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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

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,

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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, $\sigma_L^2, \sigma_Y^2, \sigma_{LY}^2$ 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

$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

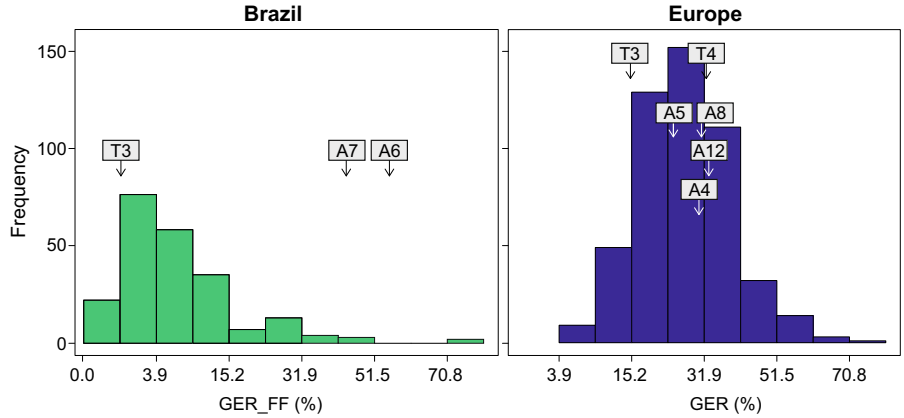

Fig. 2 Phenotypic distribution of the backtransformed Gibberella ear rot (GER) data assessed in Brazil adjusted for female flowering date (FF) and in Europe; pointing the respective tropical (T) and adapted (A) parental lines by arrows

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

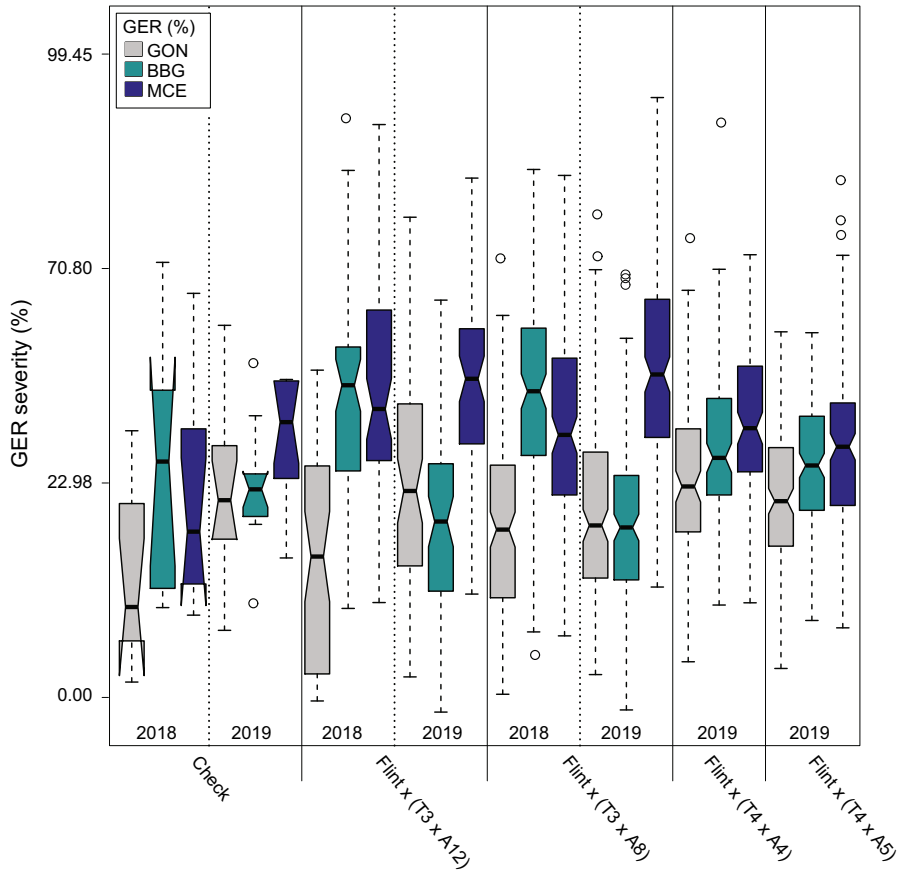

Fig. 3 Box plots for Gibberella ear rot (GER) severity (backtransformed values) of different biparental populations evaluated in Europe in six environments (year-location combinations, environments: GON = Gondelsheim/DE, BBG = Bernburg/DE, MCE = Monselice/IT; in 2018 and 2019). Horizontal lines within 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

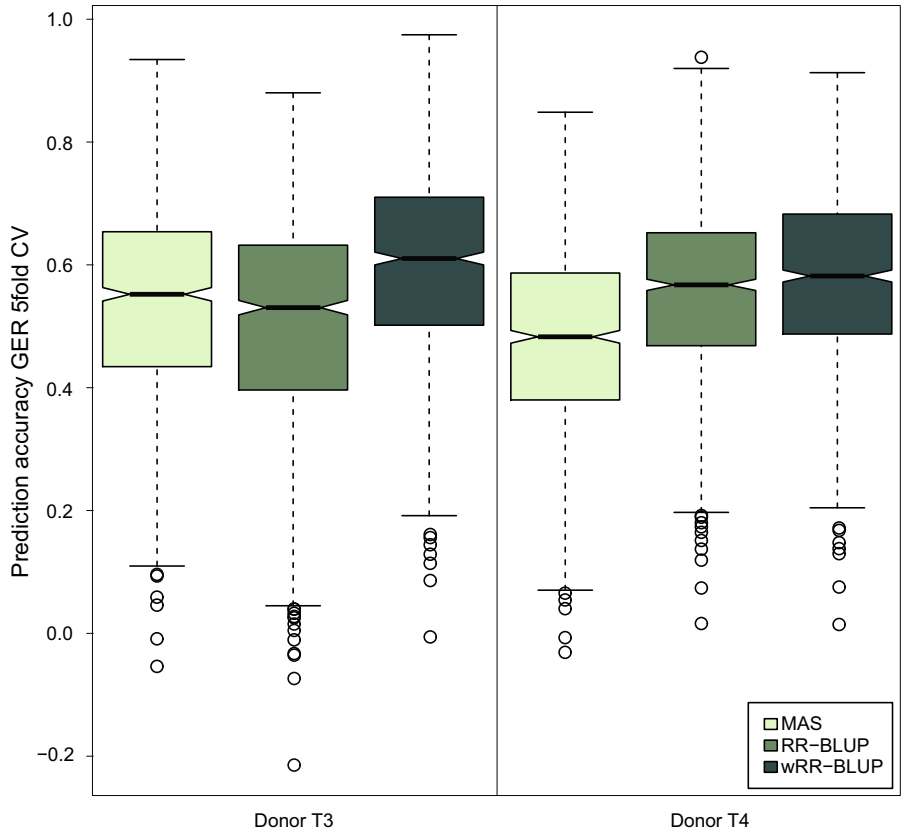

Fig. 4 Prediction accuracies obtained from marker assisted selection (MAS), genomic selection (RR-BLUP) and weighted genomic selection (wRR-BLUP) for each donor group and continent for testcrosses

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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