

# Climatic and Genotypic Effects for Grain Yield in Maize under Stress Conditions

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## ABSTRACT

Climate change is expected to affect agriculture. Yield stability across environments is a critical breeding goal when dealing with unstable climate and input reductions in farming systems. The objective of this study was to determine climatic and genetic factors contributing to genotype (G), environment (E), and genotype  $\times$  environment (GE) variability for maize (*Zea mays* L.) grain yield under some stress conditions like drought, cold, and pest attack. A large sample of Spanish maize populations was evaluated along with checks at three diverse locations during 3 yr. Factorial regression was performed to obtain a biological explanation of the G, E, and GE interaction for yield. The commercial hybrids had more yield and stability than most populations; therefore, breeding programs focusing on yield have released hybrids with high yield and stability under stress conditions. The populations with a reasonable compromise between yield and stability were Andoain and Lira. The main genotypic covariate was kernel depth, followed by ear length, although other covariates were important for each location. The main climatic covariates for yield were related to days with mean temperature over 15°C and maximum temperature in September, but they were not consistent across locations. Therefore, if yield under stress conditions is a breeding goal, several climatic variables, especially those related to high temperatures, and genotypic traits such as kernel depth and ear length should be considered.

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**Abbreviations:** E, environment; G, genotype; GE, genotype  $\times$  environment.

YIELD STABILITY is a critical breeding goal when dealing with unstable climate changes (Setimela et al., 2007; Torriani et al., 2007). Species differ for stability across environments, maize (*Zea mays* L.) being one of the most widely adapted crops in North America, Europe, and elsewhere (Chloupek and Hrstkova, 2005). Plant breeders are concerned with yield instability because they attempt to produce stable varieties for wide distribution or as an opportunity to release varieties for specific environments (Gomes et al., 2000; Abera et al., 2004; Setimela et al., 2007). Evaluations of maize varieties in multiple environments reveal variable ranges of adaptation and specific aptitudes of varieties for diverse environments (Gomes et al., 2000; Setimela et al., 2007).

Several researchers have analyzed genotype (G)  $\times$  environment (E) interaction (GE) to identify either widely adapted varieties (Ajibade et al., 2002; Abera et al., 2004) or appropriate testing locations (Fan et al., 2007; Gomes et al., 2000). Not only variety performance but also the definition of heterotic patterns is affected by environment and by GE (Castillo et al., 2005; Malvar et al., 2005; Pswarayi and Vivek, 2008). Therefore, understanding the genotypic and environmental effects involved in GE interactions for variety yield and heterosis is essential for the advance of plant breeding.

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Few studies have tried to identify the specific genotypic and environmental factors affecting yield. Epinat-Le Signor et al. (2001) studied G, E, and GE effects for grain yield in 132 French hybrids and found that flowering earliness, water balance around flowering, and mean temperature from the 12-leaf stage to the end of the grain-filling phase were determinants of the GE variation. Malvar et al. (2005) studied the G, E, and GE effects for grain yield heterosis in a diallel among nine Spanish and French maize populations and identified as the main factors affecting yield of heterotic patterns the genotypic effects earliness and vigor, the environmental effect cold temperature, and several GE effects. All these studies were conducted in conventional agricultural environments.

Some studies have shown that GE interactions are common under stress conditions, such as drought, cold, or pest attack, and make breeding progress difficult (Pswarayi and Vivek, 2008; Rodríguez et al., 2007; Butrón et al., 2008). A breeding program focusing on performance under stress can increase responsiveness of the selected populations (Johnson and Gadelmann, 1989; Bänziger et al., 2006). Therefore, a precise definition of the environmental and GE stress factors affecting yield should increase the opportunities for future improvements of grain yield.

When maize breeders think of breeding for environmental stress tolerance, they often design a breeding program for improving yield under one of the common climatic stresses, sometimes without conducting preliminary studies on which environmental factors actually limit the crop and which genetic parameters are essentially affected. Factorial regression models are lineal models that explain GE interaction by differential cultivar sensitivity to explicit external environmental variables (Vargas et al., 1999). This model incorporates genotypic and environmental covariates (Denis, 1988) that enhance biological interpretation of G, E, and GE interaction effects (Baril et al., 1995). Since both environmental and genetic factors depend on the environments and the genotypes evaluated, such a study should include a wide range of environments and a wide collection of genotypes for a given geographical region.

In this study, the objective was to determine the effects of abiotic (drought, cold) and biotic (pests) environmental stresses on the grain yield and GE interactions for grain yield on a representative sample of Spanish maize landraces.

## MATERIALS AND METHODS

### Field Trials

Seventy-eight Spanish landraces of maize and six commercial hybrid checks were used to determine genetic and environmental factors contributing to G, E, and GE variability for yield. Each year, 76 populations and 5 hybrids were evaluated in a  $9 \times 9$  triple lattice design. Two populations and one hybrid were replaced 1 yr owing to problems with seed availability; therefore, these entries were removed from the combined analyses over

environments. Evaluation included all the Spanish accessions from the European Core Collection as well as other populations with historical value, high yield, or pest resistance. Checks were commercial hybrids used by farmers in northern Spain.

The experiments were performed in 2004, 2005, and 2006 at Pontevedra ( $42^{\circ}24'20''\text{N}$ ;  $8^{\circ}38'48''\text{W}$ ; 20 m above sea level) and Corunna ( $43^{\circ}12'50''\text{N}$ ;  $8^{\circ}17'55''\text{W}$ ; 100 m above sea level), on the European Atlantic coast, and Saragossa ( $41^{\circ}41'30''\text{N}$ ;  $0^{\circ}49'30''\text{W}$ ; 250 m above sea level), in the Ebro River valley. The evaluation was made under some stress conditions, mostly a shortage of water. At Pontevedra and Corunna, trials were not irrigated, while at Saragossa reduced irrigation was applied. In all the environments the crop received between 400 and 500 mm of water. We planted early to observe the response to cold stress. No pesticide treatments were applied during the growing cycle to let pests attack without restraint. Corn borers are the main biotic stresses in Spain, and their incidence is very high in Pontevedra (Velasco et al., 2007), high in Saragossa (Malvar et al., 1993), and low in Corunna (Campo, personal communication). Pontevedra had the most stressful conditions for maize growth, because the trials were planted in a poor soil (sandy-loam soil with 4% of organic matter, pH 5.0, and 30-cm depth), with a weed incidence difficult to control; also, in this location corn borer attack was very strong. Appropriate management practices were performed to fertilize maize and to prepare the soil. Chemical preemergence control for weeds was made when necessary.

Each experimental plot consisted of two rows with 25 hills per row and two kernels per hill. The rows were spaced 0.80-m apart, and the hills were spaced 0.21-m apart. Hills were thinned after emergence to obtain a final plant density of 60,000 plants  $\text{ha}^{-1}$ . Grain yield was recorded in  $\text{kg ha}^{-1}$ , adjusted at 140 g  $\text{H}_2\text{O kg}^{-1}$ . Data on traits usually used on breeding programs and considered stable and repeatable were taken in each plot. Early vigor, recorded when plants were in the five-leaf stage by giving visual ratings that ranged from 1 (= weak) to 9 (= vigorous), days to pollen shed (days from planting until 50% of plants shed pollen), days to silking (days from planting until 50% of plants show silks), plant height (centimeters from the ground to the tassel top), ear height (centimeters from the ground to the ear node), number of leaves from the main ear to the tassel, plant appearance (on a visual scale from 1 [= poor], to 9 [= excellent]), husk appearance on a visual scale from 1 (= poor coverage of the ear) to 9 (= good coverage of the ear), number of ears per plant, total lodging (percentage of plants broken below the main ear or leaning more than  $45^{\circ}$  from the vertical), kernel moisture (percentage), ear appearance on a visual scale from 1 (= small ears with poor health and kernel filling) to 9 (= big ears with excellent health and kernel filling), ear length (centimeters), number of ear rows, kernel depth (centimeters from the ear cob to the exterior of the kernel), and kernel weight (grams measured on a mass of 100 kernels). Data were recorded on 10 plants and 10 ears per plot, except for vigor, days to pollen, days to silking, number of ears per plant, total lodging, and yield, which were recorded on the whole plot, and kernel moisture, which was recorded from a sample of five ears per plot. The environmental variables were chosen to be easy to measure and used in a breeding program, and some were related with maize optimum growth temperatures (Ellis et al., 1992). Data were recorded on weather stations at the test sites. For each month and for the whole growth period (15 April–15 October) average daily temperature, mean of daily minimum temperatures, mean

of daily maximum temperatures, number of days with maximum temperature above 25°C, number of days with average daily temperature above 15°C, and water (total amount of precipitation resulting from rainfall and irrigation) were included in the analysis.

Statistical Analyses

Analyses of variance were performed across environments for all the traits, with the lattice adjusted means. An environment was defined as the combination of a year and a location. Genotypes were considered as fixed factors. Means were compared with Fisher’s protected LSD. Genotypes were ordered based on their mean yield at each location. Comparison of ranks across locations was used as an estimate of stability (Kang et al., 1991). For yield, a factorial regression analysis was made combined over environments as well as an analysis for each location, because the stress conditions for each location were so diverse that general conclusions could be inaccurate if not supported by the individual analyses. The general form for a factorial regression model with *k* genotypic and *h* environmental covariates is (Denis, 1980):

Y<sub>ij</sub> = μ + (Σρ<sub>k</sub>G<sub>ik</sub> + α<sub>i</sub>) + (Σδ<sub>h</sub>E<sub>jh</sub> + β<sub>j</sub>) + (ΣG<sub>ik</sub>θ<sub>kh</sub>E<sub>jh</sub> + Σα'<sub>ih</sub>E<sub>jh</sub> + Σβ'<sub>jk</sub>G<sub>ik</sub> + ε<sub>ij</sub>)

where ρ<sub>k</sub> and δ<sub>h</sub> are the regression coefficients of genotypic G<sub>ik</sub>, and environmental E<sub>jh</sub> covariates, respectively; α<sub>i</sub> and β<sub>j</sub> are the residuals of genotype and environmental main effects, respectively; θ<sub>kh</sub> is the regression coefficient of the cross-product of G<sub>ik</sub> and E<sub>jh</sub> covariates; and α'<sub>ih</sub> and β'<sub>jk</sub> are the genotype *i* and environment *j* specific regression coefficient of environmen-

tal covariate E<sub>jh</sub> and genotypic covariate G<sub>ik</sub>, respectively. The term ε<sub>ij</sub> is the residual interaction effect.

All parameters were considered fixed. The covariates and their order in the factor regression model for yield data were obtained by performing a stepwise regression on genotype covariates and a second stepwise regression on environmental covariates (Denis, 1988). A correlation analysis between covariates was performed to check colinearity. After standardization of covariates, factorial regression analyses were performed by the computer package INTERA (Decoux and Denis, 1991). Genotypic and environmental terms were tested against the GE interaction.

RESULTS AND DISCUSSION

Comparisons among Genotypes

Differences among populations were significant for all traits, and the GE was significant for grain yield. The range of mean yield for populations was from 1.62 (Codoñera) to 4.45 Mg ha<sup>-1</sup> (Castellote), with the mean being 3.02 Mg ha<sup>-1</sup>; the best commercial check, ‘Maverik’, yielded 6.14 Mg ha<sup>-1</sup> (Table 1). The three locations were, however, very different, as population yields in Pontevedra ranged from 0.12 to 2.24 Mg ha<sup>-1</sup> with a mean yield of 1.49 Mg ha<sup>-1</sup>, in Corunna from 2.36 to 6.46 Mg ha<sup>-1</sup> with a mean of 4.21 Mg ha<sup>-1</sup>, and in Saragossa from 1.72 to 6.10 Mg ha<sup>-1</sup> with a mean of 3.46 Mg ha<sup>-1</sup>.

Pontevedra was the most stressful location and, hence, had the lowest yields. Moreover, the ranks of populations at different locations were more consistent between

Table 1. Means for yield and for the significant genotypic covariates contributing more than 5% of the sum of squares from the evaluation of 78 maize (Zea mays L.) varieties at three locations during 3 yr. Yield ranks for each location and across environments are included between brackets. Only populations and hybrids with relevant values for discussion are shown.

| Varieties             | Genotypic covariates |              |                  |            |              |               | Yield and rank      |           |           |           |
|-----------------------|----------------------|--------------|------------------|------------|--------------|---------------|---------------------|-----------|-----------|-----------|
|                       | Days to pollen       | Plant height | Ear appearance   | Ear length | Kernel depth | Kernel weight | Pontevedra          | Corunna   | Saragossa | Mean      |
|                       | d                    | cm           | 1–9 <sup>†</sup> | cm         | mm           | g             | Mg ha <sup>−1</sup> |           |           |           |
| Populations           |                      |              |                  |            |              |               |                     |           |           |           |
| Almonte               | 69.6                 | 163.0        | 5.46             | 11.0       | 0.89         | 0.30          | 1.90 (14)           | 3.85 (56) | 6.10 (5)  | 3.95 (7)  |
| Andoain               | 66.1                 | 169.0        | 5.95             | 14.3       | 0.85         | 0.33          | 1.79 (18)           | 6.46 (5)  | 5.00 (10) | 4.42 (6)  |
| Aranga1               | 66.6                 | 175.0        | 5.74             | 14.0       | 0.79         | 0.29          | 2.24 (5)            | 4.95 (15) | 3.88 (25) | 3.69 (13) |
| Basto                 | 75.0                 | 166.6        | 5.40             | 13.8       | 0.77         | 0.30          | 1.38 (42)           | 4.21 (44) | 5.39 (7)  | 3.66 (14) |
| Castellote            | 73.9                 | 169.2        | 5.74             | 14.7       | 0.85         | 0.33          | 1.36 (44)           | 5.89 (6)  | 6.10 (4)  | 4.45 (5)  |
| Codoñera              | 89.8                 | 130.6        | 5.63             | 12.5       | 0.59         | 0.14          | 0.12 (78)           | 2.36 (78) | 2.36 (70) | 1.62 (77) |
| Hembrilla × Queixalet | 73.9                 | 154.8        | 5.78             | 15.3       | 0.73         | 0.23          | 1.00 (67)           | 3.86 (55) | 4.68 (11) | 3.18 (35) |
| Lira                  | 65.8                 | 154.7        | 5.42             | 15.1       | 0.80         | 0.33          | 2.12 (7)            | 5.49 (10) | 4.07 (20) | 3.90 (8)  |
| Puentedeume           | 65.6                 | 170.4        | 5.69             | 13.5       | 0.81         | 0.28          | 2.11 (8)            | 5.79 (7)  | 3.65 (33) | 3.85 (9)  |
| Rastrojero            | 71.2                 | 179.9        | 5.83             | 14.2       | 0.79         | 0.30          | 1.06 (66)           | 5.00 (14) | 5.31 (8)  | 3.79 (10) |
| Sajambre              | 52.8                 | 130.3        | 4.98             | 12.0       | 0.68         | 0.25          | 0.65 (75)           | 2.41 (77) | 1.73 (77) | 1.60 (78) |
| Salvatierra           | 66.6                 | 137.4        | 5.26             | 12.0       | 0.73         | 0.26          | 0.84 (71)           | 2.85 (73) | 2.32 (72) | 2.00 (75) |
| Tuy                   | 67.1                 | 179.8        | 5.80             | 14.1       | 0.78         | 0.32          | 2.14 (6)            | 5.16 (12) | 3.55 (38) | 3.62 (16) |
| Commercial hybrids    |                      |              |                  |            |              |               |                     |           |           |           |
| Furio                 | 72.5                 | 186.9        | 5.84             | 16.7       | 0.87         | 0.28          | 2.71 (3)            | 8.34 (2)  | 7.19 (2)  | 6.08 (2)  |
| Maverik               | 79.1                 | 177.7        | 6.07             | 16.9       | 0.90         | 0.30          | 2.48 (4)            | 8.35 (1)  | 7.60 (1)  | 6.14 (1)  |
| LSD (5%)              | 2.7                  | 12.5         | 0.57             | 1.3        | 0.07         | 0.04          | 0.76                | 1.09      | 0.62      | 0.66      |
| Mean                  | 66.6                 | 161.0        | 5.58             | 13.6       | 0.75         | 0.29          | 1.49                | 4.21      | 3.46      | 3.02      |
| Range                 | 53–90                | 130–184      | 4.8–6.2          | 11–17      | 0.59–0.89    | 0.14–0.37     | 0.12–2.24           | 2.36–6.46 | 1.72–6.10 | 1.62–4.45 |

<sup>†</sup>Subjective scale from 1 (poor) to 9 (excellent).

Corunna and Saragossa than between any of these locations and Pontevedra, even though Corunna is geographically and climatically closer to Pontevedra than to Saragossa. The populations with highest yield at Pontevedra were Aranga1, Tuy, and Lira, while at Corunna were Andoain, Castellote, and Puente deume and at Saragossa, Castellote, Almonte, and Basto. The populations Castellote, Andoain, Almonte, Lira, Puente deume, and Rastrojero had the highest mean yield; their ranks across locations were very variable. Rastrojero was the population with the largest rank variations across locations, followed by Hembrilla × Queixalet. Castellote and Almonte had large rank variation among locations, while Andoain and Lira were relatively stable and Puente deume had intermediate stability. Therefore, high yield and stability were not related, with perhaps the exception of two populations: Andoain and Lira. Across locations, variety ranks for yield were very stable for two of the highest yielders, the commercial checks Furio and Maguellan, and for two of the worst yielders, the populations Salvatierra and Sajambre. The present data show that high-yielding hybrids were also the most stable genotypes, in agreement with Tollenaar and Lee (2002) who stated that modern hybrids have higher yield and stability than older populations because breeding programs focused on improvements of yield have increased stability. Obviously, stability is not guaranteed among modern hybrids, as shown by Gomes et al., 2000, Tollenaar and Lee (2002), and Fan et al. (2007), and it depends on genotypes (for example, three-way cross vs. simple cross) and stress conditions.

The populations reported here do not have an exceptional yield stability, but it might be worthwhile to purposely improve yield stability of the most promising ones to anticipate future needs in facing climatic changes (Torriani et al., 2007) or to give an agronomic stable genotype to small farmers (Fan et al., 2007). This is an important consideration for breeders looking for yield stability (Abera et al., 2004; Setimela et al., 2007). According to Kang et al. (1991), yield is sacrificed when selection is based on rank stability, but selection based on yield alone may not be adequate when GE interaction is significant because of testing in diverse environments. Therefore, both yield and stability should be considered when selecting populations for future breeding programs.

## Genotypic Effects on Yield

The variables with significant effects on yield (explaining more than 5% of the sum of squares at any location) were days to pollen, plant height, ear appearance, ear length, kernel depth, and kernel weight (Table 2). The ranges of variation for yield among populations were wider compared with other traits (Table 1). Pollen date varied from 53 to 90 d, plant height varied from 130 to 184 cm, ear appearance from 4.8 to 6.2, ear length from 17 to 11 cm, kernel depth from 0.59 to 0.89 cm, and kernel weight from 0.14 to 0.37 g. The commercial checks were within

these ranges, except for Furio, which had the tallest plants, and Maverik, which had the deepest kernels.

The significant genotypic covariates for yield were not consistent across locations except for kernel depth, which explained 14, 6, and 69% of the variability in Pontevedra, Corunna, and Saragossa, respectively (Table 2). From the combined analyses across locations, the significant covariates were only ear length (21%) and kernel depth (62%). Both covariates have positive regression coefficients with yield under stress conditions ( $\rho = 0.30$  and  $\rho = 0.40$ , respectively). Ear length and kernel depth are two yield components that have been considered by breeders for many years. Cross (1977) reported that kernel depth was stable across environments and had an important effect on yield. However, selection for a single yield component alone might not increase yield if there are negative correlations among yield components, as in the case of kernel depth and ear length (Ross et al., 2006). Among the other covariates, it is important to note that ear appearance was able to explain a high amount of the variability in Pontevedra (68%). When the environment is very stressful and no pest treatments are applied under high insect pressure, genotypes with healthy, big ears are the higher yielders. Stresses (e.g., drought, pests) severely decrease kernel growth (Godfrey et al., 1991), so genotypes with big full ears are able to produce even when resources were limited. This easy-to-measure parameter could be used in breeding programs to improve yield under low-input conditions.

When stress pressure is smaller, other genotypic covariates usually related with yield and used in breeding programs (e.g., plant height, days to pollen, kernel weight) become important, which could be the reason for the stability found on elite hybrids.

With all the genotypic variables included in this study, the residual was smaller than in previous studies (Butrón et al., 2004, 2008; Malvar et al., 2005), but still significant, explaining 7.4% of the genotypic variation, which means that other covariables should be involved.

Malvar et al. (2005) evaluated a diallel among a selected sample of populations in optimum conditions and found that days to flowering and early vigor were the main genotypic covariates involved in yield. In the stressful conditions of this study, early vigor was significant as well ( $\delta = 0.09$ ), but only explained about 0.44% of the total variation in the combined analysis. With the individual analysis for each location, early vigor explained 2.7% ( $\delta = 0.16$ ) of the total variation in Pontevedra, while in Corunna the variability explained was 2.2 ( $\delta = 0.21$ ), and in Saragossa vigor explained only 0.6% ( $\delta = -0.10$ ). It seems that this covariate is more important when the environment is close to the minimum temperature conditions for maize growth and gives the crop resources to produce under other stresses. This trait should be handled carefully, because when temperature conditions are close to the optimum for maize, vegetative development can reduce yield, as other works have shown (Revilla et al.,



**Table 2. Significant environmental (E) and genotypic (G) covariates for yield from the analyses of variance for 78 maize (*Zea mays* L.) varieties grown in Pontevedra (P), Corunna (C), and Saragossa (S) during 3 yr and combined over locations (Com). Only covariates contributing more than 5% of the sum of squares are reported.**

| Source                   | df  |     |     |      | Mean squares |       |        |        | %SS <sup>†</sup> |    |    |     | Regression coefficient <sup>‡</sup> |          |          |           |
|--------------------------|-----|-----|-----|------|--------------|-------|--------|--------|------------------|----|----|-----|-------------------------------------|----------|----------|-----------|
|                          | P   | C   | S   | Com  | P            | C     | S      | Com    | P                | C  | S  | Com | P                                   | C        | S        | Com       |
| Environment <sup>§</sup> | 2   | 2   | 2   | 8    | 86.9*        | 20.3* | 43.2*  | 13.4*  |                  |    |    |     |                                     |          |          |           |
| Water5                   | 1   |     |     |      | 173.8*       |       |        |        | 99               |    |    |     | δ = 1.06                            |          |          |           |
| Tmed6                    |     | 1   |     |      |              | 40.4* |        |        |                  | 99 |    |     | δ = 0.51                            |          |          |           |
| Tmed15                   |     |     | 1   |      |              |       | 86.4*  |        |                  |    | 99 |     | δ = 0.74                            |          |          |           |
| Tmed15-4                 |     |     |     | 1    |              |       |        | 860.0* |                  |    |    | 62  |                                     |          |          | δ = -0.85 |
| Tmax9                    |     |     |     | 1    |              |       |        | 308.1* |                  |    |    | 22  |                                     |          |          | δ = 0.58  |
| Tmed15-7                 |     |     |     | 1    |              |       |        | 152.6* |                  |    |    | 11  |                                     |          |          | δ = -0.98 |
| Residual E               | 1   | 1   | 1   | 1    | 0.38*        | 0.08  | 0.03*  | 0.01   | 1                | 1  | 1  | 1   |                                     |          |          |           |
| Genotype                 | 77  | 77  | 77  | 77   | 0.8*         | 4.2*  | 4.1*   | 6.6*   |                  |    |    |     |                                     |          |          |           |
| Days to pollen           |     | 1   |     |      |              | 21.7* |        |        |                  | 7  |    |     | ρ = 1.43                            |          |          |           |
| Plant height             |     | 1   | 1   |      |              | 47.4* | 40.2*  |        |                  | 15 | 13 |     | ρ = 0.29                            | ρ = 0.35 |          |           |
| Ear appearance           | 1   |     |     |      | 41.0*        |       |        |        | 68               |    |    |     | ρ = 0.10                            |          |          |           |
| Ear length               |     |     | 1   | 1    |              |       | 18.5*  | 107.2* |                  |    | 6  | 21  |                                     |          | ρ = 0.37 | ρ = 0.30  |
| Kernel depth             | 1   | 1   | 1   | 1    | 8.2*         | 20.9* | 215.5* | 316.1* | 14               | 6  | 69 | 62  | ρ = 0.20                            | ρ = 0.39 | ρ = 0.46 | ρ = 0.40  |
| Kernel weight            |     | 1   |     |      |              | 17.6* |        |        |                  | 5  |    |     | ρ = 0.12                            |          |          |           |
| Residual G               | 66  | 69  | 67  | 66   | 0.1*         | 2.6*  | 0.41*  | 0.57*  | 9                | 56 | 9  | 7   |                                     |          |          |           |
| G×E                      | 154 | 154 | 154 | 616  | 0.22*        | 0.45* | 0.15*  | 0.51*  |                  |    |    |     |                                     |          |          |           |
| Error                    | 408 | 336 | 460 | 1204 | 0.01         | 0.06  | 0.00   | 0.01   |                  |    |    |     |                                     |          |          |           |

\*Significant at  $P = 0.05$ .

<sup>†</sup>%SS = percentage of sums of squares within the corresponding main or interaction effect.

<sup>‡</sup>δ = regression coefficient of standardized environmental covariate; ρ = regression coefficient of standardized genotypic covariate.

<sup>§</sup>Water5: rain in May; Tmed6: mean temperature of June; Tmed15: number of days with mean temperature above 15°C during the growing cycle; Tmed15-4: number of days with mean temperature above 15°C in April; Tmax9: mean maximum temperature of September; Tmed15-7: number of days with mean temperature above 15°C in July.

2005). The other trait, days to flowering, was significant on the combined analysis, but it only explains 0.77% of the variability, with a negative regression coefficient of 0.80. In the individual analysis, this trait was only significant at Corunna, explaining 2.17% of the variability, with a negative coefficient of 1.01. Early flowering entries had been associated with higher yield under drought stress conditions by escape, but other traits should be used by breeders, since tolerance is preferred to escape because it can stabilize yield at high levels (Chapman and Edmeades, 1999). Indeed, the genotypic covariates are strongly dependent on the genotypes and environments involved in each study. Moreover, stressful environments increase the range of variation and the differences among entries, showing that ear traits become more important as stress level increases.

## Climatic Factors

The climatic variables with significant effects on yield (explaining more than 5% of the sum of squares at any location) were the rain in May (Water5), the number of days with mean temperature above 15°C during the growing cycle (Tmed15), the mean temperature of June (Tmed6), the number of days with mean temperature above 15°C in April (Tmed15-4), the mean maximum temperature of September (Tmax9), and the number of days with mean temperature above 15°C in July (Tmed15-7) (Table 3).

Factorial regression analysis across environments showed that the number of days with mean temperature above 15°C in April, June, July, September, and October with the mean temperature of August and the mean of the maximum temperatures of September can explain all the environmental variation, but only the number of days with mean temperature above 15°C in April (62% of total variability), the mean maximum temperature of September (22%), and the number of days with mean temperature above 15°C in July (11%) explained more than 5% of the sum of squares. The environmental covariates for yield were not consistent across locations (Table 2). In contrast, only one variable explained most of the variability for each location, specifically the rain in May for Pontevedra, the mean temperature in June for Corunna, and the number of days with mean temperature above 15°C during the growing cycle for Saragossa, all with positive regression coefficients.

Climatic differences among locations are important, as Pontevedra and Corunna are located in the humid regions and Saragossa in a dry region of Spain. Also, Pontevedra is warmer and wetter than Corunna. Concerning the above-mentioned variables for these trials, Pontevedra had higher rainfall and warmer temperatures in the spring, Saragossa had higher temperatures during the summer, and Corunna had intermediate values. However, we must keep in mind that the trials of Pontevedra had the worst conditions.

**Table 3. General environmental covariates and significant covariates contributing more than 5% of the sum of squares from the evaluation of 78 maize (*Zea mays* L.) varieties at three locations during 3 yr.**

| Location   | Year | Tmed† | Tmax | Tmin | Water | Environmental variables |       |          |          |          |       |
|------------|------|-------|------|------|-------|-------------------------|-------|----------|----------|----------|-------|
|            |      |       |      |      |       | Water5                  | Tmed6 | Tmed15   | Tmed15-4 | Tmed15-7 | Tmax9 |
|            |      | °C    |      |      | mm    | mm                      | °C    | no. days | no. days | no. days | °C    |
| Pontevedra | 2004 | 17.0  | 22.5 | 11.4 | 511   | 44                      | 20.4  | 134      | 4        | 29       | 18.1  |
| Pontevedra | 2005 | 17.6  | 24.2 | 11.0 | 454   | 120                     | 20.2  | 147      | 1        | 31       | 24.9  |
| Pontevedra | 2006 | 18.6  | 25.2 | 12.1 | 409   | 45                      | 19.9  | 158      | 9        | 31       | 24.4  |
| Corunna    | 2004 | 15.7  | 22.0 | 9.3  | 393   | 63                      | 18.5  | 122      | 0        | 26       | 24.3  |
| Corunna    | 2005 | 15.7  | 23.0 | 8.5  | 318   | 72                      | 17.8  | 114      | 0        | 29       | 23.9  |
| Corunna    | 2006 | 17.0  | 23.2 | 10.7 | 404   | 18                      | 17.6  | 143      | 0        | 31       | 25.5  |
| Saragossa  | 2004 | 19.9  | 27.8 | 12.7 | 424   | 38                      | 23.7  | 146      | 3        | 31       | 28.9  |
| Saragossa  | 2005 | 20.0  | 27.9 | 12.5 | 392   | 50                      | 23.6  | 136      | 4        | 31       | 27.5  |
| Saragossa  | 2006 | 20.6  | 28.2 | 13.4 | 482   | 12                      | 22.5  | 142      | 2        | 31       | 28.3  |

<sup>†</sup>Tmed: mean temperature during the growing cycle; Tmax: mean maximum temperature during the growing cycle; Tmin: mean minimum temperature during the growing cycle; Water: water by the crop; Water5: rain in May; Tmed6: mean temperature of June; Tmed15: number of days with mean temperature above 15°C during the growing cycle; Tmed15-4: number of days with mean temperature above 15°C in April; Tmed15-7: number of days with mean temperature above 15°C in July; Tmax9: mean maximum temperature of September.

Furthermore, the climate is normally more variable across years for Pontevedra than for the other locations, as shown for the six environmental variables (Table 3). For instance, during the 3 yr at Pontevedra the rain in May was 44, 120, and 45 mm in 2004, 2005, and 2006, respectively (Table 3). The soil at the field of Pontevedra (sandy loam and not very deep) might explain the large effects of rain in May. In Corunna, the main climatic variable was temperature in June, which was around the critical value for adult maize growth (18°C). Finally, in Saragossa yield increased with days with mean temperature above 15°C, especially during August, September, and October when these climatic variables were very variable across years.

These results are consistent with previous studies where water balance and mean temperature are the main yield-limiting factors (Argillier et al., 1994; Epinat-Le Signor et al., 2001; Butrón et al., 2004). Days over 15°C reduced multicollinearity problems because correlations between months are smaller than using mean temperature, according to correlation coefficients (data not shown). It seems that it could be a convenient way to measure the effect of temperature on grain yield and could be a useful parameter to be considered in a breeding program under stress conditions.

## Genotype × Environment Interaction

The factorial regression model across locations explained 92.3% of the GE interaction sums of squares (Table 4). However, the residual is still significant, and there were some significant general genotype × number of days with mean temperature above 15°C interactions in April, June, July, September, and October, as well as a significant environment × days to pollen interaction. The significant residual and general factors show that the genetic and environmental factors taken into account in this study do not thoroughly dissect all the genotypic and environmental factors affecting the variability observed for yield among these varieties. For example, environmental factors such

as soil characteristics or radiation were not analyzed, nor were genotypic traits related to physiology or plant development considered. Nevertheless, the variables recorded provided for a greater understanding of the importance of the genotypic and environmental factors and their interactions. Actually, almost all the GE interactions were significant, although most had small effect, and only about one-fifth of the interactions explained more than 1% of the interaction sum of squares, in agreement with previous reports (Malvar et al., 2005; Butrón et al., 2008).

The GE interaction components explaining more than 5% of the sums of squares were number of days with mean temperature above 15°C in April × kernel depth (9.0%,  $\theta = -0.16$ ) and × ear length (6.8%,  $\theta = -0.11$ ), followed by mean maximum temperature of September × kernel depth (4.4%,  $\theta = 0.15$ ). When considering interactions explaining more than 1% of the sums of squares, the genotypic covariates involved on more interactions were kernel depth and ear length, and the climatic covariate showing more interactions was mean maximum temperature of September, followed by number of days with mean temperature above 15°C in July and in April. Several genotypic covariates with significant effect on grain yield had no relevant interactions with environment, particularly plant height, ear appearance, and kernel weight, being good candidates for breeding for stability. Likewise, the climatic covariates with significant effects on yield and no relevant interactions with genotypes were number of days with mean temperature above 15°C in the growing period, mean temperature of June, and water in May. Apparently, water availability was not involved in GE interaction or yield stability, while several temperature-related traits explained significant and relevant proportions of the interaction.

The interpretation of so many small contributions of GE interaction factors is not straightforward, and the first conclusion is that variability for yield depends on a complicated interaction among genotypic and environmental variables.

Looking at the major interactions, days with mean temperature over 15°C during the first weeks of plant growth had a negative effect on grain yield. The regression coefficients of the interactions of the two main genetic covariates with temperature over 15°C in April were negative, as well as the regression coefficient of the interaction of this variable with early vigor. More vigorous genotypes were favored when the number of days with mean temperature was low in their first stages of development. We can speculate that the explanations for such negative effect are weeds, because competition with weeds is one of the main problems in places with wet growing seasons like Pontevedra and Corunna, although good agronomic practices were done, and can reduce yield in low-input crop systems (Posner et al., 2008). These GE interactions could be interpreted as the advantage of vigorous plants when competing with weeds at low temperatures during the first stages of development; thus, when temperatures warm up and weeds emerge and compete with maize, the maize plants are stronger than the weeds and can favorably compete for light and nutrients. Alternatively, the cause of the negative relationship could be the negative correlation between vegetative development and grain yield (Revilla et al., 2005). The interaction of the covariates' mean of maximum temperature in September and kernel length had a positive effect on grain yield, as expected, since maize development is optimum at temperatures about 25°C and September is a critical month for seed filling. Thus, yield increases more for varieties with longer kernels when maximum temperatures rise.

## CONCLUSIONS

Conventional breeding programs focusing on yield have released hybrids with high yield and also with increased stability. In the current study, the GE interaction depended on almost all the genotypic and climatic variables we evaluated. More detailed research is required, but it was concluded that some of the more important interaction components are among kernel depth and ear length with high temperature at germination and maturation. Future breeding programs dealing with stability and yield in low-input cropping systems could use some promising populations such as Andoain and Lira and could work with variables related with ear appearance, like ear length or kernel filling, to provide some stable genotypes.

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**Table 4. Significant genotype (G) × environment (E) interaction covariates for grain yield from the combined analyses of variance for 78 maize (*Zea mays* L.) varieties evaluated in three locations. Only interaction covariates contributing more than 1% of the sum of squares are reported.**

| Genotype × environment interaction covariates <sup>†</sup> | df | Mean square | % Sum of squares | Regression coefficient <sup>‡</sup> |
|------------------------------------------------------------|----|-------------|------------------|-------------------------------------|
| Kernel depth × Tmed15-4                                    | 1  | 27.904      | 9.0              | −0.16                               |
| Kernel depth × Tmed15-7                                    | 1  | 6.846       | 2.2              | −0.11                               |
| Kernel depth × Tmax9                                       | 1  | 13.819      | 4.4              | 0.15                                |
| Ear length × Tmed15-4                                      | 1  | 21.327      | 6.8              | −0.11                               |
| Ear length × Tmed15-7                                      | 1  | 7.672       | 2.5              | −0.17                               |
| Ear length × Tmax9                                         | 1  | 4.652       | 1.5              | −0.04                               |
| No. ear rows × Tmax9                                       | 1  | 4.737       | 1.5              | −0.02                               |
| Lodging × Tmed15-7                                         | 1  | 4.017       | 1.3              | 0.03                                |
| Husk appearance × Tmax9                                    | 1  | 5.957       | 1.9              | −0.05                               |
| No. ears per plant × Tmax9                                 | 1  | 6.254       | 2.0              | −0.05                               |
| Days to pollen × Tmed15-6                                  | 1  | 4.054       | 1.3              | 0.09                                |
| Days to pollen × Tmax9                                     | 1  | 4.554       | 1.5              | 0.07                                |
| Residual G × Tmed15-4                                      | 66 | 0.231       | 4.9              |                                     |
| Residual G × Tmed15-6                                      | 66 | 0.279       | 5.9              |                                     |
| Residual G × Tmed15-7                                      | 66 | 0.328       | 7.0              |                                     |
| Residual G × Tmed15-9                                      | 66 | 0.134       | 2.8              |                                     |
| Residual G × Tmed15-10                                     | 66 | 0.261       | 5.5              |                                     |
| Residual G × Tmed8                                         | 66 | 0.211       | 4.5              |                                     |
| Residual G × Tmax9                                         | 66 | 0.294       | 6.2              |                                     |
| Days to pollen × Residual E                                | 1  | 4.231       | 1.4              |                                     |
| Residual G × Residual E                                    | 65 | 0.369       | 7.7              |                                     |

<sup>†</sup>Tmed15-4, Tmed15-6, Tmed15-7, Tmed15-9, and Tmed15-10: number of days with mean temperature above 15°C in April, June, July, September, and October, respectively; Tmed8: mean temperature of August; Tmax9: mean maximum temperature of September.

<sup>‡</sup>θ = regression coefficient of standardized cross product of G and E covariates.

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