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Application of mixed models for the assessment genotype and environment interactions in cotton (*Gossypium hirsutum*) cultivars in Mozambique

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In the process of introducing cotton cultivars, it is essential to assess their productive behavior for different environments for which they will be recommended. Knowledge of the magnitude of the genotype interaction with environment allows the evaluation of the stability and adaptability of genotypes where one intends to introduce them, in addition to enabling the evaluation of the production potential and possible limitations of each environment. The study was conducted to determine the productivity, genotypic adaptability and genotypic stability of nine cotton cultivars *(Gossypium hirsutum*) in Mozambique, from 2004 to 2010 growing seasons. The genotypic stability and genotypic adaptability were assessed by Residual Maximum Likelihood (REML) and predict breeding values using Best Linear Unbiased Prediction (BLUP) methodology. The cultivars ISA 205, STAM 42 and REMU 40 showed superior productivity when they were selected by the Harmonic Mean of Genotypic Values (HMGV) criterion in relation to others. In turn, the cultivars CA 222, STAM 42 and ISA-205 were superior when selected by the Relative Performance of Genotypic Values (RPGV) and Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV). The cultivars CA 324 had the lower values for all criterions above. The cultivars CA 222 and STAM 42 will be the most recommended for farmers in cotton-growing regions and for the Cotton Breeding Program of Mozambique.

Key words: *Gossypium hirsutum*, harmonic mean of the relative performance of genotypic values (HMRPGV), relative performance of genotypic values (RPGV), harmonic mean of genotypic values (HMGV), residual maximum likelihood (REML)/best linear unbiased prediction (BLUP).

INTRODUCTION

Cotton (*Gossypium hirsutum)* is currently the leading crop in natural fiber production and is grown commercially in several environments, both in temperate as well as in tropical climate areas (Park et al., 2005; Naveed et al.,

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2007; Khadi et al., 2010). Cotton is the fifth crop for oil production, and the second for protein source in the world (Wallace et al., 2008) and the fiber´s ginning of 1.0 kg can be obtained by 1.65 kg of seed contain 21% oil and 23% protein (Benbouza et al., 2010). There are about 60 countries around the world that cultivate cotton in 34 million hectares. The countries include Australia (2, 000 kg ha^{-1}), Brazil (1, 338 kg ha^{-1}), China (1, 265 kg ha^{-1}), Mexico (1, 247 kgha^{-1}), United States of America (985 kg ha^{-1}), Uzbekistan (831 kg ha^{-1}), Pakistan (599 kg ha^{-1}) and India with 550 (kg ha^{-1}) (Fengguo et al., 2007; Khadi et al., 2010). The genus *Gossypium* includes approximately 50 species distributed worldwide, in the following continents: Asia, Africa, Australia and America, from which five are tetraploid species and belongs the subgenus viz. *Karpas* (Brubaker and Wendel, 1994; Cronn and Wendel, 2004). Among these species, only four are exploited economically: G. *herbaceum*, G. *arboretum*, G. *barbadense* and G. *hirsutum*, the latter contributing around 90% of the world output of cotton (Zhang, 2008).

The Mozambique Cotton Breeding Programs have focused mainly on the yield of cottonseed and fiber, with the CA 324 and REMU 40 cultivars are widely used by farmers, which together representing about 80% of the total cotton growing area (Bias and Donovan, 2003; IAM, 2009; Maleia et al., 2010). Although some of these introduced cultivars are already being used by producers because of incentive from fomenting companies, they only have been assessed by phenotypic stability and adaptability with balanced data (Maleia et al., 2010). In this sense, the methodology of mixed models, which allows the use of unbalanced data, and is widely used in breeding programs of perennial plants, becomes very important tools or methods to evaluate a performance of annual plants (Mora et al., 2007; Piepho et al., 2008), as cotton.

The use of mixed linear models in the advanced stages of cultivar selection such as in cultivation and use value of genotypes, which are set up in various environments, has fundamental importance, furthermore, the use of BLUP is preferable to the *Best Linear Unbiased Estimator* (BLUE) (Piepho and Möhring, 2006). In current studies, the genetic effect has been referred as random (Resende, 2007), allowing therefore the estimation of variance components, obtainment of genotypic values and the use of linear mixed linear models (Piepho et al., 2008).

The interaction of genotype and environment interferes significantly in breeding programs (Cruz and Carneiro, 2006) as an ideal cultivar, should be adapted to a broad cultivation environment (Cruz, 2005; Cruz and Carneiro, 2006). However, the interaction, in most cases, allows the release of cultivars for specific environments where they have a greater adaptation (Campbell and Jones, 2005). Therefore, knowledge of the magnitude of interaction genotype with environment is important to assess the stability and the adaptability of genotypes where they are intended to be introduced (Contreras and Krarup, 2000) also allows to evaluate the production potential, and possible limitations of these in each environment (Mora et al., 2007).

The simultaneous evaluation of stability and adaptability in the context of Mixed Linear Models (Resende, 2007) can be carried out using the HMRPGV Predicted (Silva et al., 2012). Although, the use of the REM/BLUP Methodology, the HMRPGV Method can be used for analysis of unbalanced data (Resende, 2007), non-orthogonal designs (Piepho et al., 2008), and designs with heterogeneity of variance (Mendes et al., 2012). This type of evaluation for commercial cotton cultivars is scarce in Mozambique. Therefore, the objective of this study was to evaluate the interaction between genotypes and environments, productivity, genotypic adaptability and genotypic stability of cotton cultivars in Mozambique, using the Mixed Models (REML/BLUP).

MATERIALS AND METHODS

Location of the experiments and sowing dates

The experiments were set up in the municipality of Montepuez, in the Namialo and Namapa Villages, located in the Northern Region of Mozambique, from growing season 2003/2004 to 2009/2010. In Morrumbala village, in the central region of the country, the experiments were set up from growing season 2005/06 to 2009/2010. All the locations are situated in Agro-ecological Regions 6, 7 and 8 (INIA, 2000).

The Agro-ecological Region 6 (R6) represents the semi-arid region of the Zambezi Valley and Southern of Tete Province Mozambique, which is a vast dry area. In contrast, the Agro-ecological Region 7 (R7) is region of medium-altitude in Zambezia, Nampula, Tete, Niassa and Cabo-Delgado Province Mozambique, with a variable soil texture. There is great potential for cotton production which has been practiced for several decades. The Agro-ecological Region 8 (R8) represents the Coast of Zambezia, Nampula and Cabo Delgado Provinces Mozambique, and the soils are generally sandy in some areas. The low soil fertility is one of the great limiting factors in these areas (INIA, 2000).

The municipality of Montepuez is located in the Agro-ecological Region R7, at an altitude of 555 m (medium altitude), 38º59' Longitude East and 13° 07' Latitude South, in the District of Montepuez, in the Southern of the Cabo Delgado Province Mozambique. Namialo is located between the Agro-ecological Regions R7 (medium altitude) and R8 (Coastal side) at an altitude of 157 m, 39° 59' Longitude East and 14° 55' Latitude South, in the Meconta District, Central Eastern of Nampula Province, Mozambique.

The Namapa Village is located in the Eráti District, at an altitude of 200 m (Low altitude), 13° 43'S Latitude and 39° 50' Longitude East between the R7 and R8 Agro-ecological Regions in the North of Nampula Province, Mozambique. The Morrumbala Village, in turn, is located between the Agro-ecological Regions R6 (semi-arid of Zambezi Valley) and R7 (medium altitude), at an altitude of 392 m, 35° 35' Longitude East and 17° 19' Latitude South, in the Morrumbala Village, in the Lower Region of Zambezia, Zambezia Province, Mozambique.

Climate and soil

The Namialo region is characterized by an Aw climate type

Table 1. List of cultivars assessed, origin, year of introduction, tolerance characteristics to *E. fascialis*, lint outturn, growing season.

Cultivar	Origin	Year of introduction	Tolerance to *Empoasca fascialis*	Lint outturn-GOT (%)	Growing season (days)
ALBAR SZ9314	Zimbabwe	1999	High	>42	>150
ALBAR FQ902	Zimbabwe	1999	High	41	130-150
ALBAR BC853	Zimbabwe	1999	High	37	<130
STAM 42	Senegal	1999	Low	40	130-150
CA 222	Ivory Coast	1994	Medium	39	130-150
CA 324	Ivory Coast	1994	Medium	38	130-150
IRMA 12-43	Cameron	1994	High	39	130-150
ISA 205	Ivory Coast	1994	High	39	130-150
REMU 40	Mozambique	1980	High	37	130-150

Source: IAM, 2007; Maleia et al., 2010.

(Köppen, 1948), dry sub-humid where the annual rainfall ranges from 800 to 1,000 mm and the average annual temperature of about 26°C. The soils classification ranges from sandy (ferralic arenosols and sandy textured haplic arenosols) to sandy clay and gleyic arenosols that occur alternately with hydromorphic sandy soils (MAE, 2005a). The Montepuez region has an Aw climate type (Köppen, 1948), semi-arid to sub-humid, with average annual precipitation ranging from 800 to 1,200 mm and the mean annual temperature ranging from 20 to 25°C. The hydromorphic soils predominate in this region, whose texture ranges from sandy, sandy on clay, and molic type dark-colored stratified soils gleic and dristic to halpic and luvic phaeosems (MAE, 2005b).

The Morrumbala region has an Aw climate type (Köppen, 1948), rainy tropical savanna with mean annual temperature of 22°C and 1,000 mm of rainfall. Soils are predominantly red, ranging from lightly sandy to clay, with deep ferralic lithosols (MAE, 2005c). The Namapa region has an Aw climate type (Köppen, 1948), semi-arid to sub-humid, with average annual rainfall that may exceed 1,500 mm and the mean annual temperature ranging from 20 to 25°C. The hydromorphic soils predominate, whose texture ranges from dark to gray sandy, sandy clay and stratified clay (MAE, 2005d).

Experimental design

To implement these experiments in the 19 study environments, a randomized complete blocks design was used. Each of three or four replicates (unbalanced data) consisted of set nine commercial cultivars. Table 1 shows nine cultivars and the mainly agronomic characteristics. In all experiments, plant to plant space was 0.2 m and row to row space was 1.0 m which corresponded to 50 000 plants ha^{-1} population density (Carvalho, 1996; IAM, 2007). The useful area of each plot consisted of three central rows, covering a usable area of 15 m². The experiments were set up in a non-irrigated area during the beginning of the rainy season, usually in the first two weeks of December.

Planting and other agronomic practices

The sowing was carried out manually in the hill plot (using a hoe) in rows, placing four to ten seeds per hill plot, approximately 4 cm deep. The first thinning was at fifteen days after seedling emergence, leaving two plants per hill plot. Later, at 21 days after emergence, a second thinning was performed leaving only one plant per hill plot. Weeds were controlled manually by hoeing five to six times, in order to prevent them from competing with the crop. No side dressing, mulching or fertilization was applied in order to allow experiments to simulate conditions similar to those prevailing in the rural producing fields in regions of Mozambique (Bias and Donovan, 2003). Two sprays with Endosulfan insecticide (475 g.L^{-1}), followed by three applications of Lambda-cihalothrin (50 g.L^{-1}) once in two weeks were applied (IAM, 2007), starting in the sixth week after emergence. The insecticides were applied using an ultra low volume nozzle (ULV).

Data collection

The characteristic evaluated was the total production of cottonseed harvested from all plants in the useful area of each experimental plot, with the mean value expressed in Kg.ha^{-1}.

Statistical analyses

The experimental data were test for normality and homogeneity of the errors (Levene, 1960; Shapiro and Wilk, 1965) in each environment using the SAS 9.2 software (SAS, 2009). The adaptability and stability were analyzed by the REML/BLUP Methodology (Henderson, 1975), considering the following statistical model:

$$E\begin{bmatrix} y \\ g \\ gl \\ \varepsilon \end{bmatrix} = \begin{bmatrix} Xr \\ 0 \\ 0 \\ 0 \end{bmatrix}; \quad \mathbf{var}\begin{bmatrix} \mathbf{g} \\ \mathbf{gl} \\ \boldsymbol{\varepsilon} \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_g^2 & 0 & 0 \\ 0 & I\sigma_{gl}^2 & 0 \\ 0 & 0 & I\sigma_\varepsilon^2 \end{bmatrix}$$

Where; Y is the vector of observation, *r* is the vector of fixed effects (replication) added to the overall mean and include all the repetitions of all places, g is the vector of random effects (genotypes), *gl* is the vector of effects of genotype x environment interaction (random), and $\mathcal{E}$ is the error vector (random). The X, Z and W, are associated design matrices for r, g, and gl, respectively.

The predicted genotypic values for genotype i at each site j simultaneously uses data from all environments, are given by $GV_{ij}=U_j+g_i+gl_{ij}$ where Uj is the average of location j. In this case, both g and gl are predicted because every data set is used, and the additional residues of interactions are eliminated when producing the Blup's of gl as well (Resende, 2007). The random effects are assumed to be distributed as:

$\mu \sim MVN(0;G)$ and $\varepsilon \sim MVN(0;R)$

Where, MVN (μ ; V) means multivariate normal distribution with mean μ and variance-covariance matrix V (Piepho et al., 2008).

In the simultaneous evaluation of genetic stability and adaptability of cotton cultivars was used the Harmonic Mean of the Relative Performance of the Genotypic Value (HMRPGV), as described by Resende (2007). These method is advantageous over methods such as Lin and Binns (1988) and Annicchiarico (1992), once it provides results that can be directly interpreted as genotypic values (Oliveira et al., 2005; Mora et al., 2007; Resende, 2007), allows to compute the composite character of genetic gain in the productivity, stability and adaptability (Resende, 2007). And also it does not depend on assumptions of α values associated with $Z_{(1-\alpha)}$, which refers to the percentile of the standard normal distribution function associated with a level of α, respectively (Rezende, 2007). The analysis of stability and adaptability were carried out using the software Selegen REML/BLUP (Resende, 2002).

Regarding the deviance analysis and estimation of the effect of genotypic and genotype x environment interaction, the PROC MIXED was applied (Littell et al., 2006). The estimator used for the prediction of genotypic values was the BLUP, which estimates variance's components of random factors obtained by the Method of Restricted Maximum Likelihood *(*REML) (Resende, 2007).

RESULTS AND DISCUSSION

The errors showed a normal distribution for each environment, but the variance analysis was not for overall environments allowing that the all analyzes were consider heterogeneous variances (Resende, 2007).

The Likelihood Ratio Test of the *Joint Analysis of Deviance* (Littell et al., 2006) for the productivity of cottonseed (Table 2) showed the effect of the cultivars as significant, and the coefficient of variation (CV) was 22.67%. This value of the coefficient of variation shows good precision of the experiment (Bowman, 2001), as the character cotton productivity is strongly influenced by the environment. In spite of, Maleia et al. (2010) when evaluating the adaptability and stability of the same cultivars used in this study and in 7 environments had a coefficient of variation of 18.39%. It is important to emphasize that for yield of cottonseed various authors estimate a coefficient with a range of 4.7 to 31.5%, and an average of 14.3% (Mora et al., 2007).

The genotype x environments interaction was significant (Table 2), indicating that cultivars showed different responses when exposed to different environments (local and production year) suggesting that the performance ranking of the cultivars was not constant. Table 3 shows the cultivars with their genotypic values for Namialo 2003/2004 and Namialo 2004/2005, and the overall environments analysis. It can be verified that the cultivar ALBAR BC853 showed the lowest genotypic value mean, that is, it had the worst performance to the overall environment. Considering the genotypic values (Table 3), the best cultivar in the different environments was the CA 222 cultivar. This ranking differs from that demonstrated by Maleia et al. (2010) when same genotypes were assumed as fixed effects in seven of the 19 environments assessed in this study, which concluded that the ISA 205 cultivar had the best productivity.

Table 2. Values of the Statistical Likelihood Ratio Test of the Joint Deviance Analysis and coefficient of experimental variation (CV (%) for cottonseed yield (kg ha^{-1}) in 19 environments, from 2003/2004 to 2009/2010 growing season.

Source of variation	F value
Cultivars	2.37 *
Environment x cultivars	3.98**
χ^2 Test	37.76**
CV(%)	22.67

The ISA 205 cultivar was highlighted in Namialo in 2003/2004 growing season (Table 3), while the STAM 42 cultivar showed a higher genotypic value in Namialo during 2004/2005 growing season, thus demonstrating the presence of genotype x environment interaction. Although, Maleia et al. (2010) referred that cultivar ISA 205 had the major value in the Namialo during 2003/2004 growing season, while in the Namialo environment during 2004/2005 growing season, were the cultivar STAM 42, assuming the genotypes effects as fixed.

This similarity is regarding to the normal distribution of the errors and the homogeneity of variance, consequently, the ranking of the cultivars obtained by the REML/BLUP methodology were the same with the classical methodology (Oliveira et al., 2005; Mora et al., 2007; Rezende, 2007; Piepho et al., 2008). Furthermore, Piepho and Möhring (2006) demonstrated that the use of BLUP is preferable to the BLUE.

Table 4 shows the results penalizing or capitalizing cultivars according to their performance in relation to stability (HMGV) for the overall environments. With respect to stability, it has been found that cultivars ISA 205, STAM 42 and REMU 40 had a superior HMGV, whereas the cultivars with smaller genotypic values for stability (HMGV) were cultivars CA 324, ALBAR BC853. The Maleia et al. (2010) showed a different ranking of cultivars from what obtained in present study, since ISA 205, STAM 42 and IRMA12-43 cultivars had value above 100% when estimated by the W_i confidence index, evidencing a greater phenotypic stability for cottonseed yield. In this study, the productive superiority for genotypic stability belongs to ISA 205, STAM 42 and REMU 40 cultivars. In relation to the REMU 40 cultivar, which is originated from Mozambique and widely produced (Bias and Donovan, 2003; IAM, 2009). Its superiority was obtained when selected by the HMGV method, however, Maleia et al. (2010) when using the Annichiarico (1992) method did not point out this superiority. The same authors recommended the STAM 42 cultivar for low quality environments, as it showed phenotypic adaptability restricted to those environments. In contrast, the STAM 42 cultivar showed superiority for both productive adaptability and stability in this study.

Table 3. Genotypic values obtained by the REML/BLUP methodology of cottonseed productivity (kg. ha^{-1}) in 19 environments, from 2003/2004 to 2009/2010 growing season, regarding the (u+g) predicted genotypic values, free of interaction with environments, and genotypic values predicted by environment (u+g+ge).

Cultivar	Overall environment		Namialo 2003/2005		Namialo 2004/2006	
	u + g	New mean	u+g+ge	New mean	u+g+ge	New mean
ALBAR SZ9314	1,530.52	1,542.76	837.11	871.03	1,336.53	1,411.59
ALBAR FQ902	1,521.13	1,535.43	839.58	877.82	1,439.31	1,447.51
ALBAR BC853	1,474.90	1,528.71	796.91	857.39	1,038.03	1,337.03
STAM 42	1,543.25	1,549.02	866.84	896.61	1,459.10	1,459.10
CA 222	1,554.78	1,554.78	776.10	848.36	1,256.36	1,374.40
CA 324	1,526.72	1,540.09	836.04	866.03	1,269.34	1,391.27
IRMA 12-43	1,521.79	1,537.47	859.65	887.37	1,353.11	1,426.60
ISA 205	1,542.19	1,545.82	947.51	947.51	1,444.11	1,451.61
REMU 40	1,543.06	1,547.03	875.50	911.50	1,437.35	1,444.97

Table 4. *Stability of Genotypic Values* (HMGV) for cotton cultivars evaluated in 19 environments, between 2003/2004 and 2009/2010 growing season.

Cultivar	Genotypic value (HMGV)
ISA 205	1,447.74
STAM 42	1,445.97
REMU 40	1,437.65
CA-222	1,436.61
IRMA 12-43	1,413.04
ALBAR FQ902	1,399.81
ALBAR SZ9314	1,390.68
CA 324	1,389.33
ALBAR BC853	1,312.23

It is worth emphasizing that the CA 324 cultivar in this study showed inferior productivity for both genotypic stability and adaptability (Table 5). However, it has been recommended for the quality's environments by Maleia et al. (2010). The CA 222 cultivar was not referenced in the recommendations of the Maleia et al. (2010) evaluated in seven of 19 environments assessed in this study, when evaluating the adaptability and phenotypic stability. Such facts reveal that the RPGV and HMRPGV*GM (Global Mean) are more efficient than the methods as Lin Binns (1988) and Annichiarico (1992) in the evaluation of adaptability and stability, respectively.

Table 5 shows the results penalizing or capitalizing cultivars according to their performance in relation to adaptability, stability and adaptability jointly to overall environments. Cultivars CA 222, STAM 42 and ISA 205 presented higher values when selected by the RPGV and RPGV*GM Criterion (Resende, 2002), as well as for the HMRPGV and HMRPGV*GM Method (Resende, 2002), while the cultivars with lowest values were CA 324 and ALBAR BC853 for these method. The Cultivar CA 222, STAM 42 and ISA 205 should response, in general 1.02 times above in relation to the mean of the averages of the environments where they are grown, both for RPGV and HMRPGV (Table 5).

The CA 222, STAM 42 and ISA 205 cultivars may be the most suitable and most promising for farmers in Agro-ecological regions of Mozambique and for the cotton Breeding Program of Mozambique.

Conclusions

The REML/BLUP methodology enabled to determine the genotypic stability and genotypic adaptability of the nine cultivars even with unbalanced data and heterogeneity of variances of the errors. The genotypic values were higher in overall environments for CA 222 and STAM 42 cultivars.

The cultivars ISA 205, STAM 42 and REMU 40 showed the highest values of the cottonseed yield when selected by the HMGV method, while the lowest values for the CA 324 and ALBAR BC853 cultivars. In relation to the stability and adaptability (HMRPGV) and adaptability (RPGV), the cultivars CA 222, STAM 42 and ISA-205 were superiors.

Therefore, cultivars CA 222, STAM 42 will be the most recommended for farmers in cotton-growing regions and for a cotton breeding program of Mozambique.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Table 5. Adaptability of genotypic values (RPGV and RPGV*GM), stability and adaptability of genotypic values (HMRPGV and HMRPGV*GM) for cultivars evaluated in 19 environments from 2003/2004 to 2009/2010 growing season.

Cultivar	Genotypic value			
	RPGV	RPGV*GM	HMRPGV	HMRPGV*GM
CA-222	1.03	1,568.46	1.02	1,562.43
STAM 42	1.02	1,561.87	1.02	1,558.81
ISA 205	1.02	1,561.39	1.02	1,556.90
REMU 40	1.02	1,556.23	1.02	1,551.95
RMA 12-43	1.00	1,526.87	0.99	1,517.62
ALBAR SZ9314	0.99	1,520.04	0.99	1,513.91
ALBAR FQ902	0.99	1,517.89	0.99	1,511.97
CA 324	0.99	1,516.96	0.99	1,509.30
ALBAR BC853	0.93	1,428.64	0.93	1,423.08

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