QTL mapping for maize resistance and yield under infestation with *Sesamia nonagrioides*

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Abstract

The Mediterranean corn borer (MCB) is the most important maize insect pest in the Mediterranean region. The main objective was to map QTLs for yield performance under infestation with MCB, resistance and agronomic traits in a maize RIL population derived from an inbred cross European flint × Reid.

Six QTLs for resistance traits were located: one QTL for tunnel length (bin 9.03, p = 19.8%), one QTL for stalk lodging (bin 3.07, p = 11.5%), and four QTL for ear resistance (bins 1.07. 5.03/05, and 8.04; p = 25 - 63%). Twelve QTLs for agronomic traits were located: A QTL for yield under infestation (bin 5.03, p = 15 %); two QTLs for grain moisture (bins 1.07 and 8.05), two QTLs for days to anthesis (bin 1.07 and 8.05); two QTLs for days to silking (bins 8.04 and 10.02); three QTLs for plant height (bins 5.04, 8.05 and 9.03); and two QTLs for ear height (bins 8.05 and 9.03). No genetic correlations between yield and other trait were observed. The cross validation approach showed that the estimation biases for QTLs for resistance traits were higher than those for agronomic traits.

This work stresses the importance of the region 9.03 for controlling corn borer resistance and suggests the presence of QTL with small effect on ear resistance traits. At the same genomic region, there are also genes that control plant and ear height and future works could elucidate if these genes are the same or are closely linked. The QTL for yield seem to play an important role in MCB tolerance in this genetic background. Large biases observed for QTL effects by CV was mainly due to the small sample size used and were higher for resistance traits due to their larger genetic complexity. We consider it is more appropriate to select for

grain yield under infestation instead of selecting for resistance traits because resistance to MCB could have unfavorable associations with agronomic traits.

Keywords

Quantitative trait loci, Maize, Insect resistance, Insect tolerance, Yield under infestation, Molecular markers, Corn borer, *Sesamia nonagrioides*, Marker assisted selection, Cross validation.

Abbreviations

QTL; Quantitative trait loci; MCB: Mediterranean corn borer; ECB: European corn borer; CV: Cross validation; MAS: Marker assisted selection; RIL: Recombinant inbred line; BLUP: best linear unbiased predictor; A: days to anthesis; S: days to silking; PH: Plant height; EH: Ear height; SL: Stalk lodging; KR; Kernel resistance; ShR: Shank resistance; CR: Cob resistance; TL: Tunnel length; Y: yield; GM: Grain moisture; SSR: Simple Sequence Repeat.

70

71 Introduction

72 The Mediterranean corn borer (MCB) *Sesamia nonagrioides* is the most important insect
73 pests of maize in the Mediterranean region, including Southern Europe (Malvar et al. 1993;
74 Cordero et al. 1998; Velasco et al. 2007). The larvae of the first generation feed on the leaves
75 of young plants while second and subsequent generations feed on the pith of the stem
76 provoking stalk lodging and yield reduction (Malvar et al. 1993; Meihls et al. 2012). The
77 larvae can also attack the ears favoring fungal infection and the subsequent kernel
78 contamination with mycotoxins that may affect human and animal health (Visconti et al.
79 1999; Avantaggiato et al. 2002; Butrón et al. 2006b).

80

81 There are different mechanisms of defense against insect attack: antixenosis, antibiosis and
82 tolerance. Antixenosis reduce the probability of contact between potential consumers and
83 plants and antibiosis is the ability of the plant to reduce the growth and/or development of the
84 larvae after contact has been initiated. Few works have been focused on the study of
85 antixenosis and/or antibiosis against attack by borers because these studies imply monitoring
86 ovipositional insect behavior and/or larval development (Barry and Darrah 1988; Ordas et al.
87 2002). However, most studies have evaluated insect resistance in a wide sense; it has been
88 estimated as tunnel length in the stem pith made by corn borers without paying attention to
89 insect biology. As the development of resistant varieties seemed a suitable method for
90 fighting against maize damage by *Sesamia nonagrioides*, research has been focused on the
91 search for sources of resistance in wide sense (Hudon and Chiang 1991; Malvar et al. 1993;
92 Melchinger et al. 1998; Butrón et al. 1999a; Butrón et al. 2006a), the study of the inheritance
93 of the resistance (Butrón et al. 1999a; Cartea et al. 2001) and the search of quantitative trait

94 loci (QTL) (Ordás et al. 2009; 2010) for tunnel length as previous steps for implementing a
95 breeding program (Sandoya et al. 2008) to reduce damage by corn borers (Meihls et al.
96 2012).

97

98 Nevertheless, increased resistance is often correlated to yield reduction (Butrón et al. 2012).
99 This negative relationship between resistance and yield led us to focus on another mechanism
100 of defense, tolerance which is the mechanisms by which the plants reduce the extent of
101 damage per unit of parasite present (Niks et al. 1993).

102

103 It is very difficult to detect tolerance differences among maize genotypes because it is
104 necessary to compare yield under infestation conditions with yield under protected conditions
105 and to record the level of infestation. Therefore, as this is a complicated work with large
106 experimental errors, the number of studies on true tolerance is low (Niks et al. 1993). Butrón
107 et al. (1998) studied the defense mechanisms against MCB in 10 inbred lines and the 10
108 parent diallel among these inbreds. Yield of infested and non-infested plants were computed
109 to calculate genotype yield loss which is considered as an estimation of genotype tolerance.
110 They concluded that the three mechanisms of defense to MCB attack (antixenosis, resistance
111 in a wide sense, and tolerance) were present among inbred lines and hybrids.

112

113 In addition, the correlations between yield loss and yield under infestation and no infestation
114 conditions can be low (Butrón et al. 1999b) because the high yield potential of some
115 genotypes compensate their large yield losses. Therefore, as yield under infestation
116 conditions could be a more suitable trait than yield loss for improving maize performance

under MCB attack, we developed a set of RILs from A637×EP42 in order to detect QTLs for yield under infestation with MCB. Butrón et al. (1998) found that inbred lines A637 and EP42 were tolerant and sensitive, respectively, to stem and ear damage by MCB. Inbred A637 showed a yield loss of 11.7 % versus 29.33% for EP42, and the resulting hybrid between these inbred lines had a yield loss of 2.40 %. In addition, A637 showed favorable general combining ability (GCA) effects for yield with and without infestation with MCB; while GCA effects for EP42 were not significantly different from zero.

The EP42 inbred line is an European flint inbred with very good GCA for early vigor (Revilla et al. 1999) and large yield potential, while A637 is a Reid dent inbred with similar grain yield performance under infestation and no infestation conditions.

Therefore, the heterotic pattern European flint × Reid will be explored. Previous works on QTL mapping for resistance to MCB had been carried out with other heterotic patterns: Reid × Lancaster (Ordás et al. 2009) and European flint from the North-Western Spain (humid) × European flint from Central Spain (dry) (Ordás et al. 2010) .

It is known that with a limited sample size, due to sampling effects the model selection leads to an overestimation of QTL effects and the proportion (p) of genetic variance explained by QTL and consequently to a biased assessment of the prospect of marker-assisted selection (MAS) (Utz and Melchinger 1994; Beavis 1998). The cross validation (CV) approach has been proposed by some authors as one of the best re-sampling approaches for analysis of QTL mapping data to obtain asymptotically unbiased estimates of the true QTL effects and the proportion of the genotypic variance explained by the QTL (Utz et al. 2000; Bohn et al. 2001; Melchinger et al. 2004; Schön et al. 2004). Thus, we tested our QTL results by a cross

142 validation approach (CV/G) proposed by Utz et al. (2000) to obtain a realistic picture of the
143 prospects of MAS for improving yield performance of European maize to MCB attack.
144
145 The objectives of our study were (1) to estimate the genetic correlation between yield
146 performance under infestation with MCB and resistance and agronomic traits; (2) to map and
147 characterize QTLs for yield performance under infestation, resistance and agronomic traits;
148 (3) and to determine the estimation bias of each individual QTL effect and its *p* using a cross
149 validation approach in order to know the prospects of MAS for improving yield under MCB
150 infestation and/or resistance.
151

152 Materials and methods

153 Plant material

154 A population of 146 RILs derived from the cross of the European flint inbred line EP42 and
155 the American dent inbred line A637 was developed for QTL mapping. EP42 has low
156 productivity under MCB infestation and is sensible to MCB attack while A637 shows large
157 yield under MCB infestation and is tolerant to stem and ear damage by MCB. Each F_6 RIL
158 was derived from a different F_2 plant by hand self-pollination.
159

160 Phenotypic data

161 A set of 144 RILs (two lines were discarded due to lack of seed) derived from EP42×A637
162 were evaluated at Pontevedra (42º24' N, 8º38' W, and 20 m above sea level) Spain, in 2010
163 and 2011. The parental inbred lines (EP42 and A637) and the resulting hybrid were evaluated
164 at Pontevedra in 2011 and 2012. The RILs along with some checks (parental inbreds) were
165 assayed in a 12 × 12 lattice design with two replications each year. On the other hand an

166 independent experiment was carried out in which the parental and the hybrid were assayed in
167 a randomized block design with five replications each year. The trials were hand planted and
168 each experimental plot consisted of one row spaced 0.8 m apart from the other row with 13
169 two-kernel hills spaced 0.18 m apart. Plots were overplanted and thinned, obtaining a final
170 density of ~70,000 plant ha^{-1}. The evaluations were performed under artificial infestation
171 with eggs of MCB. The eggs for inoculation were obtained at the Misión Biologica de Galicia
172 by rearing the insect as described by Eizaguirre and Albajes (1992) and Khan and Saxena
173 (1997). Five plants of each plot were infested with □ 40 MCB eggs placed between the stem
174 and the sheath of a basal leaf. Data collected were: days to anthesis (A) and days to silking
175 (S) as the days from planting to the 50 % of plants shedding pollen and showing silks,
176 respectively; ear (EH) and plant height (PH) on five representative plants as the length (in
177 cm) from the ground to the main ear and from the ground to the top of the plant, respectively;
178 stalk lodging (SL) defined as the percentage of plants in the plots with the stem broken below
179 the main ear; kernel resistance (KR), shank resistance (ShR) and cob resistance (CR) by
180 MCB larvae on the ears of the five infested plants collected at harvest, those traits were
181 measured according to a subjective visual resistance scale of 1 to 9 in which 1 indicates
182 completely damaged and 9 indicates no damage; tunnel length (TL) as mean of total length in
183 cm of stem tunnels made by borers on the five infested plants; the percentage of stalk
184 damaged by the larvae (TL/PH*100) (SD); kernel yield (Y) estimated on a plot as Mg ha^{-1} at
185 140 g H_2O kg^{-1} (infested and non-infested plants were considered); and grain moisture (GM)
186 at the harvest was measured as g of water in 100 g of kernels.
187
188 **Phenotypic data analysis**
189 The experiment of the RILs was analyzed with the SAS mixed model procedure (PROC
190 MIXED) (SAS Institute Inc 2011) considering replications, blocks within replications and

RILs as random effects. A Best linear unbiased predictor (BLUP) was obtained to estimate each line mean phenotypic value. In order to examine the shrinkage of BLUPs a second analysis was conducted in which we consider RILs as fixed effects to obtain the best linear unbiased estimator (BLUE). The resulting BLUPs and BLUEs were used to perform QTL analysis. Heritabilities ($\hat{h}^2$) across environments were estimated for each trait on a family-mean basis as described previously by Holland et al. (2003). The genetic (r_g) and phenotypic (r_p) correlations between traits were computed following Holland (2006). The experiments of the parents and their hybrid were analyzed separately using PROC GLM (SAS Institute Inc 2011) considering year, replications within years and the genotype × year interaction as random effects.

Genotypic data

DNA of one hundred forty six RILs was extracted according to Liu and Whittier (1994) with modifications. Simple sequence repeat (SSR) amplifications were performed as described by Butrón et al. (2003). SSR products were separated after amplification by electrophoresis using 1 TBE on a 6% non-denaturing acrylamide gel (approximately 250 V for 3 hours) (Wang et al. 2003). One hundred thirty polymorphic SSR were used to genotype the RILs. A linkage map was created using SSR marker data by applying the software package MAPMAKER (Lander et al. 1987). A LOD ($\log_{10}$ of the likelihood odds ratio) threshold of 2 was used to declaring significant linked two markers and a maximum distance of 50 cM was used.

QTL analysis

The QTL analysis for the 14 traits recorded was performed with 144 RILs families using the software package PlabMQTL (Utz 2012). Composite interval mapping approach (CIM) was

conducted for the QTL detection and to estimate QTL effects. According to a previously executed permutation test with 1000 random reshuffles, a LOD threshold of 2.5 was chosen to declare significant a putative QTL. The phenotypic variance explained by the QTL model was estimated by the adjusted coefficient of determination (R^2_{adj}) which accounts for the total proportion of the phenotypic variance explained by all detected QTL in the final fit. The phenotypic variance explained by an individual putative QTL *i* was calculated as:

$$R_i^2 = \frac{partR_i^2 \times R_{adj}^2}{\sum_{i=1}^{n} partR_i^2}$$

Where *n* = total of QTL *i* in the final fit and $part\ R_i^2$ = partial coefficient of determination, estimated for the *i*th QTL detected (Zhu et al. 2004). The proportion of the genotypic variance ($\hat{p}$) explained by all detected QTL in final fit was estimated from the ratio $\hat{p} = R^2_{adj}/\hat{h}^2$ and the proportion of the genetic variance explained by each individual QTL *i* was estimated as $\hat{p}_i = R_i^2/\hat{h}^2$.

Cross validation

Following Utz et al. (2000), a five-fold cross validation (CV/G) approach was employed for evaluating QTL mapping results. For each trait, CV/G was performed for the whole data set (DS) of entry BLUPs and BLUEs for the 140 RILs across environments. A total of 117 entries were used as estimation set (ES) for calibration and 29 entries were used as the test set (TS) for validation. One thousand run CV were performed in order to determine the QTL frequency and shrinkage of QTL effect estimate at the position of a QTL detected in the original data set (Melchinger et al. 2004). The proportion of the genotypic variance explained by the QTL in TS ($\hat{p}_{TS.ES}$) was calculated from the adjusted squared correlation coefficient between the phenotypic entry means observed in TS (Y_{TS}) and the predicted genotypic values

238 ($Q_{\text{TS.ES}}$) on the basis of results derived from ES, divided by the heritability of the trait under
239 study:

$$\hat{p}_{TS.ES} = \frac{R^2_{adj}(Y_{TS}, Q_{TS.ES})}{\hat{h}^2}$$

240 The magnitude of the bias of the estimation of the proportion of genotypic ($\hat{p}_i$) variances
241 explained by each individual QTL *i* due to genotypic and/or environmental samplings was
242 calculated as the difference between the averaged estimated of p obtained in ES and
243 corresponding TS ($\bar{p}_{i\,ES} - \bar{p}_{i\,TS.ES}$), and the fraction of that bias was calculated as (1-
244 $\bar{p}_{i\,TS.ES}/\bar{p}_{i\,ES}$). In the same way, we obtained the bias for the estimates of additive effect $\hat{\alpha}_i$
245 of each QTL *i* detected. The median of p_i and α_i in ES ($\tilde{p}_{i\,ES}$ and $\tilde{\alpha}_{i\,ES}$) and in the
246 corresponding TS ($\tilde{p}_{i\,TS.ES}$ and $\tilde{\alpha}_{i\,TS.ES}$), as well as the 10 and 90% quantiles of p_i and α_i for
247 ES and TS were obtained. A Grep utility (GNU 2009) was employed to extract, in each
248 CV/G run, the proportion of genotypic ($p_{i\,ES}$ and $p_{i\,TS}$) and phenotypic ($R^2_{i\,ES}$ and $R^2_{i\,TS}$)
249 variances of the ES and TS explained by each individual QTL *i* detected and also the additive
250 effects ($\hat{\alpha}_{i\,ES}$ and $\hat{\alpha}_{i\,TS}$).
251
252 The QTL × environment interaction variance was estimated with PlabMQTL software by
253 using the entry BLUPs and BLUEs from each environment and including all QTL detected
254 across environments. The mean square (MS) for genotypes obtained from the ANOVA was
255 subdivided into the variation due to regression on the QTL detected (Q) and the residual
256 variation (G:Q). Similarly the MS for the genotype × environment (E) interaction was
257 subdivide into the variation due to Q × E and the residual variation G:Q × E. The genetic
258 variance accounted by all QTL in the model was estimated by equating the MS to the
259 expected mean squares according to Bliss (1967) and Knapp (1994). The pooled error mean

square was computed as described in (Cochran and Cox 1992) $MS_e = \frac{1}{p}(\frac{MS_{e1} + MS_{e2}}{r})$. Where MS_{e1} and MS_{e2} = mean square error of the experiment at year 2010 and 2011 respectively, r = replications, and p = number of environments.

Results

Non-significant differences were found between the means of A637 and EP42 (Table 1). High heterosis has been observed for agronomical as well as for resistance traits because the hybrid F_1 significantly differed from the mid-parent for the proportion of stalk damaged, ear, shank and cob resistance, stalk lodging, yield and days to anthesis.

Genotypic variances among RILs were highly significant (P<0.01) for all agronomic traits; and for TL and SL among resistance traits (Table 1). Heritabilities were high for agronomic traits while for resistance traits ranged from values not significantly different from zero to moderate values (Table 1).

TL showed moderate to high positive genetic correlation with GM, PH and EH; and low positive correlation with A. No genetic correlation was found between ear resistance and agronomic traits (Table S1). The phenotypic correlation coefficients among KR, ShR and CR were moderate to high, but the genetic correlation coefficients were not calculated because genetic variances for these traits did not differ from zero.

The genetic map used for QTL analysis covers a length of genome of 1730.8 cM with 114 SSR markers. One hundred thirty loci were recorded on 146 RILs and 121 markers were mapped to unique positions. Seven neighbored markers among those 121 were combined by

PlabMQTL because had distances to the nearest marker smaller than 1.01 cM. No segregation distortion from the expected ratio was observed for any mapped marker loci. The 93.6% of genome had an averaged distance between consecutive markers of 20 cM.

Eighteen QTLs were mapped on six different chromosomes (1, 3, 5, 8, 9, and 10) (Table 2 and Fig. 1). For TL, the only QTL located reached the peak of LOD threshold and the maximum of the distribution of relative QTL frequency at position 48 cM on chromosome 9 (Table 2 and Fig. S1), explained the 19.8 and 6.8 % of the genotypic and phenotypic variances, respectively; the favorable allele was supplied by the inbred A637. Additive effect of QTL for TL was 0.8 cm (Table S2). Four QTLs for ear resistance (KR and ShR) were detected when BLUEs were used: one QTL for KR and three for ShR (ShR1, ShR5, and ShR8) were located on chromosomes 1, 5, and 8. They explained a substantial proportion of the genetic (26 – 63%) and phenotypic (5 – 12%) variances. The additive effect ranged from 0.3 to 0.6 in the visual scale from 1 to 9 (Table S2). For SL, a QTL located on chromosome 3 explained 5.5 and 11.5 % of the phenotypic and genotypic variances, respectively, and the allele from EP42 increased 2.7 % the SL. One significant QTL for Y was detected with high frequency between 64 and 92 cM on chromosome 5, but the maximum LOD peak was reached at 80 cM position (Fig. S2). It accounts for 15.24 % of the genotypic variance and 9.30 % of the phenotypic variance; the favorable allele came from the tolerance parent A637. Two putative QTLs for GM were detected on chromosomes 1 and 8 (GM1 and GM8), both explained a total of 30.8 and 17.9% of the genotypic and phenotypic variances, respectively. These QTLs for GM co-localized with two QTLs for days to anthesis (A1 and A8, that explained in total the 33.9 % of the genotypic variance) and also with the QTLs for ShR. The QTL for A on chromosome 8 co-localized also with other QTLs for ShR, PH, EH, and S; and they explained the 42, 7, 25, and 31% of the genotypic variances for ShR, PH, EH, and S,

309 respectively. Another QTL for S was located on chromosome 10 which explained the 9.3 %
310 of the genotypic variance.
311
312 Three QTLs on chromosomes 5, 8, and 9 were detected for PH (PH5, PH8, and PH9), they
313 explained the 32.6 % of genetic variance. The QTL PH5 was closed to the QTLs detected for
314 Y, ShR5, and KR. The QTLs PH8 and PH9 co-localized with QTLs for EH in chromosomes
315 8 and 9 and the QTL PH9 with the QTL for TL (Table 2 and Fig. 1). Therefore, there are
316 regions at chromosomes 1, 5, 8, and 9 that support QTLs for multiples traits; the allele from
317 A637 having favorable effects for TL, KR, ShR, and Y. For example, there is a region in
318 chromosome 8, that comprises from 84 to 106 cM, where QTLs for ShR, GM, A, S, PH, and
319 EH were detected, and the allele from A637 increased grain moisture, days to flowering,
320 plant and ear heights, and shank resistance to corn borer attack compared to the allele from
321 EP42 (Table S3).
322
323 The QTL × environment (QTL×E) interaction was significant (P<0.05) for SL and highly
324 significant (P<0.01) for KR, Y, GM, S, and EH (data not shown). Additive effects ($\hat{\alpha}$) and
325 genetic variances ($\hat{p}$) explained by each QTL were higher in 2011 than in 2010 for most
326 QTLs with significant QTL×E interaction (Fig. 2). Additive effects for SL and Y were highly
327 significant only in 2011.
328
329 The means and medians of $R^2_{i\,ES}$ were higher than those obtained with the whole DS except
330 for QTLs for A, S, and EH located on chromosome 8 (Table S2 and S3). There were
331 substantial reductions in the mean values of validation sets $R^2_{TS.ES}$ compared to calibration
332 sets for all traits. The mean ($\bar{p}_{ES}$) and the median ($\tilde{p}_{ES}$) proportions of genotypic variance

explained by each QTL were in good agreement for all traits (Figs. S3 and S4). The mean $\bar{p}_{ES}$ for QTLs detected ranged from 8.82 (for QTL PH5) to 66.2% (for QTL ShR8). The mean $\bar{p}_{ES}$ was higher than the $\hat{p}_{DS}$ value in most cases, except for the QTLs for A and S located on chromosome 8 (Tables S2 and S3).

Estimates of $p_{i\,TS}$ beyond theoretical boundaries (0, 100) can occur because $\hat{R}^2_{i\,adj}$ and $\hat{h}^2$ are both subjected to sampling errors (Schön et al. 2004). The mean $\bar{p}_{TS.ES}$ were substantially reduced compared to values obtained in $\bar{p}_{ES}$, especially for SL, and ranged from -1.87 (for SL) to 37.3% (for QTL ShR8) (Tables S2 and S3). Such reductions were equivalent to biases of the estimates of p and ranged from 11% (for QTL S8) to 100 % (for SL) (Tables S2 and S3). The mean $\bar{p}_{TS.ES}$ was considerably different from the median values $\tilde{p}_{TS.ES}$. Variation of 10 and 90% quantiles among TS increase in most QTLs detected compared with ES, except for the QTLs A1 and PH5 (Figs. S3 and S4). The mean $\bar{\alpha}_{i\,ES}$ and median $\tilde{\alpha}_{i\,ES}$ values were slightly different than those obtained with the whole data set $\hat{\alpha}_{DS}$ for most QTLs detected, except for Y and S8 in which the values of those parameters were the same (Tables S2 and S3).

The absolute values of the means $\bar{\alpha}_{i\,TS.ES}$ and medians $\tilde{\alpha}_{i\,TS.ES}$ obtained in TS were substantially smaller than those obtained in ES, except for QTLs for A and S located on chromosome 8. The bias of the estimation of the additive effects of QTLs detected for resistant traits ranged from 14 to 51%, while for the QTLs located for agronomic traits the bias ranged from 0 to 81 % (Tables S2 and S3). The mean values of additive effects were close to median values both in ES as in TS.

Discussion

Similarly to results by Bohn et al. (2000) and Papst et al. (2004), the heritabilities of resistance traits were lower than those of agronomic traits. In addition, genetic variance values for resistance traits were low. In a previous work, Ordas et al. (2010) found that, under infestation with MCB, the heritability of TL was higher and similar to that of Y, but those authors studied a resistant × susceptible cross, while in our study both parental inbreds were susceptible. Despite the above six QTLs for resistance traits were detected for this cross.

QTLs for resistance and agronomic traits

Most QTLs were detected when performing QTL analyses with both estimates BLUPs and BLUEs, and the associated parameters were similar for both analyses. However, QTLs for traits related to ear resistance were detected only when BLUEs were used because there was low phenotypic variability for these traits and the shrinkage of variability attained with BLUPs could mask small genetic differences. It should be stressed that a QTL with high level of occurrence has been detected for ShR although the estimate of genetic variance did not differ from zero.

In general a maximum of three QTLs were detected for each trait, this is a noticeable number of QTLs considering that both parents had similar means for all traits evaluated. The cross between EP42×A637 was chosen as the base material to develop a mapping population because EP42 did not show significant GCA effects for yield under infestation and A637 showed favorable GCA effects, although both parents did not differ for yield under infestation. Ordas et al. (2009; 2010) proposed that the low number of QTLs found in experiments with MCB is due to the aggressiveness of the insect, so most genotypes seem to

382 be susceptible, differing from experiments with European corn borer (ECB), in which the
383 number of QTLs found was larger.
384
385 The QTL for TL has been detected on chromosome 9 at the same region where other authors
386 have reported QTLs for leaf feeding and TL by corn borers. Jampatong et al. (2002) located
387 at bin 9.02 one QTL for TL ($\hat{\alpha}$ = 0.53 cm and R^2 = 10.8 %) by ECB in a set of 244 $F_{2:3}$
388 families from the cross of B73Ht×Mo47. Ordas et al. (2009) found a QTL for TL by MCB in
389 bin 9.04 with a 96.2 % occurrence in 1000 cross validation runs. Groh et al. (1998) co-located
390 in an overlapped region (bins 9.02 - 9.03) QTLs for leaf feeding damaged (LFD) by
391 Southwestern corn borer (SWCB) and by sugar cane borer (SCB) and for leaf protein
392 concentration (PC) in RILs derived from CML131×CML67. Cardinal et al. (2006) located a
393 QTL ($\hat{\alpha}$ = 0.24, 1-9 scale) on bin 9.03 for leaf blade damage (LBD) by ECB in an F_3 maize
394 population from Mo17×H99. Therefore, it is likely that genes on this region of chromosome
395 9 controlling resistance for tunnel length by corn borers could also be related with resistance
396 to leaf feeding and favourable allelic variants could be present in a wide variety of maize
397 germplasm because interesting allelic variants have been found in different materials.
398 It is known that some cell wall components are related with resistance to different corn borers
399 (Buendgen et al. 1990; Santiago et al. 2013). Several QTLs and candidate genes for cell wall
400 components have been co-localized in the region of chromosome 9 where we located QTLs
401 for resistance traits (Krakowsky et al. 2007; Truntzler et al. 2010); but the regions supporting
402 our QTLs are excessively large to propose specific candidate genes for resistance to MCB.
403 However, the genomic region at bin 9.03 could be, considered a hotspot for corn borer
404 resistance and it would be worthwhile to focus further studies on this region.
405

406 In relation to the other stem resistance trait, SL, we located a QTL for this trait in the same
407 bin (3.07) where a QTL (*bnl6.16a*) for rind penetrometer resistance (RPR) was detected by
408 Flint-Garcia et al. (2003) in a $F_{2:3}$ derived from the cross B73×Mo47). However, the possible
409 linkage between stem strength and resistance to stalk tunneling found in crosses involving
410 Reid germplasm cannot be generalized to other germplasms (Butrón et al. 2002).
411
412 We detected a QTL for Y at bin 5.03 in a neighbor region to that reported by Ordas et al.
413 (2010) who located a QTL at bin 5.05. This region on chromosome 5 have been highly
414 associated with grain yield both under stress and non-stress in several studies made by
415 Graham et al. (1997). We also found a peak of LOD threshold 2.48 value at the 126 cM
416 position on chromosome 4 near to the *umc1051* marker (bin 4.08) (data not shown), although
417 it was not declared significant. However, in this region Papst et al. (2001) detected a QTL for
418 grain yield under infestation (GYI) with ECB. So, it is likely that there are genes in this
419 region with small effect on grain yield under infestation with corn borers.
420
421 The QTLs for A and S at bin 8.05 are remarkable because were detected in the 93 and 96% of
422 the CV/G runs, respectively. These QTLs explained 26.3 and 31.6% of the σ_g^2 for A and S,
423 respectively. In addition, the biases of effect estimations obtained in CV/G for these QTLs
424 were zero. Ordas et al. (2010) also located QTL for S at the same region of chromosome 8.
425 In that study, the QTL was validated in the 92 % of the CV/G runs and the inbred EP42
426 supplied the allele with reduction effect on days to silking. In the present study, the allele
427 from EP42 reduce more than 1.8 days both traits, A and S. This QTL probably refers to the
428 mayor QTL *Vgt1* which control the transition of vegetative to reproductive phase in maize
429 and which have been previously dissected by positional cloning (Salvi et al. 2002; Salvi et al.
430 2007). On the other hand, the QTL for S located on chromosome 10 is included in a region

where other authors have previously reported genes regulating flowering time throughout photoperiod sensibility (Ducrocq et al. 2009).

QTL × E interactions

No significant G×E interaction for resistance traits was observed according with results of previous studies under infestation with MCB (Butrón et al. 1999b; Velasco et al. 2002; Ordás et al. 2010), except for KR. However, significant QTL×E interaction was observed for six agronomical traits although this interaction was of magnitude rather than of rank due to poorer experimental conditions in 2010 than in 2011.

Relationships among resistance and agronomical traits (yield is not included)

Regarding the relationship between resistance and agronomical traits, there were positive and significant genetic correlations between TL and A ($r_g = 0.40$) suggesting that late genotypes would have minor stalk resistance, this agrees with results obtained in some studies (Krakowsky et al. 2002; Ordás et al. 2010) and disagree with other studies in which a negative association between A and stem resistance traits under infestation with ECB was found (Hudon and Chiang 1991; Bohn et al. 2000; Papst et al. 2004). The relationship between flowering and resistance is complex because it depends on many factors such as the time of infestation and the material under study (Ordás et al. 2013). In our study, artificial infestation was made before flowering while contradictory results were obtained under post-flowering infestation (Hudon and Chiang 1991; Bohn et al. 2000; Papst et al. 2004). However, none of the two QTL for A was located near to the QTL detected for TL herein, although several authors have co-localized QTLs for TL by corn borers and A (Bohn et al. 2000; Krakowsky et al. 2002; 2004; Ordás et al. 2010). In addition, a selection program for earliness may negatively affect ear resistance, because QTLs for A and ShR co-localized on

chromosomes 1 and 8; but this negative effect would not be significant across all maize materials because the BS17 population was improved for earliness while ear resistance was maintained (Samayoa et al. 2012).

No QTL for TL were located near to the two QTLs for GM in this population, but the positive and significant genetic correlation between GM and TL (r_g= 0.69) obtained in our study leads us to think that there are undetected genomic regions with small additive effect on both traits. Ordas et al. (2010) co-localized QTLs for TL and GM at bin 3.05 and confirmed the positive genetic correlation (r_g = 0.61) between both traits. This means that selection for increased resistance would lead to a decrease in grain moisture.

On the other hand, QTLs for TL, PH, and EH were located at the same bin on chromosome 9. QTLs for TL and PH fell in the same marker interval (*phi065-umc1267*). The allele from EP42 significantly increased PH and EH as well as TL. The QTL for EH located at bin 9.03 herein was previously located at the same bin by Krakowsky et al. (2002) under infestation with ECB and they reported that alleles associated with increased TL were associated with increased EH. Schön et al. (1993) found a QTL for PH and another for TL by ECB on the same marker interval of chromosome 3 and the allele supplied by the same parent increased the value of both traits on 300 F_3 maize plants from B73×B52. Ordas et al. (2010) co-localized at the same region of chromosome 3 a QTL for TL by MCB and PH in a 178 RIL population obtained from a cross between European flint inbreds (EP42×EP39), and a moderate genotypic correlation (r_g = 0.51) between TL and PH was observed. Cardinal et al. (2001) also co-localized a QTL for by ECB and PH with the largest effects at bin 9.03 in a set of 200 RILs derived B73×B52. In our study, as well as in that by Ordas et al. (2010), we obtained a positive and significant genetic correlation between TL and PH (r_g = 0.66) and EH

(r_g = 0.84). These results suggest that genomic variants with additive effects for PH, EH, and TL are present in this large region (bin 9.03) of chromosome 9. Hence, we consider that it is advisable to carry out a fine mapping of this region in order to know if the same genes or linked genes are effecting plant height and tunnel length.

Yield and its relationship with resistance and other agronomic traits

Grain yield (Y) was not genetically or phenotypically associated with TL coinciding with results obtained by Bohn et al. (2000) in a F_3 maize population from D06×D408, although other authors have found a negative relationship between stalk resistance and yield (Schulz et al. 1997; Kreps et al. 1998; Butrón et al. 2012).

Relationship among agronomic traits (yield is not included)

Late anthesis was associated with an increment in EH (r_g = 0.62) and QTLs for both traits co-located on the same region of chromosome eight, as expected based on a previous study (Hallauer and Miranda 1981). However, some authors have obtained a negative, but small genetic association between A and EH (r_g = -0.36) in a 150 F_3 maize population from B73×De811 (Krakowsky et al. 2002).

QTL results supported the high and significant genetic correlation coefficients among A, S, EH and GM because QTLs with positive additive affects for A, S, and GM co-located in the same marker interval (*umc1309a-bnlg1812*) of chromosome 8 and QTLs for GM and A with positive additive effects for GM and A co-located in the same region of chromosome 1 [were linked to the same marker (*bnlg1556*)], indicating that the A637 allele increases the value for these traits.

506 **Cross validation and bias**

507 According with cross validation, three QTLs for resistance traits (TL, SL, and ShR1) and one
508 for agronomic traits (PH8) showed the highest values of estimation bias for the proportion of
509 $\hat{\sigma}_g^2$ (bias ≥ 90%) explained by each QTL. That is, the genotypic variance explained by those
510 QTLs were overestimated in a 91 and 100 %, and the estimation bias for the QTL effect (α)
511 ranged from 51and 81%. In the case of resistance traits, these results were expected, since
512 these traits showed the lowest heritability values and the estimates of heritabilities were used
513 for calculation of $p_{i\,ES}$ and $p_{i\,TS}$. On the other hand, the QTLs that showed the largest levels
514 of occurrence were more accurate, since they showed the lowest bias in the estimation of $\hat{p}$
515 and $\hat{\alpha}$. Overall, the $\hat{p}$ and $\hat{\alpha}$ for each QTL detected was notable for most traits evaluated but
516 these parameters were overestimated due to the small sample size population. Moreover, the
517 variation of the magnitude of bias of estimates of p and α observed among traits was due to
518 heritability differences as well as to differences on trait measuring complexity.
519
520 Schön et al (2004) concluded that CV yields best results when a minimum sample size (N
521 >200) and a minimum number of test environments (E > 4) are available for analysis, and the
522 large bias showed in our study for most traits corroborated these findings.
523
524 The median ($\tilde{p}_{i\,ES}$) proportions of genotypic variances explained by the QTL were higher
525 than those obtained in whole data set ($\hat{p}_{DS}$) and this inflation in $\tilde{p}_{i\,ES}$ is reflected in the
526 magnitude of the bias of p. Schön et al. (2004) got similar results when the sample size was
527 small (N<200). In addition, the large variation of the 10 and 90% quantiles of p observed in
528 TS compared with those obtained in ES, as well as the estimates of quantiles of p (10 and
529 90%) outside of theoretical boundaries (0, 100) obtained in TS herein, corroborates the
530 results obtained by Schön et al. (2004) for grain yield, who estimated quantiles of p (12.5

and 87.5 %) larger in TS and outside of theoretical boundaries when the population size was low (N=122).

Conclusions

Although QTL mapping was done in a bi-parental population involving inbreds with similar performance, significant QTLs were found for most traits. The QTLs found for TL, KR, and ShR support the existence of genes with small effect on resistance to MCB in materials classified as susceptible because the mapping population was developed from the cross between two susceptible inbreds. Based on our results and in previous results, the QTL for tunnel length by MCB attack located in chromosome 9 could be related with genes controlling stem and leaf resistance under infestation with different corn borers and could also be tightly linked with genes affecting plant and ear heights.

The region of chromosome five where we located a QTL for yield under infestation with MCB may play an important role in maize tolerance to this pest in this RIL population.

Judging the large bias of estimates of p and QTL effects for most traits we concluded that the markers associated to the located QTLs are not suitable for using in MAS. Nevertheless, our QTL for TL support the existence of genes with small effect on resistance to MCB in materials classified as susceptible because the mapping population was developed from the cross between two susceptible inbreds.

According with these results and our previous experience we considered it is more appropriate to select for grain yield under infestation with MCB instead of selecting for

555 resistance traits because resistance to MCB could have unfavorable associations with
556 agronomical traits.
557

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562

563 References

564 Avantaggiato G, Quaranta F, Desiderio E, Visconti A (2002) Fumonisin contamination of
565 maize hybrids visibly damaged by *Sesamia*. J Sci Food Agric 83:13-18
566 Barry D, Darrah LL (1988) Nonpreference resistance to European corn borer (Lepidoptera:
567 Pyralidae) in the Mo-2 ECB maize cultivar. J Kansas Entomol Soc 61 (1):72-75
568 Beavis WD (1998) QTL analyses: power, precision, and accuracy. Molecular dissection of
569 complex traits. CRC Press, Boca Raton, FL
570 Bliss CI (1967) Statistics in Biology, vol 1. McGraw-Hill, New York
571 Bohn M, Groh S, Khairallah MM, Hoisington DA, Utz HF, Melchinger AE (2001) Re-
572 evaluation of the prospects of marker-assisted selection for improving insect
573 resistance against *Diatraea* spp. in tropical maize by cross validation and independent
574 validation. Theor Appl Genet 103:1059-1067
575 Bohn M, Schulz B, Kreps R, Klein D, Melchinger AE (2000) QTL mapping for resistance
576 against the European corn borer (*Ostrinia nubilalis* H.) in early maturing European
577 dent germplasm. Theor Appl Genet 101:907-917

578 Buendgen MR, Coors JG, Grombacher AW, Russell WA (1990) European corn borer
579 resistance and cell wall composition of three maize populations. Crop Sci 30 (3):505-
580 510
581 Butrón A, Malvar R, Revilla P, Soengas P, Ordás A, Geiger H (2002) Rind puncture
582 resistance in maize: inheritance and relationship with resistance to pink stem borer
583 attack. Plant Breed 121 (5):378-382
584 Butrón A, Malvar RA, Cartea ME, Ordas A, Velasco P (1999a) Resistance of maize imbreds
585 to pink stem borer. Crop Sci 39:102-107
586 Butrón A, Malvar RA, Velasco P, Revilla P, Ordas A (1998) Defense mechanisms of maize
587 against pink stem borer. Crop Sci 38:1159-1163
588 Butrón A, Romay MC, Peña-Asin J, Alvarez A, Malvar RA (2012) Genetic Relationship
589 Between Maize Resistance to Corn Borer Attack and Yield. Crop Sci 52 (3):1176-
590 1180
591 Butrón A, Sandoya G, Revilla P, Malvar RA (1999b) Combining abilities for maize stem
592 antibiosis, yield loss, and yield under infestation and non infestation with pink stem
593 borer. Crop Sci 39 (3):691-696
594 Butrón A, Sandoya G, Santiago R, Ordas A, Rial A, Malvar RA (2006a) Searching for new
595 sources of pink stem borer resistance in maize (*Zea mays* L). Euphytica 53 (7):1455-
596 1462
597 Butrón A, Santiago R, Mansilla JP, Pintos-Varela C, Ordás A, Malvar RA (2006b) Maize
598 (Zea mays L.) genetic factors for preventing Fumonisin contamination. J Agric Food
599 Chem 54:6113-6117
600 Butrón A, Tarrío R, Revilla P, Malvar RA, Ordás A (2003) Molecular evaluation of two
601 methods for developing maize synthetic varieties. Mol Breeding 12 (4):329-333

602 Cardinal AJ, Lee M, Guthrie WD, Bing J, Austin DF, Veldboom LR, Senior ML (2006)
603 Mapping of factors for resistance to leaf-blade feeding by European corn borer
604 (Ostrinia nubilalis) in maize. Maydica 51:93-102
605 Cardinal AJ, Lee M, Sharopova N, Woodman WL, Long MJ (2001) Genetic mapping and
606 Analysis of quantitative trait loci for resistance to stalk tunneling by European corn
607 borer in maize. Crop Sci 41:835-845
608 Cartea ME, Malvar RA, Vales I, Butrón A, Ordas A (2001) Inheritance of Resistance to Ear
609 Damage Caused by Sesamia nonagrioides (Lepidoptera: Noctuidae) in Maize. J Econ
610 Entomol 94 (1):277-283
611 Cochran WG, Cox GM (1992) Experimental design. 2nd edn. Wiley, New York
612 Cordero A, Malvar RA, Butrón A, Revilla P, Velasco P, Ordás A (1998) Population
613 dynamics and life-cycle of corn borers in South Atlantic European Coast. Maydica
614 43:5-12
615 Ducrocq S, Giauffret C, Madur D, Combes V, Dumas F, Jouanne S, Coubriche D, Jamin P,
616 Moreau L, Charcosset A (2009) Fine mapping and haplotype structure analysis of a
617 major flowering time quantitative trait locus on maize chromosome 10. Genetics 183
618 (4):1555-1563
619 Eizaguirre M, Albajes R (1992) Diapause induction in the stem corn borer *Sesamia*
620 *nonagrioides* (Lepidoptera, Noctuidae). . Entomol Gen 17:277-283
621 Flint-Garcia SA, Jampatong C, Darrah LL, McMullen MD (2003) Quantitative trait locus
622 analysis of stalk strength in four maize populations. Crop Sci 43 (1):13-22
623 GNU (2009) GNU Grep: Print lines matching a pattern. 2.5.4 edn. Free Software Fundation,
624 Inc, Boston, MA.
625 Graham GI, Wolff DW, Stuber CW (1997) Characterization of a yield quantitative trait locus
626 on chromosome five of maize by fine mapping. Crop Sci 37 (5):1601-1610

627 Groh S, González-de-León D, Khairallah MM, Jiang C, Bergvinson D, Bohn M, Hoisington
628 DA, Melchinger AE (1998) QTL mapping in tropical maize III. Genomic regions for
629 resistance to *Diatraea spp*. and associated traits in two RIL populations. Crop Sci
630 1998 (38):1062-1072
631 Hallauer AR, Miranda J (1981) Quantitative genetics in maize breeding. Iowa State Univ
632 Press, Ames:869
633 Holland JB (2006) Estimating genotypic correlations and their standard errors using
634 multivariate restricted maximum likelihood estimation with SAS Proc MIXED. Crop
635 Sci 46:642-656
636 Holland JB, Nyquist WE, Cervantes-Martínez CT (2003) Estimated an interpreting
637 heritability for plant breeding: an update. Plant Breed Rev 22:9-112
638 Hudon M, Chiang MS (1991) Evaluation of resistance of maize germplasm to the univoltine
639 European corn borer *Ostrinia nubilalis* (Hübner) and relationship with maize maturity
640 in Quebec. Maydica 36:69-74
641 Jampatong C, McMullen MD, Barry D, Darrah LL, Byrne PF, Kross H (2002) Quantitative
642 trait loci for first- and second-generation European corn borer resistance derived from
643 the maize inbred Mo47. Crop Sci 42:584-593
644 Khan ZR, Saxena RC (1997) Use of surrogate stem for eliciting ovipositional response of
645 *Busseola fusca* (Lepidoptera: Noctuidae). J Econ Entomol 90:1426-1429
646 Knapp SJ (1994) Mapping quantitative trait loci. In: Phillips RL, Vasil IK (eds) DNA-Based
647 markers in plants, vol 1. Kluwer Academic Publishers Dordrecht, p 384
648 Krakowsky MD, Brinkman MJ, Woodman WL, Lee M (2002) Genetic components of
649 resistance to stalk tunneling by the European corn borer in maize. Crop Sci 42:1309-
650 1315

651 Krakowsky MD, Lee M, Holland JB (2007) Genotypic correlation and multivariate QTL
652 analyses for cell wall components and resistance to stalk tunneling by the European
653 corn borer in maize. Crop Sci 47 (2):485-488
654 Krakowsky MD, Lee M, Woodman WL, Long MJ, Sharopova N (2004) QTL mapping of
655 resistance to stalk tunneling by the European corn borer in RILs of maize population
656 B73×De811. Crop Sci 44:274-282
657 Kreps R, Gumber R, Schulz B, Klein D, Melchinger A (1998) Genetic variation in testcrosses
658 of European maize inbreds for resistance to the European corn borer and relations to
659 line *per se* performance. Plant Breed 117 (4):319-327
660 Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987)
661 MAPMAKER: An interactive computeer package for constructing primary genetic
662 linkage maps of experimental and natural populations. Genomics 1:174-181
663 Liu YG, Whittier RF (1994) Rapid preparation of megabase plant DNA from nuclei in
664 agarose plugs and microbeads. Nucleic Acids Res 22 (11):2168
665 Malvar RA, Cartea ME, Revilla P, Ordás A, Alvarez A, Mansilla JP (1993) Sources of
666 resistance to pink stem borer and European corn borer in maize. Maydica 38:313-319
667 Meihls LN, Kaur H, Jander G (2012) Natural variation in maize defense against insect
668 herbivores. Cold Spring Harb Symp Quant Biol 77:1-15.
669 doi:10.1101/sqb.2012.77.014662
670 Melchinger AE, Kreps R, Späth R, Klein D, Schulz B (1998) Evaluation of early-maturing
671 European maize inbreds for resistance to the European corn borer. . Euphytica 99
672 (2):115-125
673 Melchinger AE, Utz HF, Schön CC (2004) QTL analyses of complex traits with cross
674 validation, bootstrapping and other viometric methods. Euphytica 137:1-11

675 Niks RE, Ellis PR, Parlevliet JE (1993) Resistance to parasites. In: Hayward MD, Bosemark
676 NO, Romagosa I (eds) Plant breeding: Principles and prospects Chapman & Hall,
677 London, pp 422-447
678 Ordás B, Alvarez A, Revilla P, Butrón A, Malvar RA (2013) Relationship between time to
679 flowering and stalk and ear damage by second generation corn borers. J Econ
680 Entomol 106 (3):1234-1239
681 Ordas B, Butron A, Soengas P, Ordas A, Malvar RA (2002) Antibiosis of the pith maize to
682 *Sesamia nonagrioides* (Lepidoptera: Noctuidae). J Econ Entomol 95 (5):1044-1048
683 Ordás B, Malvar RA, Santiago R, Butrón A (2010) QTL mapping for Mediterranean corn
684 borer resistance in European flint germplasm using recombinant inbred lines. BMC
685 Genomics 11 (174):1-10
686 Ordás B, Malvar RA, Santiago R, Sandoya G, Romay MC, Butrón A (2009) Mapping of
687 QTL for resistance to the Mediterranean corn borer attack using the intermated
688 B73×Mo17 (IBM) population in maize. Theor Appl Genet 119:1451-1459
689 Papst C, Bohn M, Utz HF, Melchinger AE, Klein D, Eder J (2004) QTL mapping for
690 European corn borer resistance (*Ostrinia nubilalis* Hb.), agronomic and forage quality
691 traits of testcross progenies in early- maturing European maize (*Zea mays* L.)
692 germplasm. Theor Appl Genet 108:1545-1554
693 Papst C, Melchinger AE, Eder J, Schulz B, Klein D, Bohn M (2001) QTL mapping for
694 resistance to European corn borer (*Ostrinia nubilalis* HB.) in early maturing European
695 dent maize (*Zea mays* L.) germplasm and comparison of genomic regions for
696 resistance across two populations of F_3 families. Maydica 46:195-205
697 Revilla P, Butrón A, Malvar RA, Ordas A (1999) Relationship among kernel weight, early
698 vigor, and growth in maize. Crop Sci 39 (3):654-658

699 Salvi S, Sponza G, Morgante M, Tomes D, Niu X, Fengler KA, Meeley R, Ananiev EV,
700 Svitashev S, Bruggemann E (2007) Conserved noncoding genomic sequences
701 associated with a flowering-time quantitative trait locus in maize. Proc Natl Acad Sci
702 U S A 104 (27):11376-11381
703 Salvi S, Tuberosa R, Chiapparino E, Maccaferri M, Veillet S, van Beuningen L, Isaac P,
704 Edwards K, Phillips RL (2002) Toward positional cloning of *Vgt1*, a QTL controlling
705 the transition from the vegetative to the reproductive phase in maize. Plant Mol Biol
706 48 (5-6):601-613
707 Samayoa LF, Butrón A, Revilla P, Alvarez A, Malvar RA (2012) Five Cycles of Mass
708 Selection for Earliness and Ear Appearance under Corn Borer Infestation in the Maize
709 Synthetic BS17. Crop Sci 52 (6):2432-2437. doi:10.2135/cropsci2011.12.0651
710 Sandoya G, Butrón A, Alvarez A, Ordás A, Malvar RA (2008) Direct response of a maize
711 synthetic to recurrent selection for resistance to stem borers. Crop Sci 48 (1):113-118
712 Santiago R, Barros-Rios J, Malvar RA (2013) Impact of Cell Wall Composition on Maize
713 Resistance to Pests and Diseases. Int J Mol Sci 14 (4):6960-6980
714 SAS Institute Inc (2011) SAS 9.3 Guide to software updates. Cary, NC: SAS Institute Inc.
715 Schön CC, Lee M, Melchinger AE, Guthrie WD, Woodman WL (1993) Mapping and
716 characterization of quantitative trait loci affecting resistancie against second-
717 generation European corn borer in maize with the aid of RFLPs. Heredity 70:648-659
718 Schön CC, Utz HF, Groh S, Truberg B, Openshaw S, Melchinger AE (2004) Quantitative
719 trait locus mapping based on resampling in a vast maize testcross experiment and its
720 relevance to quantitative genetics for complex traits. Genetics 167:485-498
721 Schulz B, Kreps R, Klein D, Gumber R, Melchingeru A (1997) Genetic variation among
722 European maize inbreds for resistance to the European corn borer and relation to
723 agronomic traits. Plant Breed 116 (5):415-422

724 Truntzler M, Barrière Y, Sawkins MC, Lespinasse D, Betran J, Charcosset A, Moreau L
725 (2010) Meta-analysis of QTL involved in silage quality of maize and comparison with
726 the position of candidate genes. Theor Appl Genet 121 (8):1465-1482
727 Utz HF (2012) PlabMQTL - Software for meta-QTL analysis with composite interval
728 mapping. Version 0.9. Institute of Plant Breeding, Seed Science, and Population
729 Genetics, University of Hohenheim.
730 Utz HF, Melchinger AE (1994) Comparison of different approaches to interval mapping of
731 quantitative trait loci. Paper presented at the Biometrics in plant breeding:
732 applications of molecular markers. Proc 9th Meeting of the EUCARPIA Selection
733 Biometrics in Plant Breeding, Wageningen, The Netherlands, 6-8 July
734 Utz HF, Melchinger AE, Schön CC (2000) Bias and sampling error of the estimated
735 proportion of genotypic variance explained by quantitative trait loci determined from
736 experimental data in maize using cross validation and validation with independent
737 samples. Genetics 154:1839-1849
738 Velasco P, Revilla P, Butrón A, Ordás B, Ordás A, Malvar RA (2002) Ear damage of sweet
739 corn inbreds and their hybrids under multiple corn borer infestation. Crop Sci 42
740 (3):724-729
741 Velasco P, Revilla P, Monetti L, Butrón A, Ordás A, Malvar RA (2007) Corn borers
742 (Lepidoptera: Noctuidae; Crambidae) northwestern Spain: population dinamics and
743 distribution. Maydica 52:195-203
744 Visconti A, Marasas WFO, Miller JD, Riley R (1999) Mycotoxins of growing interest:
745 Fumonisins. . Paper presented at the The Joint FAO/WHO/UNEP International
746 Conference on Mycotoxins, Tunis,

747 Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low-cost, high-
748 throughput polyacrylamide gel electrophoresis system for genotyping with
749 microsatellite DNA markers. Crop Sci 43 (5):1828-1832
750 Zhu S, Rossnagel BG, H.F. K (2004) Genetic analysis of quantitative trait loci for groat
751 protein and oil content in oat. Crop Sci 44:254-260

752

753 Figures

754 **Fig. 1 Molecular linkage map of maize based on 121 SSR marker loci and positions of**
755 **QTL detected on 144 RILs derived from A637×EP42.**

756 Only linkage groups where QTLs were detected are shown. The black bars represent the
757 QTLs for resistance and agronomic traits, respectively. TL = tunnel length, KR = kernel
758 resistance, ShR = shank resistance, SL = stalk lodging, Y = yield, GM = grain moisture, A =
759 anthesis, S = silking, PH = plant height, EH = ear height.

760

761 **Fig. 2 Individual QTLs with significant QTL × environment interaction estimated in**
762 **140 RIL derived from A637×EP42.** Proportion of genotypic variance ($\hat{p}$) explained by each
763 individual QTL estimated in each year (2010 and 2011). Additive effects of individual QTL
764 ($\hat{\alpha}$) in different years (** means significantly different from zero at 0.01 probability level).
765 The effects are given for the next traits: KR = kernel resistance (subjective visual scale of 1 to
766 9 in which 1 indicates completely damaged and 9 indicates no damage by the larvae) SL =
767 stalk lodging (%), Y = yield (Mg ha^{-1}), GM = grain moisture (%), S = days to silking and EH
768 = ear height (cm).

Tables

Table 1 Least square means ± standard errors (RILs ± SE), heritabilities (h^2), and genetic variances (σ_G) of RILs. Means of the parental inbreds (A637 and EP42) of the RIL population, their hybrid (A637×EP42) and mid-parent are also shown

	Resistance traits						Agronomic traits					
	Tunnel length	Stalk damaged	Kernel resistance	Shank resistance	Cob resistance	Stalk lodging	Yield	Grain Moisture[a]	Anthesis	Silking[a]	Plant height[a]	Ear height[a]
	(cm)	(%)	(1-9)[b]	(1-9)[b]	(1-9)[b]	(%)	(Mg ha^{-1})	(%)	(days)	(days)	(cm)	(cm)
A637	(43.27[a])[c]	26.70[a]	7.46[b]	4.91[b]	6.91[b]	48.20[a]	2.86[b]	20.59[a]	71.60[a]	74.20[a]	165.90[a]	65.30[a]
EP42	49.47[a]	28.60[a]	7.44[b]	5.04[b]	7.71[ab]	35.37[a]	2.69[b]	18.03[a]	71.00[a]	76.20[a]	172.50[a]	69.20[a]
F_1	44.68[a]	19.58[b]	8.73[a]	8.58[a]	8.80[a]	10.55[b]	13.39[a]	18.72[a]	65.50[b]	66.20[a]	227.90[a]	104.40[a]
LSD (α = 0.05)	12.85	7.0	0.80	1.72	1.50	23.55	1.07	5.61	1.24	14.21	69.98	55.63
$\bar{P}$[d]	46.37	27.65	7.45	4.98	7.31	41.79	2.77	19.31	71.30	75.20	169.20	67.25
LSD for mid-parent heterosis (α = 0.05)	11.13	6.10	0.70	1.49	1.30	20.39	0.93	4.86	1.07	12.31	60.60	48.18
RILs ± SE	36.96	24.84	7.38	6.19	7.18	27.62	2.43	19.76	67.47	70.70	153.65	61.66
	±0.61	±0.38	±0.06	±0.09	±0.07	±1.04	±0.06	±0.14	±0.18	±0.19	±0.94	±0.64
σ_G	24.36**	4×10^{-4}[NS]	0.10[NS]	0.20[NS]	0.15[NS]	132.0**	0.52**	13.43**	13.51**	13.05**	294.1**	119.9**
h^2	0.34	0.20[NS]	0.20[NS]	0.19[NS]	0.19[NS]	0.48	0.61	0.58	0.91	0.84	0.83	0.76

Genotypic variances **(σ_G)** and heritabilities **(h^2)** for each trait estimated following Holland et al. (2006) and Holland et al (2003), respectively.

[a] No differences between F_1 and its parents was found because of the G×E interaction was highly significant.

[b] Kernel, shank and cob resistance were scored on a subjective visual scale of 1 to 9 in which 1 indicates completely damaged and 9 indicate no damaged by the larvae.

[c] Mean values with different letter were significantly different according to LSD (α = 0.05).

[d] $\bar{P}$ = Mean of the two parents A637 and EP42 (Mid-parent)

** Significant at the 0.01 probability level, [NS], non-significant.

Table 2 Location summary of QTLs mapped in the RIL population derived from EP42×A637 under MCB infestation

Trait	QTL name	Bin	Position	Confidence Interval (cM)	LOD	Flanking markers	Occurrence in cross validation (%)
Tunnel length	TL	9.03[a]	48	40-54	3.40	*phi065-umc1267*	48.3
Kernel resistance	KR	5.04/05[c]	106	102-124	4.46	*umc1221-umc1822*	48.5
Shank resistance	ShR1	1.07[c]	168	160-172	4.73	*bnlg1556-umc1147*	22.3
	ShR5	5.03[c]	92	84-102	6.42	*umc1692-umc1221*	91.4
	ShR8	8.04[c]	88	84-94	4.98	*umc1858-umc1309a*	48.9
Stalk lodging	SL	3.07[a]	162	146-166	2.50	*bnlg1449-umc1148*	45.4
Yield	Y	5.03[b]	80	64-92	3.95	*umc1692-umc1221*	72.7
Grain moisture	GM1	1.07[a]	138	126-148	2.80	*umc1335-bnlg1556*	74.2
	GM8	8.05[b]	90	86-94	4.83	*umc1309a-bnlg1812*	89.1
Anthesis	A1	1.07[a]	168	162-172	3.67	*bnlg1556-umc1147*	45.4
	A8	8.05[a]	90	86-92	10.81	*umc1309a-bnlg1812*	96.0
Silking	S8	8.05[a]	90	86-92	10.51	*umc1309a-bnlg1812*	92.9
	S10	10.02[b]	36	30-54	4.26	*umc1152-mmc0501*	56.3
Plant height	PH5	5.04[a]	102	90-108	3.02	*umc1692-umc1221*	23.8
	PH8	8.05[c]	96	88-110	3.29	*bnlg1812-bnlg240*	23.0
	PH9	9.03[a]	54	50-62	7.6	*phi065-umc1267*	89.0
Ear height	EH8	8.05[a]	98	88-106	9.02	*bnlg1812-bnlg240*	54.1
	EH9	9.03[b]	64	54-70	3.08	*umc1267-umc1492*	62.1

[a] Detected using both BLUPs and BLUEs

[b] Detected only with BLUPs

[c] Detected only with BLUEs.

Supplementary information

Fig. S1 Frequency and effect of QTL for tunnel length. (Bottom) Frequency distribution of QTL for tunnel length at 2 cM intervals on chromosome 9 derived from 1000 cross validation runs (CV/G). The solid indicates the LOD curve determined in the whole data set (DS) with composite interval mapping (CIM). The horizontal line indicates the LOD threshold of 2.5 to declare a putative QTL significant. The triangle denotes the position of the QTL, and the vertical lines the confidential interval. (Top) Mean of additive effects of putative QTLs detected at the respective position on the chromosome from estimation set (ES) and test set (TS) of CV/G run.

Fig. S2 Frequency and effect of QTL for yield. (Bottom) Frequency distribution of QTL for yield at 2 cM intervals on chromosome 5 derived from 1000 cross validation runs (CV/G). The solid indicates the LOD curve determined in the whole data set (DS) with composite interval mapping (CIM). The horizontal line indicates the LOD threshold of 2.5 to declare a putative QTL significantly. The triangle denotes the position of the QTL, and the vertical lines the confidential interval. (Top) Mean of additive effects of putative QTLs detected at the respective position on the chromosome from estimation set (ES) and test set (TS) of CV/G run.

Fig. S3 Proportion of genetic variance for resistance traits explained by each QTL in 1000 cross validation runs (CV/G). Means and medians of the proportion of genetic variance (p) for each trait explained by each QTL and 10 and 90% quantiles for p across

829 estimation (ES) and validation (TS) sets of CV/G. TL = QTL for tunnel length, KR = QTL
830 for kernel resistance, and ShR1, 5 and 8 = QTLs on chromosomes 1, 5 and 8 for shank
831 resistance.

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833 **Fig. S4 Proportion of genetic variance for agronomic traits explained by each QTL in**
834 **1000 cross validation runs (CV/G).** Means and medians of the proportion of genetic
835 variance (p) for each trait explained by each QTL and 10 and 90% quantiles for p across
836 estimation (ES) and validation (TS) sets of CV/G. SL = QTL for stalk lodging, GM1 and
837 GM8 = QTLs on chromosomes 1 and 8 for grain moisture, A1 and A8 = QTLs on
838 chromosomes 1 and 8 for days to anthesis, S8 and S10 = QTLs on chromosomes 8 and 10 for
839 days to silking, PH5, PH8, and PH9 = QTLs on chromosomes 5, 8 and 9 for plant height, and
840 EH8 and EH9 = QTLs on chromosomes 8 and 9 for ear height.

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Table S1 Genetic and phenotypic correlation coefficients among evaluated traits in the maize RIL population derived from A637×EP42

	Resistant traits					Agronomic traits					
	TL	KR	ShR	CR	SL	Y	GM	A	S	PH	EH
TL					0.22	0.32	0.69^{+}	0.40^{+}	0.31	0.66^{+}	0.84^{+}
KR	-0.27^{+}				0.22						
ShR	-0.37^{+}	0.65^{+}			0.37						
CR	-0.32^{+}	0.85^{+}	0.67^{+}		0.37						
SL	0.06	-0.05	-0.04^{+}	-0.04		0.21	-0.36^{+}	-0.09	-0.18	0.02	0.21
RL	0.02	0.13^{+}	0.12^{+}	0.11^{+}	-0.07	0.27	-0.05	0.32	0.25	0.98	0.76
Y	0	0.23^{+}	0.15^{+}	0.19^{+}	0.10^{+}		-0.04	0.14	-0.12	0.22	0.39^{+}
GM	0.07	0.02	0.11^{+}	0.01	-0.07	-0.14^{+}		0.80^{+}	0.92^{+}	0.41^{+}	0.60^{+}
A	0.10^{+}	0.11^{+}	0.21^{+}	0.13^{+}	-0.05	-0.08	0.46^{+}		0.90^{+}	0.26^{+}	0.62^{+}
S	0.06	0.01	0.12^{+}	0.01	-0.17^{+}	-0.30^{+}	0.51^{+}	0.80^{+}		0.28^{+}	0.47^{+}
PH	0.30^{+}	0.21^{+}	0.17^{+}	0.16^{+}	0.04	0.40^{+}	0.10	0.07	0		0.77^{+}
EH	0.39^{+}	0.15^{+}	0.17^{+}	0.12^{+}	0.05	0.36^{+}	0.15^{+}	0.35^{+}	0.22^{+}	0.76^{+}	

TL, tunnel length; KR, kernel resistance; ShR, shank resistance; CR, cob resistance, SL, stalk resistance; Y, yield; GM, grain moisture; A, anthesis; S, silking; PH, plant height; and EH, ear height.

The phenotypic (r_p) correlation coefficients are shown below the diagonal while the genetic (r_g) correlation coefficients are shown above the diagonal. $^{+}$ Genetic and phenotypic correlation exceeded twice its standard error. Genetic correlation coefficients were not estimated for characters with genetic variance estimations not significantly different from zero.

Table S2 Estimations of the proportion of genetic ($\hat{p}$) and phenotypic (R^2_{adj}) variances for resistance traits explained by each QTL, and their additive effects in three different sets (DS, whole data; CV_{ES}, estimation, and CV_{TS}, validation sets). The biases of estimations were calculated by cross validation

		Tunnel length	Stalk lodging	Kernel resistance	Shank resistance		
	Chromosome	9	3	5	1	5	8
R^2_{adj}	DS	6.80	5.50	9.40	4.89	11.97	8.0
	CV_{ES}	8.16/8.0[a]	7.7/7.5	9.93/9.84	6.70/6.57	12.58/12.41	8.46/8.18
	CV_{TS}	0.74/-0.75	-0.90/-1.93	3.87/1.97	0.65/0.21	7.09/5.74	3.07/2.04
$\hat{p}$	DS	19.76	11.50	47.01	25.73	62.99	42.11
	CV_{ES}	24.01/23.6	16.04/15.6	49.66/49.18	35.29/34.57	66.19/65.29	44.52/43.07
	CV_{TS}	2.17/-2.2	-1.87/-4.0	19.36/9.85	3.41/1.12	37.34/30.20	16.17/10.75
	Bias[b]	21.84(0.91)	17.91(1.0)	30.30(0.60)	31.88(0.90)	28.85(0.44)	28.35(0.64)
$\hat{\alpha}$[c]	DS	-0.75**	-2.67**	0.29**	0.29**	0.60**	0.38**
	CV_{ES}	-0.84/-0.83	-3.12/-3.1	0.31/0.31	0.37/0.40	0.59/0.59	0.40/0.40
	CV_{TS}	-0.41/0.41	-0.84/-0.9	0.22/0.23	0.07/0.08	0.51/0.51	0.25/0.28
	Bias	-0.43(0.51)	-2.28(0.73)	0.09(0.29)	0.30(0.81)	0.08(0.14)	0.15(0.38)

[a] The values before the slash (/) corresponds to the mean ($\bar{R}^2_{i\,adj}$, $\bar{p}_i$, and $\bar{\alpha}_i$) and the values after the slash correspond to the median ($\tilde{R}^2_{i\,adj}$, $\tilde{p}_i$, and $\tilde{\alpha}_i$)of the parameter.

[b] Bias of the estimation of p and $\hat{\alpha}$ calculated as the difference between $\bar{p}_{ES}$ - $\bar{p}_{TS.ES}$ and $\bar{\alpha}_{ES}$ - $\bar{\alpha}_{TS.ES}$, the values in parentheses denote the fraction of the bias of p and $\hat{\alpha}$ estimated by 1-($\bar{p}_{TS.ES}/\bar{p}_{ES}$) and 1-($\bar{\alpha}_{TS.ES}/\bar{\alpha}_{ES}$), respectively.

[c] Additive effect of each trait are given as follow: tunnel length (cm) and kernel and shank resistance (subjective visual scale of 1 to 9 in which 1 indicates completely damaged and 9 indicate no damaged by the larvae).

** Significant at the 0.01 probability level.

Table S3 Estimations of the proportion of genetic ($\hat{p}$) and phenotypic (R^2_{adj}) variances for agronomic traits explained by each QTL, and their additive effects in three different sets (DS, whole data; CV_{ES}, estimation, and CV_{TS}, validation sets). The biases of estimations were calculated by cross validation

		Yield	Grain moisture		Anthesis		Silking		Plant height			Ear height	
	Chromosome	5	1	8	1	8	8	10	5	8	9	8	9
R^2_{adj}	DS	9.30	8.41	9.48	6.97	23.92	26.51	7.29	5.75	5.67	15.65	19.21	9.32
	CV_{ES}	10.0/9.8[a]	9.0/8.8	9.76/9.46	8.14/7.92	23.54/23.31	26.14/25.92	8.92/8.75	7.32/7.32	9.02/8.78	16.93/16.93	20.03/19.4	9.59/9.36
	CV_{TS}	4.26/2.72	4.9/3.7	6.65/5.17	1.74/0.88	20.3/19.3	23.3/23.0	3.94/3.03	0.91/0.39	0.66/0.14	12.68/11.31	15.21/13.71	3.25/2.36
$\hat{p}$	DS	15.24	14.48	16.35	7.65	26.29	31.55	9.27	6.93	6.83	18.88	25.27	12.28
	CV_{ES}	16.38/16.1	15.67/15.31	16.83/16.31	8.94/8.71	25.87/25.62	31.1/30.9	10.6/10.4	8.8/ 8,8	10.87/10.58	20.39/20.0	26.36/25.58	12.61/12.32
	CV_{TS}	6.98/4.5	8.52/6.37	11.46/8.91	1.91/0.97	22.3/21.22	27.7/27.4	4.70/3.61	1.1/0.5	0.79/0.17	15.27/13.6	20.01/18.04	4.28/3.10
	Bias[b]	9.40(0.57)	7.15(0.46)	5.37(0.32)	7.03(0.79)	3.55(0.13)	3.4(0.11)	5.9(0.56)	7.72(0.87)	10.08(0.93)	5.12(0.25)	6.35(0.24)	8.33(0.66)
$\hat{\alpha}$[c]	DS	0.24**	0.61**	0.65**	1.37**	1.93**	1.81**	0.90**	-4.01**	5.47**	-7.27**	5.93**	-4.14**
	CV_{ES}	0.24/0.24	0.65/0.65	0.66/0.65	1.42/1.43	1.86/1.86	1.8/1.8	1.06/1.06	-4.71/-4.7	6.49/6.32	-7.63/-7.64	5.53/5.45	-3.62/-3.29
	CV_{TS}	0.17/0.17	0.49/0.52	0.57/0.59	0.6/0.62	1.86/1.86	1.8/1.8	0.62/0.66	-1.02/-1.12	1.21/1.15	-7.17/7.22	4.98/4.91	-1.96/-1.97
	Bias	0.07(0.29)	0.16(0.24)	0.09(0.13)	0.82(0.58)	0(0.0)	0.0(0.0)	0.44(0.42)	-3.69(0.78)	5.28(0.81)	-0.46(0.06)	1.05(0.10)	-1.66(0.46)

[a] The values before the slash (/) corresponds to the mean ($\bar{R}^2_{i\,adj}$, $\bar{p}_i$, and $\bar{\alpha}_i$) and the values after the slash correspond to the median ($\tilde{R}^2_{i\,adj}$, $\tilde{p}_i$, and $\tilde{\alpha}_i$) of the parameter.

[b] Bias of the estimation of p and $\hat{\alpha}$ calculated as the difference between $\bar{p}_{ES}$ - $\bar{p}_{TS.ES}$ and $\bar{\alpha}_{ES}$ - $\bar{\alpha}_{TS.ES}$, the values in parentheses denote the fraction of the bias of p and $\hat{\alpha}$ estimated by 1-($\bar{p}_{TS.ES}/\bar{p}_{ES}$) and 1-($\bar{\alpha}_{TS.ES}/\bar{\alpha}_{ES}$), respectively.

[c] Additive effect of each trait are given as follow: stalk lodging (%), yield (Mg ha^{-1}), grain moisture (%), silking and anthesis (days), plant and ear height (cm). Positive additive effects indicate that A637 allele increases the value of the trait.

** Significant at the 0.01 probability level