricted. NCLB shows to be more severe in the subtropics and tropics compared to temperate environments due to the shorter day length, higher humidity, and likely higher frequency of sexual reproduction of the fungus. Here, quantitative resistance to NCLB should be the main focus of resistance-breeding programs. Population improvement should favorably be accomplished by RS or multi-stage selection. For introgressing major QTL, molecular markers could accelerate the process. GS procedures might help to effectively accumulate the described small-effect QTL in high yielding maize materials.

AUTHOR CONTRIBUTIONS

ALGC conceived and wrote the manuscript. TM drafted and edited the manuscript. Both authors approved the final version to be published.

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ORIGINAL ARTICLE

Intercontinental trials reveal stable QTL for Northern corn leaf blight resistance in Europe and in Brazil

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Abstract

Key message **NCLB is the most devastating leaf disease in European maize, and the introduction of Brazilian resistance donors can efficiently increase the resistance levels of European maize germplasm.**

Abstract Northern corn leaf blight (NCLB) is one of the most devastating leaf pathogens in maize (*Zea mays* L.). Maize cultivars need to be equipped with broad and stable NCLB resistance to cope with production intensification and climate change. Brazilian germplasm is a great source to increase low NCLB resistance levels in European materials, but little is known about their effect in European environments. To investigate the usefulness of Brazilian germplasm as NCLB resistance donors, we conducted multi-parent QTL mapping, evaluated the potential of marker-assisted selection as well as genome-wide selection of 742 F_1-derived DH lines. The line per se performance was evaluated in one location in Brazil and six location-by-year combinations (= environments) in Europe, while testcrosses were assessed in two locations in Brazil and further 10 environments in Europe. Jointly, we identified 17 QTL for NCLB resistance explaining 3.57–30.98% of the genotypic variance each. Two of these QTL were detected in both Brazilian and European environments indicating the stability of these QTL in contrasting ecosystems. We observed moderate to high genomic prediction accuracies between 0.58 and 0.83 depending on population and continent. Collectively, our study illustrates the potential use of tropical resistance sources to increase NCLB resistance level in applied European maize breeding programs.

Introduction

Maize (*Zea mays* L.) is the worldwide most productive crop with 1.12 harvested billion metric tons in 2018/19 (USDA/IPAD 2020). Projections estimate more than 183 million metric tons production growth in the next decade (OECD/FAO 2019) seeking to attend the increasing demand for food, feed and fuel. In Europe, especially in Germany, maize production increased exponentially since the end of the 1970s and maize is nowadays the second largest crop in acreage, where about 85% of the production is designated to silage and biogas maize and 15% to kernel maize (Bundessortenamt 2019). NCLB was firstly observed in southern Germany in 1995 (Welz et al. 1996; Welz 1998; Hanekamp 2016) and is nowadays the most devastating maize leaf disease in the country. On a worldwide basis, harvest losses by NCLB can vary from 15 to more than 60%, especially in tropical and subtropical environments (Raymundo and Hooker 1981; Tefferi et al. 1996; De Rossi et al. 2010; Cramptom 2015; Nwanosike et al. 2015; Romero 2016). Likewise, NCLB infections can lead to a reduction in silage digestibility and pre-disposition to stalk rot, representing a significant threat to farmers and seed growers (for review, please, refer to Galiano-Carneiro and Miedaner 2017). NCLB is caused by the ascomycete *Setosphaeria turcica* (Luttrell) Leonard & Suggs (anamorph: *Exserohilum turcicum* (Pass.) Leonard & Suggs. syn. *Helminthosporium turcicum* Pass., Boln Comiz.) which grows preferably in temperatures between 15 and 28 °C and in high humidity conditions. These conditions are primarily fulfilled in the subtropics, especially in the south of Brazil. This region represents one of the most important maize production

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regions in Brazil, the third largest maize producer country worldwide (CONAB 2020). However, it has also the ideal environmental conditions for NCLB infections including long periods of dew, long nights and average temperatures (INMET 2020) exactly in the optimal range of the disease development. These favorable conditions in addition to the presence of *S. turcica* can likely trigger NCLB epidemics. With frequent epidemics, the *S. turcica* populations grow concomitantly to the potential number of pathogen mutations leading to a strong selection pressure on both, pathogen and host. This strong NCLB pressure presumably occurred in many growing seasons in South Brazil leading to more complex *S. turcica* races (Navarro, personal communication) and highly resistant host plants due to maize breeders' efforts (e.g., cultivar CDL 15, Kaefer et al. 2017).

In contrast, the three factors contributing to an epidemic (favorable environment, susceptible host and virulent pathogen) meet only sporadically in Europe. In addition, most probably due to the more recent history of the fungus in Europe, firstly recorded as an epidemic in the beginning of the 1990s in Austria (Hanekamp 2016), there was less interest for selection on highly resistant host plants, although genetic variation for NCLB resistance in Europe is present (Welz et al. 1999; Van Inghelandt et al. 2012). As fungicide application is expensive and laborious in the later stages of maize development, resistance breeding is the most economic and environmentally friendly way to reduce damage caused by *S. turcica*.

In the maize/*S. turcica* pathosystem, qualitative (race-specific) as well as quantitative, race-nonspecific resistances are known (Galiano-Carneiro and Miedaner 2017). In Europe, mainly race-specific resistances genes such as *Ht1*, *Ht2*, *Ht3* and *HtN (*"*Ht*" *refers to Helminthosporium turcicum*, former name of the pathogen*)* have been harnessed in applied breeding programs. This is expected as *Ht* genes are of practical use for breeders because the introgression of one *Ht* gene can potentially confer high resistance levels; however, this resistance can be quickly overcome by virulent pathotypes. Historically, *Ht1* described in the 1960s was the longest effective resistance gene compared to the other *Ht* genes. However, in the 1970s, race 1 overcame *Ht1* making the resistance ineffective in areas where race 1 is abundantly present (Bergquist and Masias 1974; Welz 1998). Gene pyramiding, *i.e.,* stacking multiple *Ht* genes in one genotype, is a well-known approach to increase the durability of resistance genes (Sánchez-Martín and Keller 2019). However, the emergence of complex races such as the race 123 N firstly identified in the Heilongjiang region in China (Ma et al. 2020) in addition to the strong directional selection for pathogen virulence when large acreages are sown may render also gene pyramiding ineffective (Pilet-Nayel et al. 2017).

In Brazil and in Europe, the distribution of these races is usually region specific. To exemplify this, the predominant race in Castro was race 1, while races 0 and 2 were more frequently observed in Ponta Grossa in a race monitoring conducted in 2019 (Navarro, personal communication). In Europe, races 3 N and 3 were the most common in southwest of France and north of Italy, while race 1 was the most abundant in Austria, Hungary, and the German Upper Rhine region between 2011 and 2012 (Hanekamp 2016). These race monitorings illustrate that most of the monogenic resistances mediated by *Ht* genes have already been overcome by virulent pathotypes. Therefore, race monitoring is an important tool to assist breeder's decision on the choice of the *Ht* gene to apply in each region. Moreover, these examples demonstrate that breeding for quantitative, race-nonspecific resistances should be prioritized in the NCLB pathosystem.

Genetic resources can be exploited to identify new sources of resistance alleles that can potentially increase durability of host resistance (McDonald and Linde 2002; Mayer et al. 2017). Highly resistant Brazilian maize lines with quantitative resistances to NCLB have already been identified in Brazil (Kaefer et al. 2017; Ribeiro et al. 2016) and introducing NCLB resistance from Brazilian genotypes to Europe can be a great opportunity to increase NCLB resistance levels, but little is known about the effect of these resistance sources in European environments. To investigate the potential use of Brazilian sources, three resistant Brazilian donors were each crossed with adapted elite double haploid (DH) European lines. These donors are elite lines from KWS SAAT SE & Co. KgaA breeding programs and employed here for the first time in a NCLB study. As our objective was to exploit quantitative resistance to NCLB, none of the parental lines neither the testers possessed the *Ht* genes *Ht1*, *Ht2* and *Htn1* according to markers developed and analyzed by KWS SAAT SE & Co. KGaA. To potentially discover NCLB resistance, which is durable and stable across many environments, we performed QTL mapping with a total of 742 DH lines and their respective testcrosses assessed for NCLB resistance across two locations in Brazil and 11 location-year combinations (= environments) in Europe (Austria, France, Germany and northern Italy), considering both line per se and testcross assessment. This project was a part of an applied maize breeding program, and analyzing the maximum number of genotypes and environments was desired.

In particular, our objectives were to: (1) test the potential use of Brazilian resistant germplasm to tackle NCLB infection in European conditions; (2) assess quantitative-genetic parameters for NCLB resistance in per se and testcross doubled haploid (DH) populations; (3) analyze the genetic architecture of NCLB resistance by multi-parent QTL mapping and biparental QTL mapping; (4) assess genomics-assisted

breeding strategies for an efficient introgression of NCLB resistance in adapted plant materials.

Materials and methods

Plant material and field trials

This study comprised biparental populations derived from three tropical donors (T1, T2, T5, abbreviated T) from Brazil selected to be highly resistant to NCLB. They were crossed with seven susceptible elite lines adapted to Europe (A) resulting in the following seven biparental populations: T1 × A1, T1 × A2, T1 × A10, T2 × A3, T2 × A4, T2 × A5 and T5 × A11. (Suppl. Figure 1). Four crosses derived from resistant donors T1 and T5 belong to the stiff-stalk synthetic (SSS) heterotic group and three crosses derived from resistant donor T2 to the non-stiff-stalk (NSS) heterotic group. Crosses resulted in 22–148 DH lines per population summing up to 742 unique F1-derived DH lines. Subsequently, the DH lines were crossed with line testers moderately to highly susceptible for NCLB. The testers belonged to the respective opposite heterotic group in Brazil. In Europe, one susceptible flint tester was crossed with DH lines belonging to both SSS and NSS heterotic groups to shorten maturity for the cooler European conditions. All genotypes are proprietary materials of KWS SAAT SE & Co. KgaA. Segregating plant material is available on request to this company for scientists without any commercial interest. A respective MTA must be signed in advance.

Populations showing common parents were randomized together to increase the accuracy of entry comparison (Piepho et al. 2006a). This led to four trials: (1) "trial 1″, composed by all individuals from populations T1 × A1, T1 × A2 and T1 × A10; (2) "trial 2," composed by all individuals from populations T2 × A3 and T2 × A4; (3) "trial 3," composed by population T5 × A11; (4) "trial 4," composed by all individuals from population T2 × A5, randomized separately from "trial 2" for seed logistic reasons. Populations composing trials 1 and 3 belonged to the SSS, while populations from trials 2 and 4 belonged to the NSS heterotic group.

Populations were evaluated for NCLB in the growing seasons 2019 in Brazil and 2017, 2018 and 2019 in Europe (Suppl. Figure 1). Per se performance was evaluated in one location in Brazil and four locations in up to three years (in total six environments) in Europe. Testcrosses were evaluated in two locations in Brazil and in 7 locations and up to 2 years (in total ten environments) in Europe (Supplementary Table 1). The testing environments in Brazil and in Europe will be referred as different continents for simplification. Trials were allocated in alpha-lattice designs with two replications per location, except for per se evaluation in Brazil for trials 1 and 2, and testcross evaluation for trial 3 in Europe; trials were allocated following a p-rep design where about 80% of the data was replicated to efficiently allocate the limited number of harvested seeds. Resistant and susceptible checks comprising KWS SAAT SE & Co. KgaA property DH lines and hybrids in addition to parental lines were sown in each location leading to at least eight common genotypes among trials. Testcrosses from trials 1 and 2 were evaluated in both the South of Brazil and Europe (Austria, France, Germany and Italy). Trial 2 was tested for line per se performance in both continents.

In addition to NCLB, we assessed female flowering (FF) time and plant height (PH) in Europe. These traits were assessed in alpha-lattice designs with one replication in Monselice, Italy, in 2018 and 2019, and with two replications in Neupotz, Germany, in 2018, resulting in three European environments (Supplementary Table 1).

Our experimental unit was a two-row observation plot with a length of 4.0 m and a distance between rows of 0.5 m in Brazil and one row observation plot with the same dimensions in Europe. In Europe, disease spreader rows for artificial inoculation were additionally planted in the fields between each second, fifth, seventh or tenth row depending on the field location. All entries were treated according to local best agronomic practices not affecting the development of NCLB.

Setosphaeria turcica inoculation and trait assessment

All environments in Europe and Ponta Grossa (PG) in Brazil were inoculated with *S. turcica* warranting uniform inoculum distribution. Leaves with NCLB symptoms from the respective location were collected the year before each testing season, air-dried and stored until inoculation. A parallel project was conducted to identify the races present in each field location. Differential lines were employed for the race identification, and in most of the locations, several *S. turcica* races were present (Navarro, personal communication). Subsequently, symptomatic leaves were crashed, and 1 g of the inoculum was added to the maize whorl of the spreader rows in Europe. In Brazil, the first and the last two plants of each row were inoculated. This procedure was conducted at the vegetative stage of 12–14 true visible leaves (V12–V14) and latest 10 days before tasseling as originally proposed by Hooker (1973).

About 120 days after sowing, at the phenological stage R5 to R6, the NCLB symptoms were visually assessed in a plot-wise severity scoring scale ranging from 1 to 9, where 1 = entire plot without NCLB symptoms and 9 = entire plot fully diseased (Hurni et al. 2015; Fig. 1). The NCLB plot-wise rating was assessed two to four times in an interval of 27–91 days post-inoculation where the first inoculations took place in the beginning of January in Brazil and end of

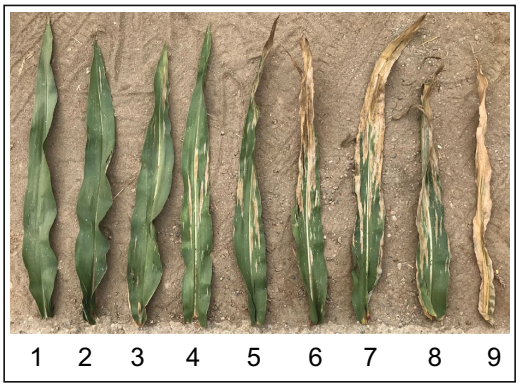

Fig. 1 NCLB damage scale 1–9, where one is a plot without NCLB symptoms and nine is a plot fully diseased, represented by one single leaf in this figure

June in Europe (dpi; Supplementary Table 2). The average ($NCLB_m$) and the final/last NCLB visual scoring ($NCLB_f$) of all evaluations were considered for further analyses.

Plant height was assessed by measuring the size of a representative plant per experimental unit from the soil level to the beginning of the tassel bifurcation. Female flowering was measured as the number of days from sowing to the day that at least 50% of the row had extruded silks.

Phenotypic data analysis

Phenotypic analyses for single environments were performed using linear mixed models and outlier detection procedures as proposed by Bernal-Vasquez et al. (2016). Combined analysis without a maximum of 15% outliers, comprising rows with lodging plants or rows with low plant density were conducted according to the mixed model:

$$y_{ijyklm} = \mu + G_i + Y_j + T_y + L_k + LY_{kj} + LT_{ky} + LTY_{kyj} + LTYR_{kyjl} + LTYRB_{kyjlm} + e_{ijyklm}.$$

where μ represents the overall mean, G_i the effect of the i th genotype, Y_j the effect of the jth year, T_y the yth trial, L_k the effect of the kth location, m^{ith} B_m the incomplete block, and its interaction terms ($LY_{kj}, LT_{ky}, LTY_{kyj}, LTYR_{kyjl} and LTYRB_{kyjlm}$), and e_{ijyklm} the heterogeneous error variance. The same model excluding the location and year effects was employed for the single location analysis.

G_i, Y_j and T_y effects were included in the fixed statement of the model to obtain the best linear unbiased estimators (BLUEs). The variance components were obtained through the restricted maximum likelihood method (REML) by including only the Y_j and T_y effects in the fixed statement of the model above. All other effects were included in the random statement of the model. The significance of the variance components was obtained by likelihood ratio test between the full and incomplete model (Stram and Lee 1994). Binary dummy variables were used to separate the effects of each population, checks and replicates as proposed by Piepho et al. (2006b). For the sake of simplicity, dummy variables were not shown in the model above.

The broad-sense heritability (H^2) was estimated following Cullis et al. (2006):

$$H^2 = 1 - \frac{\bar{\vartheta}_{\mathrm{BLUP}}}{2\sigma_G^2}$$

where $\bar{\vartheta}_{\mathrm{BLUP}}$ is the mean variance of a difference of two BLUPs and σ_G^2 is the genotypic variance.

Corrections for flowering date were conducted according to the approach of Emrich et al. (2008). The female flowering scorings were added in the fixed statement of the mixed models to obtain the $NCLB_{f\_}FF$.

Phenotypic correlations based on BLUEs of female flowering time, plant height and NCLB severity as well as the correlation of the NCLB severity between trials in the two continents were calculated with Pearson product moment correlation coefficients.

The relative efficiency of indirect selection using line per se to predict testcross performance was obtained by the following equation proposed by Falconer and Mackay (1996) and reviewed by Löffler et al. (2011):

$$\mathrm{RE} = \frac{H_{PS} \times r_G}{H_{TC}}$$

where RE is the relative efficiency, H_{PS} is the square root of the per se heritability, H_{TC} is the square root of the testcross heritability, and r_G is the genetic correlation between line per se and testcrosses.

The genetic correlation (r_G) between per se and testcross was obtained using the following formula proposed by Cooper et al. (1994):

$$r_G = \frac{r_P}{\sqrt{H_{TC} \times H_{PS}}}$$

where r_P is the phenotypic correlation between per se and testcross, H_{TC} is the square root of heritability of testcrosses, and H_{PS} is the square root of heritability of lines per se.

All analyses were conducted within the R environment (R Development Core Team 2018, version 3.5.1). Mixed-model computations were performed using the R package ASReml-R 3.0 (Gilmour et al. 2009).

Molecular data

All DH lines were genotyped at KWS molecular laboratory using an Illumina 15 k SNP chip based on the public Illumina MaizeSNP50 BeadChip. All ten chromosomes

were partitioned into bins of 0.5 cM according to the public genetic map IBM and the physical map AGPv02 (Ganal et al. 2011); therefore, we call the positions "putative cM" (putcM). Regions adjacent to centromeres were especially markedly enriched to account for the low recombination rates in this area.

The number of polymorphic markers in each population ranged from 5 to 6 k. Quality control was conducted by removing monomorphic or missing alleles for both parents, heterozygous genotypes at the parents, genotypes with more than 25% missing values, markers with more than 10% missing data and markers with minor allele frequency (MAF) lower than 5% in each population. After the quality check, 1454, 3223 and 2212 SNP markers were available for donors T1, T2 and T5, respectively.

Multi-parent QTL mapping analysis (bi-allelic model)

The T1 and T2 donor groups comprised populations that were connected through the respective resistant tropical parent. They were allocated as following: "Donor T1," comprised the individuals from populations T1 × A1, T1 × A2 and T1 × A10, included in the trial 1; "Donor T2," comprised the individuals from populations T2 × A3 and T2 × A4, included in the trial 2, and "Donor T5" comprised population T5 × A11, included in the trial 3. The population T2 × A5 did not show significant genetic variance for both NCLB traits; hence, it was not integrated into the analyses.

Multi-parent QTL mapping analysis was conducted with the R package mppR version 1.2.0 (Garin et al. 2018). Succinctly, multiple biparental populations that were connected through one resistant tropical parental, as donors T1 and T2, were analyzed jointly by the method of composite interval mapping (CIM) (Zeng 1993, 1994).

The additive effect of the QTL was obtained through the bi-allelic model of the package mppR. This model considered that alleles from different populations with the same SNP were identical by state (IBS) (e.g., model B in Würschum et al. 2012; Garin et al. 2017). To avoid false positives, population structure (Supplementary Fig. 2) was accounted by the k-model proposed by Yu et al. (2006).

QTL significance thresholds were obtained by permutation tests performing 1000 iterations (Broman and Sen 2009). QTL mapping for each model was conducted in a first step by a simple interval mapping (SIM) and the significant QTL from this analysis were applied as cofactors for the CIM. The confidence interval of each QTL was obtained by –log10 (p) value drop off interval. The contribution of each QTL to the phenotypic variance was computed by comparing the full, containing all the QTL, and incomplete models, excluding only the detected QTL of interest. Individual explained genotypic variance (p_G) was obtained following the equation proposed by Utz et al. (2000):

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

where R^2_{adj} corresponds to the adjusted R^2 that was adjusted for the number of parameters included in the linear model and H^2 the average broad-sense heritability of each population composing a donor group.

Biparental QTL mapping

Donor T5 was calculated with the CIM QTL mapping function implemented in the R package RQTL because only one population was available for this donor (T5 × A11) (Broman et al. 2003). The QTL significance threshold was defined by 1000 iterations permutation test (Broman and Sen 2009). Five markers were forward selected and used as covariates in the Haley–Knott regression (Haley and Knott 1992). Additive effects per parent component, global and partial explained phenotypic variance and QTL confidence interval were computed as described in the previous section. Each identified QTL was ordered by the type of material assessment (line per se or testcrosses) and received the nomenclature "*qx*" where "*x*" is a consecutive number of QTL. Same nomenclature indicates that QTL are co-located. However, QTL peaks identified within a large confidence interval are more likely to have many co-located QTL. In addition, the chromosome location of each identified QTL was described in chromosome bins. This refers to the interval that contains all loci delimited by two core markers. For example, QTL q4 is present on chromosome 7, region 7.03 of the maize genome within 128,175,453–156,050,469 bp (for more details, please, refer to MaizeGDB).

Marker-assisted, genomic and weighted genomic predictions

Marker-assisted predictions (MAS) were conducted with the significant QTL explaining more than 5% of the genotypic genetic variance for the trait $NCLB_f$ within donors T1, T2 and T5. Genomic prediction was carried out by ridge-regression BLUP (RR-BLUP, Whittaker et al. 2000) with the R package "rrBLUP" (Endelman 2011; Endelman and Jannink 2012) within each donor group. Missing SNP marker information was imputed for each donor group with the software LinkImpute (Money et al. 2015) and resulted in high imputation accuracies (> 97%). In addition, we performed a weighted ridge-regression BLUP (wRR-BLUP) where QTL explaining more than 5% of the genotypic variance was added to the fixed statement of the genomic

prediction model (Bernardo 2014; Zhao et al. 2014; Spindel et al. 2016). The main objective of this approach is to increase the frequency of the major effects within the breeding population (Bernardo 2014). In addition, this model has been proofed to increase the prediction accuracies in different crops (Gaikpa et al. 2020; Galiano-Carneiro et al. 2019; Herter et al. 2019).

The performance of the MAS, RR-BLUP and wRR-BLUP were evaluated by a five-fold cross-validation (CV) procedure. The data were randomly divided in five different folds where 80% of the data comprising phenotypic and molecular data were employed in the training set to predict the phenotypic values of the remaining 20% data, comprising only the molecular data, in the prediction set to assess the prediction error (Utz et al. 2000). This procedure was repeated 200 times (i.e., 1000 cross-validations), each repetition with a random composition of folds to assess CV error. For each fold composition, prediction ability was calculated as the Pearson's correlation between predicted versus observed values for each evaluated model. This procedure was also employed to compare the prediction ability of different family compositions in the training and prediction sets. For this, 60 genotypes were composing the training set and the remaining genotypes comprised the prediction set. Prediction accuracy was the prediction ability divided by the square root of the trait broad-sense heritability, composed by the average H^2 of families with common parent.

Results

The tropical donors T1, T2 and T5 were considerably more resistant than the mean of the adapted elite lines for line per se and testcross performance (Fig. 2). In Brazil, disease severity was, on average, higher for both, lines per se and testcrosses, compared to Europe. However, we had a maximum of two locations in Brazil and only testcrosses were assessed for donor T1 (Fig. 3). In Brazil, the testcrosses of donor T1 were, on average, more resistant than the lines, while in contrast the testcrosses of donor T2 were more susceptible than the lines. In Europe, all testcrosses showed a considerably higher susceptibility than the respective lines. All populations showed moderate to high broad-sense heritabilities for both NCLB ratings ranging from 0.52 to 0.90. Adjusted means indicated a quantitative distribution of $NCLB_f$ with mean severity scores ranging from 2.60 to 5.68 and high, significant ($P < 0.001$) genetic variance for $NCLB_m$, $NCLB_f$, FF and PH (Supplementary Table 3). For the sake of simplicity, we will refer in the following to the populations according to their tropical resistance donor, i.e., to populations

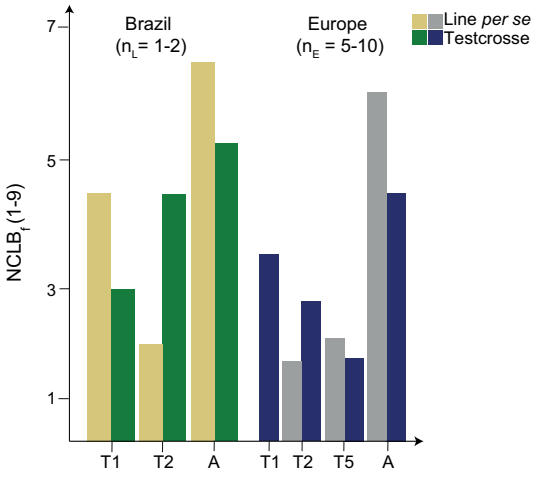

Fig. 2 Entry means of each tropical donor (T1, T2 and T5) in comparison with the mean of the adapted parent lines (A) evaluated in line per se and testcross combinations for the $NCLB_f$ in several locations (n_L) in Brazil and environments (n_E) in Europe

T1 × A1, T1 × A2 and T1 × A10 as donor T1, to populations T2 × A3 and T2 × A4 as donor T2 and to population T5 × A11 as donor T5.

Correlations among traits were also positive and significant for both, lines per se and testcrosses (Supplementary Table 4), where the traits $NCLB_f$ and $NCLB_m$ had the highest positive correlation ($r \geq 0.90$, $P < 0.001$) in both continents. Hence, we focus on $NCLB_f$ to avoid redundancy. The line per se correlations between $NCLB_f$ and FF were significant and moderate from $r = -0.40$ to $r = -0.41$ ($P < 0.001$) depending on the donor, while none of the correlations were significant between $NCLB_f$ and PH. For the testcrosses, we observed in Europe a significant, but moderate negative correlation between $NCLB_f$ and FF ranging from $r = -0.38$ to $r = -0.52$ ($P < 0.001$) depending on the resistance donor. The correlations between $NCLB_f$ and PH were significant for all donors except for donor T5. The significant correlations ranged from $r = -0.13$ ($P < 0.05$) to $r = -0.34$ ($P < 0.001$) (Supplementary Table 4). Corrections for flowering date did not reduce the correlations between $NCLB_f$ and FF.

Lines and testcrosses showed moderate and positive correlations ($P < 0.001$) in Brazil and Europe (Fig. 4). Relative efficiencies of selecting testcross performance by per se performance were low in Brazil and Europe throughout. The efficiency was slightly higher for donor T5 compared to donor T2 in Europe (Fig. 4). Between Brazil and Europe, moderate phenotypic correlations of NCLB resistance for testcrosses were observed ($r = 0.36$ for donor T1 ($P < 0.001$), $r = 0.41$ for donor T2 ($P < 0.001$), Fig. 5).

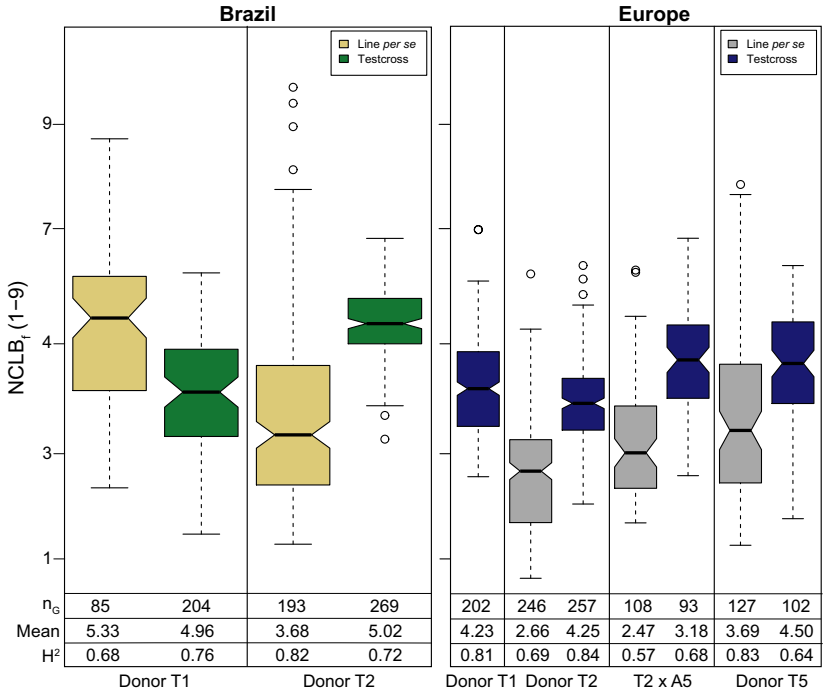

Fig. 3 Notched boxplots for NCLB final score (NCLB$_f$) evaluated as line per se and testcross in Brazil (**a**) and Europe (**b**) in a damage scale of 1–9

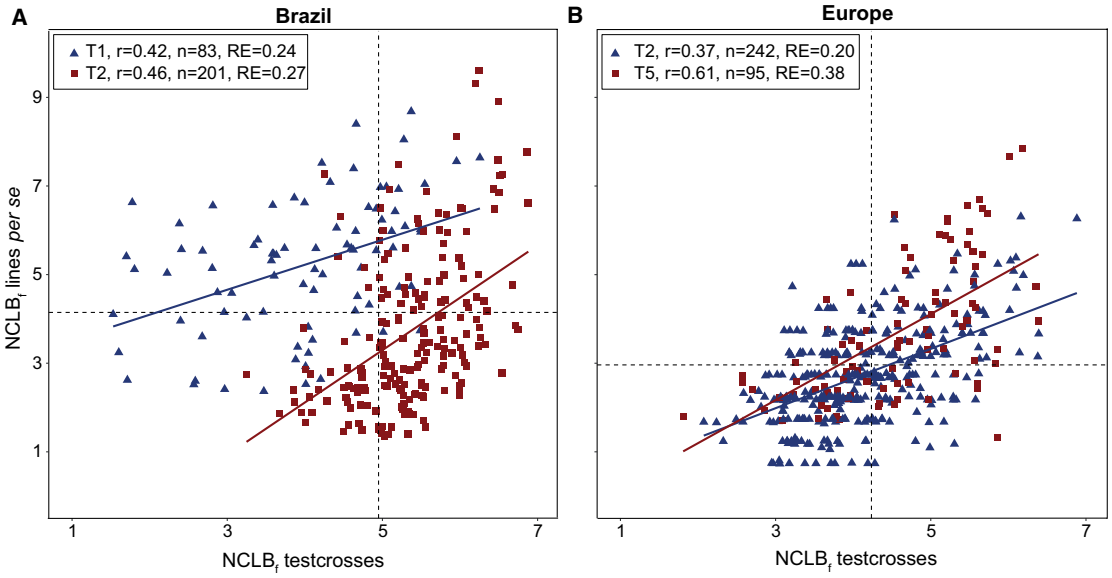

Fig. 4 Scatter plots for final NCLB score (NCLB$_f$) evaluated as line per se and testcrosses in Brazil (**a**) and in Europe (**b**) as well as the phenotypic correlation (*r*), number of genotypes (*n*) and relative efficiency (RE) of per se indirect selection for testcross performance. The dashed lines represent the mean of families for lines per se and testcrosses (4.68 and 4.98 in Brazil; 3.01 and 4.28 in Europe)

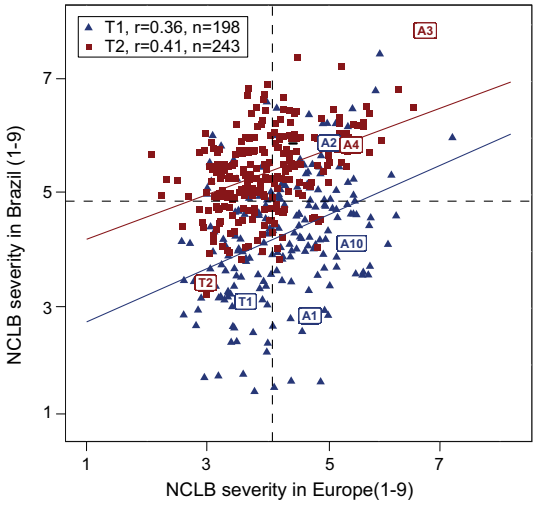

Fig. 5 Scatter plot between Brazil and Europe for $NCLB_f$ assessed for tropical donors T1 and T2. The dashed lines represent the mean of families within donors T1 and T2 scoring for each continent (4.98 for Brazil and 4.24 for Europe). Parent testcrosses were not included in the regression but are indicated in the labels

QTL mapping for $NCLB_f$ resulted in one to four QTL each for per se and testcrosses depending on resistance donor and continent, where no QTL was identified for lines per se belonging to donor T1 (Table 1). The explained genetic variances per QTL ranged from 5.4 to 13.3% in Brazil and from 3.6 to 31.0% in Europe, while the tropical parents reduced $NCLB_f$ damage from −0.29 to −2.48 scores considering both continents (Table 1; Supplementary Table 5). Four QTLs (q4, q5, q7 and q8) were co-located among per se and testcrosses within and between continents (Fig. 6).

In Europe and Brazil, QTL q4 and q5 on chromosome bins 7.03 and 9.04 were identified within the same confidence interval range. QTL q4 explained 13.29% and 16.83% of the genotypic variance in Brazil and in Europe, respectively. QTL q5 explained 10.95% and 7.10% of the phenotypic variance in Brazil and in Europe, respectively, showing a significant reduction of NCLB severity, especially when both QTL are present (Fig. 7). The QTL q4 was identified on chromosome 7 at the physical position 153.88 Mbp and in the 155.11 Mbp in Brazil and Europe, respectively (Supplementary Table 5). QTL q5 was identified on chromosome 9 at the physical position 100.37 Mbp in Europe and at the positions 107.36 and 108.35 Mbp in Brazil as two QTLs were identified within the same confidence interval (Supplementary Table 5).

For the traits FF, we detected nine and for PH we detected five QTL on chromosomes 2, 7, 8, 9 and 10 (Supplementary Table 5). Among the QTL identified for FF, five were co-localized with $NCLB_f$: q5, q6, q7, q14 and q16 which is in accordance to the moderate correlation between $NCLB_f$ and FF ranging from −0.38 to −0.53 depending on resistance donor (Supplementary Fig. 3, Supplementary Table 5). Among the overlapping QTL, QTL q23 was coding the gene GRMZM2G067921 which is known to delay flowering time (Maize 2020). The tropical lines were flowering 16 days later and were 13–32 cm higher than the adapted lines, both measured in testcross combinations in Europe, according to the allele substitution effect of the identified QTL (Supplementary Table 5). One QTL, q7, was overlapping between PH and $NCLB_f$.

Both genomic prediction methods (RR-BLUP and wRR-BLUP) showed higher prediction accuracies compared with marker-assisted selection (MAS, Fig. 8). Likewise, wRR-BLUP showed slightly higher prediction accuracies compared with standard RR-BLUP. Genomic prediction accuracies were estimated lower for Brazilian than for European environments (Fig. 8).

The prediction ability was the highest when the training and the prediction sets comprised the same family. On

Table 1 Number of QTL (n_{QTL}) identified for the NCLB final score for each donor including the number of genotypes (n_G) and markers (n_M), minimum and maximum range of confidence interval (CI, putcM), explained genotypic variance range (p_G) and allele substitution effect (α-effects) for different models. For details of each identified QTL, please refer to Supplementary Table 5

Donor	n_G	n_M	Brazil				Europe				QTL model
			n_{QTL}	CI (putcM)	p_G	α-effect	n_{QTL}	CI (putcM)	p_G	α-effect	
Per se											
T2	236	3223	3	1.2.43/20.13	10.87/13.29	−1.24/−1.11	4	1.2/39.85	10.01/15.84	−0.70/0.55	Bi-allelic
T5	129	2212	–	–	–	–	2	3.55/10.13	21.28/28.52	−0.70/−0.62	Biparental
Testcrosses											
T1	178	1454	2	1.91/111.14	8.45/12.41	−0.65/0.52	3	3.86/209.86	3.57/18.57	−0.57/0.54	Bi-allelic
T2	236	3223	1	10.81	5.43	−0.30	4	8.19/22.85	7.10/24.02	−0.74/−0.34	Bi-allelic
T5	129	2212	–	–	–	–	3	8.19/18.24	15.75/30.98	−0.42/−0.29	Biparental

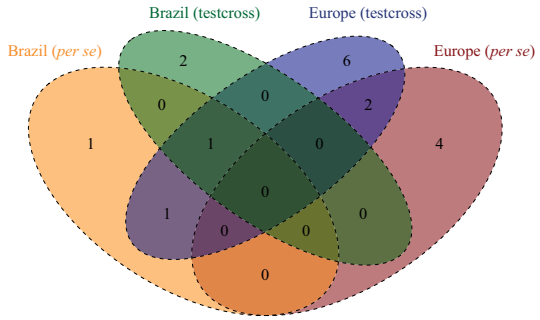

Fig. 6 Venn diagram of the co-located QTL for $NCLB_f$ score identified between and within continents

the other hand, the prediction ability was the lowest when families from different heterotic groups were composing the training and the prediction sets (Fig. 9).

Discussion

NCLB is one of the world's most devastating leaf diseases in maize. Brazilian maize is a promising source of resistant genotypes, but little is known about the effect of these tropical resistance sources in European environments. Therefore, we investigated the potential use of Brazilian donors for NCLB resistance in the phenotype and molecular levels by conducting multi-environmental trials, QTL mapping and genomic prediction.

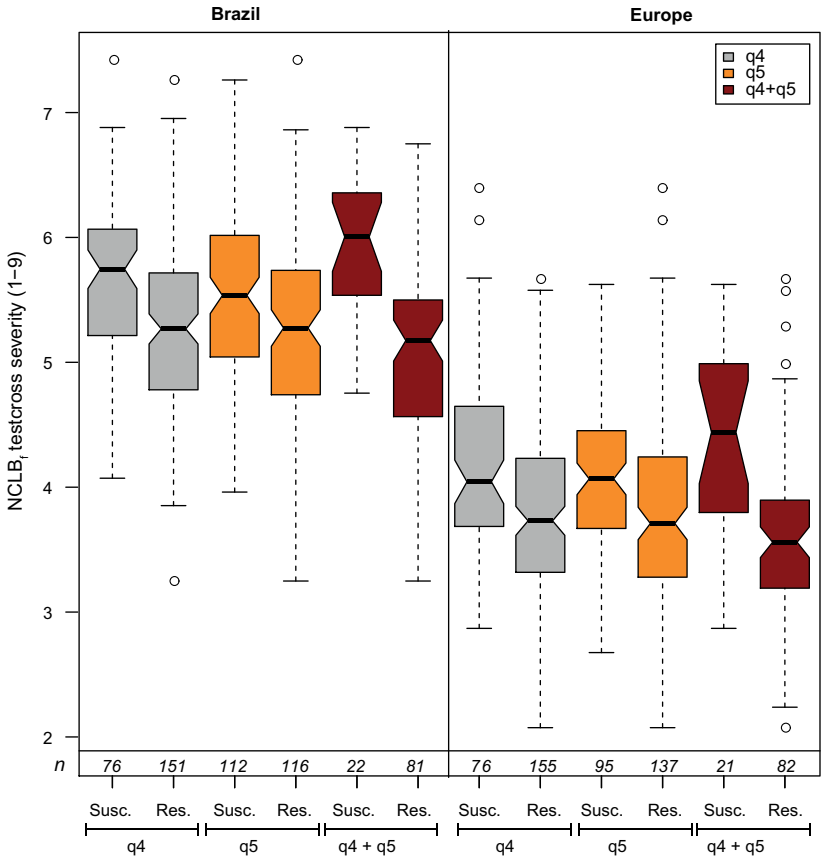

Fig. 7 Notched boxplots of allelic effects for both environmentally stable NCLB QTL q4 and q5 and their combination for final NCLB score ($NCLB_f$) severity (1–9) for donor T2 testcrosses

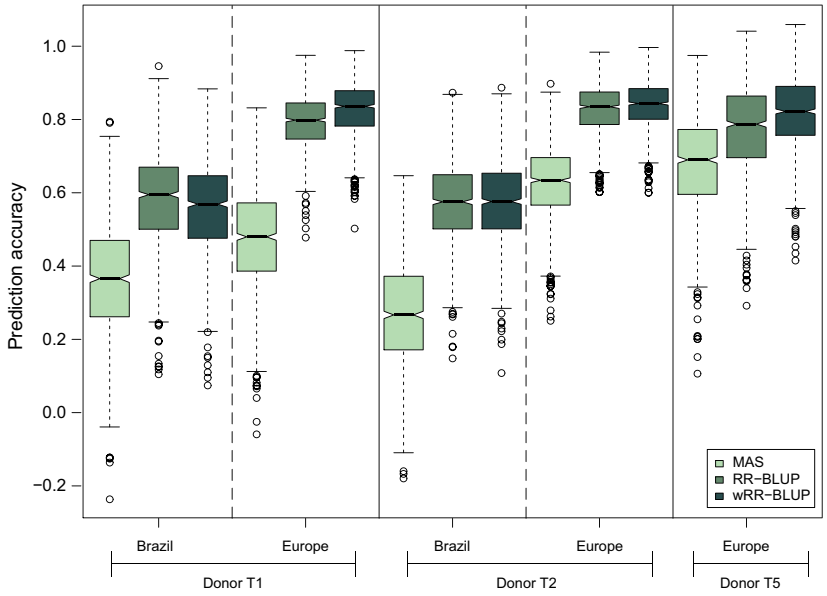

Fig. 8 Prediction accuracies obtained from marker-assisted selection (MAS), genomic selection (RR-BLUP) and weighted genomic selection (wRR-BLUP) for each donor group and continent for test-crosses

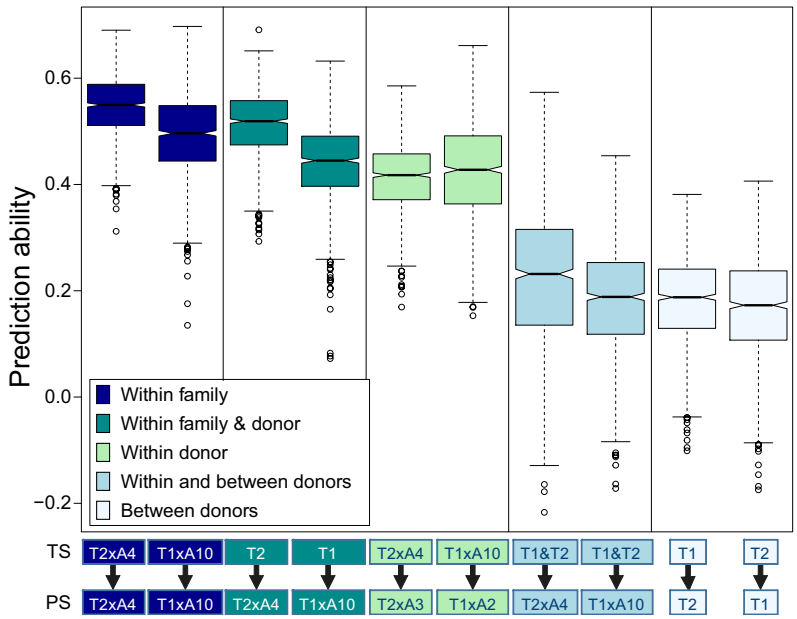

Fig. 9 Prediction accuracies within and between families from the same and different heterotic groups (T1 belongs to stiff-stalk synthetic, T2 to non-stiff-stalk heterotic group)

Assessing Brazilian resistance donors in intercontinental trials

Tropical donors were tested as per se and testcrosses in Brazil and for the first time also in Europe. Our trials were conducted during the growing season of 2019 in two locations in the South of Brazil, where the environmental conditions are favorable for NCLB infections, and during the growing seasons of 2017, 2018 and 2019 in Europe on one to 10 environments (Supplementary Table 1). The locations represented the target areas of different grain maize maturity groups with the German locations being the earliest, followed by French and Italian locations as the latest ripening group (Rüdelsheim and Smets 2011; Czarnak-Kłos and

Rodríguez-Cerezo 2010). Because of the highly differing maturity growing zones and different photoperiod response between Brazil and Europe, Brazilian donors could be tested only as testcross progeny in German and French locations.

The tropical donors, assessed both per se and in testcrosses, showed a moderate to high resistance level to NCLB in both continents, demonstrating that the Brazilian resistance sources are also resistant in the different maize maturity growing zones of Europe. However, lines per se originating from crosses between Brazilian resistance donors and susceptible European elite lines possessed higher average NCLB severity in Brazil than in Europe. This could be explained by more favorable environmental conditions for NCLB incidence in Brazil compared to Europe and/or more aggressive fungal populations. In Brazil, the average temperature between inoculation and the last field evaluation was 20.4 °C varying from 17.5 to 27.8 °C (INMET 2020, Supplementary Table 2). Considering all European locations, the average temperature between inoculation and the last field evaluation was 22.7 °C varying from 3.7 to 38.4 °C (Agrarmeteorologie Baden-Württemberg 2020; AgrarMeteorologie Bayer 2020; Ilmeteo 2020; Meteociel 2020; Time and date 2020), which indicates that the minimum and maximum temperatures in Europe were not within the ideal range of NCLB development of 15–25 °C (Carson 1999; Hanekamp 2016; Galiano-Carneiro and Miedaner 2017).

Race monitoring studies were conducted in the same field locations where our trials were located. In 2019, the predominant race in Castro was race 1 ($n = 7$ samples) and in Ponta Grossa races 0, 1, 12, 23 N and 2 occurred where races 0 and 2 were more frequently observed ($n = 14$) (Navarro, personal communication). Hanekamp (2016) collected leaf samples with NCLB symptoms from regions where our European trials were located in 2011 and 2012 and concluded that race 0 was the most abundant in the south of Germany and Austria, while race 3 N and 3 were the most common in southwest of France and north of Italy, while race 1 was the most abundant in Austria and Hungary and the German Upper Rhine region. These results indicate that a different *Setosphaeria turcica* race composition was observed in each of our field locations. Because we report in this study only QTLs identified across locations despite the different race compositions, we can conclude that these QTLs should be quantitatively inherited and not based on race-specific resistances.

The introgression of exotic, quantitatively inherited resistance QTLs by marker-assisted backcrossing (MABC) can be hampered by the lack of adaptation traits in Brazilian materials. Therefore, flowering time, which can restrict the employment of QTL in a broad number of environments, and plant height, which can lead to stalk lodging, were also assessed. These adaptation traits were solely assessed in one location in Germany in 2018 and in one location in Italy during the growing seasons 2018 and 2019. The correlations between the German and the Italian location for each donor group were high ($r > 0.91$, $P < 0.0001$; data not shown), demonstrating that the ranking of flowering time did not significantly change in contrasting environments and it was possible to compare flowering time with our NCLB ratings assessed in different environments.

The adapted elite lines assessed in testcross combination were maturing on average 16 days earlier than the tropical lines assessed in testcross combination in European environments according to the BLUEs of the parental lines (data not shown) and were, concomitantly, more susceptible than the donor lines. This justifies the negative correlations observed between $NCLB_f$ and FF. This tendency resulted in a long flowering period of testcrosses (e.g., 34 days in Italy 2019), and this can partially explain why correcting for early flowering as described by Emrich et al. (2008) did not reduce the correlation between NCLB and FF ($r = -0.51$ vs. $r = -0.67$). Van Inghelandt et al. (2012) corrected the commercial maize germplasm employed in their study by dividing the genotypes in different maturity groups where the inoculation of late maturity group started 2 weeks later than the earliest group (Bormann et al. 2004). They conducted QTL mapping for the adjusted and non-adjusted NCLB scoring for flowering time and identified different QTL for each trait. Previous research projects investigating genetic architecture of NCLB resistance within adapted US and European materials revealed very low negative correlations between NCLB symptoms and FF ranging from −0.06 to −0.14 (Balint-Kurti et al. 2010) and a moderate negative correlation of 0.53 (Van Inghelandt et al. 2012). These results are in line with the correlations observed in our study. In addition, as *S. turcica* is a hemibiotrophic pathogen the disease is expected to advance faster in necrotrophic tissues (Van Inghelandt et al. 2012, Jiang et al. 1999).

Correlations between $NCLB_f$ and PH also yielded negative values, indicating that short plants were more affected by NCLB. This is also mostly specific to our plant material since adapted lines assessed in testcross combination were on average 30 cm shorter than the tropical donors lines according to the BLUEs of the parental lines (data not shown). Moderate positive correlations between FF and PH were also observed, demonstrating that late and tall genotypes were less affected by NCLB, most probably because of the photoperiod sensitivity of the tropical donors.

Implications for NCLB resistance breeding programs

Although genotype by year and genotype by location interactions played an important role in per se and testcross assessments, moderate to high heritabilities were observed both in Brazil and in Europe. This suggests that we were consistent with our visual scoring methodology and that our material

had adequate genetic variation that can be exploited in maize resistance breeding programs of both continents.

The relative efficiency of indirect selection of lines per se for testcross performance ranged from 0.20 to 0.38 depending on the population and assuming the same selection intensity. The relative efficiency represents the expected correlated response of hybrid performance when line per se selection is applied relative to the expected direct response on hybrids (formula see in Materials and Methods). Therefore, relative efficiency below one indicates that a direct selection on hybrid performance is more efficient than the indirect selection with lines per se. However, this conclusion should be interpreted cautiously since our lines and testcrosses were not always evaluated in the same environments and our results might also be affected by the lack of adaptation traits in the tropical resistance donors. In contrast, Schechert et al. (1997) observed high per se and testcross correlations of $r = 0.94$ and 0.98 for a diallel design in three locations in the US Corn Belt and recommended selection in early stages of line development. However, they still recommended to assess NCLB resistance in hybrids since the disease showed some level of heterosis (Schechert et al. 1997). On the other hand, also the inbred lines should have a minimum resistance level to ensure seed production without or with a lower number of fungicide applications.

The choice of the tester plays an important role for NCLB resistance assessment as observed in our trials. Tropical testers A and B crossed with DH lines in Brazil are known to be moderately susceptible to NCLB with a mean score of 4 (1–9 scale) (Miranda Pires, Cambé, PR, Brazil; pers. commun.). Contrarily, the flint tester C applied in Europe was highly susceptible (NCLB scoring 7–9, Kessel, Einbeck, Germany; pers. commun.) and testcrosses revealed, on average, even a higher susceptibility than the lines per se (Fig. 3). This indicates that the highly susceptible testers are recommendable for environments with low to moderate disease severities, such as in Europe.

Brazilian genetic material is a great source of QTLs for quantitative NCLB resistance

Multi-parent QTL mapping promises to be a useful tool to dissect the genetic architecture of traits since it combines the high power to detect infrequent favorable alleles with a high mapping resolution (Würschum 2012). Connected populations are usually already available in typical breeding programs; however, each population is frequently composed by small to moderate population sizes only. As a small sample size entails a lower detection power for quantitative traits with complex/polygenic architecture (Schön et al. 2004), multi-parent QTL analysis can be an alternative to increase detection power (Han et al. 2016) in case of common QTL among families. In addition, it allows the investigation of variation in allele substitution effects, which are usually diverse at certain loci across different genetic backgrounds which increases the success rates of QTL transferability to other populations (Xu 1998; Blanc et al. 2006; Steinhoff et al. 2011; Garin et al. 2017). Hence, we conducted multi-parent QTL analysis for NCLB by means of the bi-allelic allele substitution effect models. Only donor T5 was analyzed separately in a biparental QTL mapping since it was composed by a unique family.

Our study revealed 17 QTL for $NCLB_f$ on all 10 chromosomes, while each of them explained between 3.57 and 30.98% of the genotypic variance. This complex genetic architecture was also observed in other NCLB QTL studies (Wang et al. 2018; Chen et al. 2016; Van Inghelandt et al. 2012; Poland et al. 2011; Wisser et al. 2006). To the best of our knowledge, the QTLs identified in our study and located on chromosome bins 1.07, 1.08, 2.02, 2.04, 4.03, 5.04, 8.08 and 9.04 were not yet published in the literature. These findings confirm that Brazilian resistance donors are great sources of novel alleles for NCLB resistance. Although the other nine QTLs have already been identified in the same chromosome region in other studies, different genes or alleles may confer the resistance to NCLB (Chen et al. 2016; Ding et al. 2015; Schaefer and Bernardo 2013; Van Inghelandt et al. 2012; Poland et al. 2011).

In addition to minor QTL, we identified four major QTL q8, q9, q7 and q17 on chromosome bins 1.07, 2.02, 10.04 and 6.01, resp., (> 20% explained genotypic variance) originating from our Brazilian tropical donors T2 and T5. In addition to these four QTL, the QTL q4 also showed a high explained genotypic variance of 13.3 and 16.8% in Brazil and in Europe, respectively.

Among the 17 QTL identified for $NCLB_f$, four originated from the adapted elite lines showing that some resistance for NCLB was already present in Europe. However, the QTL originating from the adapted elite lines explained only a lower proportion of the genotypic variance compared to most of the QTL originating from the tropical germplasm (3.57–12.27% vs. 5.43–30.98%). The five and the 14 QTLs identified in Brazilian and European trials, respectively, including two overlapping QTL, tend to be stable within Europe which were assessed in many environments composing different maize maturity growing zones and combinations of *S. turcica* races (Hanekamp 2016). In Brazil, more test locations would be necessary to confirm the stability of the identified QTL. We observed two co-located QTL between per se and testcrosses in Europe among the six QTL identified for line per se and 10 QTL identified for testcrosses (Fig. 6). This is in accordance to the moderate correlation between per se and testcrosses for $NCLB_f$ in Europe (0.37 for donor T2 and 0.61 for donor T5, Fig. 4) and to the lower explained genotypic variance of testcrosses compared to lines per

se for the same donor. For instance, QTL q8, which was identified for both per se and testcrosses within donor T5, had almost a twice as large allele substitution effect for line per se compared to the testcrosses. This shows that only about half of the line per se trait variance could be captured in our testcrosses which are accordance to the expectation (Melchinger et al. 1998).

The QTLs q4 and q5 for $NCLB_f$ were identified in both Brazilian and European trials on bins 7.03 and 9.04, respectively. This is in line with the moderate phenotypic correlation for NCLB severity between trials in both continents ($r = 0.36$, $P < 0.001$, for donor T1, and $r = 0.41$, $P < 0.001$, for donor T2). The two QTLs can be potentially applied in breeding programs in Brazil and in Europe to assist selection of most resistant genotypes. Fine-mapping studies are, however, advisable. They can potentially increase the precision of the QTL location which is a success factor for genomics-assisted breeding application. Conducting a fine-mapping study will also show whether there is a close linkage/pleiotropy of NCLB QTL with FF QTL (q5) or just a coincidence of two different QTL in the same chromosomal segment what could be related to the large confidence interval of this FF QTL. The possibility of a pleiotropic effect cannot be discarded as the QTL q4, q7, q11, q12 and q14 where identified in the NCLB analyses of both, non-corrected and flowering time corrected data (data not shown). In addition, both QTLs were contributed by donor T2, indicating that validation studies by observing the effect of these QTL in other genetic backgrounds would be helpful before introgressing them to other genetic material.

According to the literature, the genes *Ht1, Ht2, Ht3, HtP, HtNB, Htn1* and *rt* were identified on chromosome bins 2.08, 8.06, 7.04, 2.08, 8.07, 8.05 and 3.06, respectively (for review see Galiano-Carneiro and Miedaner 2017). Except by the chromosome bin 8.05, none of our QTL were identified in these regions where the qualitative resistance genes are located indicating that our populations most likely carry quantitative resistances. Although the QTL q14 was identified on chromosome 8.05 in our populations, it is unlikely that it represents the gene *Htn1* as none of the parents were carriers of this resistance gene. In addition, the numerous QTLs attributed to NCLB resistance in our study each explaining a small to a moderate proportion of the genotypic variance only support a quantitative inheritance in our Brazilian donor lines.

Genomics-assisted selection is a powerful breeding tool to accelerate the introgression and integration of NCLB resistance in adapted plant materials

Genomics-assisted breeding can be a good possibility to increase NCLB resistance levels in a shorter time. We investigated the applicability of these methods for our plant materials and identified high prediction accuracies for wRR-BLUP which is in accordance with studies in other pathosystems (Boeven et al. 2016; Spindel et al. 2016; Galiano-Carneiro et al. 2019; Miedaner et al. 2019; Jähne et al. 2019). RR-BLUP also presented a high prediction accuracy which is in accordance with a genomic selection (GS) assessment for NCLB resistance conducted by Technow et al. (2013) that identified prediction accuracies of 0.71 and 0.69, depending on the heterotic group. The low to moderate prediction accuracy from MAS confirms the complex genetic architecture of NCLB with many QTL with small effects only. However, accounting for epistasis can potentially increase the prediction ability as it explains a relative high proportion of the variance according to van Inghelandt et al. (2012). Conversely, the high prediction accuracies in this cross-validation study may be overestimated since our training and prediction sets were composed by closely related plant materials and were tested in the same environments, both can considerably inflate the estimates (Riedelsheimer et al. 2013; Brauner et al. 2020). The prediction accuracies for GS from tests in Brazil were lower than those from Europe, most probably due to the lower testing intensity in Brazil. Many other factors can also influence the prediction accuracy such as training set size and relationship between the training and prediction sets. We tested different training set sizes and observed a linear increase of the prediction accuracy as we increased the number of individuals within the training set (data not shown). This result is in accordance with other studies (Riedelsheimer et al. 2013; Han et al. 2016; Van Inghelandt et al. 2019). For this reason, we compared at a fixed training set of 60 individuals the prediction accuracies of materials with different genetic relationships between the training and prediction sets (Fig. 9). An increase in relatedness between training and prediction sets increased the prediction ability (Riedelsheimer et al. 2013; Han et al. 2018; Brauner et al. 2020). The lowest prediction ability was observed for predictions between heterotic pools (i.e., between T1 and T2) which is in accordance with other studies related to different traits (Han et al. 2018, Brauner et al. 2020). Van Inghelandt et al. (2019) observed an increase of the prediction ability when a mix of individuals from different pools were composing the training and prediction sets. In this study, we followed a similar approach when using populations of T1 and T2 in the training set to predict a population of the opposite donor, but this did not increase the prediction accuracy (Fig. 9). This could be due to the lower diversity of the mixed populations included in our work compared to Van Inghelandt et al. (2019)'s populations.

One aspect that can hamper the application of the identified QTLs in breeding programs in Europe is the lack of adaptation traits of the Brazilian germplasm, especially

when traits unwanted for European conditions, such as late maturity, photoperiod sensitivity and plant tallness, are located in close genomic regions to our identified QTL. The three major NCLB QTLs that could be recommended for introgression due to their high explained genetic variance and stability (e.g., q4, q8 and q9) were not linked to the QTL identified for FF and PH. Therefore, concomitant selection to reduce NCLB damage, early maturity and short plant height is feasible.

Conclusions

Quantitative resistances tend to be the best option to keep low NCLB levels durably in areas with high disease pressure. This type of resistance is especially important for NCLB resistance because *S. turcica* populations have a moderate to high evolutionary potential (McDonald and Linde 2002) leading to vulnerability of race-specific resistances. Minor and major QTL were identified for NCLB in our study explaining 3.57–30.98% of the genetic variance. Among them, two QTL were detected in Brazil and Europe explaining between 7.10 and 16.83% of the genotypic variance, which can be employed in a broad range of ecosystems.

Brazilian breeding materials were quantitatively resistant in all our European test locations and the crosses between Brazilian × European lines yielded moderate to high genetic variances for NCLB resistance. However, other resistance sources from Brazil can potentially also result in stable quantitative resistance to NCLB with even higher resistance levels. Therefore, we recommend further investigations on South American donor lines.

Before the application of these environmentally highly stable two QTLs in genomics-assisted breeding programs, QTL validation in different BC populations is recommended. This should improve the precision of the QTL location and the success rates of QTL transferability by molecular markers. For this, KASP (Kompetitive allele specific PCR) markers based on the sequences of the detected closely linked SNPs for foreground selection that allow a low-cost detection in segregating backcross generations should be generated. Finally, a genomics-assisted breeding approach can be applied for a successful introgression and integration of NCLB resistance QTL originating from tropical plant material.

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Author contribution statement TM, TP and BK planned the experiments and supervised the project. AG and BK collected phenotypic data. AG conducted all statistical analyses and wrote the manuscript; TM thoroughly revised it. All authors read the final version for publication.

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Compliance with ethical standards

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

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

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7 General discussion

Genomic tools can assist the identification and the accumulation of quantitative disease resistances in crops. This thesis focused on three important pathosystems: FHB in triticale, GER in maize, and NCLB in maize to exemplify different aspects of genomics-assisted breeding strategies. All three diseases possess devastating potential under European growing conditions. However, few studies have assessed the applicability of genomics-assisted breeding strategies to select for FHB resistance in triticale. In addition, exotic maize germplasm can potentially provide novel resistance genes and increase the durability of NCLB and GER resistances. However, exploiting exotic germplasm is challenging due to the lack of phenotypic and genotypic characterization of their benefits as well as the absence of adaptation traits. Moreover, intensive phenotyping in contrasting environments is imperative for a successful introgression of exotic into adapted germplasm. Therefore, this thesis aimed to characterize exotic germplasm in multi-environmental field trials, to investigate the potential of genomics-assisted breeding strategies to assist the introgression and the integration of exotic maize germplasm, to dissect the genetic architecture of FHB resistance in triticale and to exploit the potential of GS seeking for an efficient characterization of superior genotypes.

Genomic selection has high potential to assist selection of superior Fusarium head blight (FHB) resistant genotypes in applied triticale breeding programs

FHB in triticale reduces yield and can jeopardize farm animals' health due to DON accumulation. For instance, average DON accumulation of 12 triticale genotypes inoculated with *F. culmorum* reached more than 15 mg kg^{-1} (Gaikpa et al. 2019). This is much higher than the limit of 0.9 mg kg^{-1} DON recommended by the European Commission for swine feeding (European Commission 2006). Although DON accumulation is usually higher in inoculated disease nurseries compared to natural infections in production fields, it still reflects the devastating potential of FHB.

DON accumulation initiates after *Fusarium* spp. infection and assists FHB spread through the triticale ear (Audenaert et al. 2013; Gaikpa et al. 2019). The reduction of DON accumulation is known as type 3 resistance (Miller et al. 1985; Ollier et al. 2020), while type 1 resistance is conferring reduction of initial infection and the type 2 reduces FHB spread along the ear (Schroeder HW 1963; Ollier et al. 2020). Visual assessment of FHB is typically a combined evaluation of type 1 and 2 resistances that are correlated with type 3 resistance (Miedaner et al. 2004, 2016), being moderate in rye (r = 0.31 to 0.70) (Miedaner et al. 2003) and high in wheat (r = 0.69 to 0.82; Miedaner et al. 2003, 2004). In triticale, phenotypic correlation of type 1 and 2 with type 3 was reported to be moderate (r = 0.31 to 0.36; Miedaner et al. 2004, 2016). Thus, the German federal plant variety office decided to assess DON content to describe triticale genotypes in official trials (Miedaner et al. 2016). However, assessing DON content in an applied breeding program is costly and time-consuming. Therefore, considering large segregating populations, typical in early breeding stages, genomics-assisted breeding strategies for FHB can enhance genetic gain per breeding generation (Xu et al. 2020).

We dissected the genetic architecture of FHB based on visual severity assessment of the disease in three environments and observed a rather complex genetic architecture with few major QTL (Chapter 2). This is in line with previous studies (Kalih et al. 2014, 2015; Miedaner et al. 2016; Dhariwal et al. 2018; Ollier et al. 2020). Kalih et al. (2014) identified one QTL on chromosome 5R, explaining 48.4% of the genetic variance of FHB, but this high value was most likely due to congruency with the major dwarfing gene *Ddw1* present on the rye genome (Korzun et al. 1996). Furthermore, this QTL on chromosome 5R increased FHB incidence by 2.9%. Indeed, *Ddw1* is known to have a negative effect on FHB (Kalih et al. 2014). Accordingly, plant height was assessed in our study but low phenotypic correlation between FHB and plant height was observed and no QTL for FHB neither for plant height were identified on chromosome 5R (Chapter 2). Miedaner et al. (2016) detected a major QTL on chromosome 2A, which was overlapping among FHB severity, Fusarium damaged kernels rating and DON content. A QTL on chromosome 2A was also detected in our study (Chapter 2), but was congruent to a QTL identified for plant height and fine mapping is necessary to further explain this congruency. In fact, the majority of QTL mapping studies conducted for FHB severity in triticale detected QTL on chromosome 2B (Kalih et al. 2015; Dhariwal et al. 2018; Ollier et al. 2020), including ours (Chapter 2). QTL on chromosome 2B explained between 1.5 to 32% of the genotypic variance and reduced FHB severity by 4.8% to 29.5% depending on the study. In our study, the most prominent QTL was mapped on chromosome 5B explaining 30.2% of the genotypic variance and reducing FHB by 7.8% (Chapter 2). However, validation of this QTL in different triticale backgrounds is essential for its successful introgression and application in breeding programs. Recently, the major and most prominent QTL *Fhb1* conferring type 2 resistance in wheat, was successfully introgressed in three biparental triticale populations (Ollier et al. 2020). This QTL reduced FHB symptoms by 8 to 35% depending on the population (Ollier et al. 2020). *Fhb1* mapped on chromosome 3B was derived from "Sumai-3", a Chinese wheat line. This QTL is employed in North American wheat varieties, but is rare in European breeding programs (Steiner et al. 2017). This lower

application in Europe is mainly due to the challenge of linkage drag of the donor germplasm that may lead to lodging and decreased yield (Steiner et al. 2017). Therefore, these possible negative effects on agronomical traits should be addressed by fast recurrent marker assisted backcrossing (Cobb et al. 2019) to enable durable success of *Fhb1* in triticale breeding programs.

In fact, the identified QTL in the above studies with the largest explained genotypic variance are potential candidates for MAS. Although MAS could be an affordable solution to increase FHB resistance in triticale programs (Ollier et al. 2020), QTL with major effects for FHB are rare. As an alternative for line development, GS is a more appropriate tool to select for superior genotypes with presence of both major and minor effect QTL (Poland and Rutkoski 2016). Furthermore, GS has already resulted in promising prediction accuracies for diverse agronomical traits and FHB in triticale (Würschum et al. 2017). GS can be applied to replace phenotyping in early generations to reduce cycle length, to increase selection accuracy or to further increase selection intensity as genotyping costs continue to decrease (Xu et al. 2020). Moreover, a broad application of GS can assist the breeder in cross planning (Lehermeier et al. 2017) and opens avenues for new breeding schemes to enhance selection gain (Gaynor et al. 2017). All these applications require training of robust GS models. The potential of GS in our diverse triticale panel was assessed by 5-fold cross-validation for two GS models: RR-BLUP and weighted ridge regression-best linear unbiased prediction (wRR-BLUP). Employing RR-BLUP includes all markers in the random effect while the latter includes all four QTL explaining more than 5% of the genotypic variance in the fixed statement of the GS model (Chapter 2). The prediction accuracy of RR-BLUP was 0.55 which is similar to the prediction accuracy for FHB resistance observed in wheat (prediction accuracy = 0.6; Mirdita et al. 2015). This shows the potential of GS to select for resistances already present in elite germplasm (Steiner et al. 2017). In contrast, prediction accuracy increased to 0.78 when wRR-BLUP was employed (Chapter 2). A higher prediction accuracy by a weighted model was also observed in other small grain cereals for FHB (Herter et al. 2019; Gaikpa et al. 2020; Moreno-Amores et al. 2020).

Besides increasing prediction accuracy, this model leads to a selection pressure on selected QTL, leading to potential fixation of the favorable allele in the longterm (Bernardo 2014). However, prior application of identified QTL as fixed effects in GS models, QTL validation in independent populations is highly recommended.

Despite these encouraging results, the rather moderate correlation between FHB severity and DON accumulation must be tackled by the breeder and can limit the success of GS application in breeding programs targeting reduced DON content. Therefore, we suggested a gradual shift from visual FHB severity to DON assessment. However, while a large training set based on DON measurement is not available, we recommend a negative selection based on GS for visual FHB severity rates at initial breeding generations. The elimination of genotypes with the highest FHB infection levels allows enough variation to select for other important agronomical traits at later stages and reduces the number of genotypes for the costly assessment of DON content. In the long term, we highly encourage breeders to build GS calibrations for DON content.

Jointly, genomics-assisted breeding strategies are promising approaches to reduce FHB severity. In addition, major QTL have been identified and are potential candidates for QTL validation and subsequent implementation in GS to increase prediction accuracy. DON assessment in later breeding stages is essential to correctly classify triticale genotypes for their DON content and to select varieties that can be safely employed as on-farm livestock feed.

Gibberella ear rot (GER) in maize is highly influenced by the environment but stable QTL are also available

The correlation between GER severity and DON content in maize was reported high ($r \geq 0.90$; Löffler et al. 2011; Miedaner et al. 2015). DON

content in maize kernels can be mainly assessed by near-infrared spectroscopy (NIRS), enzyme-linked immunosorbent assay (ELISA), and indirectly through visual GER severity scoring. NIRS assessment is recommended in advanced breeding generations as it is cheaper than ELISA and the two methods are highly correlated (r = 0.89; Martin et al. 2012b, Miedaner et al. 2015). However, visual GER severity assessment is advisable because of the lower costs compared to NIRS allowing higher selection intensities (Miedaner et al. 2015; Han et al. 2018). To identify significant differences between resistant and susceptible genotypes, inoculation is essential but time-consuming (Löffler et al. 2011). *F. graminearum* inoculum is applied on individual maize cobs at optimal maturity and *in situ* structure is necessary for inoculum preparation and storage (Chapter 3). In addition, GER shows high genotype-by-environment interactions (Löffler et al. 2011; Martin et al. 2012b; Butrón et al. 2015) as confirmed in our study (Chapter 3), requiring multi-environment trials to identify stable resistances over years and locations (Reid et al. 1996; Bolduan et al. 2009; Löffler et al. 2010, 2011). High genotype-by-environment interactions can be partially explained by the quantitative nature of this trait (Martin et al. 2012a; Kebede et al. 2016), which was further confirmed by our study (Chapter 3). Indirect selection for GER severity by kernel dry down rate has been proposed (Kebede et al. 2015). However, despite significant correlation and possible pleiotropic effects with GER resistance, it was not effective (Kebede et al. 2015, 2016). Therefore, GER severity assessment is still the most efficient way to select resistant genotypes. However, the high costs of inoculation, the time necessary to accomplish this task and the laborious symptom rating can restrict phenotyping in large population sizes. In fact, genomics-assisted breeding strategies allow screening of a large number of genotypes in multi-environmental trials under budget limitations (Martin et al. 2012b). However, low resistance levels have been reported within the widely employed Flint germplasm in Europe (Löffler et al. 2011). Therefore, the introgression of exotic resistance alleles through genomics-assisted breeding strategy can improve resistance levels in European hybrids (Gaikpa and Miedaner 2019), which are mostly composed by the Flint × Dent heterotic pattern.

We dissected the genetic architecture of GER in populations derived from crosses between Brazilian exotic and European adapted double haploid (DH) lines, tested in multi-environmental field trials (Chapter 3). We identified four QTL on chromosome bins 1.02, 3.08, 5.06 and 8.05 that explained between 5.4 to 21.8% of the genetic variance while the majority of the resistance alleles originated from the Brazilian tropical parents (Chapter 3). However, each QTL was reducing GER by 0.34 to 1.33% indicating a complex genetic architecture where many QTL with small effects are necessary to substantially increase the resistance levels as observed in other studies (Martin et al. 2012b; Kebede et al. 2016). QTL on chromosome bins 3.08 and 5.06 were also identified in another QTL mapping study for GER severity employing different populations where the first QTL was identified for DON contamination additionally (Martin et al. 2012b), indicating that these genomic regions might be independent of the genetic background. Indeed, the validation of a QTL in different genetic backgrounds is essential for an efficient application of the QTL in genomics-assisted breeding strategies. To date, only few QTL for GER severity could be validated (Brauner et al. 2016; Gaikpa and Miedaner 2019). Surprisingly, the QTL located on chromosome bin 1.02 was identified in Brazil and in Europe across all six biparental populations indicating stability across different genetic backgrounds and environments (Chapter 3). Therefore, this QTL is a potential candidate for introgression in different breeding materials in diverse target environments, but fine mapping and QTL validation are necessary prior employment. Moreover, the chromosome bin 1.02 has been already identified as a region that confers resistance to a number of maize diseases such as Ear and Stalk rot (caused by multiple pathogens), Common smut (caused by *Ustilago maydis*), Gray leaf spot (caused by *Cercospora zeae-maydis*), Southern corn leaf blight, NCLB, Stewart's wilt (caused by *Erwinia stewartii* (Syn. *Pantoea stewartii*) and Common rust (caused by *Puccinia sorghi*) (Wisser et al. 2006; Chung et al. 2010). This indicates a possible linkage of QTL from different diseases or pleiotropic effects requiring further investigations (Wisser et al. 2006). Indeed, Chung et al. (2010) investigated candidate genes at this locus conferring resistance to NCLB and concluded that they enhanced the accumulation

of callose and phenolics components surrounding infection sites, a defense mechanismus that might be common among the aforementioned pathogens. In addition, pathogenesis related proteins, chitinases, xylanase inhibitors, phytoalexins, plant hormones, proteinase inhibitors and a class III peroxidase were associated with GER resistance (Chen et al. 2009; Mohammadi et al. 2011; Schmelz et al. 2011; Zhang et al. 2013; Kebede et al. 2018; Gaikpa and Miedaner 2019). For instance, significantly higher levels of PR-10 proteins, encoding intercellular proteins responsive to stress in *Aspergillus flavus* (Chen et al. 2006, 2010), were observed in the resistant genotypes to GER (Mohammadi et al. 2011; Kebede et al. 2018). In addition, higher disease severity was observed in genotypes with slow drydown, late silking, tight and full coverage of husk (Kebede et al. 2016). This reflects the significant negative correlation between female flowering time and GER or DON, and indicates that linkage or pleiotropic effects of the QTL are likely to occur (Martin et al. 2012b), but were not observed in our study in Europe (Chapter 3). Due to the significant and moderate correlation between female flowering time and GER in Brazil, we included female flowering time as a co-variable in the mixed model analysis (Emrich et al. 2008). The correction for flowering time might explain the absence of congruent QTL for GER and female flowering in our study (Chapter 3). Therefore, breeders should be encouraged to apply this model to avoid concomitant selection for GER reduction and lateness.

Epistatic effects demonstrate minor importance in this pathosystem (Martin et al. 2012a; Han et al. 2016) as observed in our study (Chapter 3). One reason might be that population sizes were not high enough to detect such effects (Han et al. 2016). Conversely, additive gene action plays an important role (Chungu et al. 1996) and sufficient GER resistance must be present in both heterotic pools to achieve sufficient resistance levels in the hybrid background (Löffler et al. 2011). Average negative mid-parent heterosis of 19% for GER has been observed (Martin et al. 2012a) indicating that significant dominance gene action may also play a role in this pathosystem (Bolduan et al. 2010; Martin et al. 2012a; Butrón et al. 2015). Moreover,

the relative efficiency of indirect selection for hybrid performance based on parental per se evaluation is rather low, indicating that the assessment of GER direct on testcrosses leads to higher progress compared to line *per se* assessment (Bolduan et al. 2010; Löffler et al. 2011; Martin et al. 2012c). In fact, this low efficiency can be related to the fact that hybrids are likely more vigorous than inbred lines for GER (Kovács et al. 1994; Bolduan et al. 2010; Martin et al. 2012a). However, a direct comparison between lines and hybrids regarding their GER levels was not possible in our study. Populations assessed for their line *per se* performance in Italian locations had poor seed set, most likely due to the lack of adaptation traits, hampering direct comparisons. This illustrates one of the challenges of evaluating exotic germplasm and emphasizes the benefits of crosses with adapted testers for the evaluation of non-adapted germplasm (Poland et al. 2011). The choice of the tester is of utmost importance to obtain satisfactory genetic variation for GER assessment (Longin et al. 2007). Employing susceptible to moderately susceptible testers is advisable to avoid masking dominant allele effects that might reduce the genetic variance of the trait (Löffler et al. 2011).

To conclude, genotype-by-environment interactions explained a large portion of the trait variation, but one QTL on chromosome bin 1.02 was decreasing GER in different genetic backgrounds evaluated in many environments. This stable QTL is a great candidate for validation and fine mapping, and subsequent introgression in European germplasm.

Exotic germplasm reveals environmentally stable resistances to Northern corn leaf blight (NCLB) in maize

NCLB can be qualitatively or quantitatively inherited (Chapter 4), but only quantitative resistance was present in our study (Chapter 5) leading to a

reduction of number and size of lesions on the leaves instead of a complete suppression or delay of disease symptoms (Chapter 4). Indeed, our inoculum, collected from naturally infected leaves, represented a mix of different *S. turcica* races (Hanekamp 2016; Navarro et al. 2021) (Chapter 5). This indicates that the resistant genotypes identified in our study (Chapter 5) were most likely resistant to a broad range of pathogen races, one of the characteristics of quantitative resistances (Chapter 4).

Our field trials were located in the South of Brazil and in Europe, comprising a wide range of climatic condtions (Chapter 5), a key requirement for the identification of stable QTL (Dingerdissen et al. 1996). To cope with the lack of adaptation traits, our genotypes were evaluated in Germany and in France solely as testcrosses (Chapter 5). Other locations allowed the assessment of line *per se* and testcross performance, which were moderately correlated (r= 0.37 to 0.61; Chapter 5). However, it is unlikely that the populations generated in our study will be directly employed in commercial breeding programs. In fact, NCLB was significantly correlated with flowering time and plant height indicating that selection for most resistant genotypes would lead to late and tall genotypes (Chapter 5).

Our research aimed to dissect the genetic architecture underlying NCLB resistance in Brazilian germplasm (Chapter 5). We identified 17 QTL distributed along the ten chromosomes of maize explaining individually 3.6 to 30.9% of the genotypic variance (Chapter 5). The majority of the alleles reducing the infections originated from Brazilian donors and reduced NCLB between 0.3 to 2.5 scores in the 1-9 severity scale (Chapter 5). Brazilian lines were more resistant than the adapted European parents for NCLB, both in Brazilian and European environments (Chapter 5). Among the identified QTL, four of them were mapped on the chromosome bins 1.08, 2.02, 8.08 and 9.03/9.04 and, to the best of our knowledge, were described for the first time in the literature (Chapter 5). These QTL were identified across a wide range of environments comprising different *S. turcica* race compositions (Hanekamp 2016; Navarro et al. 2021) and different environmental conditions for the fungal germination and infection (Chapter 5). In fact, genotype-by-environment

interaction played an important role in our experiments (Chapter 5). Despite the high influence of the environment, we identified two QTL, q4 and q5, in Brazil and in Europe within the same QTL confidence interval range. They were mapped on chromosome bins 7.03 and 9.04 and explained between 5.4 and 16.8% of the genetic variance (Chapter 5). Other studies also identified a QTL for NCLB on chromosome bin 7.03 (Welz and Geiger 2000; Poland et al. 2011), while the QTL on chromosome bin 9.04 was most likely described for the first time. However, the latter was congruent to a QTL identified for flowering time most likely due to the large confidence interval range of the QTL for female flowering time. However, fine mapping is still necessary to exclude the possibility of linkage or pleiotropic effects which would require more efforts or even hamper the introgression of these QTL. Because these two QTL were identified in Brazil and Europe, where ecosystems are highly distinct, it is likely that both QTL can be sucessfully introgressed into European germplasm and applied in Brazilian breeding programs.

The QTL q14 identified on chromosome bin 8.05 (Welz and Geiger 2000; Schaefer and Bernardo 2013) is in close location to *Htn1*, but it is unlike that our QTL is congruent with this gene according to molecular comparisons (Chapter 5). This NCLB resistance gene delays sporulation (Chapter 4) and reduces the pathogen penetration at three days post infection (dpi), characterizing the delayed susceptibility (Yang et al. 2019), and might be also present in sorghum being another host of NCLB (Zhang et al. 2020). *Htn1* encodes the wall-associated receptor-like kinase (WAKs), a protein-receptor that recognizes changes on plasma membrane structure in the first line of host defense. In the presence of WAKs, there is a reduction of defense-related benzoxazinoids, secondary methabolites such as DIMBOA (Yang et al. 2019). WAKs was also reducing bacterial blight and plant height in rice (Krattinger and Keller 2017), raising the question if WAKs would also interfere in agronomical traits in maize. The *Lr34* resistance gene, originally identified in wheat genotypes, was also proven effective in maize by most likely restricting fungal growth during the biotrophic phase of the infection (Sucher et al. 2017).

We also identified one QTL on chromosome bin 3.04 (Chapter 5) which is reported to be in a cluster of disease resistances including NCLB and ear rot diseases (McMullen and Simcox 1995; Wisser et al. 2006; Xiang et al. 2010). In addition, fine-mapping was conducted and *pan1* (Jamann et al. 2014) and remorin (Jamann et al. 2016) have been suggested as candidate genes for NCLB resistance (Yang et al. 2017). In our study, several putative genes were identified within the confidence interval of our QTL that varied between 1.2 to 209.86 cM (Chapter 5), but fine mapping is necessary to identify targeted gene functions.

In conclusion, QTL originating from Brazilian germplasm increased NCLB resistance levels and are potentially providing novel alleles to confer durable resistances (Hallauer et al. 2010). The stable QTL identified between Brazilian and European environments can be adopted in Brazilian breeding programs as well as introgressed into European germplasm, increasing resistance to NCLB in both continents.

A rapid genomics-assisted breeding approach for the introgression and integration of exotic germplasm

Novel QTL for GER and NCLB resistances were identified (Chapters 3 and 5) confirming that tropic highlands and subtropic regions are rich sources of alleles conferring disease resistances that are not present in temperate germplasm (Welz 1998; Hallauer and Carena 2014). Moreover, exploring exotic material is a great alternative to restore genetic diversity (Tanksley and McCouch 1997) but can be hampered by the lack of adaptation traits (Hallauer et al. 2010).

To assist breeding for resistant and adaptated genotypes, we proposed a rapid genomics-assisted breeding approach (**Figure 1**). This approach is

composed of two parts: (i) Trait introgression in the greenhouse from Brazilian into adapted European germplasm; and (ii) trait integration in field trials by strong selection for adaptation traits and disease resistances. The first step consists of crossing the tropical resistant donor(s) with the adapted lines that lack high levels of resistances. The objective of this step is to transfer few major genes that were fine mapped and eventually tested in different backgrounds. Subsequently, the progenies of this tropical resistant × adapted susceptible cross are backcrossed (BC) with the adapted genotypes. The number of BC generations mainly depends on the difference in adaptation traits between donor and recipient. Practically, the most divergent they are, more BC generations are necessary (Dudley 1982; Šimić et al. 2003), because linkage drag reduces with an increase of BC generations (Mahone et al. 2015).

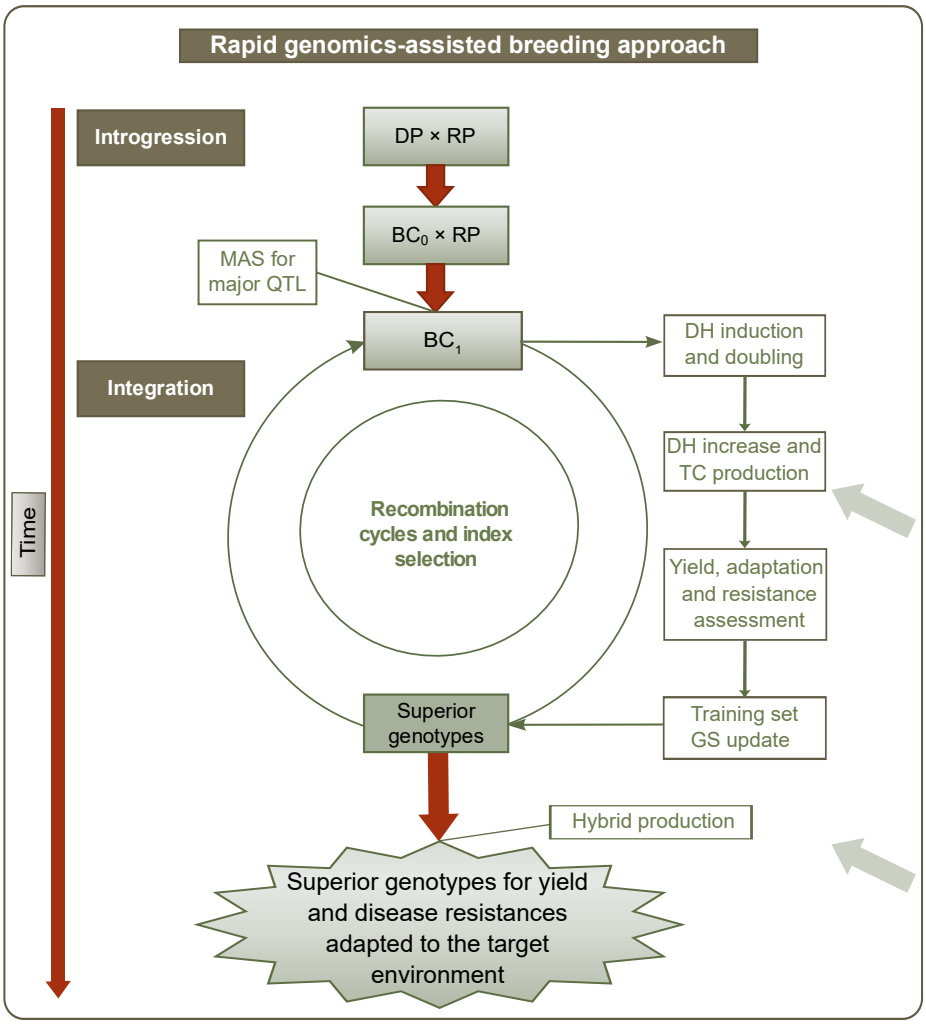

Figure 1: Schematic illustration of a rapid genomics-assisted breeding approach. Introgression and integration of resistance alleles from resistance donor parent (DP) to recurrent parent (RP) via backcross (BC) and selection of major QTL with marker assisted selection (MAS). Genomic selection is employed to select superior genotypes for adaptation traits and resistances. Double haploid (DH) and testcrosses (TC) are produced for the selection of superior hybrids for yield and resistance traits. The arrows on the right hand side indicate the inbred lines from the opposite heterotic pool

Introgression of the QTL from the resistance donor to the recipient via MAS is a well stablished approach and the primary tool to bring diversity to breeding programs, but is usually accompanied of linkage drag (Hospital 2001; Cobb et al. 2019). Especially in exotic materials, undesirable linkage drag such as lateness, lodging due to taller plants and possibily susceptibility to other diseases are expected. Positioning the flanking marker close to the target QTL can reduce the length of the donor chromosome segment (Frisch and Melchinger 2001), but cannot eliminate it. Linkage drag might be completely eliminated only if cloned genes are introgressed by gene target techniques such as clustered regularly interspaced short palindromic repeats, known as CRISPR-CAS9 (Cong et al. 2013; Arora and Narula 2017; Cobb et al. 2019). To the best of our knowledge, only the gene *Htn1* conferring resistance to NCLB has been cloned to date (Hurni et al. 2015; Yang et al. 2017) and none for GER. These genes that have not been cloned yet can be fine mapped with F2 or more advanced populations to increase the precision of the QTL or gene locations. Still, each molecular marker used for the introgression may introduce linkage drag. Therefore, few and small QTL regions should be transferred from exotic to elite germplasm.

In the integration step, DH lines should be phenotyped for NCLB and/or for GER to confirm the efficacy of resistance in the targeted backgrounds. GER sporadically occurs in Europe (Vigier et al. 2001; Mesterházy et al. 2012). Therefore, the cobs should be inoculated to guarantee high disease levels and uniform distribution of the disease. In addition, agronomical traits such as female flowering time, plant height, height of ear insertion, stalk lodging and other diseases of importance in the targeted regions should be phenotypically assessed to build a GS training set. These trials should be conducted preferentially in hot spots of the main diseases of the target regions and in multi-locations, although seed increase might be necessary. For instance, assessing Kabatiella eyespot in the north of Germany (Romero 2016) is advisible when this is the target region for the release of new varieties. Genotypes that are resistant to NCLB and/or GER and pose many adaptation traits can be selected for their GEBV index. The composition of the

training set is essential to obtain high prediction accuracies (Akdemir and Isidro-Sánchez 2019) and correctly classify superior genotypes. Therefore, different training and prediction set compositions were investigated based on testcrosses assessed for NCLB in Brazil (**Figure 2**).

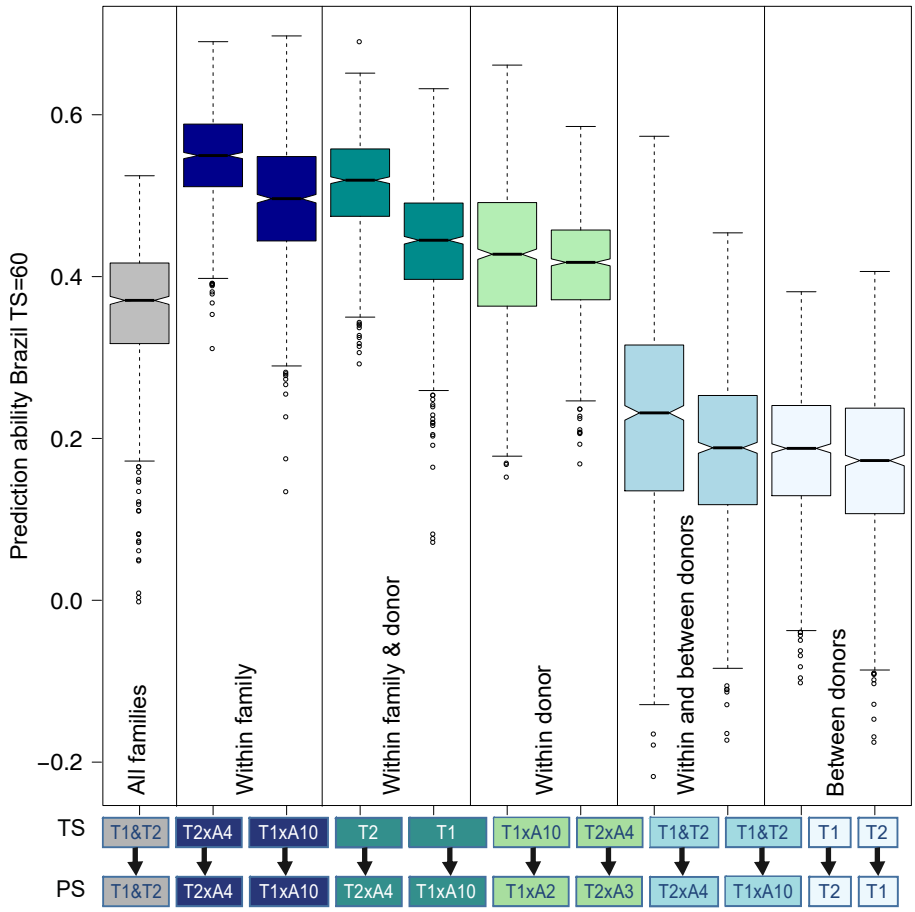

Figure 2: Prediction abilities for NCLB resistance. Testcrosses phenotyped in Brazil. Prediction abilities based on 5-fold cross validation and constant training set (TS) size of 60 individuals for different prediction sets (PS).“T2” indicates all biparental populations with the common parent T2 such as T2×A3 and T2×A4.“T1” is composed by T1×A1, T1×A2 and T1×A10

The prediction abilities were differing depending on the relatedness between training and prediction sets as well as on the size of the training set. As expected, larger training set resulted in higher prediction abilities

(Riedelsheimer et al. 2013; Han et al. 2016). Therefore we established a constant training set size comprising 60 individuals for a fair comparison among families. An increase in relatedness of training and prediction sets increased prediction ability (Riedelsheimer et al. 2013; Han et al. 2018; Brauner et al. 2020). This tendency was expected as the prediction ability depends on the genetic architecture of the trait (Würschum et al. 2017) and related families tend to share more common QTL compared to unrelated populations or half-sibs (Han et al. 2016). Nevertheless, the half-sib model led to similar or slightly lower prediction accuracies compared to the full-sib model. This might reflect the congruent QTL identified across connected families (Chapter 5). Indeed, linkage phase between the marker and the QTL is usually similar among half-sibs (Lehermeier et al. 2014) and including both full and half-sibs in the training set is advisable to increase training set size and, consequently, the prediction abilities (Brauner et al. 2020).

The most promissing genotypes can be intercrossed to new resistance donors or recipients to increase the genetic variability of the breeding population. After few cycles of recombination, testcrosses can be generated for yield, NCLB and/or GER resistance performance tests, and other critical diseases of the target location. Genotypes with high yield and NCLB/ GER performance can be employed in applied European maize breeding programs.

The release of high yielding cultivars mainly relies on an optimized design of breeding programs including germplasm and state-of-the-art breeding tools. In this research work, we investigated the potential of genomics-assisted breeding to accumulate favorable resistance alleles in three economically important pathosystems: Fusarium head blight in triticale, Gibberella ear rot in maize, and Northern corn leaf blight in maize. All populations applied in this study presented favorable QTL to decrease disease severity with rather complex genetic architectures. The strategies to identify and accumulate quantitative resistances presented in this study promise high potential to contribute to the efficient release of highly resistant genotypes to cope with global food security.

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8 Summary

Fusarium head blight (FHB) in triticale (× *Triticosecale* Wittm.), Gibberella ear rot (GER) and Northern corn leaf blight (NCLB) in maize (*Zea mays* L.) are devastating crop diseases causing yield losses and/or reducing grain quality worldwide. Resistance breeding is the most efficient and sustainable approach to reduce the damages caused by these diseases. For all three pathosystems, a quantitative inheritance based on many genes with small effects has been described in previous studies. Hence, this thesis aimed to assess the potential of genomics-assisted breeding strategies to reduce FHB, GER and NCLB in applied breeding programs. In particular, the objectives were to: (i) Dissect the genetic architecture underlying quantitative variation for FHB, GER and NCLB through different quantitative trait loci (QTL) and association mapping approaches; (ii) assess the potential of genomics-assisted selection to select superior triticale genotypes harboring FHB resistance; (iii) phenotype and characterize Brazilian resistance donors conferring resistance to GER and NCLB in multi-environment trials in Brazil and in Europe; and (iv) evaluate approaches for the introgression and integration of NCLB and GER resistances from tropical to adapted germplasm.

The genome-wide association study (GWAS) conducted for FHB resistance in triticale revealed six QTL that reduced damages by 5 to 8%. The most prominent QTL identified in our study was mapped on chromosome 5B and explained 30% of the genotypic variance. To evaluate the potential of genomic selection (GS), we performed a five-fold cross-validation study. Here,

weighted genomic selection increased the prediction accuracy from 0.55 to 0.78 compared to the non-weighted GS model, indicating the high potential of the weighted genomic selection approach. The successful application of GS requires large training sets to develop robust models. However, large training sets based on the target trait deoxynivalenol (DON) are usually not available. Due to the rather moderate correlation between FHB and DON, we recommend a negative selection based on genomic estimated breeding values (GEBVs) for FHB severity in early breeding stages. In the long-run, however, we encourage breeders to build and test GS calibrations for DON content in triticale.

The genetic architecture of GER caused by *Fusarium graminearum* in maize was investigated in Brazilian tropical germplasm in multi-environment trials. We observed high genotype-by-environment interactions which requires trials in many environments for the identification of stable QTL. We identified four QTL that explained between 5 to 22% of the genotypic variance. Most of the resistance alleles identified in our study originated from the Brazilian tropical parents indicating the potential of this exotic germplasm as resistance source. The QTL located on chromosome bin 1.02 was identified both in Brazilian and in European trials, and across all six biparental populations. This QTL is likely stable, an important feature for its successful employment across different genetic backgrounds and environments. This stable QTL is a great candidate for validation and fine mapping, and subsequent introgression in European germplasm but possible negative linkage drag should be tackled.

NCLB is another economically important disease in maize and the most devastating leaf disease in maize grown in Europe. Virulent races have already overcome the majority of known qualitative resistances. Therefore, a constant monitoring of *S. turcica* races is necessary to assist breeders on the choice of effective resistances in each target environment. We investigated the genetic architecture of NCLB in Brazilian tropical germplasm and identified 17 QTL distributed along the ten chromosomes of maize explaining 4

to 31% of the trait genotypic variance each. Most of the alleles reducing the infections originated from Brazilian germplasm and reduced NCLB between 0.3 to 2.5 scores in the 1-9 severity scale, showing the potential of Brazilian germplasm to reduce not only GER but also NCLB severity in maize. These QTL were identified across a wide range of environments comprising different *S. turcica* race compositions indicating race non-specific resistance and most likely stability. Indeed, QTL 7.03 and 9.03/9.04 were identified both in Brazil and in Europe being promising candidates for trait introgression.

These major and stable QTL identified for GER and NCLB can be introgressed into elite germplasm by marker-assisted selection. Subsequently, an integration step is necessary to account for possible negative linkage drag. A rapid genomics-assisted breeding approach for the introgression and integration of exotic into adapted germplasm has been proposed in this thesis.

Jointly, our results demonstrate the high potential of genomics-assisted breeding strategies to efficiently increase the quantitative resistance levels of NCLB in maize and Fusarium diseases in maize and in triticale. We identified favorable QTL to increase resistance levels in both crops. In addition, we successfully characterized Brazilian germplasm for GER and NCLB resistances. After validation and fine mapping, the introgression and integration of the QTL identified in this study might contribute to the release of resistant cultivars, an important pillar to cope with global food security.

9 Zusammenfassung

Ährenfusariosen (Fusarium head blight, FHB) in Triticale (× *Triticosecale* Wittm.), sowie Kolbenfusariosen (Gibberella ear rot, GER) und Turcicum-Blattdürre (Northern corn leaf blight, NCLB) im Mais (*Zea mays* L.), sind weltweit verheerende Schaderreger, die zu Ertragseinbußen und verminderter Erntegutqualität führen können. Die Resistenzzüchtung ist die effizienteste und nachhaltigste Methode, um die auftretenden Schadwirkungen dieser Pflanzenkrankheiten zu minimieren. Für alle drei Pathosysteme wurden bereits quantitative Resistenzen in der Literatur beschrieben. Die Zielsetzung der vorliegenden Arbeit war daher, das Potenzial genomisch unterstützter Zuchtstrategien zur Reduktion von FHB, GER und NCLB zu untersuchen. Insbesondere wurden dabei die nachfolgenden Ziele verfolgt: (i) die genetische Architektur von FHB, GER und NCLB mit verschiedenen QTL- und Assoziationskartierungsansätzen zu untersuchen; (ii) das Potential genomisch unterstützter Zuchtstrategien zur Selektion von Triticale Genotypen mit verbesserter FHB Resistenz zu bewerten; (iii) die Charaktierisierung und Phänotypisierung brasilianischer Resistenzdonoren für GER und NCLB in mehreren Umwelten in Brasilien und Europa; und (iv) Ansätze für die Einkreuzung und Intergration von tropischen NCLB und GER Resistenzquellen in europäisches Zuchtmaterial zu bewerten.

Die genomweite Assoziationskartierung (GWAS) für FHB Resistenz in Triticale identifizierte sechs QTL, die eine Reduktion im Befallswert zwischen 5 und 8% erklärten. Der vielversprechendste QTL wurde auf Chromosome 5B identifizert und erklärte 30% der genotypischen Varianz. Um das

Potenzial der genomischen Selektion (GS) zu evaluieren, wurde eine fünffache Kreuzvalidierung durchgeführt. Hier zeigte eine gewichtete genomische Selektion (wGS) einen Anstieg in der Vorhersagegenauigkeit von 0.55 auf 0.78, verglichen zum ungewichteten GS Modell. Dies unterstreicht das Potenzial der wGS Methode. Eine erfolgreiche Implementierung von GS benötigt eine ausreichend große Trainingspopulationen, um robuste Modelle zu kalibrieren. Hierfür ist die Datengrundlage für das Zielmerkmale Deoxynivalenol (DON) üblicherweise jedoch nicht ausreichend. Aufgrund der eher moderaten Korrelation zwischen FHB und DON empfehlen wir zunächst eine Negativselektion basierend auf genomischen Zuchtwerten für FHB Anfälligkeit in frühen Generationen. Langfristig möchten wir jedoch Züchtungsunternehmen motivieren GS Kalibrationen für DON Gehalt in Triticale zu entwickeln und testen.

Die genetische Architektur von GER, hervorgerufen durch *Fusarium graminearum* im Mais, wurde in brasilianischem Zuchtmaterial in mehreren Umwelten untersucht. Wir beobachteten eine große Genotyp-Umwelt Interkation, was Versuche in vielen Umwelten zur Identifikation von stabilen QTL voraussetzt. Wir identifizierten vier QTL die zwischen 5 und 22% der genetischen Varianz erklärten. Die meisten der identifizierten Resistenzallele hatten ihren Ursprung in den tropischen brasialinschen Eltern, was das Potenzial dieses Materials als Resistenzquelle unterstreicht. Der QTL auf dem Chromosom Bin 1.02 wurde in allen sechs Bi-parentalen Populationen sowohl in Brasilien als auch in Europa identifiziert. Dieser QTL tendiert zu einer hohen Stabilität, was eine sehr wichtige Eigenschaft für die erfolgreiche Anwendung in verschiedenen genetischen Hintergünden und Umwelten ist. Dieser stabile QTL ist daher ein Kandidat für weitere Validierungen, für eine mögliche Feinkartierung und die anschließende Einkreuzung in europäisches Zuchtmaterial. Mögliche negative Einflüsse durch Linkage Drag müssen jedoch berücksichtigt werden.

NCLB ist eine weitere wichtige Krankheit mit hoher ökonomischer Bedeutung im Mais. In Europa ist es die wichtigste Blattkrankheit im Mais. Virulente Rassen haben viele der identifizierten qualitativen Resistenzen bereits

überwunden. Daher ist ein kontinuierliches Monitoring der *S. turcica* Rassen notwendig, um Züchter in der Wahl von effektiven Resistenzen für die jeweilige Zielumwelt zu unterstützen. Daher haben wir die genetische Architektur von NCLB in tropischen brasilianischem Zuchtmaterial untersucht und identifizierten insgesamt 17 QTL, verteilt auf allen 10 Chromosomen, die zwischen 4 und 30% der genotypischen Varianz erklärten. Die meisten dieser QTL mit einem reduzierenden Effekt hatten braislianschen Ursprung und reduzierten NCLB zwischen 0.3 und 2.5 Boniturnoten auf einer Skala von 1-9. Dies unterstreicht das hohe Potenzial des brasilianischen Zuchtmaterials zur Reduktion nicht nur von GER aber auch von NCLB im Mais. Die gefundenen QTL wurden in vielen Umwelten mit verschiedenen *S. turcica* Rassen identifiziert, was eine rassenunabhängige und stabile Resistenzwirkung nahelegt. Die QTL 7.03 und 9.03/9.04 wurden sowohl in Brasilien als auch in Europa identifiziert und sind daher vielversprechende Kandidaten für Einkreuzungen.

Die identifizierten stabilen QTL, mit großen Effekten für GER und NCLB, können mit Hilfe Marker gestützter Selektion (MAS) in Elitematerial eingekreuzt werden. Ein anschließender Integrationsschritt ist jedoch nötig, um negativen Effekten durch Linkage Drag zu begegnen. Aus diesem Grund wurde ein schnelles genomisch unterstütztes Zuchtschema zur Einkreuzung und Integration von exotischen Resistenzquellen in den Elitehintergrund in dieser Arbeit vorgestellt.

Zusammenfassend zeigen unsere Ergebnisse das hohe Potenzial von genomisch unterstützten Zuchtstrategien zur effizienten Steigerung der quantitativen Resistenz von NCLB im Mais und Fusariumerkrankungen im Mais und in Triticale. Wir identifizierten vielversprechende QTL, um das Resistenzniveau in beiden Kulturarten zu erhöhen. Wir waren erfolgreich in der Charakterisierung des brasilianischen Zuchtmaterials für GER und NCLB Resistenz. Nach einer weiteren Validierung und Feinkartierung kann die Einkreuzung und Integration der identifizierten QTL einen signifikanten Beitrag zur Züchtung von Sorten mit verbesserten Resistenzeigenschaften

leisten. Dies ist ein wichtiger Baustein, um die globale Ernährungssicherheit zu gewährleisten.

10 Sumário

A fusariose (FHB) no triticale (× *Triticosecale* Wittmack), a podridão-da-espiga causada por *Fusarium graminearum* (GER) e a mancha de turcicum (NCLB) no milho (*Zea mays* L.) são doenças devastadoras que causam perdas de produção e/ou redução da qualidade dos grãos no âmbito global. O melhoramento genético de plantas é a abordagem mais eficiente e sustentável para aumentar os níveis de resistência. Para todos os três patossistemas, uma herança genética quantitativa baseada em muitos genes com pequenos efeitos foi descrita em estudos anteriores. Dessa forma, o objetivo com essa pesquisa foi avaliar o potencial do melhoramento assistido por marcadores moleculares para auxiliar o aumento da frequência de alelos de resistência ao FHB, GER e NCLB em programas de melhoramento genético. Em particular, os objetivos foram: (i) Investigar a arquitetura genética subjacente variação quantitativa do FHB, GER e NCLB através de diferentes abordagens de mapeamento de QTL; (ii) avaliar o potencial da seleção assistida por marcadores moleculares para selecionar genótipos de triticale com resistência ao FHB; (iii) realizar fenotipagem e caracterizar linhagens brasileiras quanto resistência ao GER e NCLB em ensaios multi-ambientais no Brasil e na Europa; e (iv) avaliar abordagens para a introgressão e integração de resistências ao GER e NCLB do germoplasma exótico ao adaptado.

O estudo de "Genome-wide association" (GWAS), realizado para resistência ao FHB em triticale, revelou seis QTL que reduziram de 5 a 8% dos danos causados pela doença. O QTL mais proeminente identificado

em nosso estudo foi mapeado no cromossomo 5B e explicou 30% da variação genotípica. Para avaliar o potencial da seleção genômica (GS), realizamos um estudo de validação quíntupla. Nesse caso, o modelo ponderado de predição genômica aumentou a precisão da predição de 0,55 para 0,78 em comparação ao GS, indicando o potencial desse método. O sucesso da aplicação de GS requer grandes séries de dados fenotípicos para treinar e desenvolver modelos robustos. Devido à correlação moderada entre FHB e deoxynivalenol (DON), recomendamos uma seleção negativa com base nos valores genômicos estimados de melhoramento genético (GEBVs) para a avaliaçã do FHB nas gerações iniciais de melhoramento. Ao longo prazo, no entanto, incentivamos os melhoristas a criarem e testarem calibrações de GS para o acúmulo de DON em triticale.

A arquitetura genética do GER causada por *Fusarium graminearum* no milho foi investigada no germoplasma tropical brasileiro em ensaios multi-ambientais. Observamos altas interações genótipo-por-ambiente, o que requer ensaios em muitos ambientes para a identificação de QTL estáveis. Identificamos quatro QTL que explicaram entre 5 a 22% da variação genotípica. A maioria dos alelos de resistência identificados em nosso estudo foi originária de linhagens brasileiras, indicando o potencial desse germoplasma exótico como fonte de resistência. O QTL localizado no cromossomo bin 1.02 foi identificado em ensaios brasileiros e europeus e em todas as seis populações biparentais. É provável que esse QTL seja estável, uma característica necessária para o sucesso de seu emprego em diferentes germoplasmas e ambientes. Esse QTL estável é um candidato promissor para validação e mapeamento preciso do QTL, e subsequentemente introgredi-lo no germoplasma europeu, mas deve-se considerar uma possível influência negativa de regiões adjacentes ao QTL.

NCLB é outra doença economicamente importante na produção do milho e a mais devastadora em campos de produção de milho na Europa. Raças virulentas já superaram a maioria das resistências qualitativas conhecidas. Portanto, é necessário um monitoramento constante das raças de *S. turcica*

para auxiliar os melhoristas na escolha de resistências efetivas para seus programas. Investigamos a arquitetura genética do NCLB no germoplasma tropical brasileiro e identificamos 17 QTL distribuídos ao longo dos dez cromossomos do milho, explicando de 4 a 31% da variação genotípica da doença. A maior parte do QTL que reduziu as infecções foi originário de germoplasma brasileiro e reduziu os danos causados por NCLB entre 0,3 a 2,5 na escala de severidade de 1 a 9, mostrando o potencial do germoplasma brasileiro em reduzir não apenas o GER, como também os danos causados por NCLB no milho. Esses QTL foram identificados em uma ampla gama de ambientes, compreendendo diferentes composições de raça de *S. turcica*, indicando resistência inespecífica da raça e provável estabilidade da resistência. De fato, QTL 7.03 e 9.03/9.04 foram identificados no Brasil e na Europa como candidatos promissores à introgressão de NCLB no milho.

Esses QTL estáveis, identificados para GER e NCLB, podem ser introduzidos no germoplasma adaptado por seleção assistida por marcadores (MAS). Posteriormente, é necessária uma etapa de integração para reduzir as influências negativas de regiões adjacentes ao QTL devido à falta de adaptação do germoplasma exótico. Portanto, um esquema de introgressão e integração utilizando MAS e GS para uma rápida utilização de QTL provindos de linhagens exóticas foi proposto nessa tese.

Em resumo, nossos resultados demonstram o alto potencial do melhoramento genético baseado em marcadores moleculares para aumentar eficientemente os níveis quantitativos de resistência ao NCLB e Fusariose no milho e no triticale. Identificamos QTL favoráveis para aumentar os níveis de resistências em ambas as culturas. Além disso, caracterizamos o germoplasma brasileiro para resistências ao GER e NCLB. Após validação e mapeamento preciso, a introgressão e a integração dos QTL identificados neste estudo podem contribuir para o desenvolvimento de cultivares mais resistentes doenças, um pilar importante para contribuir com a segurança alimentar global.

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Annex 3

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

Genomics-assisted breeding strategies for quantitative resistances to Northern corn leaf blight in maize ('Zea mays' L.)
and Fusarium diseases in maize and in triticale (×'Triticosecale' Wittm.)

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.

Sarstedt, 28.06.2020 _____

Place, Date Signature

Annex 4

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.

Sarstedt, 28.06.2020

Place, Date

Signature

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