Theor Appl Genet (2009) 119:1451–1459
DOI 10.1007/s00122-009-1147-6

ORIGINAL PAPER

Mapping of QTL for resistance to the Mediterranean corn borer attack using the intermated B73 × Mo17 (IBM) population of maize

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Received: 24 April 2008 / Accepted: 30 August 2009 / Published online: 16 September 2009

Abstract The Mediterranean corn borer or pink stem borer (MCB, *Sesamia nonagrioides* Lefebvre) causes important yield losses as a consequence of stalk tunneling and direct kernel damage. B73 and Mo17 are the source of the most commercial valuable maize inbred lines in temperate zones, while the intermated B73 × Mo17 (IBM) population is an invaluable source for QTL identification. However, no or few experiments have been carried out to detect QTL for corn borer resistance in the B73 × Mo17 population. The objective of this work was to locate QTL for resistance to stem tunneling and kernel damage by MCB in the IBM population. We detected a QTL for kernel damage at bin 8.05, although the effect was small and two QTL for stalk tunneling at bins 1.06 and 9.04 in which the additive effects were 4 cm, approximately. The two QTL detected for MCB resistance were close to other QTL consistently found for European corn borer (ECB, *Ostrinia nubilalis* Hübner) resistance, indicating mechanisms of resistance common to both pests or gene clusters controlling resistance to different plagues. The precise mapping achieved with the IBM population will facilitate the QTL pyramiding and the positional cloning of the detected QTL.

Communicated by M. Bohn.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1147-6) contains supplementary material, which is available to authorized users.

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Introduction

Maize stem borers are one of the most economically important pests, destroying 7% of the maize world crop annually (Europabio 2002). Although several species of stem borers [*Ostrinia nubilalis* Hübner (ECB), *Sesamia nonagrioides* Lefebvre (MCB), *Chilo partellus* Swinhoe, *Diatraea grandiosella* Dyar, *Busseola fusca* Fuller, etc.] cause economically important yield losses around the world by producing tunnels in the stalk, quantitative trait loci (QTL) analyses for stalk tunneling have been mainly focused on ECB (Bohn et al. 2000; Cardinal and Lee 2005; Jampatong et al. 2002; Krakowsky et al. 2004; Papst et al. 2004).

MCB is the most important pest in the Mediterranean area (Cordero et al. 1998; Velasco et al. 2007). MCB generally has two generations per year and stem borers of the second generation attack the maize plant at its reproductive stage. Larvae feed on the stem pith during plant development, producing tunnels that weaken the plant and, as a consequence, stalk lodging is increased and yield reduced. Yield can also be reduced by direct ear attack. Furthermore, the physical injuries to ears promote infections by *Fusarium* spp. that prejudices grain quality producing a number of toxic compounds, such as fumonisins, at levels that may affect human and animal health (Avantaggiato et al. 2002). ECB is also an important pest in the Mediterranean area and the ECB larvae feed on the plant producing tunnels as MCB do, although MCB larvae are more voracious and produce more damage (Velasco et al. 2007). Varieties resistant to ECB attack were also resistant to MCB, suggesting mechanisms of resistance common to both pests (Velasco et al. 1999).

Most of the inbreds used as female parents in temperate zones trace their parentage to Stiff Stalk Synthetic while

most of the inbreds used as males have ancestry from lines derived from the Lancaster variety (Goodman 2005; Mikel and Dudley 2006; Lu and Bernardo 2001). Within Stiff Stalk Synthetic and Lancaster the most commercially valuable inbreds are B73 and Mo17, respectively, according to the number of references of these inbreds in the patents of other inbreds (Mikel and Dudley 2006). Western European breeders have also used the heterotic pattern Corn Belt Dent × European Flint (Ordas 1991; Reif et al. 2005), using inbreds related to Stiff Stalk Synthetic or Lancaster as Corn Belt parentals.

Holland (2007) states that QTL results from two independent mapping populations can differ because of genetic heterogeneity and emphasizes that a more comprehensive analysis of the genetic architecture of a quantitative trait requires consideration of multiple populations that represent a larger sample of the standing genetic variation. In spite of its importance, a few or no experiments have been carried out to detect QTL for corn borer resistance in Stiff Stalk × Lancaster, particularly in the population B73 (or derivates) × Mo17 (or derivates). The intermated B73 × Mo17 (IBM) population serves as an invaluable source for QTL identification because it improves the resolution of QTL mapping 3–4-fold (Lee et al. 2002). Furthermore, the high number of markers included in the IBM map and the availability in 2008 of the complete and 90% of the sequence of B73 and Mo17, respectively (MaizeGDB website), facilitate the positional cloning of the QTL detected in the QTL analysis (Tuberosa et al. 2007). Therefore, the objective of this work was to identify and quantify the effects of QTL for resistance to MCB tunneling and kernel damage in the IBM population.

Materials and methods

Plant materials

A population of intermated RILs derived from B73 and Mo17 (IBM population) was employed for QTL analysis. RILs were obtained from the Maize Genetic Cooperation Stock Center (Illinois, USA). The seed of the RILs was obtained by hand self-pollination in Northwestern Spain in two consecutive years (2003 and 2004).

Phenotypic analysis

The parental inbred lines, 243 RILs, the hybrid B73 × Mo17, and another hybrid (EP39 × EP42) from which a RILs population was developed were evaluated in 2005 and 2006 in Pontevedra (42°30′ N, 8°46′ W) located in Northwestern Spain at the sea level, on the Atlantic coast. The temperatures are relatively mild all year and the average annual rainfall is around 1,700 mm. The evaluation was carried out under artificial infestation with eggs of MCB. At each environment, the treatments were arranged in a 14 × 18 α-lattice design with two replications per environment. The parental lines, B73 × Mo17 and the hybrid check were inserted twice within each replication. Each plot consisted of one row with 13 hills per plot, rows were spaced 0.80-m apart and hills were spaced 0.21-m apart. Plots were overplanted and thinned obtaining a final density of approximately 60,000 plants ha^{-1}. The seedbed preparation was made according to the standard practices of the area: a chisel plough followed by a rotary tiller. Prior to emergence a pre-emergence herbicide was applied. When the plants were about 60-cm tall, later weeds were controlled by cultivation with a shovel cultivator. Fertilization was made with 105 kg of N, 105 kg of P_2O_5, and 105 kg of K_2O. Prior to flowering we applied 55 additional kg of N. We irrigated with 60 L m^{-2} at flowering time.

For each plot, the date of silking was considered when 50% of the plants of the plot exerted the silks from within the husks. When approximately 50% of the RILs reached silking, five plants for each plot were infested with a mass of ≈40 eggs of MCB per plant. Eggs, obtained as described by Eizaguirre and Albajes (1992), were placed between the main ear and the stem (Butron et al. 1998). At harvest, stems of the infested plants from each plot were dissected and the total tunnel length (cm) of each plant was measured. In each unshelled ear of the infested plants kernel damage was recorded on a 9 point rating scale (9 = non-damaged kernels; 1 = more than 80% of kernels tunneled by MCB). The number of days from the date of planting to the date of silking was recorded for each plot.

Individual analyses of variance and adjusted mean were calculated for all traits according to a α-lattice design. Replications and treatments were considered as fixed factors and blocks within replications as a random factor. As the Bartlett's test indicated that the residual variances were homogenous a combined analysis of variance over years was computed using the adjusted means. Genetic correlations between traits were calculated following standard procedures (Mode and Robinson 1959). Computations were performed with SAS 9.1. (SAS Institute 2003).

QTL analysis

The RIL scores provided by the Maize Mapping Project (http://www.maizemap.org/) were used to construct a high-density genetic linkage map which can be found at http://www.maizegdb.org/ibm302scores.html. Information about marker and amplified sequences are available at http://www.maizegdb.org/. The original linkage map has more than 2,000 markers (SSRs, SNPs, RFLPs, microarrays, and INDEL); however, following the

recommendations of the software PLABQTL (Utz and Melchinger 2003), QTL analyses were performed using a linkage map with an average distance between loci of about 10 cM, yielding a final set of 587 markers. Composite interval mapping (Zeng 1994) was conducted with PLABQTL (Utz and Melchinger 1996) with cofactor selection performed following PLABQTL′s recommendations and using an "*F*-to-enter" and an "*F*-to-delete" value of 9. A LOD threshold of 2.69 was determined by permutation tests that ensures an experiment wise error rate of $P < 0.30$. The proportion of phenotypic variance explained by all QTL was determined by the adjusted coefficient of determination of regression (R^2_{adj}) fitting a model including all detected QTL (Papst et al. 2004). Given that $R^2_{adj} = V_M/V_P$, where V_M is the genetic variance explained by the markers and V_P is the phenotypic variance (Hospital et al. 1997), the proportion of genotypic variance explained by all QTL for one trait (p) was calculated as $p = R^2_{adj}/h^2$, where h^2 is the heritability of the trait.

Fivefold cross validation (CV/G) was performed, following the procedures described by Utz et al. (2000), to estimate the additive effects and the proportion of genotypic variance explained by the QTL. The whole dataset was randomly split into $k = 5$ data subsets. Four of these subsets were combined to form the estimation set (ES) and the remaining subset formed the test set (TS) in which predictions derived from ES were tested for their validity by correlating predicted and observed data. We used 1,000 replicated CV/G runs. For a particular QTL and its confidence interval estimated using the whole dataset, the frequency of QTL detection across the CV/G runs was calculated by counting the number of CV/G runs in which a QTL was located within that confidence interval. The frequency of QTL detection gives us an estimation of the precision of QTL localization (Utz et al. 2000).

The analysis of QTL × environment interactions was carried out by means of an analysis of variance with the following sources of variation: environment, RIL, and RIL × Environment. RIL was subdivided into QTL and residual while RIL × Environment was subdivided into QTL × Environment and residual × Environment. The values of QTL × Environment mean squares were tested for significance with a sequentially rejective Bonferroni *F* test (Utz and Melchinger 2003). The MS were used to obtain an estimate of the proportion of the genetic variance explained by the detected QTL which is adjusted for QTL × environment interactions. The proportion of genetic variance, adjusted for QTL × environment interactions, explained by the QTL for one trait (p) was calculated as: $p = 1 - \mathrm{MS(residuals)} - \mathrm{MS(residual \times environment)}/\mathrm{MS(RILs)} - \mathrm{MS(RILs \times environment)}$ (Utz and Melchinger 2003).

Results

Significant differences between the parental lines were detected for stalk tunnel length while differences were not significant for silking and kernel damage (Table 1). B73 showed values for stalk tunnel length significantly ($P < 0.05$) higher than Mo17 with the mean of the RILs falling between the two parents. Several RILs with tunneling values significantly ($P < 0.05$) higher than B73 and lower than Mo17 were observed, indicating transgressive segregation of different resistance alleles inherited from the parental lines. B73 × Mo17 exhibited more stalk tunneling than Mo17. Variation among RILs was significant for all traits and RIL × environment interactions were significant for silking and kernel damage, but not significant for stalk tunneling (Table 2). The genetic correlations between the three traits were low and not significant.

For days to silking, nine QTL explaining 48.1% of the genotypic variance was detected in all chromosomes except in chromosomes 3, 6, and 7 (Table 3). The additive effects were close to 1 day for most of the QTL and the partial R^2 values ranged from 4.1 to 8.5%. The values of additive effects estimated using the whole dataset were similar to values estimated using cross validation. The same QTL were detected in the two environments (data not shown) and, congruent with that, there was no significant QTL × environment interaction (Table 2).

One QTL, in the chromosome 8, was found for kernel damage that explained 5.5% and 8.3% of the phenotype and the genotype variances, respectively (Table 3). The allele for increased kernel damage was inherited from Mo17 (Table 3). The same QTL was detected in the two environments, although the magnitude of effect varied between environments (data not shown) and, congruent with that, there was a significant QTL × environment interaction (Table 2).

For stalk tunneling, two QTL associated with 20% of the genotype variance were detected. One QTL, located on chromosome 1, explained 7.2% of the phenotypic variance, while the other QTL, located in chromosome 9, explained the 10.8% of the phenotypic variance. The additive effect for both QTL was approximately 4 cm. The estimated QTL effects for stalk tunneling using the whole dataset were similar to those obtained by cross validation. Mo17 contributed the favorable allele for the QTL on chromosome 1, while the favorable allele of the QTL on chromosome 9 was inherited from B73. The same QTL were detected in both environments (data not shown) and, in agreement with that, the QTL × environment interaction was not significant (Table 2).

Table 1 Mean and range of B73, Mo17, and the B73 × Mo17 intermated RIL population for silking and two MCB resistance traits, as well as estimates of variance components, and heritability for those traits evaluated in 2005 and 2006 under artificial infestation

Material	Entries (no.)	Silking (days)	Kernel damage (1–9)[a]	Stalk tunnel length (cm)
Parents				
B73	2	74.3	6.0	88.3
Mo17	2	77.5	7.1	67.1
LSD[b] ($p < 0.05$)		12.9	1.7	16.2
RILs	243			
Mean		74.4	6.0	71.7
Maximum		90.3	8.6	110.5
Minimum		66.3	2.1	33.2
σ^2_g		11.9 ± 1.65	0.41 ± 0.02	104.5 ± 12.9
σ^2_{ge}		2.6 ± 1.04	0.21 ± 0.03	10.0 ± 15.7
σ^2_e		10.8 ± 0.83	1.96 ± 0.03	197.9 ± 15.9
h^2		0.70	0.51	0.77
90% C.I. on h^2		(0.54, 0.94)	(0.34, 0.85)	(0.58, 0.90)
Hybrids				
B73 × Mo17	2	69.5	6.6	91.2
EP39 × EP42	2	59.3	8.4	76.1

[a] Kernel damage was taken on a 9 point rating scale (9 = non-damaged kernels; 1 = more than 80% of kernels with injuries due to MCB larvae activity)

[b] Least significant difference

Table 2 Mean squares of the analyses of variance of the B73 × M017 intermated RIL population of maize for silking and two MCB resistance traits recorded in 2005 and 2006 under artificial infestation

Sources of variation	Silking		Kernel damage		Stalk tunnel length	
	df	MS	*df*	MS	*df*	MS
RIL	242	30.9*	240	2.81*	242	374*
QTL	9	295.7*	1	36.96*	2	7116*
Residual (R)	233	20.7*	239	2.67*	240	318*
RIL × Env	208	9.4*	196	1.37*	195	87
QTL × Env	9	6.8	1	5.90*	2	−2
R × Env	199	9.5*	195	1.35*	193	88
Error	325	5.3	282	0.85	289	103

Env environment

* $p < 0.01$

Discussion

In this study we used a population of 243 intermated RIL in which both the large number of RILs and the recombination for several generations before selfing increased the resolution of the QTL analysis. On the negative side, with such populations only additive effects can be estimated and the fact that B73 × Mo17 exhibited more stalk tunneling than Mo17 suggests that other effects different from additive ones could also play a role in the susceptibility of the hybrid. We have identified for the first time genetic factors for resistance to both kernel damage, which is related to fumonisin contamination, and stalk tunneling by MCB, the most important pest in the Mediterranean area.

Before the results of the genetic factors associated to resistance are discussed further, it is important to comment on the relationship between silking and resistance in our data. None of the 9 QTL detected for silking were located near the 2 QTL detected for stalk tunneling in concordance with the low genetic correlation that we found between these traits. Therefore, no adjustment of stalk tunneling for silking was necessary. The genetic correlation between ECB stalk tunneling and silking was low and non-significant in several experiments (Bohn et al. 2000; Cardinal et al. 2001; Krakowsky et al. 2002), but Krakowsky et al. (2004) found a highly significant genetic correlation between both traits.

The MCB larvae can produce damage on kernels and the stalk (Butron et al. 1998; Cartea et al. 2001). Studies demonstrated that both types of damage are not genetically associated (Malvar et al. 1996). The analysis of the resistance to stem borers in other experiments has been mainly focused on stalk damage; however, the larvae, by damaging the kernels, not only directly reduce the yield, but also promote infections by *Fusarium* spp. (Avantaggiato et al. 2002). We detected a QTL for kernel damage that was validated 33% of the time, although the phenotypic variance explained by the QTL computed with all data was much reduced when the phenotypic variance was calculated across validations. Therefore, a QTL for kernel damage, the first reported for this trait, at bin 8.05 seems apparent although the effect of the QTL is small and subject to environmental interactions as shown in Table 2.

Table 3 Summary of QTL detection and additive effects for silking and two MCB resistant traits for the B73 × Mo17 intermated RIL population of maize

QTL bin[a]	Confidence interval (cM)	LOD score	Flanking markers	R^2_{adj}	p[b]	â[c]	Cross validation $\hat{a}^d_{TS.ES}$			
							Median	Percentile (10, 90)	Frequency (%)	p[e]
Silking (days)										
1.10	922–940	4.83	lim78 mmp165	6.5		0.96	1.05	(0.90, 1.23)	8.2	
2.01	46–60	4.82	psb485b npi254a	6.5		−0.95	−1.07	(−0.95, −1.25)	15.5	
4.08	446–461	2.91	umc1808 umc1476	4.5		0.69	0.91	(0.81, 1.05)	3.8	
5.08	624–637	3.73	AY110413 umc1225	8.5		1.05	1.12	(0.97, 1.31)	50.6	
8.01	0–6	2.91	npi220a npi114a	4.1		0.70	1.01	(0.87, 1.15)	11.8	
8.05	357–372	3.63	ufg74 rz390a(cyb5)	5.3		−0.85	−1.11	(−0.97, −1.35)	36.7	
8.09	625–632	2.93	umc1638 AY109853	5.0		−0.77	1.11	(−0.91, −1.16)	20.5	
9.02	129–153	3.93	mmp162 mmp77	7.5		1.07	1.18	(1.03, 1.37)	55.5	
10.04	242–262	3.24	umc64a mmp16	4.9		−0.75	−1.03	(−0.88, −1.21)	16.7	
Final fit					48.1					0.7
Kernel damage (1–9 scale)										
8.05	387–423	3.08	umc1777 umc2356	5.5		0.39	0.43	(0.37, 0.49)	33.4	
Final fit					8.3					0.0
Stalk tunnel length (cm)										
1.06	521–533	4.45	AY110566 bnlg2057	7.2		−3.73	−3.81	(−3.29, −4.36)	70.0	
9.04	291–303	5.66	psr129a bnlg1012	10.8		4.46	4.31	(3.66, 5.00)	96.2	
Final fit					19.9					6.5

[a] Bin locations are designed by an *X·Y* code, where *X* is the linkage group containing the bin and *Y* is the location of the bin within the linkage group (Gardiner et al. 1993)

[b] Proportion of the genotypic variance explained by detected QTL, adjusted for QTL × environment interaction, and calculated using the whole dataset

[c] Additive effects calculated in the whole dataset. Positive additive effects indicate that the Mo17 allele increases the value of the trait

[d] Median and percentiles of the additive effects and frequency of QTL detection calculated in 200 cross-validation runs

[e] Proportion of the genotypic variance explained by detected QTL calculated as R^2_{adj}/heritability in 200 cross-validation runs

Compared with other traits without RIL × environment interactions, for kernel damage the significant ($P < 0.05$) RIL × environment interactions could decrease the power to detect QTL.

The lack of significant RILs × environment interactions for stalk tunneling is in agreement with previous research in which an elevated number of inbreds, hybrids, and open pollinated varieties were evaluated for stalk tunneling under artificial infestation with MCB (Butron et al. 1999a, b; Soengas et al. 2004). We have found less QTL associated to MCB damage—two—than the average number of QTL reported for resistance to ECB that ranged from six to nine (Cardinal et al. 2001; Jampatong et al. 2002; Krakowsky et al. 2002; Papst et al. 2004; Schon et al. 1993).

One of the possible reasons for this was the choice of parental lines, which was based on their importance for breeding and the availability of a RIL population with improved resolution and density of markers, and not on having high levels of variation for the target trait. Tuberosa et al. (2007) have pointed out that one reason for the limited applicative impact of the QTL approach is because the choice of the parental lines has prevalently disregarded their agronomic value while being mainly based on the differences for the target traits. Those authors argued that, while this approach maximizes the opportunities for identifying QTL, it does not guarantee any real progress when the beneficial QTL alleles are introgressed via marker assisted selection (MAS), because alleles for such QTL may be fixed in the elite germplasm. Therefore, it is of value that these two QTL not only segregate in the B73 × Mo17 cross, but may also segregate in crosses of lines derived from B73 and Mo17, which are the most common crosses used in maize breeding programs. Particularly, most of the hybrids cultivated across Spain are made with lines derived from B73 and/or Mo17. It is worthwhile to comment that these lines have a maturity appropriate for Central and Southern Spain and can be used in combination with European Flint lines exploiting the heterotic pattern Corn Belt Dent × European Flint in Northern Spain (Ordas 1991; Reif et al. 2005).

The two QTL detected for MCB resistance were consistent across environments, which is similar to the number of environmentally robust QTL identified by other studies of ECB resistance (Cardinal et al. 2001; Krakowsky et al. 2002, 2004). The consistency across environments is critical when the markers are used for MAS.

Varieties resistant to ECB attack are also resistant to MCB, suggesting mechanisms of resistance common to both pests (Velasco et al. 1999). One approach to directly examine the correlation between resistances to MCB and ECB could be to derive resistant, susceptible, and intermediate RILs from the IBM population and study the progression of tunneling for both pests separately. Alternatively, the comparison between QTL chromosome regions in ECB and MCB studies could be an indirect way to study the relationship between the mechanisms of resistance to both types of insects. Thus, the fact that the two QTL detected for MCB resistance were close to 2 of the 3 QTL that have been consistently found by other authors for ECB resistance (Papst et al. 2004) could be indicative of mechanisms of resistance common to both pests, although may also represent gene clusters affecting resistance to different plagues or diseases (Krakowsky et al. 2004).

The additive effects detected for QTL associated to MCB stalk tunneling (about 4 cm) were higher than the additive effects detected for QTL associated to ECB stalk tunneling in most of the populations (0.5–2 cm) (Bohn et al. 2000; Cardinal et al. 2001; Jampatong et al. 2002; Krakowsky et al. 2004). This difference in the magnitude of additive effects could be due to the difference in the level of damage produced by the two insects. Thus, in our experiment, with approximately 40 MCB larvae per plant the average tunnel length in the plants was about 70-cm long, while in other studies with 300–600 ECB larvae per plant the average tunnel length was about 15–30-cm long (Cardinal et al. 2001; Krakowsky et al. 2002, 2004). This was expected because the MCB larvae are more voracious than the ECB larvae (Velasco et al. 2007). More aggressive insects could provide better differentiation between resistant and susceptible germplasm because they produce a higher rate of damage (Jampatong et al. 2002). This could improve the detection of genetic effects in QTL experiments. Based on this argument we expected to find more QTL for stalk tunneling in our experiment with MCB than in previous experiments with ECB, however, the opposite happened. Alternatively, it is also possible that, due to the aggressiveness of the insect, most genotypes seem to be susceptible although some of them carry a low level of resistance. Selection for resistance to aggressive pests has been shown to confer resistance to less aggressive pests, although the opposite is not true (Thome et al. 1992). For that reason, although there is no direct information regarding dual resistance to ECB and MCB available, selection for resistance to MCB could confer resistance to ECB given that MCB larvae are more active feeders than ECB larvae.

QTL positions for stalk tunneling determined by composite interval mapping at bins 1.06 and 9.04 were confirmed, following Utz et al. (2000) recommendations, by a frequency of QTL detection across cross validation of 70% and 96%, respectively, that corroborate the validity of the identification. The same validation procedure was used for QTL mapping for ECB resistance only by Papst et al. (2004) that detected 2 QTL in 80% of the cross validation runs. The favorable allele for the QTL on chromosome 1 was contributed by Mo17 and the favorable allele for the QTL on chromosome 9 was contributed by B73, in agreement with the significant transgressive segregation detected. For ECB, in several experiments significant transgressive segregation and favorable alleles for resistance were also found in the two parental lines (Bohn et al. 2000; Cardinal et al. 2001; Krakowsky et al. 2004).

The QTL for stalk tunneling in the region of bin 9.04 overlapped with the stalk tunneling QTL detected in crosses of B73 with B52, De811 and Mo47, which are three lines derived from different genetic backgrounds (Krakowsky et al. 2002). Also, in the cross of two European lines (not related with the previous lines) a QTL for stalk tunneling was detected in the same region. The region

was also associated with leaf-sheath and stalk fiber and, moreover, the allele associated with highest fiber was associated with lowest ECB tunneling damage (Cardinal and Lee 2005; Krakowsky et al. 2007). Although QTL experiments relating resistance and cell wall composition were not carried out for MCB, the significant role of cell wall components of the pith and the leaf-sheath in the resistance to MCB has been shown at the phenotypic level (Santiago et al. 2006a, b). Thus, our results as well as results from other authors suggest the presence of genes at bin 9.04 associated with resistance to stalk tunneling, which is effective against different stem borers. These may be genes involved in cell wall fortification and with a pleiotropic effect on resistance. To increase the precision of the location of the QTL, the QTL analysis was repeated using all available markers in the region, but results were very similar to those obtained in previous analysis using only markers spaced 10 cM or more. The markers more tightly linked to the QTL (psr129a and bnlg1012) were used to anchor the genetic region to a physical region of chromosome 9 which is 4-Mb long and includes part of contigs 382 and 383 (http://www.maizegdb.org). A total number of 84 genes were annotated, based on transcript evidence and mapping of putative rice homologs, in contigs 382 and 383 (Table S1; http://www.plantgdb.org/ZmGDB/DisplayGeneAnn.php). From these, the gene *GRMZM2G116452* (AC203571.3: 39903–41496 bp, http://www.maizesequence.org/Zea_mays2/geneview?db=core;gene=GRMZM2G116452) encodes a protein which is homolog of a peroxidase 57 precursor found in *Arabidopsis thaliana* (AT5G17820, http://www.arabidopsis.org/servlets/TairObject?id=134912&type=locus) and rice (OS09G15500; http://bioinformatics.psb.ugent.be/plaza/genes/view/OS09G15500). This peroxidase belongs to the Class III peroxidase family which is involved in cell wall synthesis and defense against pathogens in rice (Passardi et al. 2004) and maize (Mika et al. 2008). In addition, in a region close to the QTL (contig 380, chromosome 9), a *4-coumarate-Coa ligase (4CL)* gene has been found (*GRMZM2G057594*, AC216185.2: 36746-43269 bp) using as query a sequence that showed homology to 4CL genes in Arabidopsis (http://www.polebio.scsv.ups-tlse.fr/MAIZEWALL/afficheseq.php?type=cg&id=75). 4CL are genes from the phenylpropanoid pathway that catalyze the biosynthesis of monolignols (Guillaumie et al. 2007) and have been associated to digestibility of cell wall in maize (Andersen et al. 2008). The two genes constitute reasonable candidate genes for resistance to stalk tunneling that could be validated by an association study (Sneller et al. 2009).

The proportion of phenotypic variance for stalk tunneling explained by each QTL was similar to that reported in other studies. However, the total genotypic variance explained by the QTL, estimated using the whole dataset, was lower than that found by other authors (Cardinal et al. 2001; Jampatong et al. 2002; Krakowsky et al. 2004) due to the smaller number of QTL detected. The total genotype variance explained by the QTL was also calculated by cross validation, following recommendations made by Utz et al. (2000), for obtaining unbiased estimates and a realistic assessment of the prospects of MAS. Similar to the results of Papst et al. (2004) the estimate of the genotypic variance explained by the QTL is much lower when calculated by cross validation. The proportion of genotypic variance explained by the detected QTL following cross validation in our experiment was somewhat lower than that found by Papst et al. (2004) for ECB stalk tunneling (6.5 vs. 10.6%). Given the low number of detected QTL and the small proportion of genotypic variance explained, it is likely that the trait be regulated by many QTL of small effect, but for detecting them the power of the experiment has to be increased. This could be done using larger mapping populations or increasing the heritability of the trait (Bernardo 2002) which, in turn, can be achieved by increasing the number of replications or environments (Hallauer and Miranda 1988). Due to the low value for the proportion of genotypic variance explained, the theoretical expectation of the efficacy of MAS is low.

The high degree of consistency between environments, coupled with the advantages of the IBM population stated in Sect. Introduction, has allowed two QTL for resistance to stalk tunneling to be mapped to narrowly defined regions with a high degree of confidence. These QTL could be combined with QTL detected by other authors by QTL pyramiding strategies (Dekkers and Hospital 2002). This process is facilitated by the precise mapping achieved with the IBM population because the effectivity of MAS depends on the linkage between markers and genes. In addition, the fact that B73 and Mo17 would be adapted lines reduces the probability of deleterious genes located around the target QTL and, therefore, increases the efficiency of the selection. The precise mapping with the IBM population facilitated the selection, within the marker interval, of one candidate gene for positional cloning. The genetic improvement through MAS would be most effective if the genetic architecture of a quantitative trait was completely transparent. In this case MAS could be based on gene sequences (or SNPs) that would permit pyramiding of beneficial alleles directly (Dekkers and Hospital 2002; Papst et al. 2004).

Acknowledgments This research was supported by the National Plan for Research and Development of Spain (Project Cod. AGL2006-13140). B Ordas and R Santiago acknowledge a contract from the Spanish National Research Council (I3P Program) and G Sandoya acknowledges a fellowship from the Ministry of Education and Science (Spain).

References

Andersen JR, Zein I, Wenzel G, Darnhofer B, Eder J, Ouzunova M, Lübberstedt T (2008) Characterization of phenylpropanoid pathway genes within European maize (*Zea mays* L.) inbreds. BMC Plant Biol 2008(8):2–13

Avantaggiato G, Quaranta F, Desiderio E, Visconti V (2002) Fumonisin contamination of maize hybrids visibly damaged by *Sesamia*. J Sci Food Agric 83:13–18

Bernardo R (2002) Breeding for quantitative traits in plants. Stemma Press, Woodbury, Minnesota, USA

Bohn M, Schulz B, Kreps R, Klein D, Melchinger AE (2000) QTL mapping for resistance against the European corn borer (*Ostrinia nubilalis* H.) in early maturing European dent germplasm. Theor Appl Genet 101:907–917

Butron A, Malvar RA, Velasco P, Revilla P, Ordas A (1998) Defense mechanisms of maize against pink stem borer. Crop Sci 38:1159–1163

Butron A, Malvar RA, Cartea ME, Ordas A, Velasco P (1999a) Resistance of maize inbreds to pink stem borer. Crop Sci 39:102–107

Butron A, Malvar RA, Velasco P, Vales MI, Ordas A (1999b) Combining abilities for maize stem antibiosis, yield loss, and yield under infestation and non infestation with pink stem borer. Crop Sci 39:691–696

Cardinal AJ, Lee M (2005) Genetic relationships between resistance to stalk-tunneling by the European corn borer and cell-wall components in maize population B73 × B52. Theor Appl Genet 111:1–7

Cardinal AJ, Lee M, Sharopova N, Woodman WL, Long MJ (2001) Genetic mapping and analysis of quantitative trait loci in maize for resistance to stalk tunnelling by the European corn borer. Crop Sci 41:835–845

Cartea ME, Malvar RA, Vales MI, Butron A, Ordas A (2001) Inheritance of resistance to ear damage caused by *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in maize. J Econ Entomol 94:277–283

Cordero A, Malvar RA, Butron A, Revilla P, Velasco P, Ordas A (1998) Population dynamics and life-cycle of corn borers in south Atlantic European coast. Maydica 43:5–12

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nature Rev Genet 3:22–32

Eizaguirre M, Albajes R (1992) Diapause induction in the stem corn borer *Sesamia nonagrioides* (Lepidoptera, Noctuidae). Entomol Gen 17:277–283

Europabio (2002) EuropaBio′s biotechnology information kit. Genetically modified maize. http://www.europabio.be/module_10.htm

Goodman MM (2005) Broadening de US maize germplasm base. Maydica 50:203–214

Guillaumie S, San-Clemente H, Deswarte C, Martinez Y, Lapierre C, Murigneux A, Barriere Y, Pichon M, Goffner D (2007) Maizewall. Database and developmental gene expression profiling of cell wall biosynthesis and assembly in maize. Plant Physiol 143:339–363

Hallauer AR, Miranda JB (1988) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa, USA

Holland JB (2007) Genetic architecture of complex traits in plants. Curr Opin Plant Biol 10:156–161

Hospital F, Moreau L, Lacoudre F, Charcosset A, Gallais A (1997) More on the efficiency of marker-assisted selection. Theor Appl Genet 95:1181–1189

Jampatong C, McMullen MD, Barry BD, Darrah LL, Byrne PF, Kross H (2002) Quantitative trait loci for first- and second-generation European corn borer resistance from the maize inbred line Mo47. Crop Sci 42:584–593

Krakowsky MD, Brinkman MJ, Woodman-Clikeman WL, Lee M (2002) Genetic component of resistance to stalk tunnelling by the European corn borer in maize. Crop Sci 42:1309–1315

Krakowsky MD, Lee M, Woodman-Clikeman WL, Long MJ, Sharopova N (2004) QTL mapping of resistance to stalk tunnelling by the European corn borer in RILs of maize population B73 × De811. Crop Sci 44:274–282

Krakowsky MD, Lee M, Holland JB (2007) Genotypic correlation and multivariate QTL analyses for cell wall components and resistance to stalk tunnelling by the European corn borer in maize. Crop Sci 47:485–490

Lee M, Sharopova N, Beavis WD, Grant D, Katt M, Blair D, Hallauer A (2002) Expanding the genetic map of maize with the intermated B73 × Mo17 (IBM) population. Plant Mol Biol 48:453–461

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Malvar RA, Butron A, Cartea ME, Ordas A (1996) Ear resistance to pink stem borer in maize inbred lines. In: XVIIth Conference of genetics, biotechnology and breeding of maize and sorghum, Thesaaloniki, Greece, p 96

Mika A, Buck F, Lüthje S (2008) Membrane-bound class II peroxidases: identification, biochemical properties and sequence analysis of isoenzymes purified from maize (*Zea mays* L.) roots. J Proteomics 71:412–424

Mikel MA, Dudley JW (2006) Evolution of North American dent corn from public to proprietary germplasm. Crop Sci 46:1193–1205

Mode CJ, Robinson HF (1959) Pleiotropism and genetic variance and covariance. Biometrics 15:518–537

Ordas A (1991) Heterosis in crosses between American and Spanish populations of maize. Crop Sci 31:931–935

Papst C, Bohn M, Utz HF, Melchinger AE, Klein D, Elder J (2004) QTL mapping for European corn borer and forage quality traits of testcross progenies in early-maturing European maize (*Zea mays* L.) germplasm. Theor Appl Genet 108:1545–1554

Passardi F, Longet D, Penel C, Dunand C (2004) The class III peroxidase multigenic family in rice and its evolution in land plants. Phytochemistry 65:1879–1893

Reif JC, Hallauer AR, Melchinger AE (2005) Heterosis and heterotic patterns in maize. Maydica 50:215–223

Santiago R, Butron A, Arnason JT, Reid LM, Souto XC, Malvar RA (2006a) Putative role of pith cell wall phenylpropanoids in *Sesamia nonagrioides* (Lepidoptera: Noctuidae) resistance. J Agric Food Chem 54:2274–2279

Santiago R, Butron A, Reid LM, Arnason JT, Sandoya G, Souto XC, Malvar RA (2006b) Diferulate content of maize sheaths is associated with resistance to the Mediterranean corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae). J Agric Food Chem 54:9140–9144

SAS Institute (2003) Version 9.1.3. SAS Institute, Cary, NC

Schon CC, Lee M, Melchinger AE, Guthrie WD, Woodman WD (1993) Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70:648–659

Sneller CH, Mather DE, Crepieux S (2009) Analytical approaches and population types for finding and utilizing QTL in complex plant populations. Crop Sci 49:363–380

Soengas P, Butron A, Revilla P, Ordas A, Malvar RA (2004) Performance of crosses among flint maize populations under infestation by *Sesamia nonagrioides* (Lepidoptera: Noctuidae). J Econ Entomol 97:1438–1443

Thome CR, Smith ME, Mihm JA (1992) Leaf-feeding resistance to multiple insect species in a maize diallel. Crop Sci 32:1460–1463

Tuberosa R, Salvi S, Giuliani S, Sanguineti MC, Belloti M, Conti S, Landi P (2007) Genome-wide approaches to investigate and improve maize response to drought. Crop Sci 47:S120–S141

Utz HF, Melchinger AE (1996) PLABQTL. A computer program to map QTL. J Quant Trait Loci 2:1

Utz HF, Melchinger AE (2003) A computer program to map QTL. http://www.uni-hohenheim.de/~ipspwww/soft.html

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

Velasco P, Malvar RA, Revilla P, Butron A, Ordas A (1999) Ear resistance of sweet corn populations to *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Lepidoptera: Pyralidae). J Econ Entomol 92:732–739

Velasco P, Revilla P, Monetti L, Butron A, Ordas A, Malvar RA (2007) Corn borers (Lepidoptera: Noctuidae, Crambidae) in Northwestern Spain: population dynamics and distribution. Maydica 52:195–203

Zeng Z (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468