

**Effects of selection for resistance to *Sesamia nonagrioides* on maize yield,
performance and stability under infestation with *Sesamia nonagrioides* and
Ostrinia nubilalis in Spain**

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Abbreviations:

E: Environmental main effects

G: Genotype main effects

GE: Genotype × environment interaction

GGE: G plus GE interaction

SREG: Sites Regression Model

MCB: Mediterranean Corn Borer

ECB: European Corn Borer

Abstract. A selection program to improve resistance to the Mediterranean corn borer (MCB, *Sesamia nonagrioides* Lef) while maintaining yield was carried out in a maize synthetic population. The objectives of this research were to investigate if yield and yield stability of the maize synthetic population named EPS12 were affected by selection for MCB resistance, and to determine, which genotypic and environmental covariates could explain the Genotype (G), Environment (E), and Genotype \times Environment (GE) effects for yield under corn borer infestation. Plants from three cycles of selection and their testcrosses to three inbred testers (A639, B93, and EP42) were evaluated at two locations in two years, under MCB and ECB infestations. After selection EPS12 was a more stable genotype. Hybrids derived from crosses between B93 and inbreds obtained from the initial cycles of selection could be recommended for cultivation in northern Spain. The yield of crosses between cycles of selection and testers increased with when there were fewer days with mean temperatures $> 25^{\circ}\text{C}$ and higher mean maximum temperatures. Differences for yield among these genotypes were mostly explained by resistance to corn borer attack. In general, among EPS12 derived materials, genetic characteristics that contribute to increased grain yield were also responsible for increased abiotic stress tolerance.

Key words: maize, yield, stability, GGE interaction, *Sesamia nonagrioides*, *Ostrinia nubilalis*

1 **Introduction**

2
3 Insect herbivores cause high yield losses in crops. In maize (*Zea mays* L.), yield losses
4 due to insect pest attacks are in average 16% (Oerke, 2006). In southern Europe, there
5 are two species of corn borer which attack maize, namely European corn borer (ECB),
6 *Ostrinia nubilalis* Hbn (Lepidoptera: Crambidae), and Mediterranean corn borer
7 (MCB), *Sesamia nonagrioides* Lef, (Lepidoptera: Noctuidae) (Cordero *et al.*, 1998;
8 Velasco *et al.*, 2007). In Spain, MCB seems to be the most damaging pest of maize
9 (Castañera, 1986), specifically, in northwestern Spain where periodic samplings of
10 maize indicated that there were 1.2 larvae of MCB and 0.12 larvae of ECB per plant
11 (Cordero *et al.*, 1998). Larvae of the second and subsequent generations of these borers
12 attack maize near flowering time by entering into the stem, causing stem-tunneling that
13 weakens plants, provokes lodging and reduces yield.

14 In Spain, maize is produced in two well-differentiated regions - northern Spain
15 and inland Spain. Northern Spain has an Atlantic climate with mild temperatures and
16 high rainfall, whereas inland Spain has a dry climate, low rainfall and warmer
17 temperatures during the growing season. Mediterranean corn borer is more abundant in
18 coastal northwestern Spain, whereas ECB and MCB populations are equally important
19 in central Spain (Malvar *et al.*, 1993; Cordero *et al.*, 1998; Velasco *et al.*, 2007).

20 Because insect damage impacts maize yield, it is important to select genotypes that
21 perform stably across environments with different climates and borer species
22 populations. Identifying superior cultivars for the target region(s) should be done by
23 conducting regional performance trials. Superior cultivar performance in a particular
24 environment is a combination of environment main effects (E), genotype main effects
25 (G), and genotype \times environment interaction effects (GE). When high levels of

variation are due to environmental factors, use of the Sites Regression (SREG) method is recommended (Crossa & Cornelius, 1997). This multiplicative method considers G and GE effects simultaneously (Yan *et al.*, 2000) and has been used by several authors to study yield potential and stability (Malvar *et al.*, 2005; Castillo *et al.*, 2005; Fan *et al.*, 2007; Setimela *et al.*, 2007).

In addition, the Factorial Regression approach can be used to obtain a biological explanation of genotype (G) and environmental (E) main effects, and GE interaction effects (Denis, 1988; Vargas *et al.*, 1999). Epinat-Le Signor *et al.* (2001) studied the grain yield stability of 132 hybrids across 12 years and different locations in France and they determined that most of the GE variability was due to differences for earliness and yield limiting factors. Malvar *et al.* (2005) studied the performance of crosses among French and Spanish populations across eight environments, concluding that effects of G, E, and GE for grain yield were mainly due to earliness, vigor effects, and/or environmental factors related to cold stress. Neither genotypic covariates nor environmental covariates related to resistance to stem borers and level of infestation, respectively, were taken into account in these studies.

Butrón *et al.* (2004) studied G, E, and GE for yield under natural infestation by *S. nonagrioides* and concluded that GE effects were mainly due to earliness, vigor effects, and environmental factors such as average minimum temperature and percentage of air humidity.. However, the study was conducted with inbreds from different backgrounds evaluated under natural conditions, a situation that could preclude detection of relationship between resistance and yield. In the present study, three selection cycles developed by classical plant breeding that shared a common background, and their crosses to testers were used. Therefore any possible

overestimation or underestimation of resistance was avoided because genotypes were evaluated under artificial infestations with MCB and ECB eggs.

A selection program was carried out in the EPS12 maize synthetic population to improve resistance to MCB while maintaining yield by using the S_1 intrapopulational recurrent selection method. Stem damage was reduced significantly whereas yield did not significantly decrease (Sandoya *et al.*, 2008). However, the study of the effects of selection on yield and yield stability should be done together because genotype \times environment effects for yield could be as important as main genotype effects. This type of study should address the selection cycle that is the best in terms of yield performance and stability as well as resistance to insect attack. Since the final goal of maize breeding is the development of better hybrids, it is important to determine the possible biological causes for yield variation in the crosses of cycles of selection with testers. Therefore, the objectives of this research were to investigate if yield and yield stability were affected by selection for MCB resistance and to determine which genotypic and environmental covariates explain the Genotype (G), Environment (E), and Genotype \times Environment (GE) effects for yield under corn borer infestation using crosses between selection cycles with testers.

Materials and methods

The Selection Program of EPS12 maize synthetic population

The S₁ recurrent selection program used to improve resistance of EPS12 against MCB was initiated in 1993 with about 150 S₁ families. In 1994, 100 S₁ progenies were evaluated under artificial infestation with eggs of *S. nonagrioides*, and the 10 lines that showed the shortest stem tunnel length and yield above the mean of the 100 families were selected. In 1995, selected families were recombined, and the first cycle of recurrent selection EPS12(S)C1 was established in 1996, 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 S₁ families were obtained from EPS12(S)C1-Syn1 before recombination to obtain EPS12(S)C1.

Plant material and methods

Twelve genotypes were used to study the changes in stability due to selection while nine of these genotypes were used to find the relationships between genotypic and environmental covariates and yield. These genotypes were the three cycles of selection *per se* derived from EPS12 (used only to study changes in stability), and their testcrosses with three different inbred testers (EP42, B93 and A639 representing the humid Spain, the Lancaster and the Reid heterotic groups, respectively). Evaluations were made in two different types of experiments, one infested with MCB eggs and another one with ECB eggs, at two locations in two years, resulting in eight different trials.

In each trial, the twelve populations studied were arranged in a randomized complete block design with three replications. The experiments were conducted in two well-differentiated Spanish locations: 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 two years - 2003 and 2004. In Pontevedra, each experimental plot was hand-planted and consisted of two rows spaced 0.80 m. apart with 25 two-plant hills spaced 0.21 m. apart. Plots were overplanted and thinned to leave 1 plant per hill, the final density being $\approx 60,000$ plants ha^{-1} . In Zaragoza, plots were machine-planted and consisted of two rows spaced 0.75 m apart with 27 two-plant hills spaced 0.18 m apart. Plots were overplanted and thinned, and the final density was $\approx 74,000$ plants ha^{-1} . In each location and year, trials infested with MCB and ECB eggs were adjacent.

At flowering, artificial infestations with MCB eggs were carried out in ten adjacent and competitive plants per plot. Infestations were made by laying a mass of about 40-50 eggs between the main ear and the stem, as described by Butrón *et al.* (1998). The MCB rearing method used was described by Eizaguirre & Albajes (1992), the rearing methodology was carried out in the insect rearing laboratory at the Mision Biologica de Galicia (Pontevedra – Spain). ECB eggs were supplied by the Institute National de la Recherche Agronomique (France).

Traits recorded

The traits recorded were as follows: tunnel length (at harvest, stalks of ten infested plants per plot were longitudinally split to measure the total length in cm per plant of tunnels made by borers), percentage of stem damaged (estimated as tunnel length divided by plant height), yield (expressed as Mg ha^{-1} at $140 \text{ g H}_2\text{O kg}^{-1}$), visual ratings for ear, cob, shank, grain, and husk damages (on a 9 point subjective scale determined

as follows: 1 = > 90% damaged, 2 = 81 to 90% damaged, 3 = 71 to 80% damaged, 4 = 61 to 70% damaged, 5 = 41 to 60% damaged, 6 = 31 to 40% damaged, 7 = 21 to 30% damaged, 8 = 1 to 20% damaged, and 9 = 0%), early vigor (at approximately five-leaf stage, on a subjective scale from 1= the least vigorous to 9= the most vigorous plants], plant and ear heights (recorded on ten competitive plants, length from the surface to the node of the male inflorescence and to the insertion of the main ear, respectively), days to pollen shedding (days from planting to 50% of plants shedding pollen), days to silking (days from planting to 50% of plants showing silks), ear-row number, ear length (cm), and 100-kernel weight (g).

Tunnel length and visual ratings for ear, cob, shank, grain and husk damages were recorded in the infested plants, whereas, plant and ear height, ear-row number, ear length, and 100-kernel weight were taken in ten randomly-chosen plants per plot. Days to pollen shedding and to silking as well as yield were recorded on per plot basis. All traits except grain yield were considered genotypic covariates.

To obtain a biological explanation for the E and GE effects, some environmental variables defining environmental conditions during maize growth period were recorded. These included number of surviving larvae of MCB and ECB per stem and ear (values recorded in the ten infested plants), stem tunnel length estimated in cm in each environment, daily mean minimum, maximum and daily mean temperatures in °C, rainfall in mm, percentage of air humidity, and the number of days with daily mean temperatures > 30 °C, > 25 °C, <15 °C, and <10 °C. Meteorological data were recorded by stations that were < 500 m from trials.

Statistical Analysis

SREG Method

Each environment was defined as the combination of a year, a location, and infestation with a particular borer species; resulting in eight different environments under study. The SREG method was used to study the GGE component of yield variability among cycles of selection and crosses of cycles to testers.

The fixed-effect two-way model for the analysis of multienvironmental genotype trials by the SREG model is as follows (Cornelius *et al.*, 1996; Crossa & Cornelius, 1997):

$$Y_{ij} = \mu + \beta_j + \sum \xi_{ik}^* \eta_{jk}^*$$

where k goes from 1 to r , with r = number of principal components (PCs) required to approximate the original data. Y_{ij} is the mean grain yield of genotype i in the environment j ; μ is the mean value, β_j is the environmental main effect and, ξ_{ik}^* and η_{jk}^* are the i th genotype and the j th environmental scores on the k th PC, respectively. The analysis was performed with plot data as is shown by the degrees of freedom. SREG analysis was computed by a SAS (SAS Institute, 2007) program which was developed by Burgueño & Crossa (2003).

With the SREG method, PC analysis is made on residuals of an additive model with environmental effects being the only main ones. Therefore, the term $\sum \xi_{ik}^* \eta_{jk}^*$ contains the variation due to G and GE interactions. A two-dimensional biplot (Gabriel, 1971) called GGE biplot (G plus GE interaction) of the first two PCs was used to display the genotypes and the environments simultaneously (Yan *et al.*, 2000). Each genotype and environment was defined by its respective score on the two PCs. The which-won-where view method of the GGE biplot (Yan *et al.*, 2000) was also used to predict which genotype is particularly favored in each environment.

Factorial regression method

For this analysis, each environment was considered as the combination of a location and a year because all environmental covariates detected as significant by the stepwise method were indeed meteorological variables that were common to both MCB and ECB infested trials. Therefore only four environments were taken into account.

Cycles *per se* were not included in this analysis. As cycles and cycles testcrossed to testers have different levels of heterosis, the inclusion of both types of genotypes will presumably bring information about those genotypic covariables that better distinguish between cycles and crosses. However, our goal is to obtain a biological explanation on G and GE variability among crosses because they represent different heterotic patterns.

The general formula for a factorial regression model with K genotypic and H environmental covariates is (Denis, 1980; Vargas *et al.*, 1999):

$$Y_{ij} = \mu + [\sum \rho_k \cdot G_{ik} + \alpha_i] + [\sum \delta_h \cdot E_{jh} + \beta_j] + [\sum G_{ik} \cdot \theta_{kh} \cdot E_{jh} + \sum \alpha'_{ih} \cdot E_{jh} + \sum \beta'_{jk} \cdot G_{ik} + \varepsilon_{ij}]$$

where ρ_k and δ_h are the regression coefficients of genotypic G_{ik} , and environmental covariates E_{jh} , respectively; α_i and β_j are the residuals of genotype and environmental main effects respectively, respectively; θ_{kh} is the regression coefficient of the cross-product of covariates G_{ik} and E_{jh} ; α'_{ih} and β'_{jk} are the genotype i and environment j specific regression coefficients of genotypic covariate G_{ik} and environmental covariate E_{jh} , respectively; and ε_{ij} is the residual genotype \times environment interaction effect. All parameters of this model were considered fixed effects. The covariates and their order in the factorial regression model for grain yield were obtained by performing a stepwise regression on genotypic covariates and a second stepwise regression on environmental covariates (Denis, 1988). After standardization of covariates, factorial regression analyses were performed by the software INTERA (Decoux & Denis, 1991). All factors

1 were tested against residual experimental error.

Results

SREG

The SREG analysis showed that E and GGE variation for yield were highly significant (Table 1). The most important sum of squares for grain yield under infestation was E, which explained 42% of the total variation for yield while GGE explained 38%. 51% of the proportion explained by GGE was accounted for by GE while the remaining 49% was due to G effects.

From the eight principal components (PCs) obtained after singular value decomposition of location-centered yield, the first three PC's were highly significant and explained 89.89% of GGE variation; the remaining components were not significant. The first two PCs of the SREG explained 81.63% of GGE variation (Table 1). Mean values for grain yield were significantly higher in Pontevedra than in Zaragoza, and yield values in 2003 were significantly lower than in 2004 (Data not shown). In general, the three cycles of selection and their testcrosses to A639 showed negative values for the projections of the scores on the new abscissa *axe* (Fig. 1); meanwhile, the highest abscissa values were presented by EPS12C0 × B93 and EPS12C3 × EP42.

The ordinate values for the cycles of selection *per se* were low; but the initial cycle of selection presented a higher value than the next cycles. Among testcrosses, EPS12C0 × B93 and EPS12C0 × EP42 showed the lowest ordinate values.

According to Figure 1, the EPS12C0 × B93 would be favored compared to the other genotypes in Pontevedra in 2003 under MCB and ECB infestations, and in Zaragoza in 2004 under ECB infestation; meanwhile EPS12C2 × B93 showed the

highest positive interaction with Pontevedra in 2004 under MCB and ECB infestations, and in Zaragoza in 2004 under MCB infestation, $\text{EPS12C3} \times \text{EP42}$ would be favored in Zaragoza in 2003 under ECB infestation, and $\text{EPS12C0} \times \text{A639}$ in Zaragoza in 2003 under MCB infestation (Figure 1).

Factorial regression

The genotypic covariates detected as significant by the stepwise method were days to pollen shedding (PS), plant height (PH), ECB in the ear (ECB), tunnel length (TL), stalk lodging (SL), and days to silking (S); whereas number of days with daily mean temperatures $> 25^\circ\text{C}$ (TM25) and mean maximum temperature (Tmax) were detected as significant environmental covariates (Table 3).

Genotypes showed highly significant variation for S and ECB, while differences for PS, PH, SL, and TL were significant. Variability for PS, PH, SL, ECB, S and TL explained approximately 90% of the G variation. The regression coefficients of G variation for yield on PS, PH and ECB were positive ($\rho_{\text{PS}} = 4.64$, $\rho_{\text{PH}} = 0.05$, $\rho_{\text{ECB}} = 0.31$, respectively) and on SL and TL were negative ($\rho_{\text{SL}} = -0.20$ and $\rho_{\text{PS}} = -0.32$, respectively).

The environmental covariates (TM25 and Tmax) explained almost the 100% of the variation for E among testcrosses. The regression coefficients of the environmental covariates were positive for Tmax ($\delta_{\text{Tmax}} = 0.55$) and negative for TM25 ($\delta_{\text{TM25}} = -1.61$).

Three cross-products between genotypic covariates and the environmental covariate TM25 were significant, $\text{PH} \times \text{TM25}$ (highly significant), $\text{ECB} \times \text{TM25}$ (significant) and $\text{SL} \times \text{TM25}$ (highly significant), and explained approximately the 65% of the sum of squares for GE. The regression coefficients for the cross-products $\text{PH} \times \text{TM25}$, $\text{ECB} \times \text{TM25}$ and $\text{SL} \times \text{TM25}$ were -0.58, 0.09, and 0.62, respectively. The

cross-products between the genotypic covariate PS and the environmental covariable Tmax was significant and explained approximately the 4% of the sum of squares for GE. The regression parameters of yield on $PS \times Tmax$ was negative (-1.35).

The interaction of three genotypic covariates, ECB, TL, and S, with the residual environmental variation (Env) were significant for ECB and TL and highly significant for S, they explained the 20% of the sum of square for the interaction. The interactions of the environmental covariates (Tmax and TM25) with the residual genotype variation were not significant and explained less than 1.5% of the GE variation.

Discussion

The percentages of the total sum of squares for grain yield explained by E and GGE were similar to those reported by other authors, although the geographical proximity among locations and genetic variability were very different across studies (Butrón *et al.*, 2004; Malvar *et al.*, 2005). In the present study, artificial infestation, necessary to adequately estimate genotype resistance, could partly have homogenized the conditions across environments.

Percentage of variation due to G was lower than that reported by Butrón *et al.* (2004) for a set of 45 hybrids and by Malvar *et al.* (2005) for a diallel among 12 populations. Genotypes included in this study had similar genetic background because all were derived from the same population, EPS12. Therefore, although genotypic differences were diminished compared to other studies, the relationship between stability and genotype yield performance, as well as between genotypic covariates and GGE, were less biased by background differences than in previous studies.

The high percentage of GGE variation in the first two PCs of the SREG suggests that a biplot of PC1 and PC2 adequately approximates the environment-centered data (Yan *et al.*, 2007). The PC1 reflects the mean performance plus the noncrossover GE interaction if the primary effects of sites from the SREG model are all of the same sign in the two dimensional biplot (Yan *et al.*, 2000; Crossa *et al.*, 2002). PC2 represents the disproportionate yield differences across environments.

We used the symmetric scaling method for the biplot drawing because it has intermediate properties between the genotype and the environmentally-focused scaling method. This method does not show the genotypes with the largest yield at each

environment, but it does show the one particularly favored by these conditions compared to the other genotypes, independent of its mean value for yield.

Besides, we have also used the GGE biplot recommended by Yan *et al.* (2001) which forces the abscissas to present the genotype main effect and is, therefore, more interpretable in terms of mean performance and stability (In Figure 1, the black dotted lines become the x and y abscissas). Hence, new axes were obtained by using the average environment, which forces the abscissa to present the genotype main effect and, consequently, facilitates the interpretation of the biplot in terms of mean performance and stability of the genotypes (Yan, 2002). The two-dimensional biplot showed that cycles *per se* were grouped together as the yield worst producers.

Although it was not statistically significant, it was previously shown that the yield performance of genotypes from the EPS12 selection cycles was negatively affected by the selection process (Sandoya *et al.*, 2008), however, stability seemed to improve during selection. The higher genotype mean effect for yield was accompanied by lower stability across cycles *per se* or crossed to A639 and EP42. That suggests that higher heterosis is associated to lower stability. Nevertheless there was a positive relationship between stability and heterosis among cycles of selection crossed to B93,. Therefore, for the entire target region, inbreds will be preferentially obtained from the initial cycle of selection in order to obtain promising hybrids, in terms of yield performance and stability, when crossed to B93. Alternatively, a breeding program to improve the specific combining ability between EPS12 and B93 could be performed.

In Pontevedra the genotypes showed a better performance for yield and less cross-over interaction than in Zaragoza. This could be consequence of having multiplied and improved the synthetic population from which EPS12 was released, EPS7 (Vales *et al.*, 2001), and EPS12 itself at Pontevedra for more than 20 years. Adaptation to mild

climate conditions, such as those present in northern Spain, has probably been enhanced in parallel to the intended increases for yield and resistance to MCB attack. Therefore, we discourage any cultivar recommendation for Central Spain because these materials, although descendant from populations collected in Central Spain, no longer show adaptation to those environments. These results emphasize the preliminary nature of our study and reinforce the importance of choosing the target environment and performing selection based on performance across locations with well differentiated climatic characteristics if the target environment covers an extensive geographical region (Setimela *et al.*, 2005). Hence we will limit our recommendations to northern Spain.

Differences for yield between both infestation species were only significant in 2003 because temperatures were higher than usual, favoring pest development, especially MCB, which has an African origin (Sandoya *et al.*, 2008). The cross EPS12C0 \times B93 would be the best cultivar for years with exceptionally warm temperatures, such as those observed in 2003, but the cross EPS12C2 \times B93 would perform better across years with mean temperatures more similar to the average temperature of the last 25 years. Our main suggestion is to initiate a breeding program in EPS12C2 in order to improve its specific combining ability with B93 under infestation with MCB eggs.

The two regression coefficients of yield G variation on the genotypic covariates plant height (PH) and ECB in the ear (ECB) were positive, indicating that taller genotypes with higher presence of ECB in the ears are more productive, whereas stalk lodging (SL) and tunnel length (TL), both indirect and direct consequences, respectively, of corn borer attack were detrimental to yield. The effect of maturity on yield was low because regression coefficients for days to pollen shedding and to silking were similar in value and with opposite direction. Several authors (Argillier *et al.*, 1994;

Epinat-Le Signor *et al.*, 2001; Butrón *et al.*, 2004; Malvar *et al.*, 2005) reported that G variation for grain yield was mainly due to earliness and vigor. However, in the present study, all genotypes shared a common background and were infested with MCB and ECB eggs to avoid any possible escape of borer attack. In this scenario, precocity reduces considerably its influence on yield and, in consequence, genotypic characteristics related to heterosis, such as plant height, and characteristics related to resistance to borers, such as tunnel length and stem lodging, appeared as the most determinant for yield.

The factorial regression analyses showed that yield increased when the number of days with temperatures $> 25^{\circ}\text{C}$ (T) diminishes and mean maximum temperature (T_{max}) increases. Maize is a tropical crop and optimum temperatures during the growing period from sowing to tassel initiation could vary between 22 and 31°C , depending on the genotype (Ellis *et al.*, 1992). However, maize plants under high temperatures exhibit decreased leaf area index, less total biomass production, and loss of grain yield (Westgate *et al.*, 2004). Shaw (1988) suggested that, during reproductive development, each 1°C increase in temperature above optimum (25°C) results in reduction of 3 to 4% in grain yield. Cheik & Jones (1994) showed that kernels exposed to short-term (four days) heat stress exhibited a recovery in kernel growth, but kernel fresh and dry matter accumulation was severely reduced by long-term heat stress. Therefore, the number of days with mean temperature higher than 25°C would characterize heat stress conditions better than the daily maximum temperatures. In addition, the development of the MCB would be favored by more days with high mean temperatures because this species is a tropical moth. Once the unfavorable effects of heat stress and insect pressure are removed, warmer environments, characterized by higher mean maximum temperatures, would be more favorable for maize development.

The regression coefficients of yield on the cross-product between genotypic and environmental covariates showed that characteristics favorable for increased yield, except reduced stalk lodging, were more favorable under environments with higher stress. This positive interaction between yield-related traits and environmental stress explained more than 50% of variability for GE and agrees with the idea that genetic characteristics that contribute to increased grain yield could also be responsible for increased abiotic stress tolerance (Lee & Tollenaar, 2007). The positive SL \times TM25 interaction suggests that there is a competition between using resources to resist insect biotic stress (insect attack) and abiotic stress (heat).

Conclusions

Selection for corn borer resistance increased the stability of the maize synthetic EPS12 under artificial infestation with MCB and ECB. A positive correspondence between stability and yield performance was observed only when plants from cycles of selection were crossed to B93. Therefore, for the target region of the European Atlantic coast, we suggest initiating a breeding program with EPS12C2 to improve its specific combining ability with B93 under infestation with MCB eggs. Yield differences among these genotypes were mainly due to differences for resistance to corn borer attack; while, heat stress was the most yield limiting environmental factor. In EPS12, genetic characteristics that contribute to increased grain yield could also be responsible for increased abiotic stress tolerance.

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Table 1. Analysis of variance of the SREG multiplicative model for yield of the three cycles of selection *per se* of EPS12 and its testcrosses with three different testers evaluated under artificial infestations with MCB and ECB.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Cumulated % Variability
E †	7	288.50	41.21 **	
GGE ‡	88	263.15	2.99 **	
PC1	17	175.40	10.32 **	66.66
PC2	15	39.39	2.63 **	81.63
PC3	13	21.76	1.67 **	89.89
PC4	11	10.89	0.99	94.03
PC5	9	8.64	0.96	97.32
PC6	7	5.86	0.84	99.54
PC7	5	1.09	0.22	99.96
PC8	3	0.12	0.04	100.00
Error	192	136.32	0.71	

***,** Significant at 0.05 and 0.01 probability level, respectively**

† E = Environmental main effects, where one E is the combination of a location – year – infested specie

‡ GGE = Genotype + genotype × environment interaction effects

- 1 **Table 2. Means for grain yield (Mg ha⁻¹) in each environment and averaged across environments, and PC1 and PC2 values from the**
- 2 **SREG analysis of cycles of selection of EPS12 synthetic and their testcrosses evaluated under MCB and ECB artificial infestations.**

Environments†											
Genotypes	Mean	PoMCB2003	PoECB2003	ZaMCB2003	ZaECB2003	PoMCB2004	PoECB2004	ZaMCB2004	ZaECB2004	PC1	PC2
EPS12C0	5.83	4.98	6.08	3.95	4.70	6.72	6.56	5.70	7.93	-0.79	-0.39
EPS12C2	5.72	5.10	5.03	4.68	4.93	6.45	7.30	5.97	6.32	-0.95	-0.37
EPS12C3	5.38	4.76	5.52	4.28	3.95	5.90	5.29	6.43	6.88	-1.41	-0.28
EPS12C0×EP42	6.81	7.86	8.26	4.77	5.10	7.39	7.58	5.44	8.04	0.38	0.28
EPS12C2×EP42	6.87	7.07	8.13	4.12	4.72	8.41	8.00	6.29	8.23	0.49	-0.44
EPS12C3×EP42	7.36	7.43	8.10	5.90	6.31	7.72	8.83	6.02	8.56	0.73	0.60
EPS12C0×A639	6.61	6.67	7.28	5.26	6.85	6.66	6.41	6.22	7.57	-0.20	0.99
EPS12C2×A639	6.28	6.51	6.78	5.95	4.70	6.35	6.30	6.49	7.15	-0.56	0.52
EPS12C3×A639	6.18	6.58	6.81	5.17	4.67	6.76	6.57	6.06	6.84	-0.46	0.18
EPS12C0×B93	7.61	7.37	8.71	5.77	6.79	9.42	8.43	6.70	7.66	1.05	0.44
EPS12C2×B93	7.14	7.83	8.85	3.73	4.87	9.06	8.98	6.37	7.41	0.98	-0.67

EPS12C3×B93	7.14	6.38	7.73	4.79	4.93	9.34	9.54	6.79	7.63	0.76	-0.83
Mean		6.55	7.27	4.86	5.21	7.52	7.48	6.21	7.52		
Environments											
PC1		1.11	1.42	0.14	0.56	1.38	1.38	0.13	0.48		
PC2		0.57	0.29	1.03	1.08	-0.67	-0.69	-0.10	0.19		

1

2 †Environments are referred as PoMCB2003 (Pontevedra 2003 under MCB infestation), PoECB2003 (Pontevedra 2003 under ECB

3 infestation), ZaMCB2003 (Zaragoza 2003 under MCB infestation), ZaECB2003 (Zaragoza 2003 under ECB infestation) PoMCB2004

4 (Pontevedra 2004 under MCB infestation) PoECB2004 (Pontevedra 2004 under ECB infestation) ZaMCB2004 (Zaragoza 2004 under

5 MCB infestation), and ZaECB2004 (Zaragoza 2004 under ECB infestation), respectively

Table 3. Factorial regression analysis for yield of three cycles of selection and their testcrosses evaluated at two locations in two years. Environmental and genotypic covariates were previously detected with the stepwise method.

Source of variation	DF	Mean squares	Regression coefficient [†]	Variability explained [‡]
Genotypes (gen)	8	0.93**		
Days to pollen shedding (PS)	1	0.67 *	$\rho_{PS} = 4.64$	9.0
Plant height (PH)	1	0.55 *	$\rho_{PH} = 0.05$	7.3
ECB in the ear (ECB)	1	2.82 **	$\rho_{ECB} = 0.31$	37.8
Tunnel length (TL)	1	0.41 *	$\rho_{TL} = - 0.32$	5.5
Stalk lodging (SL)	1	0.59 *	$\rho_{SL} = - 0.20$	7.9
Days to silking (S)	1	1.85 **	$\rho_S = - 4.55$	24.8
Residual gen	2	0.29		7.8
Environment (env)	3	12.00 **		
TMed 25 (TM25) ‡	1	34.34 **	$\delta_{TM25} = -1.61$	95.4
T Maximun (Tmax) ‡	1	1.66 **	$\delta_{Tmax} = 0.55$	4.6
Residual env	1	0.01		
Gen × Env	24	0.49**		
PS × TM25	1	0.45		3.8
PH × TM25	1	5.00 **	$\theta_{PH-TM25} = 0.58$	42.5
ECB × TM25	1	0.46 *	$\theta_{ECB-TM25} = 0.09$	3.9
TL × TM25	1	0.07		0.5
SL × TM25	1	2.19 **	$\theta_{SL-TM25} = 0.62$	18.6
S × TM25	1	0.17		1.5

PS × Tmax	1	0.51 *	$\theta_{PS-TM25} = - 1.35$	4.3
PH × Tmax	1	0.01		0.1
ECB × Tmax	1	0.02		0.2
TL × Tmax	1	0.17		1.5
SL × Tmax	1	0.01		0.1
S × Tmax	1	0.04		0.3
PS × Env	1	0.03		0.3
PH × Env	1	0.02		0.2
ECB × Env	1	0.47 *		4.0
TL × Env	1	0.56 *		4.8
SL × Env	1	0.12		1.0
S × Env	1	1.35 **		11.5
Tmed25 × Gen	2	0.01		0.1
Tmax × Gen	2	0.07		1.1
Residual GE	2	0.05		0.9
Error	160	0.12		

*,** Significant at 0.05 and 0.01 probability level, respectively

[†] ρ_k and δ_h are the regression coefficients of genotypic G_{jk} and environmental covariates E_{jk} , respectively. θ_{kh} is the regression coefficient of the cross-product of covariates G_{ik} and E_{jh}

[‡] Percentage of the variability of the corresponding main or interaction effect explained by the covariate.

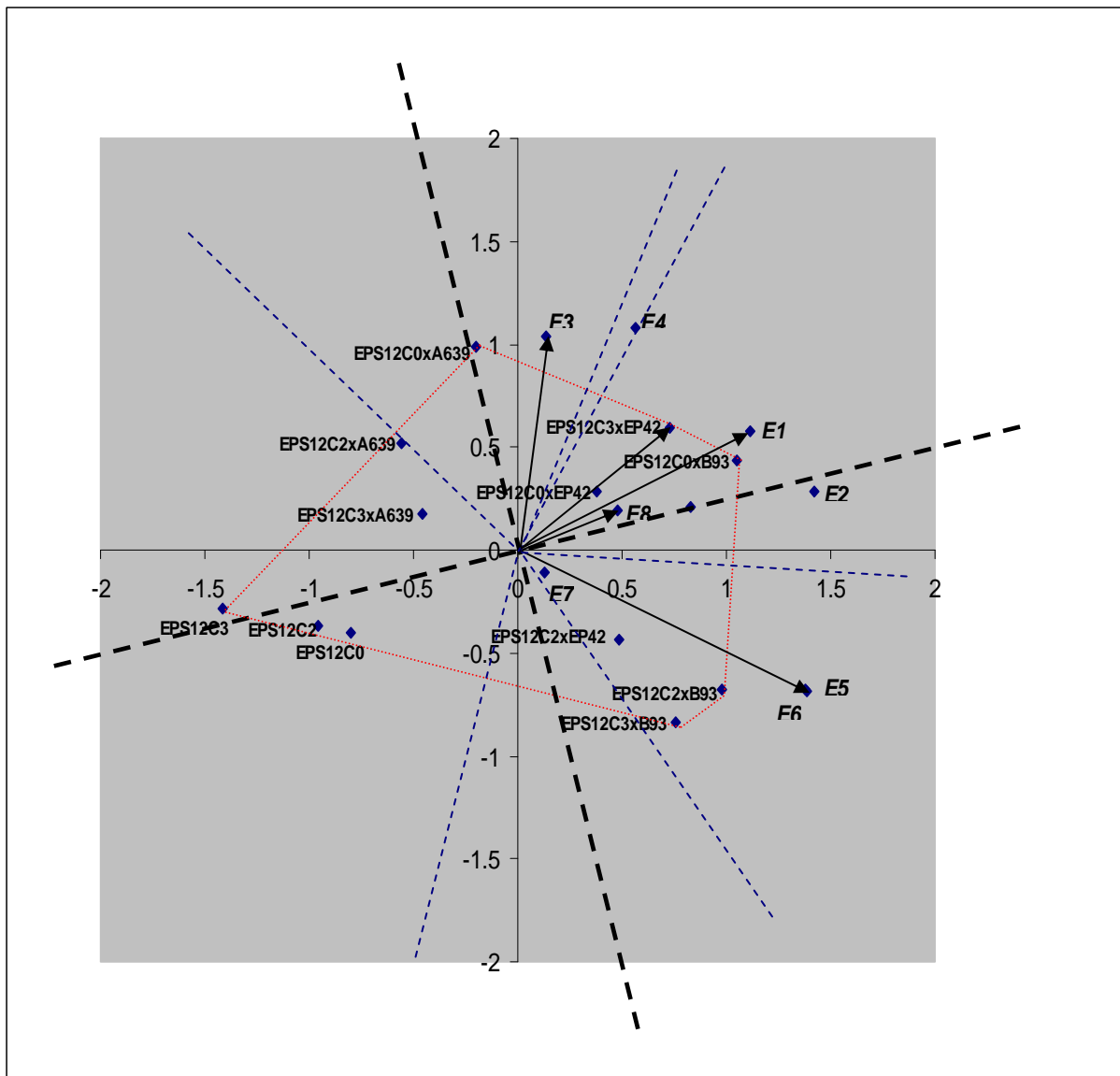


Fig. 1. The GGE biplot based on the yield performance of cycles of selection *per se* derived from the maize synthetic EPS12 and their testcrosses under MCB and ECB artificial infestations in two locations and two years. Environments are E1, E2, E3, E4, E5, E6, E7, and E8 for Pontevedra under MCB infestation in 2003, Pontevedra under ECB infestation in 2003, Zaragoza under MCB infestation in 2003, Zaragoza under ECB infestation in 2003, Pontevedra under MCB infestation in 2004, Pontevedra under ECB infestation in 2004, Zaragoza under MCB infestation in 2004, and Zaragoza under ECB infestation in 2004, respectively. The red dotted lines represent the polygon made

1 with the genotypes which are on vertex. The blue dotted lines are the perpendicular lines
2 to each side of the polygon, it shows which genotype(s) were grouped together as the
3 most promising in an specific environment(s). The black dotted lines represent the new
4 biplot according to Yan *et. al.* (2002) in which abscissas axe was forced to pass trough
5 the origin and the average genotype points.