




Genetic progress, adaptability and stability of maize cultivars for value of cultivation and use trials

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ABSTRACT. Maize breeding programs conduct multi-environment trials every year to assess the performance of new cultivars in pre-releasing tests. The data are combined across sites and seasons to perform a joint analysis in order to obtain information that will help breeders to select the best cultivars for different environments. Beyond this, it is essential to understand the different factors that can hamper the selection and genetic progress (i.e., genetic variability, selection intensity and genotype-by-environment interactions). In this study, the genetic progress (GP) was estimated and the adaptability and stability of 81 maize genotypes were evaluated in a series of trials for the value of cultivation and use (VCU) between the 2010/11 and 2014/15 growing seasons. The genotypes were composed of open-pollinated varieties, topcross hybrids, intervarietal hybrids, and single, double and three-way cross hybrids and were assessed in 117 environments in the central region of Brazil, from which 22 presented environmental stresses. For grain yield, an annual GP of 331.5 kg ha⁻¹ was observed, thus showing efficiency in the selection of superior cultivars. Additionally, it was observed that some low-cost seed cultivars showed yield potential, adaptability and stability estimates that were compatible with commercial hybrids, thus making them quite attractive for cultivation in environments with or without abiotic stresses.

Keywords: mixed model; genetic gain; biplot.

Received on October 30, 2017.

Accepted on April 10, 2018.

Introduction

In several countries, genotypes are evaluated in independent trial experiments (Smith, Ganesalingam, Kuchel, & Cullis, 2015). In Brazil, such pre-commercial tests are known as value for cultivation and use (VCU) trials. In these VCU tests, current network commercial materials and candidate genotypes are evaluated and further compared with each other.

A key point to maximize the profitability and food security is to provide reliable information to farms on the potential of cultivars in order to enable them to choose the best cultivars for the most diverse environmental conditions.

In this sense, the data reliability and models chosen for analysis become essential in multiple environment trials (MET). MET data refers to a large number of trials covering a wide range of geographic locations and seasons. Often, several traits are measured in these experiments, but the main one is grain yield.

In MET tests conducted over the several years, it is important to evaluate the genetic progress (GP). One way to evaluate the GP is to measure the performance of previous and current cultivars in the same test or those estimated by genotypic means in different trials. These biased means are influenced by the different environmental and experimental conditions, which can mask the true value of the actual GP.

The mixed-model approach for the analysis of MET experiments has become widely used (Stefanova & Buirchell, 2010). These models include components of variance models (Talbot, 1984; Frensham, Barr, Cullis, & Pelham, 1998; Cullis, Gogel, Verbyla, & Thompson, 1998), mixed models with regression on environmental variables (Theobald, Talbot, & Nabugoomu, 2002), mixed models with regression on environmental averages (Finlay & Wilkinson, 1963; Gogel, Cullis, & Verbyla, 1995; Nabugoomu, Kempton,

& Talbot, 1999), and the multiplicative mixed models (Smith, Cullis, & Thompson, 2001; Smith, Cullis, & Thompson, 2005; Crossa & Cornelius, 2002; Kelly, Smith, Eccleston, & Cullis, 2007). Stefanova and Buirchell (2010) emphasized that, generally, regressions on environmental variables or averages can be considered explanatory and non-descriptive models. Among the most common multiplicative mixed models (MMM), the factor analytic models (FA), the additive main effects and multiplicative interaction (AMMI) or the model of main effects of genotypes plus GE interaction (GGE) approach can be highlighted.

Several authors have used the methodologies of mixed linear models for adaptability and stability studies related to genotypic effects in several crops and in different situations. They have observed the flexibility of these models in addressing unbalanced data, thus achieving accurate rankings of treatments on this condition.

Based on the above, in this study, we propose the use of mixed multi-environment models to study the performance of maize cultivars under VCU tests (unbalanced) in order to estimate the genetic progress (GP) and the genotypic gain (GG) in unbalanced trials.

Material and methods

Description of environments and cultivars

In this study, we used data from VCU tests of maize low-cost seed cultivars that were coordinated by Embrapa Maize and Sorghum. The field evaluations were performed across the 2010/11 to 2014/15 crop seasons and covered trials that were spread all over the central region of Brazil. On average, 23 environments were used per year, thus totaling 117 environments. The respective meteorological data were obtained from the Meteorological Database for Teaching and Research (INMET, 2015).

Each crop season had an average of 39 genotypes and some were evaluated in more than one crop season, which totaled 81 cultivars. From this total, most (72) were low-cost seed cultivars, such as open-pollinated varieties (56), intervarietal hybrids (8) and topcross hybrids (8). Some others (9) were used as checks, such as double-cross hybrids (3), three-way cross hybrids (3), and single-cross hybrids (3).

From the 117 studied environments, 95 were evaluated in the first season (regular crop conditions). The remaining 22 environments were submitted to some environmental stress. That is, 17 environments were second crop seasons and the other five presented some nutritional deficiency (low nitrogen, low phosphorus or low fertilization).

Cropping practices were adopted according to the recommendation for each region and the crop conditions. The experimental design was a randomized complete block with two replicates with plots composed of two lines that were 4 m long with 0.80 m between them. The evaluated trait was grain yield, which was expressed in $\text{kg}\cdot\text{ha}^{-1}$ and corrected for 13% moisture.

Genetic-statistical models and estimation of parameters

Statistical analysis was performed in two stages. In the first stage, a multi-environment mixed model using an unstructured covariance matrix was used to estimate the components of the genetic covariance and to predict the genotypic values. Additionally, the heterogeneity of residual variance was assumed. From the best linear unbiased predictions (BLUP) for each environment, the stability and adaptability were measured via the singular value decomposition (SVD) of the principal effect of the genotypes plus the genotype-by-environment interaction (GEI) matrix, which is similar to the GGE-biplot model of Yan, Hunt, Sheng, and Szlavnic (2000).

First stage analysis

A multivariate mixed model was assumed for the MET analysis by taking into account the data from all 117 environments. From this data set, a joint analysis was performed based on the mixed model approach with an unstructured covariance matrix for the genotypes and a diagonal design for the residual variance by considering the following linear model:

$$y = X\beta + Zu + e \quad (1)$$

where: y is the raw data vector in each environment; β and μ are the fixed effect (related to the blocks and environments confounded with seasons) and the random genotype vectors, respectively; e is the random vector of errors; and X and Z are the incidence matrices for the fixed and random effects, respectively. In this data set, it was assumed that $e \sim N(\mathbf{0}, R = D \otimes I)$ and $u \sim N(0, \Sigma = G \otimes I)$, where G is an unstructured matrix and R is a diagonal matrix D that considers the heterogeneity of variances.

The $\hat{\beta}$ and \hat{u} solutions via Henderson's equation system are given by:

$$\begin{bmatrix} \hat{\beta} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} XR^1X & XR^1Z \\ ZR^1X & ZR^1Z + \Sigma^1 \end{bmatrix} \begin{bmatrix} XR^1y \\ ZR^1y \end{bmatrix} \quad (2)$$

Assume that:

$$C = \begin{bmatrix} XR^1X & XR^1Z \\ ZR^1X & ZR^1Z + \Sigma^1 \end{bmatrix} \begin{bmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{bmatrix} \quad (3)$$

The empirical estimators of fixed effects (EBLUE) and the empirical predictors of random effects (EBLUP) are given by:

$$\hat{\beta} = (X'V^{-1}X)^{-1}X'V^{-1}y \quad (4)$$

The Henderson mixed models equations calculate the solution for

$$\hat{u} = (Z'R^{-1}Z + \Sigma^{-1})^{-1}Z'R^{-1}(y - X\hat{\beta}) \quad (5)$$

The estimates of the residual variance components were obtained following Nuvunga et al. (2015):

$$\sigma_{ii}^2 = \frac{e_i'e_i + tr(KC^{-1}K')}{n} \quad (6)$$

where: $K=X||Z$ and i corresponds to the phenotypic vector related to i -th environment.

The estimates of the genotypic covariance among the environments and genetic variances were respectively given by:

$$\sigma_{u_i}^2 = \frac{[u_i'u_i + tr(C_{ii}^{-1})]}{n_a} \quad (7)$$

$$\sigma_{u_{ij}}^2 = \frac{[u_i'u_j + tr(C_{ij}^{-1})]}{n_a} \quad (8)$$

Second stage - Determination of stability and adaptability measures

Given the multi-environment EBLUP matrix obtained in the joint analysis, the singular value decomposition (SVD) was applied (Gauch, Piepho, & Annicchiarico, 2008) to the confounded GGE matrix. On this framework, principal component analysis based on the SVD is similar to the GGE/SREG2 model (Yan et al., 2000) in which the interpretation considers the first PC explaining the adaptability and the second one explaining the stability.

Genetic progress

Given the estimated parameters in the first stage, the GP and GG were estimated using the following criterion:

a) Environmental effect (EE): $EE = \mu CG_{(i+1)} - \mu CG_{i0}$

where: μCG_i and $\mu CG_{(i+1)}$ are the genotypic averages of common cultivars between years i and $i+1$, respectively, and $i = 1, 2, \dots, 5$.

b) Renewal rate (RR): $RR(\%) = \frac{GN}{NG} \times 100$

where: GN is new genotypes introduced in the year and NG is the total number of genotypes.

c) Genetic progress due to introduction (GPI) in the last pair of years:

$$GPI_{(i+1)} = MGI_{(i+1)} - MGM_{(i;i+1)}$$

where: $GPI_{(i+1)}$ is the genetic progress due to introduction in year $i+1$ for $i = 1, 2$; $MGM_{(i;i+1)}$ is the mean of the genotypic values (BLUPs) of the selected genotypes from year i to year $i+1$; and $MGI_{(i+1)}$ is the mean of the genotypic values of the genotypes introduced in year $i+1$ for $i = 1, 2$. This criterion measures the gains of the introduced genotypes over the best genotypes that were selected in the previous VCU tests.

d) Advancement genetic progress (AGP) or differential of selection (DS) in contiguous years:

i) $AGP_{(i;i+1)} = MGM_{(i;i+1)} + MGE_i$

where: $AGP_{(i;i+1)}$ is the advancement genetic progress of the cultivars from year i to year $i+1$ for $i = 1, \dots, 5$; and MGE_i is the mean of BLUPs related to the genotypes that were not selected in year i for $i = 1, \dots, 4$.

ii) Progress based on the AGP.

Here, the progress related to the AGP was calculated using the linear relationship $AGP_{(i,i+1)} = b_0 + b_1x$

Where x is the independent variable that corresponds to years, b_0 is the intercept, and b_1 is the slope of the line that will indicate the program efficiency in selecting cultivars.

i) Total genetic progress:

This criterion was used to estimate the genetic progress using the cumulative AGP over the five years of evaluation. In this linear regression, b_1 indicates the annual average genetic progress.

Results and discussion

In more than 70% of the 117 environments under study, the genetic variances were higher than the residual ones (Figure 1). This result allows one to infer that the environmental factors had a low influence on the cultivars' rankings based on grain yield in the majority of trials.

In addition to the natural imbalance of data commonly observed in maize breeding programs, in this study, it was possible to verify the heterogeneity of the residual variances, which ranged from 0.34 (environment 33) to 2.62 (environment 100). This justifies the use of mixed models that present different residual variances (Figure 1).

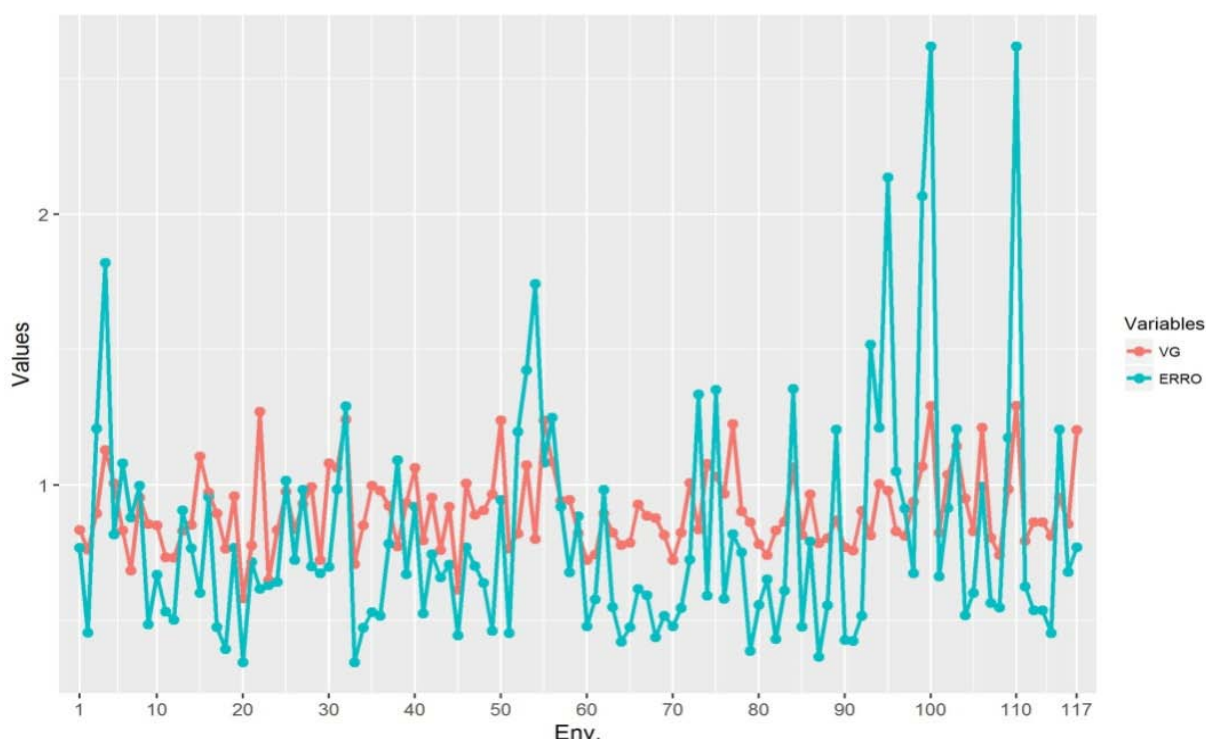


Figure 1. Estimates of genetic (GV) and residual (Error) variance components for the 117 environments under study.

Genetic progress

The annual genotype renewal rate measures the proportion of new genotypes included in each year in the experiments. In a sense, this rate reflects the dynamism of a breeding program.

During the covered period (2010/11 - 2014/15 crop seasons), a renewal rate of genotypes of approximately 31% was observed, which indicates good dynamism of the breeding program (Table 1).

One of the issues to be taken into account in the estimates of genetic progress is the environmental influence on the genotypic values. In this sense, it is argued that the genetic progress could be calculated using the current and old cultivars in the same trial. The other alternative is to correct the genotypic values using the common genotypes that were evaluated in the contiguous year. Since this information was available for the twelve genotypes, it was possible to correct the genotypic gains over the years. The common genotypes assessed across the five years had a genotypic mean of 6,556 kg ha⁻¹ (Table 1) with the highest environmental effects occurring in the 2011/12 (1,050 kg ha⁻¹) and 2012/13 (-1,345 kg ha⁻¹) crop seasons. In this way, genetic progress was calculated based on the genetic values free of the environmental effect (BLUPs).

Table 1. Renewal rate (RR%) in the harvests under study (considering the total number of genotypes (TNG) and the new genotypes (GN) evaluated every year, the genotypic averages of the twelve common genotypes in five harvests (μ_{gc} , kg ha⁻¹) and the environmental effect (EE, in kg ha⁻¹) for the VCU data of the low-cost maize cultivars).

Harvest	μ_{gc}	EE	TNG	GN	RR (%)
2010/11	6404	-	43	-	
2011/12	7455	1050	38	8	21.1
2012/13	6109	-1345	35	6	17.1
2013/14	6504	395	28	9	32.1
2014/15	6310	-194	34	18	52.9
Mean	6556	-23.5	36	10	30.8

The GPI was calculated based on information related only to the last crop season. (For the other seasons, this information was not available.) The genetic gain (-178 kg ha⁻¹) was not observed for the introduced cultivars when compared to the best ones that were evaluated in the 2014/2015 crop season (Table 2). Although this was not expected, this result may be justified because the introduction of cultivars is not based solely on the grain yield trait. For example, some genotypes inserted in VCU trials were selected due to their secondary traits such as precocity, nutrient use efficiency, and stress tolerance under limiting environmental conditions, which generally decrease the potential grain yield.

Table 2. Introductory genetic progress in VCU tests of maize varieties based on the BLUP averages (kg.ha⁻¹) of the 2014/15 harvest.

MGI	MGM	IGP
-76	102	-178

MGI: mean BLUP of genotypes introduced in the 2014/15 harvest; MGM: mean BLUP of genotypes maintained in the 2013/14 harvest for 2014/15 harvest; and IGP: Introductory genetic progress.

Although the cultivars introduced in the last crop season had negative genetic values, it is worth mentioning that the G79 topcross hybrid (HTCMS707) introduced in the last crop season (2015/2016) showed a high grain yield and stability similar to the best single-cross hybrid G22 (BRS1055) that was used as a check in the analysis (Table 2 and Figure 4).

In the results of the advancement genetic progress among pairs of years (AGP), it was observed that, except for the year 2014 that corresponds to the genetic progress of cultivars selected in the 2012/13 harvest (year 2013) for 2013/14, the pairs of the remaining harvests had positive AGPs, thus indicating the program's efficiency in increasing the differential of selection across the years (Figure 2a).

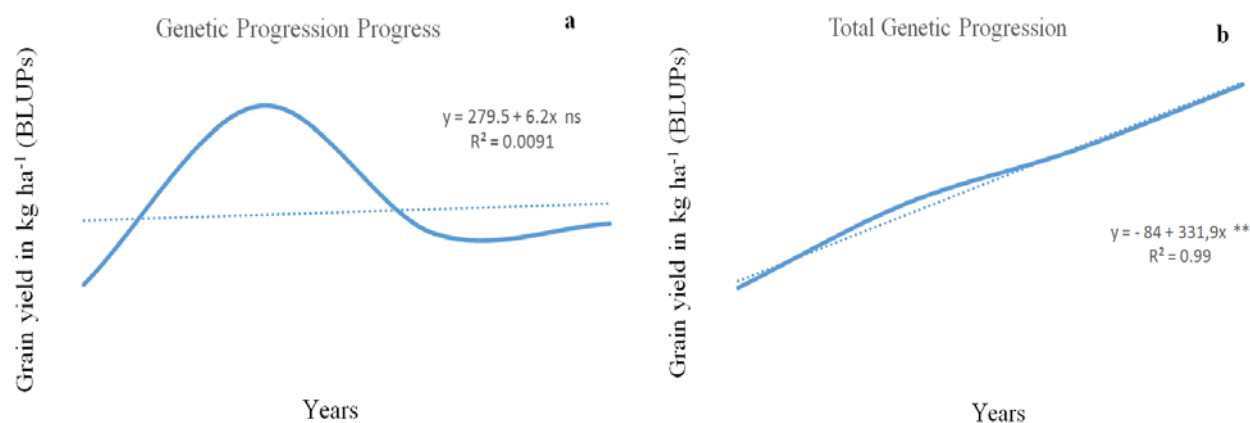


Figure 2. The advancement genetic progress (2a) and the total genetic progress (2b) of the maize cultivars evaluated in pairs of consecutive years in tests of the value for cultivation and the use of maize low-cost cultivars for the years from 2012 (2010/11 to 2011/2012 harvest) to 2015 (2013/14 to 2014/15 harvest). ns - not significant and ** - significant at the 1% level using a t test.

Regarding the total genetic progress, it was verified that the program was efficient in the selection of cultivars throughout the years, thus observing an average annual gain of 332 kg.ha⁻¹ (Figure 2b). Therefore, it can generally be inferred that the selected cultivars and those introduced over the five studied harvests tend to increase the favorable alleles for grain yield in the VCU tests of low-cost maize cultivars that were coordinated by Embrapa Maize and Sorghum.

The coefficients b_1 and b_0 of Figure 2a in the fifth year agree with the annual increases in grain yield observed in Figure 2b. In it, the value of 279.5 kg ha⁻¹ corresponds to the actual differential of selection, while the value of 6.2 kg ha⁻¹ reflects how much this parameter increased across the years. Therefore, in the fifth year, a differential of selection of 310 kg ha⁻¹ was expected, which was equivalent to the angular coefficient related to the expected gain (331.9 kg ha⁻¹).

Adaptability and stability

Grain yield can be considered the target trait for maize selection (Spinelli et al., 2010; Rocha et al., 2012). From the 81 cultivars evaluated in the present study, 30 showed an average yield higher than the general mean (6,227 kg ha⁻¹) (Figure 3). Among these cultivars, twenty were open-pollinated varieties (OPV) (such as PC 0904, Sint 10783, PC 0905, and Sint 10781), eight were topcrosses (HTC) and intervarietal hybrids (HI) (three HTCs - HTCMS707, HTCMS699, and HTCMS781 - and five HI - 11934, 2K1265, DSS HI 01, MC 6028, and DSS HI 02), and the remaining varieties corresponded to some hybrids that were used as checks (such the single-cross hybrid 2B707), which can be highlighted because of their highest genotypic values for grain yield.

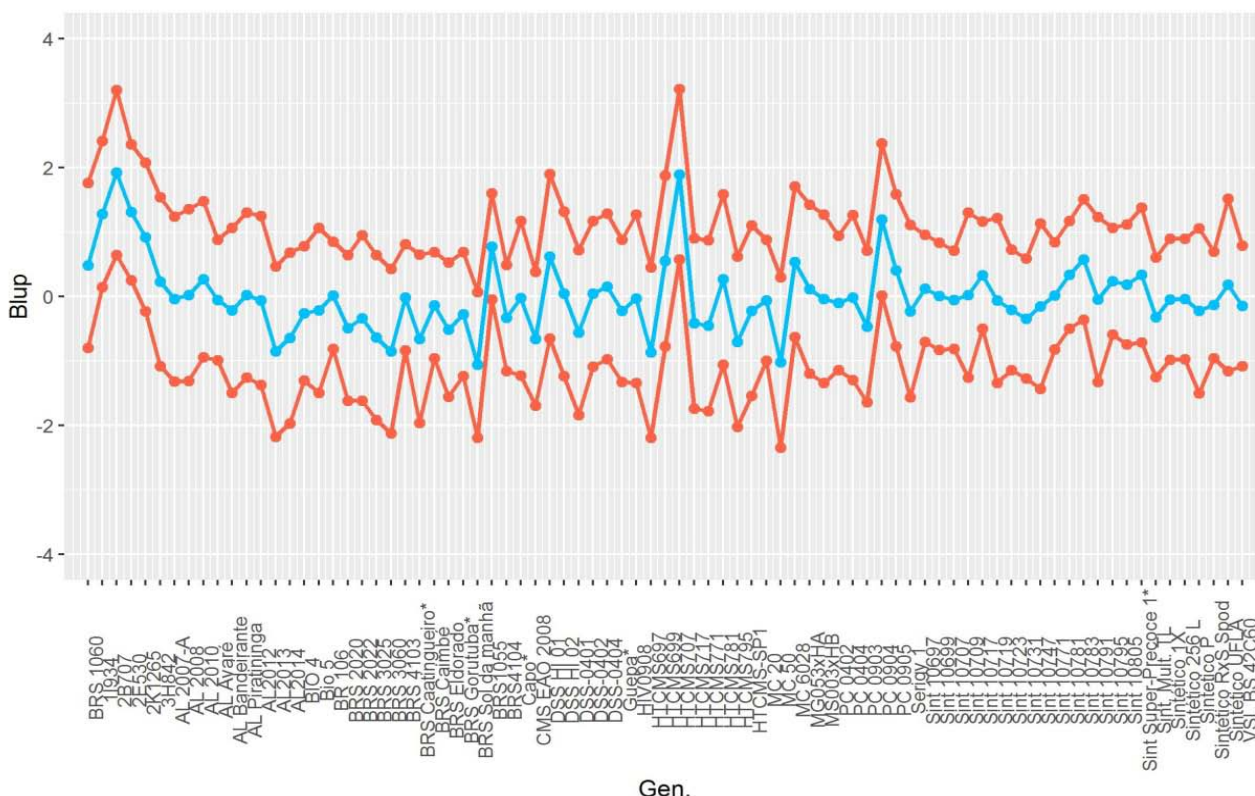


Figure 3. Confidence intervals (CI-red) and probability values (Prob-blue) for the genotypic values (BLUPs) of the 81 maize cultivars in the study.

Those cultivars that showed confidence intervals that did not include zero can be considered different from the general mean. In the top genotype group, five cultivars can be observed, which is highlighted by the hybrids HTCMS707 and 11934 that occupied the second and fourth places, respectively (Figure 3).

The GGE biplot with all the data of this study (117 environments and 81 genotypes) allowed the formation of three mega-environments. However, one of the inherent advantages of the method (the visual identification of environments that compose the multi-environments) was compromised due to the high overlap of points that confer the environments in this graph (Figure 4). It is still possible to identify the ideal genotypes for each mega-environment. For instance, the single-cross hybrid G22 (BRS1055) may be considered ideal for the first (top-to-bottom) mega-environment, the single-cross hybrid G26 (2B707) was assigned to the second mega-environment, and the topcross hybrid G79 (HTCMS707) and the OPV G42 (Sint 10783) were respectively assigned to the third and the fourth mega-environments.

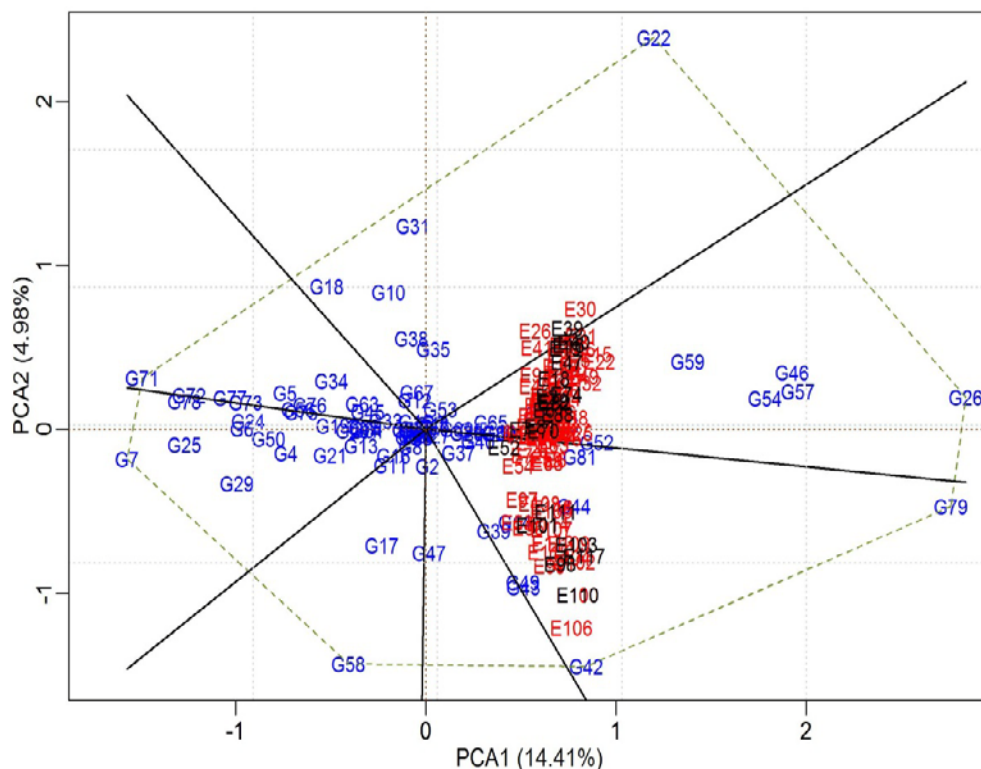


Figure 4. The GGE-biplot with the first two principal components (PC1 and PC2) corresponding to the 81 maize cultivars in the 117 environments. The G_ represents the cultivars (in blue) and the E_ corresponds to the environments (in red are the environments under normal cultivation conditions, and in black are the environments with some edaphoclimatic restriction) evaluated during the harvests from 2010/11 to 2014/15 in Brazil.

It is worth highlighting that G79 and G42 (HTCMS707 and Sint 10783) are low-cost seed cultivars and were winners in the mega-environments that include environments that depict the regular crop season (summer season) and some environments with poor-quality soil and edaphoclimatic constraints (Figure 4).

The use of OPVs is very attractive for low-income farms since they present a broad genetic background, low seed costs, lower requirements for the application of inputs and the ability to store seeds harvested on the farm for sowing in later years, thus reducing production costs (Romano, Verburg, & Andrade, 2007).

The biplot resolution was affected due to the number of environments and genotypes that were used in this study. To overcome the high overlap among environments and the low explanation of G+GE in the first two principal components (19.4%) in the joint analysis (Figure 4), the data was split into two subsets. The first one was composed of 81 cultivars that were evaluated in 22 environments with some edaphoclimatic stress, and the second consisted of 49 cultivars that were evaluated in 46 environments in the last two years (corresponding to the 2013/2014 and 2014/2015 harvests).

It was verified that in these scenarios, the GGE-biplot explained a greater proportion of variation due to G+GE with respect to the joint analysis, with values of 56.34% and 42.72%, respectively (Figure 5a and b). Similar results were reported by Balestre, Von Pinho, Souza, and Oliveira (2009) and by Oliveira, Von Pinho, Balestre, and Ferreira (2010) when they observed values of 53.82% and 51.5% for the variation due to G+GE that was explained by the first two principal component GGE biplots of the respective studies.

The set of 22 environments constituted by the experiments assessed in the second crop season (E7, E19, E40, E40, E47, E61, E70, E74, E79, E81, E82, E88, E98, E101, E103, and E117) and those evaluated with some nutritional deficiency (E2, E16, E52, E110, and E111) were divided into four environmental strata (Figure 5a). However, it was not possible to observe a clear concordance between the estimated mega-environments based on the environmental characteristics (second season environments and edaphoclimatic stress). This type of result was reported by Figueiredo, Von Pinho, Silva, and Balestre (2015) who verified the overlap of environments that were evaluated in the same crop season, even under different edaphoclimatic conditions. For example, the existence of environments with altitudes varying from 11 m (E81: Campos dos Goytacazes - season 2) to 732 m (E110: Sete Lagoas- Low fertilization) was observed in the third mega-environment. Regarding rainfall, it was also possible to observe a large amplitude within this mega-environment, with

rainfall varying from 236 mm (E52: Nossa Senhora das Dores - Low fertilization) to 1116 mm (E110: Sete Lagoas - Low fertilization) (Unpublished edaphoclimatic data).

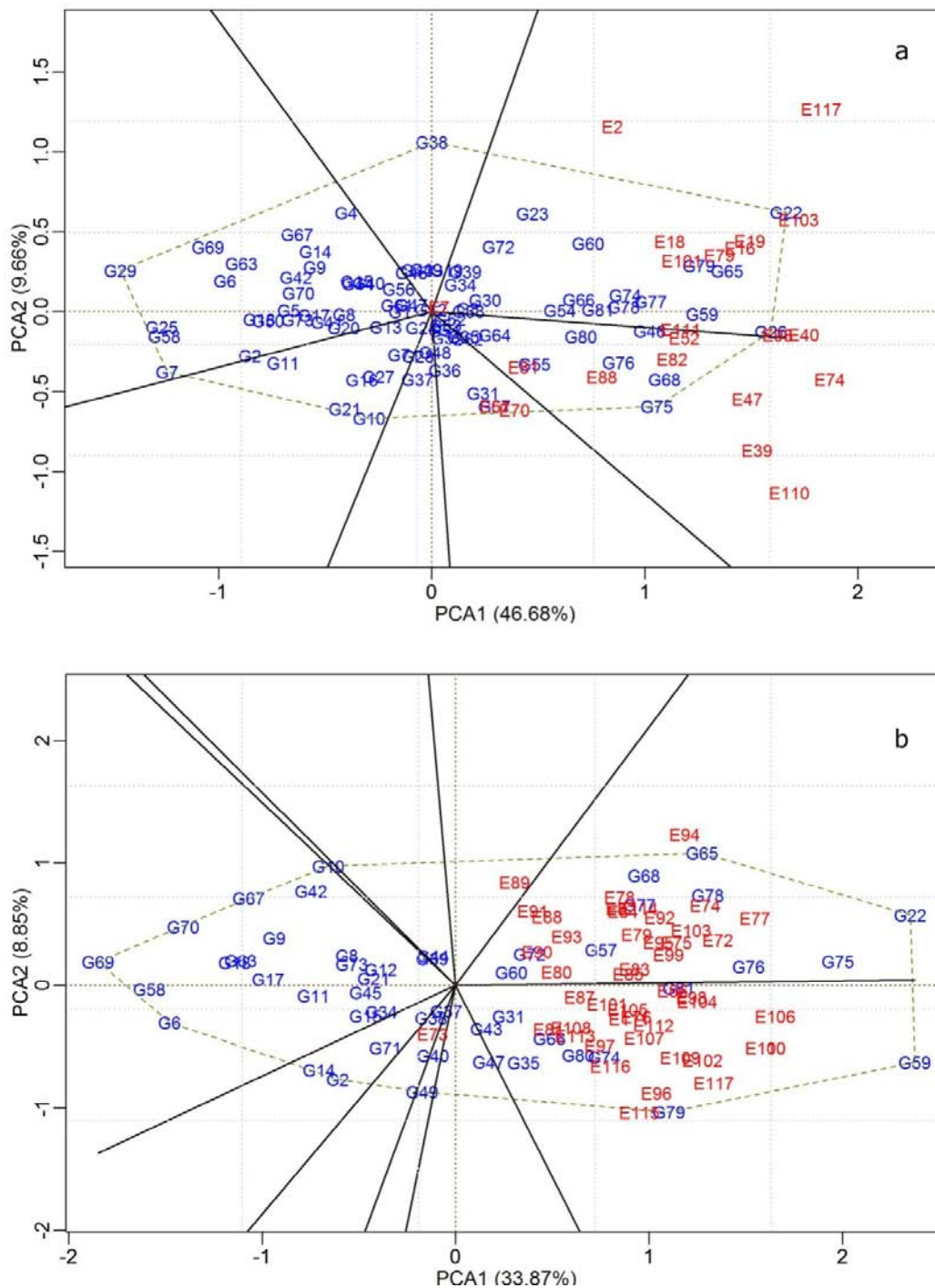


Figure 5. GGE-biplots with the first two principal components (PC1 and PC2) corresponding to the 81 maize cultivars that were evaluated in the 22 environments with some edaphoclimatic restriction during harvests from 2010/11 to 2014/15 (5a) and the 49 cultivars that were evaluated in the 46 environments during the 2013/14 and 2014/15 harvests (5b). In blue (G) are the cultivars and in red (E) are the evaluated environments.

With the exception of the last mega-environment (E61 and E70), it was possible to identify at least one ideal cultivar in the other three environmental strata. The single-cross hybrids G22 (BRS1055) and G26 (2B707) were considered ideal for mega-environment 2. The HTC hybrid G75 (HTCMS771) showed a relative superiority in this set of environments, which was considered the ideal cultivar for the second environmental stratum.

In the first mega-environment (composed only by E7), the synthetic variety G38 (Sint 10707) was the cultivar with the greatest adaptability, whereas in the last environmental stratum only open-pollinated

varieties were recommended (e.g., G7 (BRS Sol da Manhã), G31 (AL Alvaré), and G36 (Sint 10719)). In the second mega-environment where the single-cross hybrids G22 and G26 were assumed to be the best, it is also possible to recommend low-cost seed cultivars, such as the intervarietal hybrid G59 (2K1265), the topcross G79 (HTCMS707) and the varieties G60 (AL 2010) and G66 (AL 2012).

Since the open-pollinated varieties presented a satisfactory performance in mega-environments 1 and 4, it might be suggested that, for environments presenting restrictive conditions (i.e., showing lower values for the first principal component in the GGE-biplot), they become interesting recommendation alternatives (Figure 5a).

For the analysis of the harvest data of the 2013/14 and 2014/15 seasons, it was also possible to build four environmental strata (Figure 5b). In the principal environmental strata, it was possible to verify that the environments present in different seasons (E81: Campos dos Goytacazes - 2013/14 and E98: Dourados - 2014/15), the environments presenting nutritional restrictions (E110: Sete Lagoas - Low fertilization and E111: Sete Lagoas - Low phosphorus), and the environments presenting regular conditions hindered the perception of the pattern adopted for the formation of this multi-environment (Figure 5b).

With the exception of the first mega-environment composed of E89 (where there are no recommended cultivars), it was possible to observe the best cultivars for the other mega-environments. In the second environmental stratum, some hybrids that were used as checks were considered ideal, such as the single-cross hybrid G22 (BRS1055) and the three-way cross hybrid G65 (3H842). However, in the third and fourth environmental strata, low-cost seed cultivars were considered the best genotypes, namely, the intervarietal hybrid G59 (2K1265), the topcross G79 (HTCMS707), and the synthetic variety G49 (Sint 10781) showed greater adaptability and stability for these strata.

As observed in the analysis of the 22 environments, the recommendation of only open-pollinated varieties (G37-Sint 10697 and G40-Sint 10805) for the stratum represented by environment E73 support the use of these cultivars for environments where there are limiting factors to plant development. Additionally, it is highlighted that both in the second and third mega-environments, it is possible to recommend at least one open-pollinated variety, such as varieties G72 (AL 2013) and G60 (AL 2010) in the second mega-environment and G35 (Sint 10771) and G66 (AL 2012) in the third mega-environment.

Given the formed mega-environments, it is necessary to emphasize the dynamic and provisional characteristic of the environmental stratifications obtained by the descriptive statistical approaches over the GE interaction estimated in (VCU) trials. This is due to the dynamism of the breeding program and the process of the development and release of cultivars that lead to the continuous and periodical replacement of genotypes. This particularity may result in changes in the environmental strata across crop seasons since stratification depends on the GE interaction with the predominance of the complex interactions (Negash, Mwambi, Zewotir, & Taye, 2013). However, even in this imbricate system of trials, it was possible to obtain the genetic progress across the years and the selection of stable low-cost seed cultivars, thus showing the efficiency of the VCU net coordinated by Embrapa Maize and Sorghum.

Conclusion

For grain yield, an annual genetic gain of 331.5 kg ha⁻¹ was observed, thus showing the efficiency in the selection of superior maize cultivars for the central region of Brazil.

Some low-cost seed maize cultivars have grain yield potential, adaptability and stability compatible with commercial hybrids, thus making them quite attractive for cultivation in environments with or without stress conditions.

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