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Multi-parental QTL mapping of resistance to white spot of maize (*Zea mays*) in southern Brazil and relationship to QTLs of other foliar diseases

María Belén Kistner[1,3,4] [ID] | Ana Luísa Galiano-Carneiro[1,2] | Bettina Kessel[2] |
Thomas Presterl[2] | Thomas Miedaner[1] [ID]

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

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

[3]Estación Experimental Pergamino, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina

[4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CABA, Buenos Aires, Argentina

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

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Abstract

Maize white spot (MWS) is one of the most important foliar diseases in Brazil causing significant yield losses. Breeding genotypes with MWS resistance is the most sustainable alternative for managing such losses; however, their genetic control is poorly understood. Our objectives were to identify genomic regions controlling MWS resistance and to explore the presence of common regions controlling resistance to MWS, grey leaf spot (GLS) and northern corn leaf blight (NCLB). We performed a multi-parental QTL mapping for MWS and GLS resistance with a total of 474 testcrosses and phenotypic data collected in southern Brazil. Six QTLs for MWS resistance on bins 1.03, 1.04, 6.02, 8.05, 1.03, and 10.06 were detected. These findings were compared with previously reported QTLs for NCLB in the same populations, and a common QTL region (bin 8.05) controlling MWS and NCLB resistances was identified. Our findings contribute to a better understanding of MWS resistance by revealing three QTLs (bin 6.02, 1.03, and 10.06), to the best of our knowledge, not yet described in the literature, that are valuable for improving MWS resistance and one promising candidate region for multiple disease resistance.

KEYWORDS
Cercospora spp, *Exserohilum turcicum*, grey leaf spot (GLS), multiple disease resistance (MDR), northern corn leaf blight (NCLB), *Pantoea ananatis*

1 | INTRODUCTION

Maize is the world's most produced crop and one of the most important cereals for human and animal nutrition. However, diseases can threat maize production and consequently global food security (Ali & Yan, 2012; Yang, Balint-Kurti, & Xu, 2017). Maize white spot (MWS) is considered one of the most aggressive foliar diseases causing losses up to 60% of maize production (Escanferla et al., 2018; Paccola-Meirelles et al., 2002). The bacterium *Pantoea ananatis* (synonym

Erwinia ananas) is the causal agent of MWS (Paccola-Meirelles et al., 2001), but its aetiology has been a topic of discussion (Amorim et al., 2016; Bomfeti et al., 2008; Gonçalves et al., 2013). Firstly, the disease was denominated *Phaeosphaeria* leaf spot caused by the fungus *Phaeosphaeria maydis* (Henn) Rane, Payak & Renfro (Paccola-Meirelles et al., 2001). Currently, the evidence strongly supports *P. ananatis* as the principal causal agent of MWS, and other species occur as opportunistic fungi on MWS leaf spots (Bomfeti et al., 2008; Gonçalves et al., 2013; Lanza et al., 2013). MWS symptoms appear

initially as dark-green water-soaked spots on the leaves that later become small (.3–1 cm diameter), round or oval straw-coloured necrotic lesions. In advanced disease stages, lesions may coalescence and appear irregularly on dead tissue (Bomfeti et al., 2008; Gonçalves et al., 2013; Lanza et al., 2013; Paccola-Meirelles et al., 2001). *P. ananatis* occurs in America, Europe and South Africa (Alippi & López, 2010; Carson et al., 2005; Derera et al., 2007; Krawczyk et al., 2010; Paccola-Meirelles et al., 2001; Pérez-y-Terrón et al., 2009), but currently MWS is economically important only in South America (De Rossi et al., 2017; Escanferla et al., 2018). In Brazil, the third biggest maize producer in the world (FAO, 2020), MWS is an endemic major disease being present across all growing regions and favoured by high humidity and moderate temperatures (Escanferla et al., 2018; Paccola-Meirelles et al., 2002). In Argentina, the disease was also registered more frequently and more severely in recent years (De Rossi et al., 2017, 2019).

Maize is affected by other foliar diseases such as grey leaf spot (GLS) and northern corn leaf blight (NCLB). They are among the most damaging and widely distributed foliar diseases of maize and constitute a major concern for South America (Brunelli et al., 2008; De Rossi et al., 2017; Kuki et al., 2018). In Europe, GLS has been observed, but is of no relevance at present (Ramos Romero, 2016), whereas NCLB represents the most important foliar disease here (Galiano-Carneiro et al., 2020; Hanekamp, 2016). GLS is caused by the sibling species *Cercospora zeina* Crous & U. Braun and *C. zeae-maydis* Tehon & E. Y. Daniels (Nyanapah et al., 2020) with the first being predominant in Brazil (Kuki et al., 2018). The pathogens causing GLS are necrotrophic fungi that produce greyish rectangular lesions on maize leaves. Disease development is favoured by warm (around 27°C) and wet (>90% relative humidity) weather conditions (He et al., 2018; Lennon et al., 2016). NCLB is caused by the hemibiotrophic fungus *Exserohilum turcicum* [Pass.] Leonard and Suggs (Galiano-Carneiro & Miedaner, 2017). Symptoms are initially elliptical grey-green lesions, which later turn to large tan brown lesions, sometimes coalescing. Moderate temperatures between 15° and 25°C, high relative humidity (90%–100%) and dew periods of at least 4 h are major factors favouring disease development (Galiano-Carneiro & Miedaner, 2017; Hanekamp, 2016). A common feature among MWS, GLS and NCLB is that their causal agents survive on maize debris allowing inoculum to build up from year to year in agricultural fields (Galiano-Carneiro & Miedaner, 2017; Kuki et al., 2018; Sauer et al., 2015). Because minimum or even no-tillage and continuous maize growing became a common agronomic practice in the last decades, the importance of these diseases has drastically increased (De Rossi et al., 2017; Kuki et al., 2018; Lennon et al., 2016) with resistance breeding being one of the most sustainable control mechanisms.

The genetic nature of MWS and GLS resistances, as in most economically important maize diseases, is quantitative rather than qualitative (Du et al., 2020; Rossi et al., 2020; Yang, Balint-Kurti, & Xu, 2017). However, for NCLB, qualitative resistance based on major race-specific *Ht* genes is also available, but quantitative resistance is preferred being more durable and effective against all races of the pathogen (Galiano-Carneiro & Miedaner, 2017). In recent years,

researchers have focused on the study of multiple disease resistance (MDR), that is, plants being resistant to more than one disease (Jamann et al., 2016; Lopez-Zuniga et al., 2019; Miedaner & Juroszek, 2021; Qiu et al., 2020; Wisser et al., 2011; Yang, He, et al., 2017). QTL studies are still useful approaches to investigate the genetic bases of quantitative traits. To counteract their major drawback that only two alleles per population can be studied, multi-parental populations derived from more than two parents were established to explore a wider proportion of the variation in more genetic backgrounds in one run (Bardol et al., 2013; Garin et al., 2017).

The main objective of this study was to identify genomic regions controlling resistance to MWS by multi-parental QTL mapping. Specifically, we aimed for (1) assessing MWS phenotypically in southern Brazil, (2) performing multi-parental QTL mapping for MWS resistance and (3) exploring the presence of common regions for MWS, GLS and NCLB resistances in the same maize populations. For these purposes, 474 maize testcrosses were phenotyped in replicated field experiments at up to three locations in southern Brazil and genotyped with a 15-k single nucleotide polymorphism (SNP) array.

2 | MATERIALS AND METHODS

2.1 | Plant materials

In this study, five biparental populations of maize (*Zea mays* L.) based on seven parents (Table 1) and jointly comprising 474 testcrosses were evaluated. Each biparental population derived from a cross between a tropical Brazilian line (denoted as 'T') and an adapted European line (denoted as 'A'). Three biparental populations (T1 × A1, T1 × A2 and T1 × A10) were connected by the tropical donor T1 and belonged to the stiff-stalk synthetic (SSS) heterotic group, whereas the other two biparental populations (T2 × A3 and T2 × A4) were connected by the tropical donor T2 and belonged to

TABLE 1 Parental performance for maize white spot (MWS) and grey leaf spot (GLS) severity (1-to-9, where 1 indicates complete resistance) at the test locations in southern Brazil

| Parent | MWS (1-to-9) | | | GLS (1-to-9) |
	CL	CA	PA	CL
T1	3.65	4.15	4.71	2.96
A1	5.96	8.85	9.82	5.26
A2	5.17	7.29	7.88	4.43
A10	6.87	8.89	8.12	1.97
T2	3.12	2.31	3.54	NA
A3	3.60	5.57	7.11	NA
A4	2.51	4.44	5.57	NA
LSD$_{5\%}$	1.90	2.36	2.51	.32

Abbreviations: CA, Castro; CL, Campo Largo; NA, not available; PA, Palmeira.

non-stiff-stalk (NSS) heterotic group. Crosses resulted in a different number of F1-derived double haploid (DH) lines per population (see Table 2) that were afterwards crossed with a Brazilian tester line from the respective opposite heterotic group to produce testcrosses. For further details, please refer to Galiano-Carneiro et al. (2020). For simplification, all populations connected by T1 (i.e. T1 × A1, T1 × A2 and T1 × A10) will be denominated as 'T1 donor population', and populations connected by T2 (i.e. T2 × A3 and T2 × A4) as 'T2 donor population'. The germplasm employed in this study was provided by KWS SAAT SE & Co. KGaA, Einbeck, Germany.

2.2 | Field trials and MWS assessment

MWS was assessed during the season 2018/2019 in three locations in southern Brazil: Campo Largo (CL), Castro (CA) and Palmeira (PA). In all locations, the experiments were sown in an alpha-lattice field design with two replications. 'T1 and T2 donor populations' were randomized in separated but adjacent trials. The parents were included in the trials. The experimental unit was a plot of approximately 4 m length with a single-row plot in CL and a two-row plot in CA and PA where one row comprises approximately 20 plants. The disease severity of MWS was scored plotwise on a 1-to-9 scale (Agroceres, 1992), where 1 was assigned to genotypes without symptoms and 9 to completely diseased genotypes. The assessment was performed under natural infection at the phenological stage of R5.

2.3 | Phenotypic data analyses for MWS

The phenotypic analyses were firstly performed separately for single locations using a linear mixed model and the outlier detection procedure developed by Bernal-Vasquez et al. (2016). Genotype and trial were considered as fixed effects in order to obtain the best linear unbiased estimators (BLUEs) of genotypes. The BLUEs were used to calculate the Pearson correlation coefficient and to test the correlation among locations. Then, a combined analysis across locations for MWS was performed based on the following mixed model:

$$\gamma_{ijklm} = \mu + G_i + L_j + T_k + LTR_{jkl} + LTRB_{jklm} + GL_{ij} + e_{ijklm}, \qquad (1)$$

where γ_{ijklm} is the severity value of MWS with μ as the general mean, G as the effect of the ith genotype, L as the effect of the jth location, T as the effect of the kth trial and LTR_{jkl} is the effect of the lth complete replication nested into kth trial and jth location and their interactions. We assumed heterogeneous variances among locations. The G_i, L_j and T_k effects were considered as fixed to obtain the BLUEs of genotypes. The same model dropping the L_j effect and its interactions was used for the single location analysis. The variance components and entry-mean heritabilities were estimated by adding to the above model (1) the population effect and considering genotype as random effect. We used dummy variables to separate the genotypes into populations and estimated the parameters for each of them (Piepho et al., 2006), but for simplicity, this is not written in the model.

TABLE 2 Summary of statistics for maize white spot (MWS) severity (1-to-9, where 1 indicates complete resistance) per donor (T1 and T2) and per biparental population (T1 × A1, T1 × A2, T1 × A10, T2 × A3 and T2 × A4) across three locations (CL, CT and PA) and for grey leaf spot (GLS) severity (1-to-9) for donor T1 and per biparental population (T2 × A3 and T2 × A4) in location CL

Trait	MWS (1-to-9)					GLS (1-to-9)		
Donor	**T1**			**T2**		**T1**		
Population	**T1 × A1**	**T1 × A2**	**T1 × A10**	**T2 × A3**	**T2 × A4**	**T1 × A1**	**T1 × A2**	**T1 × A10**
Minimum	3.24	2.83	3.00	.95	1.09	1.47	.98	.98
Median	6.44	5.32	5.78	3.24	3.05	3.11	3.45	2.91
Mean	6.35	5.15	5.88	3.17	3.04	3.24	3.41	2.98
Maximum	8.20	7.38	9.27	4.94	5.05	6.51	6.44	6.52
n	34	58	113	106	163	34	58	112
LSD$_{5\%}$	1.30	1.30	1.30	1.30	1.30	.32	.32	.32
Variance component								
σ^2_G	1.03[***]	1.20[***]	1.25	.20[**]	.23[***]	.99[***]	1.37[***]	1.10[***]
$\sigma^2_{G \times L}$	.18[*]	.24[**]	.002	.41[***]	.22[***]	-	-	-
σ^2_e	1.18	1.18	1.18	1.07	1.07	.91	.91	.91
Heritability[a]	**.87**	**.89**	**.93**	**.34**	**.47**	**.60**	**.75**	**.80**

Notes: Genotypic variance (σ^2_G), genotype-by-location interaction variance ($\sigma^2_{G \times L}$) and pooled error variance (σ^2_e). For GLS, minimum, median, mean, maximum and least significant difference values at $P < .05$ (LSD$_{5\%}$) are given in back-transformed units, because variance components are square root transformed.

[a]For GLS, values correspond to repeatability.

[*]Significantly different from zero at the .05 probability level.

[**]Significantly different from zero at the .01 probability level.

[***]Significantly different from zero at .001 probability level.

The entry-mean heritability (H^2) for each population was estimated using the approach proposed by Cullis et al. (2006):

$$H2C = 1 - \frac{\overline{\vartheta BLUP}}{2\sigma_G^2}, \qquad (2)$$

where the mean variance of a difference of two best linear unbiased predictors (BLUPs) is denoted as $\overline{\vartheta BLUP}$ and σ_G^2 is the genotypic variance (Piepho & Möhring, 2007). Repeatability estimates for the analysis of single environments were calculated accordingly. All phenotypic analyses were done using the software package ASReml-R 3.0 (Butler, 2009). The BLUEs obtained from the multi-environment analysis were employed in QTL mapping.

2.4 | Multi-parental QTL mapping for MWS

A total of 177 (out of 205) and 228 (out of 269) DH lines from T1 and T2 donor populations, respectively, were genotyped with a 15-k SNP chip based on the public Illumina MaizeSNP50 BeadChip at the KWS molecular laboratory. Regions adjacent to centromeres were especially marked enriched to account for the low recombination rates in this area. To construct the genetic map, the 10 maize chromosomes were partitioned into bins of .5 cM and translated to the public genetic map IBM and the physical map AGPv02 (Ganal et al., 2011). For that reason, the positions are called 'putative cM' (putcM). A marker quality control was done in order to ensure suitable genotypic information, excluding monomorphic markers, markers fully missing in the parents and markers that showed a missing rate greater than .1 or a minor allele frequency (MAF) lower than .05. Genotypes with a missing rate higher than .25 were also excluded. After the quality control, 2131 SNPs and 176 genotypes for T1 donor population and 3223 SNPs and 228 genotypes for T2 donor population were kept for the analysis.

QTL mapping was performed, for T1 and T2 donor populations independently, by composite interval mapping (CIM) with a multi-QTL effect model (MQE) implemented in the R package mppR Version 1.2.2 (Garin et al., 2018; Zeng, 1993, 1994). MQE allows a different incidence matrix for each tested loci where cross-specific, parental or biallelic QTL effects are selected with a forward selection procedure (Garin et al., 2017). Cross-specific effects correspond to the disconnected model proposed by Blanc et al. (2006) where effects are estimated within crosses. Parental effects correspond to the connected model (Blanc et al., 2006), which, unlike the previous model, connects the biparental population by assuming that common parental lines share the same allele substitution effect. Finally, the biallelic effects connect the biparental populations by assuming that parental lines with the same SNP score transmit the same allele (Model B; Garin et al., 2018; Würschum et al., 2012). Cofactors were selected based on a simple-interval mapping genome scan. Significance thresholds were obtained by averaging the thresholds calculated for single-effect models, based on 1000 permutation tests by taking the highest LOD score from the 90th percentile from the QTL significance threshold

null distribution (Churchill & Doerge, 1994; Garin et al., 2018). The confidence interval of each QTL was obtained by the $-\log10(P)$ value drop-off interval of 20 putcM. The contribution of each single QTL to the phenotypic variation (partial R^2) was calculated through the difference between the R^2 full model, including all QTL positions, and the R^2 reduced model, without the particular QTL position. Additionally, we performed QTL mapping for single-biparental populations with CIM QTL mapping function implemented in the R package R/qtl (Broman et al., 2003). Five markers were forward selected and used as covariates in the Haley–Knott regression, and QTL significance threshold was determined by 1000 permutations test.

The performance of the QTLs detected and their effects were evaluated in pseudo-independent populations by a cross-validation procedure implemented in the package mppR. Briefly, the procedure includes a random partitioning of the dataset into five subsets within crosses, where one subset is defined as training set (TS) and used to detect QTLs and evaluate the proportion of explained phenotypic variance in the TS (R_{TS}^2). The remaining four subsets are defined as validation set (VS) and employed to predict the proportion of phenotypic variance in the VS (R_{VS}^2). The R_{VS}^2 is the squared Pearson correlation between the observed and the predicted value and is calculated within each cross. Afterwards, a measurement for the whole population is estimated with the weighted average of the within cross values R_{VS}^2 accounting for the cross sizes. A relative bias between the R_{TS}^2 and the R_{VS}^2 is calculated. The CV procedure was iterated 1000 times by replicating the five subsets 200-fold.

The physical positions of QTLs were determined by aligning the markers in the B73 reference genome Version 2 in MaizeGBD database. The QTLs were designated with the prefix "*qMws*" followed by the chromosome bin location of the identified QTL. The chromosome bins are chromosomal segments of approximately 20 cM that are useful for comparisons between QTL identified in different studies (Wisser et al., 2006). The same designation, but with the prefix "*qGls*" or "*qNclb*" was later employed for GLS and NCLB, respectively.

2.5 | MDR data analysis

An exploratory study for resistance to GLS was conducted with phenotypic information collected in one location (CL) for 204 genotypes from T1 donor population. The GLS phenotypic data were transformed with a square root transformation to obtain a normal distribution of the residuals. BLUE estimation and the entry-mean heritability were determined using the same methodology as described above for MWS except that the statistical model was circumscribed to one trial and one location. The methodology for QTL detection, LOD threshold determination, confidence interval and the cross-validation used was exactly as described above for MWS. NCLB BLUEs and QTLs were obtained in a separate study based on the same populations (Galiano-Carneiro et al., 2020). Both GLS and NCLB were assessed with the same 1-to-9 scale used for the assessment of MWS (see Section 2.2); therefore, no scale adaptations were necessary. The BLUEs of genotypes for MWS, GLS (back-transformed) and NCLB were used to

calculate the Pearson correlation coefficient testing correlations between the different diseases. Genotypes were considered resistant to all three diseases when the BLUE scores for MWS, GLS and NCLB were lower than 3.5 units on the 1-to-9 scale. The presence of overlapping genomic regions was determined by comparing the confidence intervals of the QTLs identified for each disease.

3 | RESULTS

3.1 | Phenotypic data analysis

In all the locations, a considerable level of MWS natural infection was observed (Table 1 and Figure S1). Location PA showed the highest level of infection with a severity mean of 5.45 on the 1-to-9-scale, followed by CT (4.20) and CL (3.20) (data not presented). This trend was also confirmed by the parental means (Table 1). Correlations between MWS severity across locations were all positive and significant ranging from .60 to .76 (Figure S1). For T1 donor population, the tropical parent was considerably more resistant than the adapted parents (A1, A2 and A10) (Figure 1a). For T2 donor population, the difference among the parents was lower. MWS severity was normally distributed for both donors, but T1 donor population showed a higher disease severity and broader variance (Figure 1a). The BLUE scores ranged between 3 and 9 in T1 donor population and between 1 and 5 in T2 donor population on the 1-to-9 scale. All populations presented significant ($P < .01$) genotypic

FIGURE 1 Frequency distribution for (a) maize white spot (MWS) BLUEs across locations for donor T1 and T2 and (b) grey leaf spot (GLS) BLUEs (back-transformed values) in location CL for the donor T1. Triangles indicate the mean scores of the parental lines (T, tropical Brazilian parent; a, adapted European parent) [Color figure can be viewed at wileyonlinelibrary.com]

variance (σ_G^2) (Table 2). However, the estimates were larger for T1 donor population than for T2 donor population, although the genotype-by-location interaction variances ($\sigma_{G \times L}^2$) were much smaller, resulting in higher heritabilities for T1 donor population.

GLS square root transformed phenotypic data were quantitatively distributed and presented a genetic variation ranging from 1 to 7 on the 1-to-9 scale (Figure 1b). The tropical donor T1 had a similar disease severity than the adapted parent A10, whereas the other two adapted parents were more susceptible than the donor T1. The σ_G^2 was significant for all biparental populations, and the entry-mean heritabilities ranged from .60 to .80 (Table 2).

3.2 | Multi-parental QTL mapping for MWS

The MQE model detected more QTL and explained a larger proportion of the phenotypic variance than biparental QTL mapping. Additionally, all QTLs detected in the biparental QTL mapping were also detected by the MQE model (Table S1). In T1 donor population, we identified six QTLs, but after the cross-validation procedure, two of them were discarded because of a low recovery rate (<5%) and a high bias (>80%) (data not presented). Then, four QTLs with different type of effects located on bins 1.04, 6.02, 8.05 and 10.06 explaining together 44% of the phenotypic variance for MWS resistance were considered in the results (Table 3). In T2 donor population, the joint analysis detected two parental QTLs on bins 1.03 and 10.03, explaining together 21% of the total phenotypic variance (Table 3). No overlapping QTLs for MWS between T1 and T2 donor populations were identified. The majority of the adapted parents were carrying alleles that increase the disease severity (Table 3). In T1 donor population, only seven testcrosses carried all four QTL with the alleles conferring resistance to MWS, contrarily in T2 donor population almost half of the testcrosses carried the two favourable QTL alleles (Figure S2b). We compared the QTLs for MWS identified in this study with previous studies (Figure 2a). QTLs on chromosomes 1, 6 and 8 have been previously reported controlling MWS resistance. On chromosome 8, two QTLs were mapped in the same confidence interval where we found *qMws8.05* and two more in the vicinity. On chromosome 1, *qMws1.03* and *qMws1.04* mapped close to previously reported QTLs. No previous study reported a QTL within the bin of *qMws6.02*, and no region on chromosome 10 had been formerly associated with MWS resistance.

4 | MDR

Disease severities were low, but significantly ($P < .05$) and positively correlated in T1 and T2 donor populations with Pearson's correlation coefficients ranging from .17 and .24 (Table 4). In T1 donor population, four out of 205 testcross progenies were resistant to all three diseases simultaneously (Table 5).

For GLS resistance, three biallelic QTLs were detected in T1 donor population on bins 2.02, 3.05 and 7.01 explaining jointly 30%
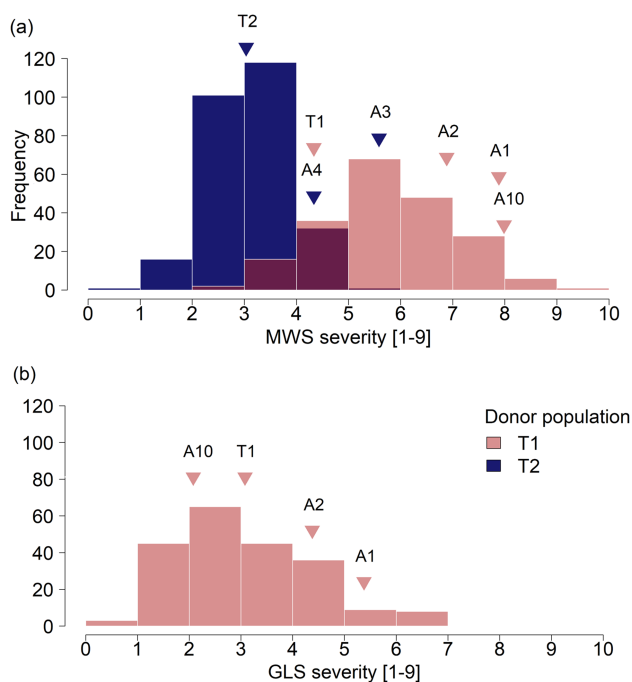
TABLE 3 QTL detected for maize white spot (MWS) by multi-parental QTL mapping and co-localizing QTL between diseases in the same population

Donor	QTL	Bin	Position (putcM)	Range (putcM)	LOD	$R^2_{adj.}$	α-Effect[b]			Cross-validation Recov. (%)	R^2_{TS}(%)	R^2_{VS}(%)	Bias
Maize white spot A1 A2 A10													
T1	qMws1.04 (par)	1.04	120.84	40.82	6.99	11.47	.10	−.13*	−.49***	43.1	11.9	8.5	.3
T1	qMws6.02 (biall)	6.02	51.21	16.31	11.80	19.91	.52***	.52***	.52***	34.2	15.6	11.6	.3
T1	**qMws8.05 (par)**	**8.05**	121.94	31.20	4.28	5.43	.73*	−.16	.38***	63.0	9.5	4.5	.5
T1	qMws10.06 (par)	10.06	134.36	17.56	4.98	7.49	.03	.23*	.36***	40.4	7.5	5.0	.3
Maize white spot A3 A4													
T2	qMws1.03 (par)	1.03	85.79	25.62	3.55	7.66	.24***	.19***		20.4	9.1	4.3	.5
T2	qMws10.03 (par)	10.03	72.16	9.62	5.80	13.29	.18**	.32		21.8	13.0	11.4	.1
Northern corn leaf blight A1 A2 A10													
T1	**qNclb8.05 (biall)**[a]	**8.05**	121.12	13.34	8.32	14.11	.57***	.57***	.57***	NA	NA	NA	NA

Notes: QTL name and their respective type of effect (QTL), QTL physical position (bin), QTL position, QTL confidence interval range, significant LOD threshold score, the adjusted explained phenotypic (R^2_{adj}) and the allelic effect (α-effect). Cross-validation results for each QTL are presented as the percentage of times the QTL has been detected in 1000 cross-validations (= recovery rate, recov.), the explained phenotypic variance in the training set (R^2_{TS}) and in the validation set (R^2_{VS}) and the relative bias between R^2_{TS} and R^2_{VS}. Highlighted QTLs indicate that they co-localized between diseases in the same population.
[a]q14 in Galiano-Carneiro et al. (2020).
[b]Allelic effect should be interpreted according to their type of effect; for biallelic QTLs, it represents the additive effect of one copy of the minor allele with respect to T1 or T2, whereas for parental QTLs, it represents a deviation with respect to T1 or T2.
*Significantly different from zero at the .05 probability level.
**Significantly different from zero at the .01 probability level.
***Significantly different from zero at .001 probability level.

of the total phenotypic variance. The allelic additive effects were around −.10 for GLS-favourable alleles and in the same order of magnitude for one unfavourable allele (data not presented). Only 17 testcrosses carried all favourable QTL alleles (Figure S2b). The bins where qGls2.02, qGls3.05 and qGls7.01 mapped have been repeatedly detected by different authors (Figure 2b).

The comparison of QTL locations for disease resistances reveals a common region between MWS and NCLB resistances in T1 donor population (Table 3). The QTL qMws8.05 was identified within the same confidence interval range and at approximately the same map position (<1 putcM difference) as qNclb8.05. Overlapping QTLs were neither identified between MWS and GLS resistances, nor between GLS and NCLB resistances.

5 | DISCUSSION

The growing importance of emerging and re-emerging diseases in maize is forcing the development of ecological alternatives to mitigate disease impact and ensure global food security (De Rossi et al., 2017;

Savary et al., 2019; Yang, Balint-Kurti, & Xu, 2017). Therefore, we investigated genomic regions associated with MWS resistance, a potential threat for maize production in Brazil, and did a preliminary study of the same genetic materials for resistance to GLS. Finally, we combined these results with previous results for NCLB resistance analysed in the same populations to explore the presence of common genetic regions harbouring resistance to multiple diseases.

5.1 | Phenotypic assessment of diseases

Reliable phenotypic data are crucial for the success of QTL mapping studies (Gaikpa & Miedaner, 2019; Rivero Do Vale et al., 2001; Yang, Balint-Kurti, & Xu, 2017). Our phenotypic data were collected in southern Brazil, where both diseases, MWS and GLS, are frequently observed in maize fields (Escanferla et al., 2018; Kuki et al., 2018). In our study, naturally infected maize fields presented high disease pressure of MWS and GLS. Environments with a high and uniform level of natural inoculum distribution like in this study are advantageous for the breeder as no extra resources are dispended with
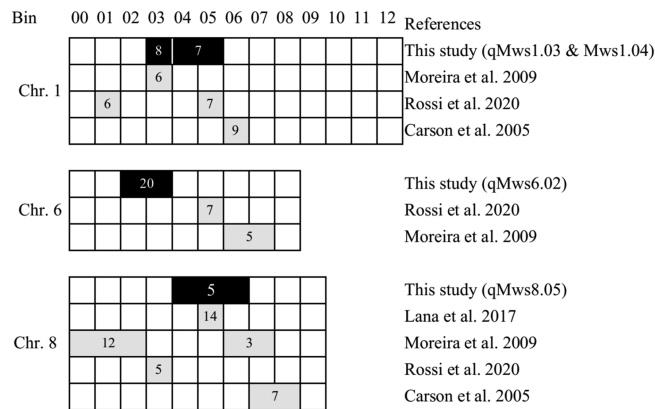
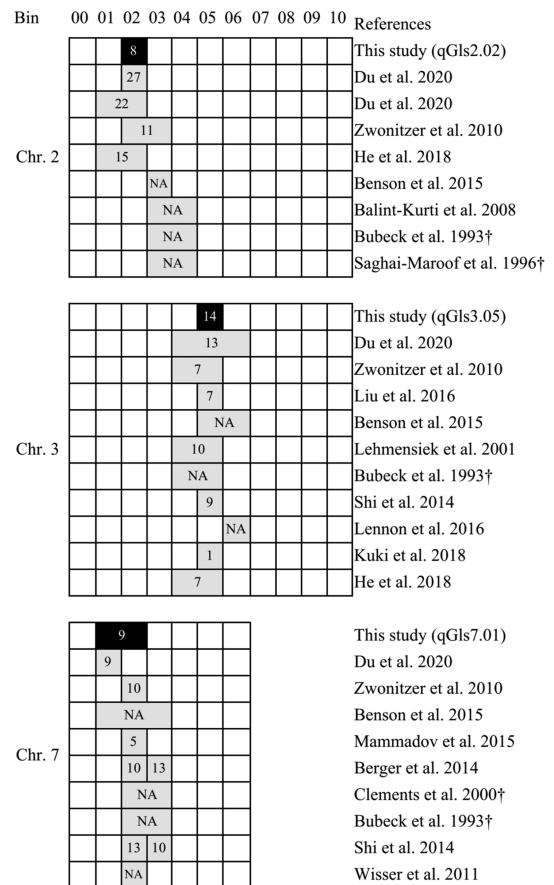(a)   Maize white spot (MWS)   (b)   Gray leaf spot (GLS)

FIGURE 2   Bin positions (including confidence intervals) of QTL for (a) maize white spot (MWS) resistance and (b) grey leaf spot (GLS) resistance detected in this study compared with other studies. The markers of the confidence interval were aligned with the B73 reference genome to determine the bins of the QTLs of this study. The numbers within the bins indicate the proportion of phenotypic variation explained by the position in percent. NA indicates no available information
†Information extracted from Berger et al. (2014)

TABLE 4   Pearson's coefficients of correlation among resistances to maize white spot, grey leaf spot and, northern corn leaf blight in T1 donor population and to maize white spot and northern corn leaf blight in T2 donor population

Donor population	Disease	Grey leaf spot	Northern corn leaf blight
T1	Maize white spot	.24[**]	.17[*]
T1	Grey leaf spot		.24[**]
T2	Maize white spot	NA	.18[**]

Note: GLS values in back-transformed units were used for the correlation.
[*]Significantly different from zero at the .05 probability level.
[**]Significantly different from zero at the .01 probability level.

TABLE 5   BLUE values for maize white spot (MWS), grey leaf spot (GLS) and northern corn leaf blight (NCLB) in four genotypes resistant to three diseases simultaneously, that is, severity score < 3.5 on the 1-to-9 scale

Genotype	Maize white spot	Grey leaf spot	Northern corn leaf blight
T1 × A1_064	3.24	2.45	3.05
T1 × A2_014	3.15	1.47	3.27
T1 × A2_018	3.20	1.01	3.21
T1 × A2_054	3.31	2.51	2.66
T1 donor population mean	**5.75**	**3.15**	**3.92**

inoculation. In combination with a suitable scoring method, we achieved high heritabilities with the T1 donor population allowing a sound mapping study.

Using testcrosses, the outcome highly depends on the inheritance of the trait and the resistance of the testers used (Hallauer et al., 2010). The tester used for T1 donor population was rather susceptible to MWS, leading to significant genotypic variation for MWS and high entry-mean heritabilities that were comparable with previous studies (Juliatti et al., 2013; Lana et al., 2017; Moreira et al., 2009; Rossi et al., 2020). In contrast, the tester used for the T2 donor population was more resistant to MWS. Therefore, only a restricted genetic variation, a higher proportion of genotype-by-environment interaction and consequently lower heritabilities were detected. GLS repeatability was similar as reported by several authors (Benson et al., 2015; Lopez-Zuniga et al., 2019; Mammadov et al., 2015; Qiu et al., 2020), reflecting that genetic variation for this trait can be easily exploited in breeding programmes.

5.2 | Detection of QTLs for MWS

We performed QTL mapping for MWS and detected six QTLs for resistance to MWS on chromosomes 1, 6, 8 and 10 that showed a low bias in the cross-validation study. These results suggest that MWS resistance is controlled by several genomic regions across the genome, each of them explaining a small to moderate proportion of the phenotypic variance, which is in accordance to the quantitative resistances with mainly additive effects observed in previous studies (Carson et al., 2005; Juliatti et al., 2013; Lana et al., 2017; Moreira et al., 2009; Rossi et al., 2020). Most of the resistance QTLs were found within T1 donor population. This was expected due to the larger genotypic variance and higher heritability compared with T2 donor population. Two genomic regions on chromosome 10 (qMws10.03 and qMws10.06) and one on chromosome 6 (qMWS6.02) associated with MWS resistance have, to the best of our knowledge, not yet been described in the literature. The cross-validation allowed to obtain more realistic QTL identification by excluding those that are not precisely estimated and by reducing the overestimation of the proportion of genotypic variance (Han et al., 2016; Utz et al., 2000; Würschum et al., 2012).

Multi-parental populations are derived from several parents and potentially contain more alleles with differing effects (Garin et al., 2017). Several models have been proposed to better capture the allelic diversity of a multi-parental population; however, their performance seems to vary across populations and traits (Blanc et al., 2006; Garin et al., 2017; Würschum et al., 2012). Indeed, we identified QTLs with both parental and biallelic allele substitution effects in our QTL mapping study for MWS resistance. The biallelic QTL effect identified for QTL qMws6.02 showed the largest allele substitution effect. After fine mapping and QTL validation in other genetic backgrounds, it is potentially suitable for marker-assisted backcrossing to increase resistance to MWS in adapted elite backgrounds (Galiano-Carneiro et al., 2020).

5.3 | Exploiting MDR

Another objective of this study was to investigate the relationship between MWS, GLS and NCLB in the same populations. In southern Brazil, these three diseases are serious threats for maize production. Therefore, the selection of multiresistant genotypes represents a highly valuable goal for plant breeding. We identified four testcross progenies simultaneously resistant to MWS, GLS and NCLB (Table 5). After assessment of yield and other important traits for the release of new varieties, these testcrosses could be potentially employed in subtropical regions in Brazil as parents or as donor lines for the introgression of MDR in temperate germplasm.

GLS is a disease of worldwide concern, and, therefore, more QTL studies are available than for MWS. So far, more than 100 QTLs across all chromosomes have been mapped, most of them explaining a small portion of the phenotypic variance (<15%) (Benson et al., 2015; Berger et al., 2014; Du et al., 2020; Kuki et al., 2018; Shi et al., 2014). In this study, qGls2.02, qGls3.05 and qGls7.01 coincided with hotspot regions for GLS resistance; however, none of them overlapped with QTLs for MWS or NLCB. We found that MWS and NCLB resistances were positively and significantly correlated, which may be a first evidence of the presence of MDR loci in this germplasm (Qiu et al., 2020; Zwonitzer et al., 2010). A common region for resistance to MWS and NCLB at bin 8.05 has been identified. Previous studies already associated this bin with MWS resistance (Lana et al., 2017), NCLB resistance (Hurni et al., 2015) and MDR for NCLB and southern corn leaf blight (Zwonitzer et al., 2010). A fine mapping would be necessary to improve the precision of the QTL locations and to explore whether this region is conditioned by several clustered resistance genes or by a pleiotropically acting gene.

In conclusion, our findings provide an important resource for improving MWS resistance, and this is the first study that tries to explore the relationship among MWS, GLS and NCLB, the most important foliar diseases for Brazilian maize production. Our results suggest the presence of an MDR region against unrelated pathogen species, which could be exploited in future to systematically develop multiresistant germplasm.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

TM, TP and BK designed the experiments and supervised the project. BK collected the phenotypic data. MBK conducted all statistical analyses and drafted the manuscript. ALGC supported the statistical analyses. ALGC and TM revised the manuscript. All co-authors reviewed and approved the final version.

DATA AVAILABILITY STATEMENT

Data available within the article and in the supplementary material

ORCID

María Belén Kistner https://orcid.org/0000-0002-0947-6414
Thomas Miedaner https://orcid.org/0000-0002-9541-3726

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