**Plant Breeding**

*Short communication*

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

J. Barros*, R.A. Malvar, A. Butrón, and R. Santiago

Misión Biológica de Galicia, Spanish Council for Scientific Research. CSIC

Apartado 28, 36080

Pontevedra-Spain.

Phone +34986854800

Fax +34986 841362

* Corresponding author jbarros@mbg.cesga.es

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 resistance to MCB, measured as tunnel length, over seven cycles of selection for resistance (unpublished data).

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

## 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 $H_2O$ $kg^{-1}$), 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 the node cross section on one side of the stalk surface, after removing the sheath (see Figure 1A in Santiago et al., 2003). Natural MCB infestation was evaluated as tunnel length at two sowing dates (early and late). Stems of five plants were split into longitudinally and tunnel length was measured.

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

## 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.

However, the resistant by resistantCM151×EP39 hybrid had the lowest (5.94 cm) mean tunnel length.

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

Concerning MCB damage, just natural infestation was possible in order to prevent pest dispersion. To clarify the results, only the crosses CM151 (resistant) × EP39 (resistant) and EP47 (susceptible) × EP42 (susceptible) are discussed. Damage was insignificant (means around 0.5 cm) for one location, therefore only results for two locations and the three years evaluated are presented (Table 2). In general, the cross between resistant lines (CM151×EP39) showed less damage than the cross between susceptible lines (EP47×EP42), although just differences were significant in 2008 early sow and evaluation in location 1, and 2009 late sow and evaluation in location 2 (Table 2). The results suggest, as previously mentioned, that LIBR and MCB resistance could be negatively linked in agreement with previous research (Santiago et al. 2003).

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

In conclusion, the importance of additive effects and the stability over different environments of LIBR for the inbred lines studied indicate that LIBR can be effectively modified and acceptable gain from selection can be expected. In the inbred lines studied, we expect that selection for increasing LIBR would increase yield and other important agronomic traits such as plant height and silking, but also could increase susceptibility to MCB. Future studies should establish if the LIBR-yield relationship is direct or is dependant on other traits related with yield such as plant height or vigor. With regard to corn borer resistance, these preliminary results suggest that LIBR may be useful in breeding programs for MCB resistance.

## Acknowledgments

This research was supported by the National Plan for Research and Development of Spain (Projects Cod. AGL2006-13140, AGL 2009-09611). J. Barros acknowledges a grant from the Ministry of Science and Innovation (Spain). R. Santiago acknowledges a postdoctoral contract "Juan de la Cierva" partially financed by the European Social Fund.

## References


Butrón, A., R.A. Malvar, P. Revilla, A. Ordás, and A. Álvarez, 1999: Resistance of maize inbreds to pink stem borer. Crop Science **39**, 102-107.

Butrón, A., R.A. Malvar, P. Revilla, P. Soengas, and A. Ordás, 2002: Rind puncture resistance in maize: inheritance and relationship with resistance to pink stem borer attack. Plant breeding **121**, 378-382.

Griffing, B., 1956: Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Science **9**, 463-493.

Holland, J.B., 2006: Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. Crop Science **46**, 642-654.

Jung, H.G., Morrison, T.A., Buxton, D.R., 1998: Degradability of cell-wall polysaccharides in maize internodes during stalk development. Crop Science **38**, 1047–1051.

Kiesselbach, T.A., 1999: The structure and reproduction of corn. 50th Anniversary ed. Cold Spring Harbor Laboratory Press, New York.

Klenke, J.R., W.A. Russell and W.D. Guthrie, 1986: Grain yield reduction caused by second-generation European corn borer in BS9 corn synthetic. Crop Science **26**, 859 - 863.

Malvar, R.A., A. Butrón, B. Ordás and R. Santiago, 2008: Causes of natural resistance to stem borers in maize, In: E. N. B. a. P. V. Williams, (ed.) Crop Protection Research Advances. Nova Science Publishers, Inc., pp 57-100.

Morrison, T.A., Jung, H.J.G., Buxton, D.R., Hatfield, R.D, 1998: Cell-wall composition of maize internodes of varying maturity. Crop Science **38**, 455-460.

Nyhus, K.A., W.A. Russell and W.D. Guthrie, 1989: Changes in agronomic traits associated with recurrent selection in two corn synthetics. Crop Science **29**, 269-275.

Ordás, B., A. Butrón, P. Soengas, A. Ordás, and R.A., Malvar, 2002: Antibiosis of the pith maize to *Sesamia nonagrioides* (Lepidoptera: *Noctuidae*). Journal of Economic Entomology **95(5)**, 1044-1048.

Russell, W.A., G.D. Lawrance, and W.D. Guthrie., 1979: Effects of recurrent selection for European corn borer resistance on other agronomic characters in synthetic cultivars of maize. Maydica **24**, 33-47.

SAS Institute, 2007: The SAS System. SAS Online Doc. HTML Format Version Eight. Cary, North Carolina, SAS Institute Inc.

Sandoya, G., A. Butrón, A. Álvarez, A. Ordás, and R.A. Malvar, 2008: Direct response of a maize synthetic to recurrent selection for resistance to stem borers. Crop Science **48**, 113-118.

Santiago, R., X.C. Souto, J. Sotelo, A. Butrón, and R.A. Malvar, 2003: Relationship between maize stem structural characteristics and resistance to pink stem borer (Lepidoptera: *Noctuidae*) attack. Journal of Economic Entomology 96, 1563-1570.

Velasco, P., P. Revilla, L. Monetti, A. Butrón, A. Ordás, and R.A. Malvar, 2007: Corn borers (*Lepidoptera : Noctuidae; Crambidae*) in

Northwestern Spain: Population dynamics and distribution. Maydica **52**, 195-203.

Zhang, Y., and M.S. Kang, 1997: DIALLEL-SAS: A SAS Program for Griffing's Diallel Analyses. Agronomy Journal **89**, 176-182.

Table 1. Estimates of General Combining Ability (GCA) and means for tunnel length, LIBR and grain yield from a complete diallel of four inbred lines of maize.

| | *General Combining Ability (GCA)* | | |
|---|---|---|---|
| **Inbred** | **Tunnel length (cm)** | **LIBR (mm)** | **Grain Yield (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* |
| | *Means* [1] | | |
| **Hybrid** | **Tunnel length (cm)** | **LIBR (mm)** | **Grain Yield (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 |

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

[1] Least Significant Difference (LSD) for LIBR was 0.25 mm and LSD for grain yield was 1.03 Mg ha$^{-1}$.

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

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

| | | | **Tunnel length (cm)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Year 2007** | | **Year 2008** | | **Year 2009** | |
| **Trials** | **Sowing Time** | **Tunnel Length Evaluation** | **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* |

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