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ORIGINAL PAPER

QTL mapping for reaction to Phaeosphaeria leaf spot in a tropical maize population

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Abstract Phaeosphaeria leaf spot (PLS) is an important disease in tropical and subtropical maize (*Zea mays*, L.) growing areas, but there is limited information on its inheritance. Thus, this research was conducted to study the inheritance of the PLS disease in tropical maize by using QTL mapping and to assess the feasibility of using marker-assisted selection aimed to develop genotypes resistance to this disease. Highly susceptible L14-04B and highly resistant L08-05F inbred lines were crossed to develop an F_2 population. Two-hundred and fifty six F_2 plants were genotyped with 143 microsatellite markers and their $F_{2:3}$ progenies were evaluated at seven environments. Ten plants per plot were evaluated 30 days after silk emergence following a rating scale, and the plot means were used for analyses. The heritability coefficient on a progeny mean basis was high (91.37%), and six QTL were mapped, with one QTL on chromosomes 1, 3, 4, and 6, and two QTL on chromosome 8. The gene action of the QTL ranged from additive to partial dominance, and the average level of dominance was partial dominance; also a dominance × dominance epistatic effect was detected between the QTL mapped on chromosome 8. The phenotypic variance explained by each QTL ranged from 2.91 to 11.86%, and the joint QTL effects explained 41.62% of the phenotypic variance. The alleles conditioning resistance to PLS disease of all mapped QTL were in the resistant parental inbred L08-05F. Thus, these alleles could be transferred to other elite maize inbreds by marker-assisted backcross selection to develop hybrids resistant to PLS disease.

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Introduction

Phaeosphaeria leaf spot disease (PLS) in maize, caused by the ascomycete *Phaeosphaeria maydis* (Henn.), is considered a serious foliar disease in tropical and subtropical growing areas because it has the potential to cause high yield losses (Das and Chattopadhyay 1984; Paccola-Meirelles et al. 2001; Carson 2005; Derera et al. 2007). Grain yield losses due to PLS disease are caused by accelerated leaf senescence, reduced development period, and decrease in grain size and weight (Paccola-Meirelles et al. 2001). In Brazil, reported grain yield losses varied greatly, but losses up to 60% have been documented (Fernandes and Oliveira 1997; Paccola-Meirelles et al. 2001). Furthermore, only 32% of the most cultivated maize hybrids were resistant to PLS disease showing that the germplasm is highly vulnerable and, thus, there is an increased concern about the high economic losses that PLS disease could cause to the Brazilian maize production (Pereira 1995). Also, in India (Das and Chattopadhyay 1984) and South Africa (Derera et al. 2007) most of the maize genotypes assessed were susceptible to the PLS disease and, therefore, there are concerns on the potential of this disease to cause substantial yield losses in maize production in these countries. In the United States, Carson (1999) reported that most of

the hybrids and inbred lines related to Mo17 type are highly resistant and that there was no indication that PLS disease should represent an immediate threat to the central maize belt, although in a later study he reported yield losses from 11 to 13% as a result of PLS disease (Carson 2005).

Reports on the reaction of maize to PLS disease have shown that it is a quantitatively inherited trait. Generation means and diallel crosses analyses have shown that additive effects were more important than dominance and epistatic effects in both tropical and temperate maize germplasm, and that the heritability coefficient is quite high (Carson 2001; Lopes 2003; Silva and Moro 2004; Derera et al. 2007); nevertheless, Das et al. (1989a, b) reported that dominance effects were more important than additive effects. To the author′s knowledge, only two studies on QTL mapping of reaction to PLS disease have been reported, one with tropical (Lopes 2003) and the other one with temperate maize germplasm (Carson et al. 2005). Lopes (2003), assessing $F_{2:3}$ progenies and using the composite interval mapping (CIM), mapped five QTL, with one QTL on chromosomes 1, 2 and 5, and two QTL on chromosome 4. The phenotypic variance explained by each QTL ranged from 9.81 to 32.87% and all QTL explained 44.29% of the phenotypic variance, and partial dominance was the average level of dominance. Carson et al. (2005), assessing recombinant inbred (RI) lines and using CIM, also mapped five QTL, with one QTL on chromosomes 1, 4, and 8, and two QTL on chromosome 7. The phenotypic variance explained by each QTL ranged from 4.1 to 11.0% and the set of mapped QTL explained 47.3% of the phenotypic variance; an additive × additive epistatic effect was detected involving one QTL on chromosome 7 and a QTL on chromosome 8. Only one QTL on chromosome 4 was mapped in the same genomic region by both authors. The number, positions, and effects of QTL mapped for a trait are expected to differ because of the genetic background of the populations assessed, the type of population assessed (F_2, $F_{2:3}$, RIL and BC), the saturation of the genetic map and the statistical method used (Lima et al. 2006).

Although PLS disease has the potential to cause high economic losses in tropical areas, there is limited information on its inheritance in maize, mainly on QTL mapping and, therefore, it is necessary to map QTL in other maize populations to increase our understanding of the genetic architecture of this trait. PLS disease trait could be very difficult to assess because it depends on the presence of the pathogen to allow the discrimination and selection of resistant genotypes and then the detection of QTL conditioning resistance to PLS disease could be a valuable tool in maize breeding programs. Thus, the objectives of this study were to map QTL for reaction to PLS disease in a tropical maize population and to assess the feasibility of using marker-assisted selection to increase the efficiency of breeding programs aimed to develop maize genotypes resistant to PLS disease.

Materials and methods

Genetic material

Parental inbreds L-14-04B and L-08-05F were derived from the Brazilian early-flowering maize populations BR-106 (yellow dent kernels) and IG-1 (orange flint kernels), respectively. These populations and their derived inbreds are from different heterotic groups. BR-106 was released by EMBRAPA/Milho e Sorgo and IG-1 was released by the Department of Genetics/ESALQ-University of São Paulo, and both populations were derived only from tropical germplasm (Sibov et al. 2003). The inbred L-14-04B is highly susceptible and inbred L-08-05F is highly resistant to PLS disease. The parental inbreds were crossed, and three F_1 plants, previously screened against the inbred parents with microsatellite markers to assess their genetic identity, were selfed to develop the F_2 reference population. In the 2000/2001 agricultural season, the F_2 plants were selfed to develop 256 $F_{2:3}$ progenies.

Experimental evaluation

The 256 $F_{2:3}$ progenies were evaluated at four locations in the 2002/2003 and at three locations in the 2003/2004 cropping seasons. In 2002/2003, the experiments were carried out at the Experimental Stations Department of Genetics, Caterpillar, Areão and Anhembi, and in 2003/2004 at the Experimental Stations Department of Genetics, Caterpillar and Areão. These experimental stations were near the city of Piracicaba, the state of São Paulo, Brazil. The experimental design was a 16 × 16 lattice with two replications per environment, where environment is a combination of cropping season and location. Plots were 4.0 m long spaced 0.8 m between rows, and they were overplanted and thinned to 20 plants per plot (62,500 plants ha^{-1}). Artificial inoculation was not used, but to facilitate the spread of the disease plots of the highly susceptible parental inbred L-14-04B was sown at each set of 16 plots and surrounding the experiments. Also, plots of the parental inbreds were sown at the beginning and at the end of each replication in the experiments. Ten plants per plot were visually rated 30 days after silking using a scale rating system from 1 to 9 to record the degree of disease

infestation, where 1 = 0% (no symptoms), 2 = 1%, 3 = 1 to 10%, 4 = 10–20%, 5 = 20–30%, 6 = 30–40%, 7 = 40–60%, 8 = 60–80%, and 9 >80% leaf area spotted, respectively, and the plot means were used for analyses. Natural infection and similar rating scale have been used in studies on the inheritance of maize reaction to PLS disease (Carson 1999, 2001; Lopes 2003; Carson et al. 2005; Derera et al. 2007).

Analyses of variance and covariance

Analyses of variance were computed for each environment according to the lattice design. The least square means of each environment and the effective error mean squares were used to compute the joint analyses of variance. Individual and joint analyses of covariance between the environments were also performed following the same procedure for the analyses of variance. All effects of the mathematical model, except the mean, were considered random, and the analyses were computed using PROC GLM from SAS software (SAS Institute 2001). Before the joint analyses were computed, the error variances of the experiments were tested for their homogeneity using the Bartlett's test ($P \leq 0.05$) (Sokal and Rohlf 1995), and no significance was found. Genetic (σ^2_G) and genetic by environment (σ^2_{GE}) variances were estimated by equating the computed mean squares to their respective expectations and solved as $\hat{\sigma}^2_G = (MSG - MSGE)/RE$ and $\hat{\sigma}^2_{GE} = (MSGE - MSE)/R$; and the phenotypic variance was estimated as $\hat{\sigma}^2_{Ph} = MSG/RE$, i.e., $\hat{\sigma}^2_{Ph} = \hat{\sigma}^2_G + (\hat{\sigma}^2_{GE}/E) + (\hat{\sigma}^2/RE)$. Here, MSG, MSGE and MSE are the mean squares of the $F_{2:3}$ progenies, of the genotype by environment interaction and of the error; R and E are the number of replications and environments, respectively. Broad-sense heritability coefficient on a progeny mean basis was estimated as $\hat{h}^2 = \hat{\sigma}^2_G/\hat{\sigma}^2_{Ph}$ (Hallauer and Miranda Filho 1988), and 95% confidence intervals were computed for the variance components and heritability estimates following the Burdick and Graybill (1992) procedures. Genetic ($\hat{Cov}_{G(E_iE_j)}$) covariance between pair-wise environments (E_i, E_j) were estimated from the joint analysis of covariance following the same procedures to estimate the respective variances; and genotypic correlations between environments were computed as $\hat{r}_{G(E_iE_j)} = \hat{Cov}_{G(E_iE_j)}/\hat{\sigma}_{G_i}\hat{\sigma}_{G_j}$, where $\hat{\sigma}_{G_i}(\hat{\sigma}_{G_j})$ are the square roots of the estimates of the genotypic variances at environments i and j, respectively (Mode and Robinson 1959). Standard errors of the genetic correlations were computed following the Falconer and Mackay (1996) procedures. Distribution histograms from the progeny means and from the standardized residuals were tested for normality using the Shapiro–Wilk W test (Shapiro and Wilk 1965) at $P \leq 0.05$.

Genetic map

The genetic map used, and the procedures used to develop it, was previously described by Sibov et al. (2003). Briefly, the F_2 plants that gave rise to the $F_{2:3}$ progenies were genotyped with microsatellite markers. The genetic map was developed using the MAPMAKER/EXP version 3.0b (Lincoln et al. 1992) with a LOD threshold of 3.0 and a maximum distance between adjacent markers of 50 cM; i.e., 0.38 as the maximum recombination frequency, to establish the linkage groups, and the Kosambi's (1944) mapping function was used to convert recombination frequencies into map distances. Twenty-six new microsatellite markers were added to the map of Sibov et al. (2003), for a total of 143 markers distributed along the ten linkage groups. The genetic map spanned 1,844.80 cM in length with an average interval of 13.00 cM between adjacent markers.

QTL mapping

The multiple interval mapping (MIM) (Kao et al. 1999) was used to map QTL and to search for epistatic interactions. The least square means of the trait across environments were used for QTL mapping. The underlying mixture model is:

$$\begin{aligned} Y_i = \mu &+ \sum_{j=1}^{m} a_j x^*_{ij} + \sum_{j=}^{m} d_j z^*_{ij} + \sum_{j\neq k}^{t} a_j a_k x^*_{ij} x^*_{ik} \\ &+ \sum_{j\neq k}^{t} a_j d_k x^*_{ij} z^*_{ik} + \sum_{j\neq k}^{t} d_j a_k z^*_{ij} x^*_{ik} \\ &+ \sum_{j\neq k}^{t} d_j d_k z^*_{ij} z^*_{ik} + e_i \end{aligned}$$

where Y_i is the phenotypic mean of the i*th* progeny ($i = 1 \ldots 256$); μ is the mean of the model; a_j and d_j are the additive and dominance effects of the j*th* QTL; $a_j a_k$, $a_j d_k$, $d_k a_j$, and $d_j d_k$ are the additive by additive, additive by dominant, dominant by additive, and dominant by dominant epistatic effects between the j*th* and k*th* QTL; x^*_{ij} (x^*_{ik}) is an indicator variable of the j*th* (k*th*) QTL taking values of 1, 0 or −1 for genotypes QQ, Qq, and qq, respectively, with probabilities depending on genotypes of the markers flanking the putative QTL and the recombination frequencies between the QTL and the markers; $z^*_{ij}(z^*_{ik})$ is an indicator variable of the j*th* (k*th*) QTL taking values of −1/2, 1/2, and −1/2 for genotypes QQ, Qq and qq, respectively, with probabilities depending on genotypes of the markers flanking the putative QTL and the recombination frequencies between the QTL and the markers; and e_i is the residual of the model. The analysis of QTL mapping was performed using the Windows QTL Cartographer software, version 2.5 (Wang et al. 2005). The selection of the model, including and/or excluding the main effects and the epistatic effects of the QTL, was performed using the

Bayesian Information Criterion (BIC), as recommended by Zeng et al. (1999), with the penalty function $c(n) = \log(n)$, with $n = 256$. Thus, starting with the software defaults, the final selection of the model included several round steps until the number and position of the QTL and epistatic effects remained unchanged in the model. Once the complete model was fitted, the main effects, epistatic effects, the coefficients of determination (R^2) of each QTL and of all QTL were automatically computed by the software. Also, the genotypic mean of each progeny based on the mapped QTL was computed by the software.

Parental inbred L-08-05F is highly resistant to PLS disease and the negative additive effects of the QTL indicated that the favorable (resistance) alleles were from this inbred. Levels of dominance were estimated as LD = $[d]/[a]$ ratio for each QTL, and the average level of dominance (ALD) was computed weighing each *LD* ratio by its respective R^2 value. Following several reports (Bohn et al. 1996; Sibov et al. 2003; Lima et al. 2006), gene action was characterized as follows: additive (A) $0.00 \leq \text{LD} \leq 0.20$; partial dominance (PD) $0.21 \leq \text{LD} \leq 0.80$; dominance (D) $0.81 \leq \text{LD} \leq 1.20$; and overdominance (OD) $\text{LD} \geq 1.21$.

Responses to selection

Selection was applied on the phenotypic means and on the genotypic means using 10% (26 selected progenies) and 5% (13 selected progenies) selection intensities, and the number of progenies selected simultaneously by the two types of means was computed.

Expected responses to selection based on phenotypic means were computed as $R_S^{\text{Ph}} = \text{Sh}^2$ (Falconer and Mackay 1996) and on the genotypic means were computed as$R_S^G = \text{SR}^2$(Kao et al. 1999), where S, h^2 and R^2 are the selection differential (mean of the selected progenies minus the general mean), heritability coefficient and the phenotypic variance explained by all mapped QTL, respectively.

Results

Means, heritability and correlations

The *W* test performed for the distribution histogram of the data and of the standardized residuals were non-significant showing that both histograms presented normal distribution. In the individual analyses of variance (not shown), highly significant differences ($P \leq 0.01$) were detected for progenies, and the average level of infestation varied greatly among the seven environments; i.e., from 2.60 at Experimental Station Departamento de Genética to 5.39 at the Experimental Station Anhembi in the 2002/2003 growing season. The experimental coefficients of variation of the individual analyses ranged from 5.44 to 14.22%, and they were similar to values already reported in tropical regions (Lopes 2003; Silva and Moro 2004). In the joint analysis of variance (Supplementary Table 1), highly significant differences were detected for environments, progenies, and for progenies by environment interaction, and the experimental coefficient of variation was 11.59%. The overall means of the parental inbreds differed significantly ($P \leq 0.05$), with ratings of 7.28 for the highly susceptible line L-14-04B and 2.00 for the highly resistant line L-08-05F, and the means of the progenies assessed ranged from 2.54 to 5.99. Estimates of genetic variance ($\hat{\sigma}^2_{\text{G}}$), genetic by environment interaction variance ($\hat{\sigma}^2_{\text{GE}}$), and broad-sense heritability coefficient ($\hat{h}^2$) differed significantly from zero ($P \leq 0.05$). Also, the estimate of $\hat{\sigma}^2_{\text{G}}$ was significantly greater than the estimate of $\hat{\sigma}^2_{\text{GE}}$; $\hat{\sigma}^2_{\text{G}}$ estimate was about three times $\hat{\sigma}^2_{GE}$ estimate, and the magnitude of the heritability coefficient was quite high ($\hat{h}^2 = 91.37\%$) (Table 1). The genetic correlations of progenies between environments were highly significant ($P \leq 0.01$) ranging from 0.67 to 1.04; this latter value was not expected but it could occur due to the error of the estimates of the components of variance and covariance (Table 2).

Table 1 Estimates of variance components, heritability coefficient, means and ranges of the $F_{2:3}$ progenies for reaction to phaeosphaeria leaf spot disease in maize

Parameters	Estimates	Confidence intervals
$\hat{\sigma}^2_G$	0.37	[0.32; 0.44]
$\hat{\sigma}^2_{GE}$	0.12	[0.10; 0.14]
$\hat{\sigma}^2_{Ph}$	0.41	[0.36; 0.48]
$\hat{h}^2$	0.91	[0.90; 0.93]
Mean L-08-5F	2.00	[1.66; 2.34]
Mean L-14-04B	7.28	[6.69; 7.88]
Mean $F_{2:3}$	4.40	[4.06; 4.74]
Range $F_{2:3}$	2.54; 5.99	–
CV%	11.59	–

Confidence intervals at the 95% probability level

QTL mapping

Six QTL were mapped for PLS reaction on five chromosomes. One QTL was mapped on each of the following chromosomes 1, 3, 4 and 6, and two QTL were mapped on chromosome 8. The locations of the QTL were as follows: *Ph1* on the short arm of chromosome 1 in bin 1.03; *Ph3* on the long arm of chromosome 3 between bins 3.07–3.08; *Ph4* on the long arm of chromosome 4 in bin 4.08; *Ph6* on the long arm of chromosome 6 between bins 6.06–6.07; *Ph8a* and *Ph8b* on the short and on the long arm of the

Table 2 Genetic correlations for the progenies evaluated at seven environments for phaeosphaeria leaf spot disease

Environments/crop season	Caterpillar 02/03	Areão 02/03	Anhembi 02/03	Genética 03/04	Caterpillar 03/04	Anhembi 03/04
Genética 02/03	0.96	0.93	0.67	0.95	0.87	0.81
Caterpillar 02/03		1.03	0.79	0.98	0.95	0.90
Areão 02/03			0.86	1.04	0.91	0.88
Anhembi 02/03				0.81	0.85	0.79
Genética 03/04					0.90	0.89
Caterpillar 03/04						0.90

All correlations are highly significant ($P \leq 0.01$)

Table 3 Genomic positions, gene actions, average levels of dominance, directions and coefficients of determination (R^2) for the QTL mapped for reaction to phaeosphaeria leaf spot disease

QTL	QTL position			Gene effect		Gene action		Direction	R^2(%)
	Bin	cM	Marker interval	Additive	Dominance	LD	Type		
Ph1	1.03–1.03	65.11	umc1073–umc1021	−0.25	0.17	0.70	PD	L-08-05F	5.77
Ph3	3.07–3.08	139.60	umc1659–umc1320	−0.25	0.00	0.00	A	L-08-05F	5.77
Ph4	4.08–4.08	126.07	bnlg2162–umc1086	−0.30	0.09	0.31	PD	L-08-05F	6.49
Ph6	6.06–6.07	157.80	umc1520–bnlg1759	−0.20	−0.04	0.18	A	L-08-05F	5.40
Ph8a	8.00–8.02	21.87	phi420701–phi0119	−0.31	−0.09	0.29	PD	L-08-05F	11.86
Ph8b	8.06–8.07	131.14	bnlg1607–bnlg1823	−0.18	−0.11	0.62	PD	L-08-05F	2.91
Ph8a x *Ph8b*	–	–	–	–	0.56	–	D × D	L-08-05F	3.41
					ALD	0.32	PD	Total	41.62

QTL names are indicated as *Ph* (phaeosphaeria) followed by the chromosome number and by a word for more than one QTL on the same chromosome

Gene action type see material and methods

Direction indicates the parental which contributes to decrease the trait

chromosome 8 between bins 8.00–8.02 and 8.06–8.07, respectively (Supplementary Figs. 1, 2). Notice that QTL *Ph8a* and *Ph8b* are not linked, they are 109.27 cM away from each other. The additive effects of all QTL were negative, three QTL displayed negative dominance effects, two positive dominance effects, and one had no dominance effects. Two QTL displayed additive gene action, four QTL displayed partial dominance, the QTL *Ph8a* and *Ph8b* displayed positive dominance × dominance digenic epistasis, and the average level of dominance was partial dominance (ALD = 0.32). The phenotypic variance explained by each QTL (R^2) ranged from 2.91% (*Ph8b*) to 11.86% (*Ph8a*), the epistasis between the QTL *Ph8a* and *Ph8b* explained 3.41%, and collectively the QTL and the epistasis explained 41.62% of the total phenotypic variance. The QTL mapped on chromosome 8 explained 18.18% of the phenotypic variance (Table 3).

The predicted mean genotypic values of the progenies based on the effects of the mapped QTL ranged from 3.37 to 5.29, whereas the mean phenotypic values ranged from 2.54 to 5.99, and the overall mean was 4.40. The correlation between the genotypic and phenotypic means was positive and significant ($r = 0.70$). The expected responses to one cycle of selection (R_s) based on the phenotypic values were $R_S^{\mathrm{Ph}} = -1.08(-24.55\%)$ and -1.23 (-27.95%) and on the predicted genotypic values were $R_S^{\mathrm{G}} = -0.30(-6.82\%)$ and -0.35 (-8.04%) for 10 and 5% of selection intensities, respectively.

Discussion

The level of infestation of PLS disease varied greatly among the environments. The highly significant differences detected in the individual analyses of variance for progenies indicated that PLS disease developed sufficiently in all seven environments. This allowed discrimination of the progenies assessed, and then, the natural infection and the rating scale used to assess the level of PLS infestation of the progenies and of the parental inbreds were effective. The joint analyses of variance showed that the environments differed in relation to the level of infestation of the

PLS disease as mentioned above. There was genetic variation among progenies for reaction to PLS and the progenies showed differential levels of PLS infestation across the environments. Because of the high genetic divergence of the parental inbreds in relation to the PLS disease, the genetic variation among progenies was already expected. However, transgressive progenies for resistance to PLS disease did not occur, i.e., none of the progenies assessed presented levels of resistance higher than the resistant parental inbred. This result likely suggests that the favorable alleles of the loci that confer resistance to the PLS disease are concentrated in the parental inbred L-08-05F.

The magnitude of the coefficient of heritability was very high ($\approx$90%) because the G $\times$ E interaction ($\hat{\sigma}^2_{GE}$) variance was low compared to the genetic variance ($\hat{\sigma}^2_{G}$) estimate, and also because the number of environments ($E = 7$) and replications ($R = 2$) used for evaluation of the progenies reduced the contribution of the G $\times$ E interaction and of the error variance to the phenotypic variance. The reported coefficients of heritability on a progeny mean basis for PLS reaction have ranged from 65.00 to 92.80% (Carson 2001; Silva 2002; Lopes 2003; Carson et al. 2005; Derera et al. 2007), and can be considered of high magnitudes. Also, in these reports the magnitudes of the genetic variances were consistently higher than the G $\times$ E interaction variances or the interactions were not significant. Thus, our results are in agreement with previous studies conducted with temperate (Carson 2001; Carson et al. 2005; Derera et al. 2007) as well as with tropical germplasm (Silva and Moro 2004; Lopes 2003).

The magnitudes of the genetic correlations of progenies between environments were high and of the genetic variances varied greatly among the environments. Thus, the G $\times$ E interaction variance was primarily due to differences of the magnitudes of the genetic variances among the environments, not because of the lack of genetic correlations between environments. Therefore, the set of genotypes selected for PLS disease in one environment will be similar in other environments. These results suggested that the populations of the pathogen in the environments where the experiments were evaluated did not differ in genetic variability, i.e., in relation to the different races of the pathogen. Thus, the level of infestation of the PLS disease varied greatly among environments probably because the differences in temperature and/or precipitation caused differences in the growth and in the spore spread of the pathogen.

QTL mapping

The additive effects of all QTL were negative showing that the favorable alleles, i.e., the alleles that increase the resistance to PLS disease, were in the resistant parental inbred L-08-05F, which is consistent with the non occurrence of transgressive progenies above mentioned. The dominance effects presented negative and positive signs showing that the dominance effects were not unidirectional, and as the average level of dominance was partial dominance, likely the magnitude of inbreeding depression and of heterosis for PLS reaction will be of low magnitude. In fact, Carson (2001) used generation mean analysis and reported that the mean of the F_2 generation was slightly higher than the mean of the F_1 generation, which is consistent with our results and interpretation of the dominance effects of the QTL. Digenic epistatic effect was detected only between QTL at chromosome 8, which presented positive value and then will contribute to an increase in susceptibility. Only 41.62 and 45.55% of the phenotypic and genotypic variances were accounted for by the set of QTL mapped. Lima et al. (2006) reported that these results could be due to (1) failure of the model to map QTL with very small effects, (2) greater distances between flanking markers reduce the power of QTL mapping, and in our map greater distances were on chromosomes 2, 4, 9, and 10, and (3) as the model allows to map only one QTL per interval, sets of genes in the same interval with different genetic effects could have the total effect canceled or highly reduced and thus not being mapped as an unique QTL with small effects. Then, new markers should be added to those regions to allow that new QTL could be mapped and, consequently, the proportion of the phenotypic variance explained by them will increase.

Considering our results and those reported by Lopes (2003) and by Carson et al. (2005), QTL for PLS reaction were not mapped only on chromosomes 5, 9, and 10. Three out of the six QTL mapped in our study were mapped in the same bin or in the vicinity of the QTL mapped by the authors above mentioned; e.g., the QTL *Ph1* was mapped at the same genomic region (bin 1.03) of a QTL mapped by Lopes (2003); the QTL *Ph4* (bin 4.08) was mapped in the vicinity of a QTL mapped by Lopes (2003) and by Carson et al. (2005) (bins 4.07–4.08), and the QTL *Ph8b* (bin 8.06–8.07) was mapped in the vicinity of a QTL reported by Carson et al. (2005) (bins 8.07–8.08). Thus, only the QTL *Ph4* was mapped on the same genomic region in our study and in the Lopes (2003) and Carson et al. (2005) studies. Because of the different genetic backgrounds of the populations, different genetic maps, and methods used for QTL mapping, one could expect that different QTL should be mapped by different studies. Although few coincident QTL were reported in these three studies, the results suggest that the genomic region of the QTL *Ph4* has a locus or loci related to PLS disease that could be stable across populations and environments.

Several reports on the inheritance of PLS disease in maize using diallel analysis and generation mean

analysis are in close agreement with the results of this study and with those reported by Lopes (2003) and Carson et al. (2005). These reports have shown that the additive effects are more important than dominance effects, and when detected, epistatic effects were of minor importance on the inheritance of PLS disease in maize (Paterniani et al. 2000; Carson 2001; Pegoraro et al. 2002; Silva and Moro 2004; Derera et al. 2007). Also, Carson (2001) and Pegoraro et al. (2002) reported that three to four effective factors (genes) would be involved in the inheritance of PLS disease.

McMullen and Simcox (1995) reported that genes controlling different diseases and pests in maize are not randomly distributed in the genome, but clustered in some genomic regions. More recently, Wisser et al. (2006) reviewed fifty publications on QTL mapping for several diseases in maize and reported the presence of clusters of genomic regions for multiple diseases. However, they did not include information on QTL mapping for PLS disease. All mapped QTL in our study were in the same genomic regions of QTL controlling other diseases reported by these authors: *Ph1* (bin 1.03) is in the QTL region controlling bacterial leaf blight (*Erwinia stewartii*), multiple fungal diseases causing ear and stalk rot (*Aspergillus flavus, Fusarium moliniforme, Giberella zeae* and *Colletotrichum graminicola*), common rust (*Puccinia sorghi*), common smut (*Ustilago maydis*), gray leaf spot (*Cercospora zeae-maydis*), and southern (*Cochliobolus heterostrophus*) and northern (*Exserohilum turcicum*) corn leaf blight; *Ph3* (bins 3.07–3.08) is in the QTL region controlling common rust, common smut, southern and northern corn leaf blight, and southern rust (*Puccinia polysora)*; *Ph4* (bin 4.08) maps in the QTL region controlling multiple viral diseases (High plains virus, Maize chlorotic dwarf virus, Maize dwarf virus, Maize mosaic virus, Maize streak virus, Sugarcane mosaic virus, and Wheat streak mosaic virus), multiple fungal diseases causing ear and stalk rot, common smut, common rust, gray leaf spot, and southern and northern corn leaf blight; *Ph6* (bins 6.06–6.07) maps in the QTL region controlling multiple viral diseases, multiple fungal diseases, common rust and northern corn leaf blight; *Ph8a* (bins 8.00–8.02) maps in the QTL region controlling common smut, common rust, and gray leaf spot; and *Ph8b* (bins 8.06–8.07) is in the QTL region controlling multiple viral diseases, aflatoxin, common smut, common rust, gray leaf spot and northern corn leaf blight. Carson et al. (2005) also reported that four of the five QTL mapped were in the same bin or in the vicinity of QTL mapped for reaction to other diseases. Although these reported QTL related to reaction to several diseases were mapped in the same genomic regions of the QTL mapped in our study, there is no evidence that they could be considered as the same QTL. Nonetheless, our results coupled with that of Carson et al. (2005) are in line with both McMullen and Simcox (1995) and Wisser et al. (2006) hypothesis.

Implications for selection

The coefficient of determination indicates that only 49% of the variation of the progenies phenotypic means could be explained by the predicted progenies genotypic means. Consequently, only part of the selected progenies based on the phenotypic values and on the genotypic values coincided. For instance, for 10% (26 selected progenies) of selection intensity only 12 progenies were selected simultaneously by both phenotypic and genotypic means. Expected responses to one cycle of selection based on the mean phenotypic values will be about 3.5 times more efficient than that based on the genotypic values to reduce the level of the PLS disease infestation on the improved population. The predicted mean genotypic values of the progenies were not computed with all QTL that control the trait but with those that were mapped which explained less than half of the genotypic variance, indicating that these means could not be considered as precise as those obtained from the mean phenotypic values. Thus, selection applied on the phenotypic values was expected to be more precise than that applied on the predicted genotypic values which could explain the result above mentioned.

The results of this research and those already reported (Paterniani et al. 2000; Carson 2001; Pegoraro et al. 2002; Lopes 2003; Silva and Moro 2004; Carson et al. 2005; Derera et al. 2007) showed that the reaction to PLS disease presents high heritability, low magnitude or no G × E interaction, additive effects are more important than non-additive effects, and that dominance effects of the QTL were not unidirectional. Thus, the magnitudes of heterosis should be low and could not be exploited in crosses. Also, the magnitudes of inbreeding depression should be low and coupled with the high magnitude of the coefficient of heritability simple selection procedures as individual phenotypic selection could be used to develop inbred lines resistant to the PLS disease.

Although marker-assisted selection (MAS) applied directly on the selection of the progenies will not be as effective as phenotypic selection in a per cycle basis as already shown, MAS coupled with or not coupled with phenotypic selection, could be used to design more efficient breeding programs aimed to develop new resistant inbred lines or to improve elite inbred lines not resistant or presenting low levels of resistance to PLS disease. Then, the following aspects should be considered: (1) phenotypic selection could be conducted only during the growing season since it depends on the pathogen presence, but as MAS does not depend on its presence it could be conducted under both growing and

off-growing season speeding-up the breeding programs; (2) in population improvement two generations were required for one cycle of phenotypic selection because selection for PLS disease could be practiced only after flowering, while with MAS genotypes with alleles for resistance to PLS disease could be identified before flowering requiring only one generation for each selection cycle; (3) MAS could be used to transfer favorable alleles from mapped QTL in elite and non-elite germplasm to susceptible elite inbred lines via marker-assisted backcross selection to increase their resistance or to transform them from susceptible to resistant elite inbred lines. Several reports have shown that the marker-assisted backcross selection method is efficient to transfer QTL from elite or non-elite germplasm to elite inbred lines (Stuber and Sisco 1992; Toojinda et al. 1998; Benchimol et al. 2005; Neereja et al. 2007; Garzón et al. 2008).

Our research contributed to increase the knowledge on the inheritance of the PLS disease and how to use these information to implement an effective maize breeding program aimed to develop genotypes resistant to PLS disease. It could also contribute to develop hybrids resistant to PLS disease as the inbred line L-08-05F is an important source of resistance to this disease and, therefore, it could be used as a donor parent in marker-assisted backcross selection to transfer the favorable alleles of the mapped QTL to other elite inbreds not resistant to this disease. Thus, this research could effectively be used to alleviate the damages and economic losses that the PLS disease could cause in tropical maize growing areas.

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