

1 **Genetic relationship between maize resistance to corn borer attack and yield**

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12 **Abstract** - Selection for reduced stalk tunnel length by the Mediterranean corn borer (MCB,  
13 *Sesamia nonagrioides* Lef.), while maintaining yield under infestation, has already been evaluated.  
14 Significant reductions for tunnel length were reported, but yield showed a non significant  
15 tendency to decrease with selection that could be due to increased inbreeding or significant  
16 genetic correlation between yield and resistance. Simultaneously, the presence of major QTLs for  
17 resistance in chromosome 4 has been suggested. In the present study, we investigated the genetic  
18 relationship between yield and stalk tunneling resistance by conducting one generation of  
19 selection each for yield alone, resistance alone, and yield and resistance combined in a broad-  
20 based population. In addition, we tested the effectiveness of marker assisted selection at two  
21 markers on chromosome 4 for reducing tunnels by MCB. Evaluations were made for two years at  
22 two locations under MCB infestation. Unfavorable genetic correlation between stalk tunnel  
23 length and yield has been confirmed and could prevent detection of major QTLs for tunnel  
24 length by using selection mapping. Markers phi076 and umc1329 could be linked to QTLs for  
25 plant height, but are not useful markers for reducing tunnel length and hence damage caused by  
26 borers. Finally, results advise against selection for reduced tunnel length by MCB or any index  
27 involving yield and tunnel length.

28

29 **Keywords:** Maize, *Sesamia nonagrioides*, Resistance, Yield, Breeding

30

## 31 **Introduction**

32

33 Host plant resistance has been presented as one of the main strategies for arthropod pest  
34 management in organic farming which is one of the fastest growing sectors of agriculture due to  
35 the increasing demand for organically produced food and feed (Zehnder et al. 2007). Varieties  
36 with partial pest resistance may not be cost effective in conventional farms where pesticides and  
37 GMO organisms are currently used, but are advisable to maintain low-level pest densities that  
38 support natural enemy populations. In the Mediterranean area the Mediterranean corn borer  
39 (MCB, *Sesamia nonagrioides* Lef.), also named pink stem borer or West African pink borer, is the  
40 main pest threat for maize cultivation and non-*Bt* resistant cultivars appear as one promising  
41 control method for organic farming. Our group developed a maize synthetic (EPS7) intercrossing  
42 four local Spanish landraces which showed resistance to stalk tunneling by the Mediterranean  
43 corn borer (Ordás 1991; Malvar 1993; Vales et al. 2001) and a selection program was initiated to  
44 improve resistance to MCB, while maintaining high yield during selection, using the breeding  
45 population EPS12 derived from EPS7 (Butrón et al 2005). The breeding scheme used has been  
46 presented in detail by Sandoya et al. (2008). Following three cycles of  $S_1$  recurrent selection,  
47 molecular changes during selection were monitored (Butrón et al. 2005) and direct and indirect  
48 responses to selection were determined (Sandoya et al. 2008; 2010). In the evaluations, crop yield  
49 showed a non significant tendency to decrease with selection, while stalk tunneling significantly  
50 decreased ( $-1.8 \text{ cm cycle}^{-1}$ ), as well as days to silking and plant height. Accompanying the  
51 agronomic changes, allele frequencies for two SSR molecular markers located on chromosome 4  
52 showed directional changes due to selection suggesting the presence of QTLs for resistance to  
53 tunneling by MCB in this region.

54 Sandoya et al. (2009) also concluded that selection could continue for more cycles  
55 because no reduction of additive variance for tunnel length was observed after three cycles of  
56 recurrent selection. However, effects on yield would be unpredictable because no information on  
57 additive correlation between yield and the length of tunnels made by MCB could be estimated as  
58 additive variance for yield did not significantly differ from zero. The non significant tendency of  
59 yield to decrease with selection could be due to increased inbreeding or to significant genetic  
60 correlation between yield and tunnel length. Inbreeding could be avoided using alternative  
61 breeding schemes in which the number of families selected is increased. High genetic correlation  
62 between yield and tunnel length by MCB, however, would misadvise efforts to increase resistance  
63 to MCB attack except when performing marker-assisted selection with markers linked to  
64 important QTLs for resistance and unlinked to QTLs for yield. In order to study the genetic  
65 relationship between yield and resistance, we compared three alternative breeding methods: (1)  
66 Selection for reduced tunnel length (L), (2) Selection for increased yield under artificial infestation  
67 (Y), and, (3) Selection for reduced tunnel length while maintaining yield (I), as done during the  
68 previous four cycles of selection. Simultaneous to the phenotypic selection for improved  
69 resistance to MCB, one cycle of divergent marker-assisted selection was carried out in EPS12  
70 using those markers for which allele frequency changes due to selection were significant. The  
71 goal was to confirm if those markers were indeed linked to major QTLs for tunnel length and  
72 unlinked to QTLs for yield.

73 In the present study, we investigated the genetic relationship between yield and stalk  
74 tunneling resistance by conducting one generation of selection each for yield alone, resistance  
75 alone, and yield and resistance combined in a broad-based population. In addition, we tested the  
76 effectiveness of marker assisted selection at two markers on chromosome 4 for reducing tunnel  
77 length by MCB.

78

79 **Materials and methods**

80

81 EPS12(T)C2, EPS12(T)C3, and EPS12(T)C4 were derived from EPS12 through  $S_1$  recurrent  
82 selection. The 10% families with the least tunnel length and higher yield than the average yield of  
83 families evaluated were selected (Sandoya et al. 2008). Synthetics EPS12(L)C1, EPS12(Y)C1, and  
84 EPS12(T)C5 were obtained following one cycle of  $S_1$  recurrent selection in EPS12(T)C4 for  
85 reduced tunnel length, increased yield under infestation or simultaneous selection for both traits,  
86 respectively. In 2005,  $S_1$  families were obtained from EPS12(T)C4 and, in 2006, 90 families were  
87 evaluated in a simple lattice 9 x 10 under infestation with MCB. In this selection trial,  
88 heritabilities and genotypic and phenotypic correlation coefficients were computed following  
89 Holland (2003, 2006). The estimated heritabilities on a family mean basis ( $\pm$  standard error) for  
90 yield and tunnel length were 0.78 ( $\pm$  0.05) and 0.32 ( $\pm$  0.15), respectively. The genotypic and  
91 phenotypic correlation coefficients ( $\pm$  standard error) between both traits were 0.41 ( $\pm$  0.24) and  
92 0.22 ( $\pm$  0.08), respectively. In 2007, ten families with the highest yield or shortest tunnel length  
93 were recombined to constitute EPS12(Y)C1Syn 1 and EPS12(L)C1Syn1, respectively. Seed of  
94 each selected progeny was sown in a 15-plant row. In each row, five plants were used as males  
95 and another five plants as females. Bulked pollen from the 50 male plants was used to pollinate  
96 the five female plants in each row. The resulting ears contributed equally to the bulk of 1,000  
97 seeds named EPS12(Y)C1Syn1 and EPS12(L)C1Syn1. Similarly, EPS12(T)C5Syn1 was the result  
98 of recombining 10 families with the shortest tunnel length and higher yield than the average yield  
99 of families. In 2008, a second recombination was made within each population  
100 [EPS12(Y)C1Syn1, EPS12(L)C1Syn1, and EPS12(T)C5Syn1] in order to reach linkage  
101 equilibrium: 300 seeds were planted from each population, thinned to 150 plants, and crosses  
102 were made using each plant only once as male or female. At harvest, at least 50 ears were  
103 obtained from each population and an equilibrated bulk of 1,000 seeds was collected establishing

104 the synthetics EPS12(Y)C1, EPS12(L)C1, and EPS12(T)C5, respectively. In the same year,  
105 EPS12, EPS12(T)C2, EPS12(T)C3, and EPS12(T)C4 were multiplied to obtain homogeneous  
106 seed for evaluations.

107 In a previous work, marker *phi076* segregated for three alleles, arbitrarily coded as 1, 2,  
108 and 3; and *umc1239* segregated for two alleles, coded 1 and 2; and frequencies of alleles 1 and 3 at  
109 marker *phi076* and alleles 1 and 2 at marker *umc1329* showed directional changes due to selection  
110 (Butrón et al., 2005). In order to check whether markers *phi076* and/or *umc1329* could be useful  
111 markers for marker-assisted selection toward reducing tunnel length by MCB, five individuals  
112 carrying a determined combination of alleles at both markers were selected in EPS12 and  
113 intercrossed to constitute populations EPS12-1111, EPS12-1112, EPS12-1122, EPS12-1311,  
114 EPS12-1312, EPS12-1322, EPS12-3311, EPS12-3312, and EPS12-3322 (first two digits after  
115 dash refer to *phi076* alleles and the other two to *umc1329* alleles, presence of both alleles at a  
116 determined maker does not mean all individuals are heterozygote but each allele is present at a  
117 frequency equal to 0.5). The genotypes at other loci were not controlled because marker-assisted  
118 selection would only be effective if these markers are linked to major QTLs and genetic  
119 background should not obscure effects of major QTLs.

120 Genotypes were evaluated using triple-lattice designs at Pontevedra (42°24' N, 8°38' W,  
121 20 m above sea level), and Zaragoza (41° 44' N, 0° 47' W, 230 m above sea level) in Spain in 2009  
122 and 2010. In Pontevedra, the experiment was hand-planted and consisted of two rows spaced  
123 0.80 m. apart with 29 plants within each row spaced 0.21 m. apart. Plots were overplanted and  
124 thinned to obtain a final population density of about 60,000 plants ha<sup>-1</sup>. In Zaragoza, plots were  
125 machine-planted and consisted of two rows spaced 0.70 m apart with 25 plants within each row  
126 spaced 0.20 m apart. Plots were overplanted and thinned to a final population density of 70,000  
127 plants ha<sup>-1</sup>. At flowering, ten adjacent and competitive (equally spaced apart from adjacent  
128 plants)plants per plot were infested by placing egg masses of about 40-50 eggs between the upper

129 ear and the stem. The MCB rearing method used has been described by Eizaguirre & Albajes  
130 (1992).

131 Observations were recorded for days to pollen shed (days from planting when 50% of  
132 plants had shed pollen), days to silking (days from planting to when 50% of plants had silks  
133 emerged), plant height (recorded in Pontevedra on ten competitive plants as the distance from  
134 the ground to the top of the plant), stem height (recorded in Zaragoza on ten competitive plants  
135 as the distance from the ground to the flag leaf), stem lodging (percentage of plants in the plot  
136 with the stem broken below the main ear), root lodging (percentage of plants in the plot leaning  
137 more than 45° to the vertical), kernel moisture (g of water in 100g of kernels), yield (Mg ha<sup>-1</sup> of  
138 kernels at 140 g H<sub>2</sub>O kg<sup>-1</sup>), tunnel length (total length in cm per plant of stem tunnels made by  
139 borers), and visual ratings for kernel, shank, and cob damages (on a 9 point subjective scale  
140 determined as follows: 1 = > 90% damage, 2 = 81 to 90% damage, 3 = 71 to 80% damage, 4 = 61  
141 to 70% damage, 5 = 41 to 60% damage, 6 = 31 to 40% damage, 7 = 21 to 30% damage, 8 = 1 to  
142 20% damage, and 9 = no damage).

143 Combined analysis of variance was done considering genotypes as fixed effects and  
144 environments (each location-year combination was considered an environment) as random (SAS  
145 2007). The relationship between *phi076* and *umc1329* markers and agronomic traits was computed  
146 considering environments as random effects and markers as fixed effects. Mean comparisons  
147 were made using Fisher's protected LSD.

148

## 149 **Results and discussion**

150

151 The combined analyses of variance showed significant differences among genotypes for days to  
152 anthesis and silking, stem and root lodging, kernel moisture, plant and stem heights, yield and

153 tunnel length (Table 1). In our experiments, significant differences among EPS12, EPS12(T)C2,  
154 EPS12(T)C3, EPS12(T)C4, and EPS12(T)C5 were not detected for tunnel length (Table 2), but  
155 there was a tendency for decreasing tunnel length with selection, agreeing with results obtained  
156 previously (Sandoya et al. 2008, 2009). The non-significant tendency to reduce yield during the  
157 first three cycles of selection became significant in the following two cycles with plant height  
158 changes also becoming undesirable. These undesirable changes could be the result of inbreeding  
159 depression due to genetic drift or consequence of negative correlation between resistance to stem  
160 tunneling and yield. Comparisons among EPS12(L)C1, EPS12(Y)C1, and EPS12(T)C5  
161 developed from EPS12(T)C4 using three alternative breeding criteria [tunnel length, yield or both  
162 traits (applying more selection pressure on tunnel length)] confirmed that resistance to stem  
163 tunneling and yield could be genetically related. As new genetic variability has not been  
164 introduced, changes due to increased inbreeding would not be recovered, but original yield and  
165 plant height were recovered after one cycle of selection made exclusively for yield, suggesting that  
166 inbreeding increase due to genetic drift was not responsible for yield and plant height reductions.  
167 In addition, one cycle of selection made exclusively for yield tended to increase tunnel length,  
168 meanwhile one cycle of selection made exclusively for tunnel length reduced tunnel length,  
169 resulting in EPS12(L)C1 that was the only cycle significantly different from EPS12 for tunnel  
170 length. These results give enough evidence for a strong and negative genetic correlation between  
171 yield and resistance to stem tunneling, although the estimated genetic correlation coefficient  
172 between yield and tunnel length ( $0.41 \pm 0.24$ ) was not significantly different from 0. Previously,  
173 Ordás et al (2010) co-localized QTLs of small effect for increased yield and stalk tunneling by  
174 MCB in a population of recombinant inbred lines derived from the cross EP39 x EP42, although  
175 they did not detect significant genetic correlation between both traits. Genetic linkage between  
176 yield and stalk tunneling by another stem borer, *Ostrinia nubilalis* Hübner (European corn borer),  
177 seems to be a common phenomenon (Schulz et al. 1997; Kreps et al. 1998) and this negative  
178 association has made that selection for reduced tunnel length resulted in significant yield



179 reductions (Klenke et al. 1986). In addition, minor improvement for resistance to European corn  
180 borer has achieved when selection is done simultaneously for agronomic performance and  
181 resistance to borers (Bohn et al. 2003).

182 One cycle of selection for yield significantly increased productivity of EPS12(T)C4, while  
183 one cycle of selection for reduced tunnel length did not significantly change EPS12(T)C4  
184 resistance as expected based on estimated heritabilities for yield ( $0.78 \pm 0.05$ ) and tunnel length  
185 ( $0.31 \pm 0.15$ ).

186 Marker-assisted selection has proved an useful tool for improving resistance to the  
187 European corn borer (Flint-Garcia et al. 2003) using the bi-parental mapping approach. The  
188 QTL mapping approach (selection mapping) used by Butrón et al. (2005) identified alleles whose  
189 frequencies significantly changed with selection and Wisser et al. (2008) has suggested that this  
190 kind of mapping identifies the loci responsible for the strongest selection response and allow the  
191 discovery of superior alleles at these loci. However, as genotypic classes for markers *phi076* and  
192 *umc1329* did not significantly differ for tunnel length (Table 3), markers *phi076* and *umc1329* were  
193 totally discarded as useful markers for marker-assisted selection for improving resistance to  
194 tunneling by MCB. The homozygote '33' for *phi076* was significantly taller than the homozygote  
195 '11' [Butrón et al. (2005) reported that allele '1' was fixed in EPS12(T)C3] and the homozygote  
196 '22' for *umc1329* was significantly (at 0.10 probability level) less productive and tended to be  
197 smaller than '11' [Butrón et al. (2005) reported that allele '2' was fixed in EPS12(T)C3]. Therefore  
198 QTLs linked to *umc1329* could contribute to the yield reduction trend observed during selection  
199 and markers *phi076* and *umc1329* could be used to partially explain the indirect responses in traits  
200 such as stem height that was significantly reduced within the first three cycles of the recurrent  
201 selection program. These results agree with the fact that previously reported additive genetic  
202 correlation between plant height and tunnel length depends on the environment, Sandoya et al.  
203 (2009) reported a correlation coefficient close to zero in one year and higher than 0.7 in another

204 year. Therefore, markers potentially linked to plant height and/or stem height such as *phi076* and  
205 *umc1329* could have some effect on tunnel length under determined conditions and become  
206 irrelevant for resistance to stem tunneling under other conditions.

207         In conclusion, unfavorable genetic correlation between stalk tunnel length by MCB and  
208 yield has been confirmed and could prevent detection of major QTLs for resistance to stalk  
209 tunneling when selection mapping has performed in a selection program using both traits as  
210 selection criteria. Markers *phi076* and *umc1329* could be linked to QTLs for plant height, but are  
211 not useful markers for reducing tunnel length and hence damage caused by borers. Finally, results  
212 advise against selection for reduced tunnel length by MCB or any index involving yield and  
213 tunnel length. Selection for increased yield under infestation with MCB is recommended to  
214 increase maize tolerance to MCB attack. In the future, phenotypic selection could be combined  
215 with marker-assisted selection for reduced tunnel length, once makers linked to important QTLs  
216 for resistance and unlinked to QTLs for yield are identified.

217

## 218 **Acknowledgment**

219

220 Research supported by the Plan Nacional I+D+I (AGL2009-09611) and the ‘Deputación  
221 Provincial de Pontevedra’. M.C. Romay and J Peña-Asín acknowledge their fellowships from the  
222 Ministry of Education and Science of Spain. We thank Raquel Díaz and Silvana Poceiro for  
223 technical assistance.

224

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274 Table 1. Mean squares (MS) and degrees of freedom (DF) of the combined analysis of variance of maize breeding varieties evaluated in four  
 275 environments.

Source		Days to											
of		Days to	pollen	Stem	Root	Kernel	Plant	Stem		Ear	Shank	Cob	Tunnel
variation <sup>†</sup>		silking	shedding	lodging	lodging	humidity	height	height	Yield	damage	damage	damage	length
G	MS	31.36**	39.78**	126**	112**	27.85**	391**	309**	4.24**	0.33	0.48	0.86	75**
	DF	24	24	24	24	24	24	24	24	24	24	24	24
GE	MS	3.13**	2.40**	60**	41**	6.97**	71*	24	0.40**	0.22*	0.49*	0.50**	49
	DF	44	64	64	64	64	20	20	64	44	44	20	44
Error	MS	0.48	0.36	27	24	0.30	35	21	0.08	0.14	0.30	0.19	36
	DF	134	170	185	158	212	91	79	182	152	152	106	134

276 † G refers to genotype and GE to genotype x environment

277 \*. \*\* Significant at 0.05 and 0.01 probability level, respectively

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280 Table 2. Mean comparison among breeding populations for agronomic traits evaluated at two locations for two years.

Genotype	Days to								
	Days to silking	pollen shedding	Stem lodging	Root lodging	Kernel humidity	Plant height	Stem height	Yield	Tunnel length <sup>†</sup>
Genotype	Days	Days	%	%	%	cm	cm	Mg ha <sup>-1</sup>	cm
<b>Phenotypic selection</b>									
EPS12	65	63	28	24	20	234	135	6.1	33.1
EPS12(T)C2	68	64	22	14	22	234	127	5.4	29.7
EPS12(T)C3	67	64	21	16	20	225	121	5.2	30.7
EPS12(T)C4	64	62	19	19	19	217	118	4.8	27.0
EPS12(T)C5	64	62	16	16	20	196	105	3.5	26.4
EPS12(L)C1	64	62	16	11	19	209	112	4.4	23.2
EPS12(Y)C1	65	63	27	10	19	230	132	5.6	31.9
<b>Marker-assisted selection</b>									
EPS12-1111	64	64	30	20	21	216	113	5.9	31.1

EPS12-1112	65	63	32	17	20	217	126	5.5	34.2
EPS12-1122	64	63	33	20	20	217	126	5.9	36.1
EPS12-1311 <sup>‡</sup>	67	65	32	20	22	241	135	6.4	31.2
EPS12-1312	66	64	28	18	19	226	122	5.9	40.0
EPS12-1322	65	63	27	25	21	226	129	5.2	38.2
EPS12-3311	68	66	24	25	22	250	135	5.6	34.7
EPS12-3312	65	63	21	26	21	238	130	5.8	36.0
EPS12-3322	66	64	29	15	19	246	132	5.1	32.3
LSD (0.05)	3	2	12	10	2	19	9	1.0	9.7

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282 <sup>†</sup>Total length in cm of stem tunnels made by borers.

283 <sup>‡</sup> Presence of both alleles at a determined maker does not mean all individuals are heterozygote but each allele is present at a frequency equal to 0.5.



284 Table 3. Differences in agronomic traits among genotypic classes for SSR markers *phi076* and  
 285 *umc1329*<sup>†</sup>.

Marker	Allele <sup>‡</sup>	Days to						
		Tunnel length	Yield	Days to silking	pollen shedding	Kernel humidity	Plant height	Stem height
		cm	Mg ha <sup>-1</sup>	Days	Days	%	cm	cm
<i>phi076</i>	33	34.1 a	5.4 a	66 a	64 a	20 a	245 a	133 a
	13	36.5 a	5.8 a	66 a	64 a	21 a	231 b	129 ab
	11	33.8 a	5.7 a	64 b	63 b	20 a	216 c	122 b
<i>umc1329</i>	11	32.3 a	6.0 a <sup>§</sup>	66 a	65 a	22 a	236 a	128 a
	12	36.8 a	5.7 ab	65 a	63 b	20 b	225 a	125 a
	22	35.5 a	5.4 b	65 a	63 b	20 b	229 a	129 a

286 <sup>†</sup>For each marker and trait, means followed by the same letter did not differ at 0.05 probability  
 287 level

288 <sup>‡</sup>Butrón et al. (2005) reported that frequency of allele '1' increased at marker *phi076* and  
 289 decreased at marker *umc1329* during the first three cycles of selection for reduced tunnel length,  
 290 while maintaining yield. Presence of both alleles at a determined maker does not mean all  
 291 individuals are heterozygote but each allele is present at a frequency equal to 0.5

292 <sup>§</sup> Differences were significant at 0.10 probability level.