Genetic relationship between maize resistance to corn borer attack and yield

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

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

30

31 **Introduction**

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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.

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

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

Materials and methods

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

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

In a previous work, marker *phi076* segregated for three alleles, arbitrarily coded as 1, 2, and 3; and *umc1239* segregated for two alleles, coded 1 and 2; and frequencies of alleles 1 and 3 at marker *phi076* and alleles 1 and 2 at marker *umc1329* showed directional changes due to selection (Butrón et al., 2005). In order to check whether markers *phi076* and/or *umc1329* could be useful markers for marker-assisted selection toward reducing tunnel length by MCB, five individuals carrying a determined combination of alleles at both markers were selected in EPS12 and intercrossed to constitute populations EPS12-1111, EPS12-1112, EPS12-1122, EPS12-1311, EPS12-1312, EPS12-1322, EPS12-3311, EPS12-3312, and EPS12-3322 (first two digits after dash refer to *phi076* alleles and the other two to *umc1329* alleles, 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). The genotypes at other loci were not controlled because marker-assisted selection would only be effective if these markers are linked to major QTLs and genetic background should not obscure effects of major QTLs.

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

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

Observations were recorded for days to pollen shed (days from planting when 50% of plants had shed pollen), days to silking (days from planting to when 50% of plants had silks emerged), plant height (recorded in Pontevedra on ten competitive plants as the distance from the ground to the top of the plant), stem height (recorded in Zaragoza on ten competitive plants as the distance from the ground to the flag leaf), stem lodging (percentage of plants in the plot with the stem broken below the main ear), root lodging (percentage of plants in the plot leaning more than 45° to the vertical), kernel moisture (g of water in 100g of kernels), yield (Mg ha⁻¹ of kernels at 140 g H₂O kg⁻¹), tunnel length (total length in cm per plant of stem tunnels made by borers), and visual ratings for kernel, shank, and cob damages (on a 9 point subjective scale determined as follows: 1 = > 90% damage, 2 = 81 to 90% damage, 3 = 71 to 80% damage, 4 = 61 to 70% damage, 5 = 41 to 60% damage, 6 = 31 to 40% damage, 7 = 21 to 30% damage, 8 = 1 to 20% damage, and 9 = no damage).

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

Results and discussion

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

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

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

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

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

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

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

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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 variation [†]		Days to	pollen	Stem	Root	Kernel	Plant	Stem		Ear	Shank	Cob	Tunnel
		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.

	Days to		Stem	Root	Kernel	Plant	Stem	Yield	Tunnel
	Days to silking	pollen shedding							
Genotype	Days	Days	%	%	%	cm	cm	Mg ha ⁻¹	cm
Phenotypic selection									
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
Marker-assisted selection									
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 [‡]	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 [†]Total length in cm of stem tunnels made by borers.

283 [‡] 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.

Table 3. Differences in agronomic traits among genotypic classes for SSR markers *phi076* and *umc1329*[†].

Marker	Allele [‡]	Days to						
		Tunnel length	Yield	Days to silking	pollen shedding	Kernel humidity	Plant height	Stem height
		cm	Mg ha ⁻¹	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 [§]	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

[†]For each marker and trait, means followed by the same letter did not differ at 0.05 probability level

[‡]Butrón et al. (2005) reported that frequency of allele '1' increased at marker *phi076* and decreased at marker *umc1329* during the first three cycles of selection for reduced tunnel length, while maintaining yield. 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

[§] Differences were significant at 0.10 probability level.