Indirect response to selection for improving resistance to the Mediterranean corn borer (*Sesamia nonagrioides* Lef) in maize

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Short Title: Indirect response to MCB (*Sesamia nonagrioides* Lef) selection

Abstract Mediterranean corn borer (MCB) (*Sesamia nonagrioides* Lef) and European corn borer (ECB) (*Ostrinia nubilalis* Hbn) are the most important biotic stresses of maize in Europe. The first selection program to improve stalk resistance to MCB was carried out in the maize population EPS12. It has shown that selection was effective to improve stalk resistance to MCB and European corn borer (ECB), while yield was not significantly diminished. The objective of this research was to determine if correlated changes in EPS12 occurred due to selection for resistance to MCB. Cycles of selection *per se* and testcrosses to three testers were evaluated under MCB and ECB artificial infestation at two different Spanish locations during two years. Selection has significantly reduced cob damage, days to silking, plant and ear height, and 100-kernel weight; meanwhile early vigor was increased. These changes could rather be a consequence of unconscious selection and/or the genetic correlation of these traits with resistance than a consequence of genetic drift.

Key words: insect resistance, intrapopulation Mediterranean Corn Borer, maize, recurrent selection, *Sesamia nonagrioides* Lef

Introduction

European corn borer (ECB) (*Ostrinia nubilalis* Hbn) is one of the most destructive maize (*Zea mays* L.) pests in North America and northern and central Europe while Mediterranean corn borer (MCB) (*Sesamia nonagrioides* Lef), also known as pink stem borer, is the most destructive pest in the Mediterranean area, causing important stalk damage and decreases on maize yield. Several selection programs have been carried out to improve maize resistance to ECB (Russel et al., 1979; Klenke et al., 1986; Nyhus et al., 1989; Anglade et al., 1996). There is only one report about selection done for improving resistance to MCB, three cycles of S_1 intrapopulational recurrent selection have improved resistance to MCB while yield was maintained in EPS12 synthetic (Sandoya et al., 2008).

Selection could have positive or negative effects in other resistance-related and agronomic traits. Previous research indicated that recurrent selection for resistance to ECB resulted in decreased yielding ability (Russell et al., 1979; Klenke et al., 1986) and changes in important agronomic traits (Russell et al., 1979). In selection for resistance to first generation ECB, Russell et al. (1979) reported that favorable changes in plant height, ear height, and grain yield were associated with selection for resistance to insect attack. Klenke et al. (1986) found that four selection cycles reduced the damage by both the first and second generation of ECB attack but decreased grain yield, suggesting that yield should be included in the selection criteria in a selection program. There are no current studies regarding changes due to selection for resistance to MCB in agronomic and resistance-related traits.

In general, these changes occur because the alleles involved in pest resistance could have pleiotropic effects or could be in linkage disequilibrium with alleles controlling other quantitative traits of agronomic importance. Moreover, unfavorable changes in agronomic traits in a recurrent selection program may be the result of sampling variation for alleles affecting nonselected traits (genetic drift) if few individuals are selected to form the parents of subsequent cycles (Nyhus et al., 1989).

Maize resistance to corn borers is usually measured by reduced stalk tunneling, while ear resistance is rarely considered. Damaged ears could increase the secondary infection by microorganisms and accumulation of mycotoxin (Muñoz et al., 1990; Butrón et al., 2006b). Therefore the indirect response in ear resistance should be considered when stalk resistance is improved. The objective of this research was to determine correlated changes in agronomic and ear-resistance traits due to selection for resistance against MCB attack in the maize synthetic population EPS12.

Materials and Methods

Selection program of EPS12

The S_1 recurrent intrapopulational recurrent selection program for improving Mediterranean corn borer (MCB) resistance in the maize synthetic population EPS12 started after obtaining three cycles of recurrent selection for yield in EPS7 (Vales et al., 2001). EPS7 was originally made using four landraces from the Ebro Valley and eastern Spain (Ordás, 1991).

The selection program used to improve resistance of EPS12 against Mediterranean corn borer (MCB) attack started in 1993 when approximately 150 S_1 families were derived from the EPS12 maize synthetic. In 1994, 100 S_1 families were evaluated in field conditions under artificial infestation with MCB eggs; the families with the shortest tunnel made by MCB and the yield up to the average were selected. In 1995 selected families were recombined and the first cycle of recurrent selection EPS12(S)C1 was established in 1996. In a similar manner, EPS12(S)C2 and EPS12(S)C3 were obtained in 1999 and 2002, respectively. Unfortunately, EPS12(S)C1 seeds were accidentally mixed with seeds from another maize synthetic, and they could not be included in the present study. Nevertheless, the selection process was not affected because S_1 families were obtained from EPS12(S)C1-Syn1 before recombination to obtain EPS12(S)C1. More details about this selection program can be found in details in Butrón et al. (2005) and Sandoya et al. (2008).

In 2002, seeds from the synthetics EPS12(S)C0, EPS12(S)C2 and EPS12(S)C3 were multiplied and testcrossed to inbred lines A639, B93 and EP42. The inbred lines A639 and B93 were reported as resistant to MCB, and EP42 as susceptible (Butrón et al., 1999a; 2006a).

Evaluation of the cycles of selection and testcrosses to testers

All field trials were conducted during 2003 and 2004 in two different regions of Spain, Pontevedra (42º24' N, 8º38' W, at the sea level) and Zaragoza (41º 44' N, 0º47ºW, 230 m above sea level). In each year and location, two adjacent trials were arranged, one infested with MCB eggs and the other one with ECB eggs. The three cycles of selection *per se*, and the testcrosses of the cycles of selection to the inbred lines A639, B93 and EP42 (belonging to Lancaster, Reid and humid Spain heterotic groups) were grown in a complete block design with three repetitions.

In Pontevedra, plots were hand-planted in rows spaced by 0.80 m apart containing 25 two-kernel hills separated by 0.21 m. Plots were thinned to obtain a final density of ≈ 60,000 plants ha $^{-1}$. In Zaragoza, plots were machine-planted in rows 0.75 m apart with two-kernel hills, with 0.21 m between plants; plots were thinned to a final density ≈ 74,000 plants ha^{-1}. All agronomic practices were made according to standards practiced by producers of maize in Spain.

Rearing of MCB larvae was carried out with the methodology proposed by Eizaguirre and Albajes (1992), whereas ECB eggs were provided by the INRA (Institute National de la Recherche Agronomique) located in the region of Poitou-Charentes, France. Artificial infestations were carried out according to Butrón et al. (1999b). At flowering, 10 plants per plot were infested with a mass of 50 eggs of MCB or ECB, depending on the trial, between the main ear and the stalk.

Recorded traits

Important agronomic traits were considered. At approximately five-leaf stage, early vigor was registered on a subjective scale (from 1= less vigorous to 9= the most vigorous plant). Days to anthesis and silking were recorded as the number of days from planting until approximately 50% of the plants on a plot were shedding pollen or were receptive, respectively. Plant and ear insertion heights were measured in cm as the distance from the base of the plant to the insertion of the tassel and the node bearing upper ear, respectively. Before harvesting, in each plot, the percentage of plants leaning greater than 45º from the vertical was registered as root lodging. As borer attack could cause stalk lodging, the percentage of the stalk-lodged plants was also taken into account; plants were considered stalk-lodged if corn stalks were broken below the ear.

At harvest time, the tunnel length made by borers was recorded in the ten infested plants. Plants were longitudinally split and the total length was measured in cm. Grain yield on the plot was registered, adjusted to kernel moisture of 140 g H_2O kg^{-1} and expressed as Mg ha^{-1} and ear length (cm), ear-row number and 100-kernel weight (g) were also recorded. Other data observed were traits related to ear resistance to corn borer, namely husks, grain, shank and cob appearance scored on a 9 point subjective scale from 1 = wholly damaged to 9 = without injury (Butrón et al., 2009).

Statistical analysis

Combined analysis of variance was made considering infested-species and genotypes as fixed effects. In addition, a combined analysis of variance was computed considering each combination of infested-species, environment and location as one environment; all factors were considered as random effects except genotypes. The sums of squares of genotypes were orthogonally divided into: cycles *per se*, testcrosses to A639, B93 and EP42, and among groups. The main effects and interactions of the main effects were tested by the appropriate interactions with environments or by the error mean squares when the interactions were not significant. Furthermore, sums of squares were divided into sums of squares due to linear regression, according to Carmer and Seif's (1963) formula. The rate of response per cycle of selection was the linear coefficient of regression. Mean comparisons were made using Fisher's protected LSD (Steel and Torrie, 1980).

Phenotypic correlations between traits under selection (yield and tunnel length) and remaining traits were estimated following Johnson et al (1955).

$$r_a = \frac{M_{12}}{\sqrt{(M_{11})\,(M_{22})}}$$

M_{12} is the genotype mean product of both characteristics and M_{11} and M_{22} are the mean squares of genotypes for each trait. All statistical analyses were computed using SAS Institute Software (2007).

Results

The tunnel length made by MCB was not significantly different from ECB tunnel length. In addition, no significant infested species × environment interaction nor significant infested species × genotype interaction were found for most traits (data not shown). Instead of considering each infested species as a fixed effect; we included the infested species within the environment. Therefore the combination of one infested species – location – year was considered as one environment.

Tunnel length was significantly and positively associated with anthesis and 100 kernel-weight ($P \leq 0.05$), plant and ear height, and ear length ($P \leq 0.01$). On the other hand, grain yield was positively associated with anthesis ($P \leq 0.05$), plant height, ear length, ear-row number and 100 kernel-weight, and grain appearance ($P \leq 0.01$) (Table 1).

There were high significant differences among cycles of selection for early vigor, days to anthesis and silking, ear height, and 100 kernel-weight, and cob appearance ($P \leq 0.01$) (data not shown). The linear regression coefficients for those traits on cycles of selection were also highly significant for early vigor, anthesis, ear height, 100 kernel-weight, and cob appearance ($P \leq 0.01$), and significant for days to silking ($P \leq 0.05$). In addition, differences for plant height, root lodging, and ear-row number were significant at the $P \leq 0.05$. The cycle *per se* × environment interaction was only significant ($P \leq 0.05$) for early vigor.

Average mean values for early vigor increased from 5.75 in EPS12C0 to 7.00 in EPS12C3. In addition, EPS12C3 showed shorter plant and ear heights, higher precocity, and lower root lodging and ear-row number and less 100-kernel weight and cob damage than EPS12C0 (Table 2).There were few significant differences among testcrosses to testers. Cycles crossed to the tester A639 significantly differed for early vigor, plant height (*P≤0.01*) and ear height (*P≤0.05*) (Table 2); the testcrosses × environment interaction was significant for anthesis, 100 kernel weight (*P≤0.05*) and silking (*P≤0.01*) (data not shown). Testcrosses to B93, showed significant differences for early vigor, precocity, and plant and ear heights (*P≤0.01*); while the testcrosses × environment interaction was not significant for any agronomic trait. Finally no significant differences for agronomic traits were found among testcrosses to EP42, except for early vigor (*P≤0.01*). No differences among any testcrosses were detected for ear-resistance traits, but testcrosses × environment interactions were significant for husk and cob appearance (data not shown).

Discussion

Selection process in the maize synthetic EPS12 was made taking into account as main selection criteria the shortest stem tunneling due to the feeding by MCB. This pest is more abundant than ECB which is also a harmful pest in the coastal region of Pontevedra (Cordero et al., 1998; Velasco et al., 2007). Similarly, although ECB had been reported as an important pest in the Ebro valley where Zaragoza is located, MCB causes higher yield losses in Zaragoza (Malvar et al., 1993). In the present study damage was mainly due to MCB, although the presence of both species was reported at the two locations. The extremely high rate of natural infestation of MCB could have masked the damage differences between the two borers (Velasco et al., 2007).

Selection for stem resistance in the maize synthetic population EPS12 significantly modified other agronomic traits. The selection process has improved early vigor and cob appearance, has increased precocity, and has reduced the 100 kernel-weight.

Early vigor is very important in places where temperatures are low during planting and early developmental stages. Under low temperatures, plants with less vigor could not survive or show reduced ability to compete for soil nutrients; consequently yield would be reduced (Revilla et al., 1999).

Earliness could be negative depending on the aims of breeding, because earlier genotypes usually have reduced yield. Nevertheless an early genotype is desirable when maize is grown under short growing season conditions as those present in central and northern Europe. Other authors have reported that S_1 intrapopulation recurrent selection programs for resistance to insects have increased earliness (Nyhus et al., 1989; Butrón et al., 2000), but in contrast to our results, yield was also significantly reduced.

The changes reported in plant and ear height, ear row number and 100-kernel weight were negative. Sandoya et al. (2008) did not detect significant changes due to selection for yield under infestation of MCB; but if agronomic changes reported continue in the future, yield could be negative and significantly affected, since plant height, ear length; ear-row number and 100-kernel weight are highly correlated with yield.

Changes identified in this study regarding agronomic traits could be due to three main factors: increased inbreeding by genetic drift or allele fixation by selection, genetic correlation with the trait(s) under selection, and unconscious selection for the trait studied. Russell et al. (1979) pointed out that if differences between initial and improved populations did not persist in testcrosses, then the differences were likely due to inbreeding depression. In general, testcrosses to the three testers had similar responses to selection as the cycles of selection *per se*, except for 100-kernel weight. Therefore inbreeding should not be the cause of the phenotypic changes observed for agronomic traits such as plant and ear height, ear length, precocity, and 100-kernel. Sandoya et al. (2009) found genetic correlation between those traits and resistance to MCB, therefore genetic correlation between resistance and agronomic traits could play a more important role than inbreeding depression. In a QTL study using recombinant inbred lines derived from the cross EP39 (resistant) × EP42 (susceptible), QTLs in repulsion linkage phase were found for yield and tunnel length by MCB (Ordás et al., 2010).

Early vigor increased with selection program. The reason could be unconscious selection for this trait since the weakest plants are removed as consequence of thinning

As mentioned, selection in EPS12 was carried out to improve stem resistance, so it is not surprising that cycles did not differ for ear-resistance traits with the exception of cob appearance. Many reports have already pointed out that ear resistance and stem resistance are not related when damage is done by ECB (Pounders et al, 1975; Grier and Davis, 1980) or by MCB (Butrón et al., 1999b). As most ear-resistance traits were not improved, and damaged ears could increase the secondary infection by microorganisms and accumulation of mycotoxins, in the future, ear resistance traits could be included in the selection criteria.

Despite having some unfavorable changes, the resistance to MCB in the maize synthetic EPS12 has been improved while maintaining yield by S_1 intrapopulation recurrent selection. In addition, cob appearance and early vigor have changed favorably. Similar studies have found some unfavorable changes in important agronomic traits, such as yield, when a genotype was improved for resistance to *Helicoverpa zea* (Butrón et al., 2000).

Recurrent selection has been made by plant breeders for improving a desirable characteristic of a crop, such as yield, resistance to abiotic and biotic stresses, or/and quality; identifying traits modified by the selection process is important in order to include them as selection criteria, when they could be genetically and unfavorably correlated to traits under selection, or to increase the effective size of the population to avoid changes due to random genetic drift. In the future, we propose to include kernel health as a new selection criteria in order to diminish kernel damage by stem borer and reduce the risk of mycotoxin contaminations, harmful to livestock and consequently to human health mainly where maize is used a source of food.

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Table 1. Phenotypic correlations between traits under selection and agronomic and ear resistance traits in the maize composite EPS12 evaluated in two locations and two years under corn borer infestation.

Traits	**Tunnel length**	**Yield under infestation**
Early vigour	0.17	0.22
Anthesis	0.62 *	0.63 *
Silking	0.53	0.52
Plant height	0.79 **	0.88 **
Ear height	0.86 **	0.37
Root lodging	0.11	0.13
Ear length	0.82 **	0.69 **
Ear row number	0.39	0.80 **
100-kernel weight	0.65 *	0.71 **
Stalk lodging	0.18	0.31
Husk appearance	0.30	0.40
Grain appearance	0.32	0.62 *
Shank appearance	0.09	-0.07
Cob appearance	-0.20	0.02

*,** Significant at the 0.05 and 0.01 probability levels, respectively

Table 2. Means and linear coefficients† of cycles of selection *per se*‡ and testcrossed to testers for agronomic traits evaluated in eight different trials.

EPS12	Early vigor	Anthesis	Silking	Heights plant	Heights ear	Root lodging	Cob appearance	Ear-row number	100-kernel weight	Tunnel length[1]	Yield[1]
	(Scale 1 – 9)§	days		cm		%	(Scale 1 – 9)		g	cm	Mg ha^{-1}
C0	5. 75 b	59.29 a	62.95 a	170.7 a	96.4 a	22.50 a	5.64 b	11.30 a	33.27 a	61.54 a	5.83
C2	6. 79 a	60.20 a	63.21 a	162.5 b	85.4 b	24.87 a	6.38 a	11.38 a	31.07 b	63.21 a	5.72
C3	7. 00 a	58.75 b	62.08 b	162.9 b	81.9 b	15.81 b	6.33 a	10.78 b	30.89 b	54.84 b	5.38
LSD (0.05)	0. 61	0.73	0.70	6.6	5.00	6.61	0.50	0.47	1.27	4.40	
Linear	0. 43	-0.31	-0.23	-2.83	-4.90		0.35		-0.84	-1.80	
C0 × A639	5.13 b	57.67	60.17	175.9 a	81.0 a	19.50	6.73	11.87	31.07	63.70 a	6.61
C2 × A639	6.00 a	56.58	59.21	163.9 b	74.8 b	25.86	6.31	11.73	30.61	51.17 b	6.28
C3 × A639	6.38 a	56.42	59.50	162.5 b	74.7 b	14.19	6.71	11.63	30.85	58.87 b	6.19
LSD (0.05)	0.62			6.6	5.0					4.4	
Linear	0.42			-4.70	-2.26					-1.85	
C0 × B93	6.38 b	63.96 a	66.08 a	185.2 a	92.9 a	20.38	5.95	13.43	32.45	71.21 a	7.61
C2 × B93	6.92 a	62.83 b	65.08 b	175.3 b	82.1 b	20.33	6.40	13.24	33.70	62.03 b	7.14

C3 × B93	7.08 a	62.92 b	65.29 b	174.1 b	82.9 b	19.23	5.87	13.22	32.58	64.54 b	7.14
LSD (0.05)	0.38	0.73	0.70	6.6	5.0					4.4	
Linear	0.24	-0.38	-0.30	-3.87	-3.61					-2.56	
C0 × EP42	6.46 c	60.54	62.75	177.0	109.5	25.43	6.44	11.77	34.95	68.45	6.81 b
C2 × EP42	6.96 b	60.92	63.04	175.9	105.7	23.86	6.01	11.53	33.74	73.12	6.87 b
C3 × EP42	7.38 a	60.58	62.79	181.5	106.9	18.55	6.82	11.83	34.56	66.87	7.36 a
LSD (0.05)	0.38										0.41
Linear	0.16										0.16
Mean	6.52	60.11	62.68	172.3	89.51	13.44	6.30	11.98	32.48	63.80	6.57
CV%	10.23	2.13	1.96	6.71	6.82	5.04	13.8	6.92	6.88	12.0	12.9

† Linear coefficient were included when a linear contrast was significant on the analysis of variance (not shown)

‡ Cycle 1 is not included

§ Subjective scale from 1= less vigorous to 9= the most vigorous plant

¶ Subjective scale from 1= wholly damaged to 9= without injury

1 Data published in Sandoya et al (2008)

Means followed by the same letter are not different significantly at the 0.05 probability level, according to Fisher protected LSD method