1 **Plant Breeding**

2 *Short communication*

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4 Combining Abilities in Maize for the Length of the Internode Basal Ring,
5 the Entry Point of the Mediterranean Corn Borer Larvae

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Abstract

Length of the internode basal ring (LIBR) in maize (*Zea mays* L.) is a morphological character that has been associated with resistance to Mediterranean corn borer (MCB), *Sesamia nonagrioides* Lef. The present study is the first research to evaluate the usefulness of this trait in breeding programs. Six maize hybrids, from a complete diallel set of four inbred lines (two resistant and two susceptible to MCB), were evaluated under early and late sowing conditions at three locations in Northwestern Spain. General and specific combining ability (GCA and SCA, respectively) for LIBR were estimated, and LIBR correlations with grain yield and other important agronomic traits were evaluated. Hybrid by environment interactions were not significant for LIBR and the sums of squares partitioning indicated a greater GCA effect (95%), suggesting that this trait is stable and shows important additive effects for this set of hybrids. Correlation coefficients indicate that selection for increasing LIBR could enhance grain yield and other related plant traits (height and silking), but also an increase in the MCB susceptibility. Based on the limited number genotypes evaluated, LIBR could be modified by selection; however, if LIBR is used as an indirect selection criterion to improve MCB resistance, some negative effects on yield may be expected.

Keywords: *Zea mays*, *Sesamia nonagrioides*, diallel cross combining ability, intercalary meristem, structural borer resistance.

Introduction

The Mediterranean corn borer (MCB), *Sesamia nonagrioides* Lef., also called pink stem borer, is the most important pest of maize in Northwestern Spain (Velasco *et al.*, 2007). There is ample evidence to suggest that morphological and structural maize defenses have a role as resistant factors to borer attack (Malvar *et al.*, 2008).

Length of the internode basal ring (LIBR) refers to the area located between the node complex and the pulvinus line in the internode. The rind tissue corresponding to this area is light green or white in color, contrasting with the darker green color of the rest of the internode (Kiesselbach, 1999). This region arises from the intercalary meristem at the base of the internode and includes the youngest, least differentiated tissues. Cell walls of the lower internode are physiologically younger and less developed (less lignified) than those of the upper internode (Morrison *et al.*, 1998; Jung *et al.*, 1998).

Neonate larvae of MCB feed around the basal area of the internode before penetrating into the stem (Butrón *et al.*, 2002). Impact of LIBR was evaluated previously by Santiago *et al.* (2003) who observed differences between MCB susceptible and resistant inbred lines. Susceptible inbreds had the largest LIBR, suggesting that the size and/or properties of this area could be related to the ability of the larvae to enter the plant. Furthermore, this character was highly related ($r = 0.84$, $P < 0.01$) with stem damage, measured as tunnel length (Santiago *et al.*, 2003). Current studies with the synthetic variety EPS20 (originating from the US Corn Belt population “Reid”) showed a significant simple positive correlation (0.95) of LIBR and

68 resistance to MCB, measured as tunnel length, over seven cycles of
69 selection for resistance (unpublished data).

70 The relationship of LIBR with corn borer resistance suggests that
71 LIBR may be a useful indirect selection trait for resistance to MCB. There is
72 no information about inheritance of LIBR or its relationship with other
73 economically important traits. In addition, in the previous study LIBR was
74 only evaluated in a single environment. Therefore, the current study was
75 conducted to assess the usefulness of this trait in future selection programs.
76 Three objectives were evaluated: first, to estimate the general and specific
77 combining ability (GCA and SCA, respectively) of LIBR in a set of inbreds
78 previously evaluated for this trait; second, to determinate the stability of
79 LIBR among different environments; and third, to evaluate the relationship
80 of LIBR with important agronomic traits, such as grain yield, and pest
81 resistant traits such as MCB tunnel length.

82

Materials and Methods

Four unrelated inbred lines of maize (CM151, EP39, EP42 and EP47) from the germplasm collection of the Misión Biológica de Galicia (MBG), previously evaluated by Santiago et al. (2003) for LIBR, were selected for this study. Lines resistant to MCB (CM151 and EP39) had lower LIBR values, while susceptible lines (EP42 and EP47) had higher LIBR values in the previous study. Inbred lines were mated in 2006 to produce a complete diallel set of crosses excluding reciprocals. The six hybrids were evaluated in a randomized complete block design with three replicates. Field trials were grown in Pontevedra (Northwestern Spain) in 2007 at three different locations (20, 50 and 300 meters above sea level) and two sowing dates (early and late). Each plot had two rows, with 15 plants per row, for a density of approximately 60,000 plants ha⁻¹.

Data collected for each plot were days to 50 % silking (days from sowing to the date when 50% of plants showed silks), plant height (cm), kernel moisture at harvest (%), grain yield (weight of grain expressed as Mg ha⁻¹, adjusted to a kernel moisture of 140 g H₂O kg⁻¹), LIBR (mm), and MCB tunnel length (cm). Tunnel length data were measured in the same hybrids and similar field trials in 2008 and 2009. Five typical plants from each experimental plot were randomly selected for recording observations on plant height and kernel moisture. The LIBR area was identified by its light green or white color, in contrast to the darker green color of the rest of the internode (Kiesselbach, 1999), and the LIBR was measured at the base of the fourth internode above ground with a Vernier caliper for 10 typical plants. The LIBR measurement was made in the middle of the long axis of

108 the node cross section on one side of the stalk surface, after removing the
109 sheath (see Figure 1A in Santiago et al., 2003). Natural MCB infestation
110 was evaluated as tunnel length at two sowing dates (early and late). Stems
111 of five plants were split into longitudinally and tunnel length was measured.

112 Combined analyses of variance across environments were performed
113 considering environment, replication, genotype, and their interactions as
114 sources of variation. Each sowing date-location was considered one
115 environment. Genotypes were considered as fixed effects, while the
116 remaining sources of variation were considered as random effects. Variation
117 among hybrids of the diallel was further partitioned into GCA and SCA.
118 Griffing's Method 4, Model I (fixed effects) (Griffing, 1956) was used to
119 determine GCA, SCA, and their interactions with environment for LIBR,
120 days to silking, plant height, kernel moisture, and grain yield. The diallel
121 was analyzed using a program developed by Zhang and Kang (1997). Mean
122 comparisons were accomplished using Fisher's protected least significant
123 difference (LSD) method. The phenotypic and genotypic correlations, and
124 their standard errors between pair of traits, were calculated as suggested by
125 Holland (2006). All analyses were performed using the SAS software
126 package (SAS Institute, 2007)

127

Results and Discussion

The combined analysis of variance of the diallel indicated significant GCA effects for all traits except tunnel length. Because maize plants in the trials were not artificially infected with MCB larvae, the lack of significance of GCA for tunnel length was expected. Specific combining ability effects were significant ($P < 0.05$) for silking and plant height. Interactions of GCA by environment were significant for days to silking, plant height, and grain yield while SCA by environment interactions were significant for silking, plant height, and kernel moisture (data not shown).

Orthogonal subdivision of GCA and SCA effects showed that SCA explained 5% of the sum of squares due to hybrids while GCA effects explained 95% (data not shown). Consequently, most of the variation for LIBR among this set of hybrids was controlled by genes with additive gene action as opposed to other types of gene action (dominance, epistasis, overdominance, etc). In addition, there were no significant interactions with environment, indicating that genotypic differences for LIBR were environmentally stable.

All hybrids obtained from the susceptible inbred line EP47 showed high values for LIBR, with the maximum (5.59 mm) observed in the resistant by susceptible EP39×EP47 hybrid. The LIBR values for that hybrid were not significantly different from the CM151×EP47 and EP42×EP47 hybrids. Hybrids obtained from resistant inbred line CM151 showed the lowest values for LIBR, with lowest LIBR observed in resistant by resistant hybrid CM151×EP39. There were no significant differences in mean tunnel length among the hybrids under these natural infestation conditions.

153 However, the resistant by resistant CM151×EP39 hybrid had the lowest
154 (5.94 cm) mean tunnel length.

155 The hybrids EP42×EP47 and EP39×EP47 had the highest grain
156 yields (up to 7.34 Mg ha⁻¹), and CM151×EP47 was not different from these
157 two highest yielding hybrids. The hybrid CM151×EP39 had the lowest yield
158 (5.5 Mg ha⁻¹). The inbred EP47 was a parent in all the highest yielding
159 crosses and CM151 was a parent in the lowest yielding crosses. Inbred line
160 EP47 was the only inbred that had a significant positive GCA for LIBR
161 (0.49 mm), whereas CM151 had a significant negative GCA (-0.26 mm).
162 Previous resistance evaluations of these inbreds to MCB showed that
163 CM151 exhibited significantly less *S. nonagrioides* damage, lower larvae
164 incidence, and reduced larval growth than EP47 (Butrón *et al.*, 1999; Ordás
165 *et al.*, 2002); however, GCA effects for tunnel length were not significant in
166 the current study. Significant GCA effects for yield were detected for the
167 inbreds EP47 and CM151. Compared to the hybrid average, crosses
168 including EP47 and CM151 increased (1.02 Mg ha⁻¹) and reduced (0.62 Mg
169 ha⁻¹) grain yield (Table 1), respectively. Although the inbred line CM151
170 may be a good parent in hybrid combination relative to MCB damage, its
171 negative effects on yield must be considered.

172 Concerning MCB damage, just natural infestation was possible in
173 order to prevent pest dispersion. To clarify the results, only the crosses
174 CM151 (resistant) × EP39 (resistant) and EP47 (susceptible) × EP42
175 (susceptible) are discussed. Damage was insignificant (means around 0.5
176 cm) for one location, therefore only results for two locations and the three
177 years evaluated are presented (Table 2). In general, the cross between

178 resistant lines (CM151×EP39) showed less damage than the cross between
179 susceptible lines (EP47×EP42), although just differences were significant in
180 2008 early sow and evaluation in location 1, and 2009 late sow and
181 evaluation in location 2 (Table 2). The results suggest, as previously
182 mentioned, that LIBR and MCB resistance could be negatively linked in
183 agreement with previous research (Santiago et al. 2003).

184 Both genotypic and phenotypic correlation coefficients among traits
185 were calculated. Maize LIBR had significant positive genotypic correlations
186 with grain yield (1.00) and silking (0.95). Phenotypic correlations were
187 significant and positive between LIBR and grain yield (0.49), plant height
188 (0.49), and silking (0.45); but no significant correlations of LBIR with
189 tunnel length were detected. Several studies showed that selection to
190 improve maize resistance to insect pests has been associated with
191 unfavorable responses in grain yield (Russell *et al.*, 1979; Klenke *et al.*,
192 1986; Nyhus *et al.*, 1989; Butrón *et al.*, 2002; Sandoya *et al.*, 2008). The
193 correlation coefficients indicate that selection for increasing LIBR could
194 enhance yield and its components (plant height and silking); however, the
195 resulting genotypes should be more susceptible to MCB (Santiago *et al.*,
196 2003). However, the resistant lines may be inherently lower yielding due to
197 factors not related to resistance to stem borers or length of LIBR. Evaluation
198 of more resistant and susceptible genotypes will be needed to determine if
199 the observed correlations are causal or simply due to limited genetic
200 sampling.

201 In conclusion, the importance of additive effects and the stability
202 over different environments of LIBR for the inbred lines studied indicate

203 that LIBR can be effectively modified and acceptable gain from selection
204 can be expected. In the inbred lines studied, we expect that selection for
205 increasing LIBR would increase yield and other important agronomic traits
206 such as plant height and silking, but also could increase susceptibility to
207 MCB. Future studies should establish if the LIBR-yield relationship is direct
208 or is dependant on other traits related with yield such as plant height or
209 vigor. With regard to corn borer resistance, these preliminary results suggest
210 that LIBR may be useful in breeding programs for MCB resistance.
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273 Table 1. Estimates of General Combining Ability (GCA) and means for
 274 tunnel length, LIBR and grain yield from a complete diallel of four inbred
 275 lines of maize.

<i>General Combining Ability (GCA)</i>			
Inbred	Tunnel length	LIBR	Grain Yield
	(cm)	(mm)	(Mg ha⁻¹)
CM151 (R)	-0.30	-0.26*	-0.62*
EP39 (R)	0.16	-0.13	-0.30
EP42 (S)	0.95	-0.09	-0.08
EP47 (S)	-0.80	0.49*	1.02*
<i>Means¹</i>			
Hybrid	Tunnel length	LIBR	Grain Yield
	(cm)	(mm)	(Mg ha⁻¹)
CM151 (R) × EP39 (R)	5.94a	4.73c	5.38c
CM151 (R) × EP42 (S)	10.22a	4.88c	6.11bc
CM151 (R) × EP47 (S)	8.10a	5.53a	6.62ab
EP39 (R) × EP42 (S)	9.23a	5.12b	6.31bc
EP39(R) × EP47(S)	8.98a	5.59a	7.34a
EP42(S) × EP47(S)	7.60a	5.53a	7.43a

276

277 * GCA effects, significantly different from zero at $P < 0.05$.

278 ¹ Least Significant Difference (LSD) for LIBR was 0.25 mm and LSD for
 279 grain yield was 1.03 Mg ha⁻¹.

280 Means within a column not sharing a common letter are significantly
 281 different ($P \leq 0.05$).

282 Table 2. Means for natural tunnel length (cm) in two locations for early and late sowing and evaluation in the resistant by resistant (CM151 ×
283 EP39) and susceptible by susceptible (EP42×EP47) hybrids during three years.

Trials	Sowing Time	Tunnel Length Evaluation	Tunnel length (cm)					
			Year 2007		Year 2008		Year 2009	
			CM151 × EP39 (R × R)	EP42 × EP47 (S × S)	CM151 × EP39 (R × R)	EP42 × EP47 (S × S)	CM151 × EP39 (R × R)	EP42 × EP47 (S × S)
Location 1	Early	Early	5.3	21.0	3.3*	13.7*	13.3	16.4
	Early	Late	1.1	1.6	10.1	9.3	16.4	28.9
	Late	Early	3.3	1.0	5.1	11.5	12.5	15.9
	Late	Late	5.8	10.3	13.9	20.7	22.7	41.7
Location 2	Early	Early	9.0	14.0	12.5	14.3	5.2	9.7
	Late	Early	12.6	14.1	8.3	5.7	3.7	8.7
	Early	Late	18.0	15.0	2.7	7.2	2.2	15.1
	Late	Late	24.9	17.0	7.3	7.7	8.9*	20.9*

284 * Hybrid means within year, location, sowing time, and evaluation were statistically significant different at 5% level of probability