Simultaneous selection for cowpea (*Vigna unguiculata* L.) genotypes with adaptability and yield stability using mixed models

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ABSTRACT. The aim of this study was to select erect cowpea (*Vigna unguiculata* L.) genotypes simultaneously for high adaptability, stability, and yield grain in Mato Grosso do Sul, Brazil using mixed models. We conducted six trials of different cowpea genotypes in 2005 and 2006 in Aquidauana, Chapadão do Sul, Dourados, and Primavera do Leste. The experimental design was randomized complete blocks with four replications and 20 genotypes. Genetic parameters were estimated by restricted maximum likelihood/best linear unbiased prediction, and selection was based on the harmonic mean of the relative performance of genetic values method using three strategies: selection based on

the predicted breeding value, having considered the performance mean of the genotypes in all environments (no interaction effect); the performance in each environment (with an interaction effect); and the simultaneous selection for grain yield, stability, and adaptability. The MNC99542F-5 and MNC99-537F-4 genotypes could be grown in various environments, as they exhibited high grain yield, adaptability, and stability. The average heritability of the genotypes was moderate to high and the selective accuracy was 82%, indicating an excellent potential for selection.

Keywords: *Vigna unguiculata* L.; BLUP/REML; Genetic parameter

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a legume crop that is grown in several regions of the world. In Brazil, the crop is one of the main sources of protein in the diet, and is grown in the Midwest, North, and Northeast regions. Brazil is the third largest cowpea producer, with an area and average production of 1.4 million ha and 514,000 tons, respectively, and an average grain yield of 369 kg/ha (Filho et al., 2011). However, this yield is low when compared to its productive potential, which in experimental conditions has reached levels higher than 3000 kg/ha of dry beans (Hall, 2012).

Given the wide range of cowpea cultivation regions, it is necessary to study adaptability and yield stability, because these provide detailed information on the performance of genotypes under different environmental conditions. In general, high grain yield has been used as a criterion for genotype recommendation, which can harm or favor genotypes with specific adaptations to particular environments (Cysne and Pitombeira, 2012). Therefore, the use of more refined genetic and statistical procedures, such as mixed models, in plant breeding is increasing, because its success arises from the selection of superior individuals based on genetic merit (Pimentel et al., 2014).

A major limitation in the genetic breeding of plants is the effect of the environment on the phenotype, and environmental and genotypic values overlap. Mixed modeling using restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) is the most efficient method for the selection of superior genotypes (Resende and Duarte, 2007), because it simultaneously involves the prediction of breeding values and the estimation of variance components (Borges et al., 2009).

The simultaneous selection for yield, stability, and adaptability using mixed models can be accomplished by the harmonic mean of the relative performance of genetic values (HMRPGV) method (Carbonell et al., 2007; Gonçalves et al., 2014). This method, which was first proposed by Resende (2004), is similar to the methods of Annicchiarico (1992) and Lin and Binns (1988), and allows the simultaneous selection for yield, stability, and adaptability and considers phenotypic effects as random; therefore, it provides genotypic stability and adaptability. Furthermore, it takes into account correlated errors within sites, as well as the stability and adaptability of individuals within progeny. Moreover, it provides breeding values without instability and can be applied in any number of environments. Lastly, it generates results within the range of the trait measured, which can be directly interpreted as breeding values (Verardi et al., 2009; Freitas et al., 2013). The HMRPGV method has been used in

perennial and semi-perennial crops, such as coffee (Pereira et al., 2013; Rodrigues et al., 2013; Carias et al., 2014), eucalyptus (Rosado et al., 2012), cashew (Maia et al., 2009), and passion fruit (Santos et al., 2015a). Its use in annual crops is still in its early stages, although an increasing number of studies have proved its effectiveness in crops such as cowpea (Torres et al., 2015) and rice (Regitano Neto et al., 2013).

Studies of adaptability and stability in the cowpea have been conducted using traditional methods that were based on analysis of variance and regression (Almeida et al., 2013; Nunes et al., 2014; Santos et al., 2015b. The use of the HMRPGV method is an advance, because it is based on an analysis of predicted genotypic values using mixed models. Therefore, this study aimed to select erect cowpea genotypes simultaneously for high adaptability, stability, and grain yield in Mato Grosso do Sul, Brazil using mixed models.

MATERIAL AND METHODS

The experiments were conducted in 2005 and 2006 in the municipalities of Aquidauana, Chapadão do Sul, and Dourados (State of Mato Grosso do Sul), and Primavera do Leste (State of Mato Grosso), the edaphoclimatic characteristics of which are presented in Table 1. We used a randomized complete blocks design with 20 treatments and four replications. The experimental unit consisted of four 5-m rows, with 0.5 m between rows and 0.25 m between plants within each row. In each experimental unit, grain yield was evaluated in two central rows, corrected to 13% moisture, and transformed to kg/ha.

Table 1. Characteristics of trials with 20 upright cowpea genotypes evaluated in six environments in the States of Mato Grosso (MT) and Mato Grosso do Sul (MS).

Environment	Municipalities/State	Crop	Latitude	Longitude	Altitude	Climate[1]
A1	Aquidauana/MS	2005	22º01'S	54º05'W	430 m	Aw
A2	Dourados/MS	2005	20º03'S	55º05'W	147 m	Cwa
A3	Primavera/MT	2005	15º33'S	54°17'W	636 m	Aw
A4	Aquidauana/MS	2006	22º01'S	54º05'W	430 m	Aw
A5	Chapadão do Sul/MS	2006	18º05'S	52º04'W	790 m	Aw
A6	Aquidauana/MS	2006	22º01'S	54º05'W	430 m	Aw

[1]According Koppen classification.

The treatments consisted of 20 cowpea genotypes (MNC99-537F-1, MNC99-537F-4, MNC99-541-F5, MNC99-541-F8, IT93K-93-10, Pretinho, Fradinho-2, MNC99-519D-1-1-5, MNC00-544D-10-1-2-2, MNC00-544D-14-1-2-2, MNC00-553D-8-1-2-2, MNC00-553D-8-1-2-3, MNC00-561G-6, EV X 63-10E, MNC99542F-5, EV X 91-2E-2, MNC99-557F-2, BRS-Guariba, Patativa, and Vita-7) from the cowpea genetic breeding program of Embrapa Meio-Norte. Therefore, genotypic effects were considered random according to Resende and Duarte (2007), who recommended treating genotypic effects as random when the number of treatments is equal to or greater than 10.

For assessing genetic x environmental interaction effects, we used 54 statistical models in the Selegen-REML/BLUP program (Resende, 2007) that corresponded to $y = Xb + Zg + Wc + e$, wherein y, b, g, and c correspond, respectively, to the fixed effects of data vectors (means of blocks by environment), genotype effects (random), genotype x environment interaction effects (random), and random errors; and X, Z, and W are incidence matrices for b, g, and c, respectively. The assumed distributions and structures of the means (E) and variances (Var) were obtained by Equations 1 and 2, respectively:

$$E \begin{bmatrix} y \\ g \\ c \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

(Equation 1)

$$Var \begin{bmatrix} g \\ c \\ e \end{bmatrix} = \begin{bmatrix} I\sigma_g^2 & 0 & 0 \\ 0 & I\sigma_c^2 & 0 \\ 0 & 0 & I\sigma_{ge}^2 \end{bmatrix}$$

(Equation 2)

The model fit was obtained from Equation 3:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z+I\lambda_1 & Z'W \\ W'X & W'Z & W'W+I\lambda_2 \end{bmatrix} \times \begin{bmatrix} \hat{b} \\ \hat{g} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix}$$

(Equation 3)

Where λ_1 and λ_2 were, respectively:

$$\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{1-h_g^2-C^2}{h_g^2}$$

(Equation 4)

$$\lambda_2 = \frac{\sigma_e^2}{\sigma_c^2} = \frac{1-h_g^2-C^2}{C_g^2}$$

(Equation 5)

where h_g^2 corresponds to broad-sense individual heritability in the block and was defined by:

$$h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_c^2 \quad \sigma_e^2}$$

(Equation 6)

and χ^2 corresponds to the coefficient of determination of genotype x environment interaction effects, being defined by:

$$C^2 = \frac{\sigma_c^2}{\sigma_g^2 + \quad \sigma_c^2 \quad \sigma_e^2} \qquad \text{(Equation 7)}$$

σ_g^2 is the genotypic variance between the cowpea genotypes; σ_c^2 is the variance of the genotype x environment interaction; σ_e^2 is the residual variance between plots; and r_{gloc} corresponds to the genotypic correlation of genotypes by the environment and was defined by:

$$r_{gloc=} \frac{ó_c^2}{ó_g^2 + ó_c^2} = \frac{h_g^2}{h_g^2 + c^2} \qquad \text{(Equation 8)}$$

Iterative estimators of variance components by REML, via an maximum estimation (ME) algorithm, were:

$$\sigma_e^2 = \frac{\left| y'y - \hat{b}'X'y - \hat{g}'Z'y - \hat{c}W'y \right|}{\left[N - r(x) \right]} \qquad \text{(Equation 9)}$$

$$\hat{\sigma}_g^2 = \frac{\left| \hat{g}'\hat{g} + \hat{\sigma}_e^2 trC^{22} \right|}{q} \qquad \text{(Equation 10)}$$

$$\hat{\sigma}_c^2 = \frac{\left| \hat{c}'c + \hat{\sigma}_e^2 trC^{33} \right|}{s} \qquad \text{(Equation 11)}$$

where C^{22} and C^{33} is derived from:

$$c^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix}^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix} \qquad \text{(Equation 12)}$$

C being the coefficient matrix of the mixed model equations; tr is the matrix trace operator; r(x) is the rank of matrix X; and N, q, and s are the total number of data, number of genotypes, and number of genotype x environment combinations, respectively. Using this model, empirical BLUP predictors (eBLUP or REML/BLUP) were obtained of genotypic values free of the interaction, given by $\hat{\mu} + \hat{g}_i$, where $\hat{\mu}$ is the average estimate of all environments and $\hat{g}_i$ is the genotypic effect free of the genotype x environment interaction. For each environment j, genotypic values (V_g) were predicted by:

$$\hat{\mu}_j + \hat{g}_i + (\hat{g}e)_{ij} \qquad \text{(Equation 13)}$$

where $\hat{\mu}_j$ is the average estimate of the environment j, $\hat{g}_i$ is the genotypic effect of genotype i in environment j, and $(\hat{g}e)_{ij}$ is the genotype x environment interaction effect concerning genotype i. The prediction of genotypic values capitalizing the average interaction (gem) in the different environments was given by:

$$gem = \hat{\mu} + \frac{\left(\dfrac{\hat{\sigma}_g^2 + \hat{\sigma}_c^2}{n}\right)}{\hat{\sigma}_g^2}\hat{g}_i \qquad \text{(Equation 14)}$$

where $\hat{\mu}$ is the overall mean estimate for all environments, n is the number of environments, and $\hat{g}_i$ is the genotypic effect of genotype i. Selection that simultaneously considered the yield, stability, and adaptability of the erect cowpea genotypes was obtained by the HMRPGV method:

$$MHPRVGi = \frac{n}{\displaystyle\sum_{j=1}^{n} \frac{1}{Vg_{ij}}} \qquad \text{(Equation 15)}$$

where n is the number of locations in which genotype I was evaluated and Vg_{ij} is the genotypic value of genotype i in environment j, which was expressed as a proportion of the mean of that environment.

RESULTS AND DISCUSSION

The coefficient of experimental variation (CV_e) was 26.88% (Table 2), which is similar to that found in previous studies on the cowpea (Almeida et al., 2012; Santos et al., 2014a,b; Torres et al., 2015; Barroso et al., 2016). Because it was grain yield this value was expected, since this trait was significantly influenced by the soil and climatic features of each environment (Table 1). In contrast, high coefficient of genotypic variation (CV_g) values indicated that a significant proportion of the total variance was based on genotypic effects ($\hat{\sigma}_g^2$) and not phenotypic variance ($\hat{\sigma}_f^2$). The joint analysis of CV_g and CV_e is given in the statistic, $\hat{r}_{g\hat{g}}$ (Resende and Duarte,

2007). The accuracy obtained (74%) indicates experimental quality, and therefore security and credibility in the selection of superior genotypes for grain yield.

Table 2. Variance components (individual REML) for the grain yield of 20 upright cowpea genotypes grown in four environments in the States of Mato Grosso and Mato Grosso do Sul.

Estimates of variance components (individual REML)			
$\hat{\sigma}_g^2$	7,396.87	$\hat{r}_{g\hat{g}}$	0.74
$\hat{\sigma}_c^2$	29,712.81	c^2	0.43
$\hat{\sigma}_e^2$	31,709.49	$\hat{r}_{gloc}$	0.20
$\hat{\sigma}_f^2$	68,819.17	CV_g (%)	12.98
$\hat{h}_g^2$	0.11 ± 0.04	CV_e (%)	26.88
$\hat{h}_{mg}^2$	0.54	μ (kg/ha)	662.47

$\hat{\sigma}_g^2$: genotypic variance; $\hat{\sigma}_c^2$: genotype x environment interaction variance; $\hat{\sigma}_e^2$: residual variance between parcel; $\hat{\sigma}_f^2$: individual phenotypic variance; $\hat{h}_g^2$: coefficient of individual heritability in the broad sense; $\hat{h}_{mg}^2$: heritability of the mean of genotype; $\hat{r}_{g\hat{g}}$: accuracy in the selection of genotypes; c^2: coefficient of determination of the genotype x environment interaction effects; $\hat{r}_{gloc}$: genotypic correlation through environments; CVg: coefficient of genetic variation; CVe: coefficient of experimental variation; μ: overall mean.

The average heritability of genotypes ($\hat{h}_{mg}^2$) is based on the averages of the blocks as criteria for evaluation and/or selection (Resende, 2004). Therefore, in view of the value obtained (0.54), the selection of cowpea genotypes based on their predicted genotypic values was reliable. In estimating broad-sense individual heritability (h_g^2) we considered the total genetic dispersion, because we aimed to investigate all of the genetic variance between the cowpea genotypes. Torres et al. (2015) obtained similar results for the parameters h_{mg}^2 and $\hat{h}_g^2$ using mixed models for selecting semi-prostrate cowpea genotypes in Mato Grosso do Sul. The $\hat{\sigma}_g^2$ parameter was lower than the variance of the genotype x environment interaction effect ($\hat{\sigma}_c^2$). This generated low genotypic variation that was equivalent to 12.98% of the overall average and 10.75% of the total phenotypic variability, which was represented by the heritability of the individual plot (h_g^2). Similar results were obtained by Maia et al. (2009) and Rosado et al. (2012) when they estimated genetic parameters by REML/BLUP in cashew and eucalyptus clones, respectively.

Due to the greater or lesser degree of adaptability/genetic stability of individuals, the variance of the genotype x environment interaction ($\hat{\sigma}_c^2$) can inflate the phenotypic expression of a trait (Bastos et al., 2007). This measure estimates the proportion of the total variation accounted for by variation in the genotype x environment interaction. Low values of $\hat{\sigma}_c^2$ indicate that the interaction has little influence on the phenotypic value (Maia et al., 2009). Therefore, a genotype that produces a high yield in one environment would tend to maintain the same level in different environments, because the genotype responds favorably to environmental effects (high correlation between genotypic values through environments), in addition to having high predictability of environmental variations. The $\hat{\sigma}_c^2$ value was moderate, and was equivalent to 43.18% of the total phenotypic variation. This value was high compared to that of h_g^2 (0.11), resulting in a low phenotypic correlation ($\hat{r}_{gloc}$) in all of the environments. This reflects the importance of evaluating the adaptability and stability of upright cowpea genotypes, in order to provide accurate information to producers in Mato Grosso do Sul.

The measurement of a trait in field trials refers to its phenotypic value, and consists of genetic and environmental effects and their interaction. In this regard, the sum of genotypic and residual between-plot variances and interactions results in the value of $\hat{\sigma}_f^2$. Despite the residual dispersion between plots being 46.08% of this amount, genetic progress was obtained (Table 3). Maia et al. (2009) and Rosado et al. (2012) found that the residual variance between plots accounted for 60% of the individual phenotypic variance when assessing genotype x environment interaction effects on cashew and eucalyptus clones, respectively, with mixed models; their results are similar to those obtained in this study.

Table 3. Genotypic values of grain yield (kg/ha) of 20 upright cowpea genotypes in four environments and the mean environment.

Genotype	A1	A2	A3	A4	A5	A6	Mean environment
MNC99-537F-1	1231	1075	1436	298	318	609	719
MNC99-537F-4	1426	1102	1497	377	375	653	870
MNC99-541-F5	1308	1115	1067	266	349	695	712
MNC99-541-F8	1345	967	1303	229	249	674	652
IT93K-93-10	1195	911	979	365	284	625	529
Pretinho	1176	956	925	219	211	662	455
Fradinho-2	1333	946	1030	272	257	690	641
MNC99-519D-1-1-5	1207	1053	1106	355	242	712	671
MNC00-544D-10-1-2-2	1320	1020	1048	236	230	601	608
MNC00-544D-14-1-2-2	1358	1063	1183	283	265	704	716
MNC00-553D-8-1-2-2	1297	929	1213	334	224	725	644
MNC00-553D-8-1-2-3	1286	1041	1128	290	236	631	652
MNC00-561G-6	1390	987	1364	251	311	566	688
EV X 63-10E	1218	1176	1155	277	335	585	681
MNC99542F-5	1404	978	1557	401	401	638	861
EV X 91-2E-2	1374	1088	1252	261	296	644	716
MNC99-557F-2	1245	1008	952	244	274	555	511
BRS Guariba	1259	1136	1086	256	325	593	667
Patativa	1273	1031	1524	307	305	575	745
Vita-7	1155	997	1005	319	217	617	512
Mean	1290	1029	1191	292	285	638	662

The mean genotypic correlation of the performance of the genotypes in different environments ($\hat{r}_{gloc}$) indicates the reliability of how constant was the "ranking" of cowpea genotypes over the tested environments. As shown in Tables 2 and 3, there were changes in the ordering of the genotypes due to the low value of $\hat{r}_{gloc}$ (0.20). This indicates the presence of a genotype x environment interaction, and reflects the difficulty of selecting the most widely adapted genotypes.

The genotypes MNC99542F-5 and MNC99-537F-4 had the highest mean genotypic values in all of the environments, in addition to being selected in at least three environments (Table 3). The genetic gains obtained by selecting MNC99542F-5 and MNC99-537F-4 were 18.79% and 18.04%, respectively. These genotypic values were similar to those in other environments with a standard genotype x environment interaction. According to Maia et al. (2009), this occurs because mixed models penalize the predicted genotypic values. Therefore, the same performance of the genetic means ($\hat{\mu} + \hat{g}$) for grain yield is expected when the above genotypes are in different environments.

The genotypic value for the mean of environments ($\hat{\mu} + \hat{g}$) generated results similar to those generated by methods that select for adaptability (RPGV) and adaptability and stability (HMRPGV) simultaneously (Table 4). According to Maia et al. (2009), this interaction chooses the most stable genotypes that are adapted to a range of environments. The two genotypes

chosen by genetic means free of the interaction criterion ($\hat{\mu} + \hat{g}$) were MNC99-542F-5 and MNC99-537F-4. This indicates that these genotypes had high adaptive synergism in the four environments analyzed and exhibited good predictability, i.e., the yield was maintained in different environments. These results are similar to those obtained by Maia et al. (2009) and Regitano Neto et al. (2013), who verified yield maintenance in the ordering of cashew clones and rice genotypes by the genotypic values of the annual average ($\hat{\mu} + \hat{g}$), HMGV, RPGV, and HMRPGV. They attributed their results to genotypic correlation through the local ($\hat{r}_{gloc}$), which was positive and of a similar magnitude to that found in this study.

Table 4. Stability of genetic values (MHVG), adaptability of genetic values (PRVG and PRVGμ), and stability and adaptability of genetic values (MHPRVG and MHPRVGμ) of 20 upright cowpea genotypes as predicted by BLUP the analysis.

Genotype	MHVG	PRVG	PRVGμ	MHPRVG	MHPRVGμ
MNC99-537F-1	476	0.79	521	1.08	717
MNC99-537F-4	616	1.11	738	1.34	888
MNC99-541-F5	466	1.03	681	1.08	713
MNC99-541-F8	266	0.74	492	0.77	513
IT93K-93-10	399	1.05	694	0.82	541
Pretinho	114	1.43	948	0.38	252
Fradinho-2	384	1.06	705	0.93	618
MNC99-519D-1-1-5	409	1.00	665	0.99	654
MNC00-544D-10-1-2-2	278	0.95	631	0.77	511
MNC00-544D-14-1-2-2	405	0.96	639	1.03	679
MNC00-553D-8-1-2-2	358	1.04	692	0.89	592
MNC00-553D-8-1-2-3	368	0.82	545	0.93	616
MNC00-561G-6	395	1.05	697	0.95	632
EV X 63-10E	458	0.95	633	1.03	680
MNC99542F-5	648	0.61	401	1.32	876
EV X 91-2E-2	423	0.91	603	1.04	689
MNC99-557F-2	295	0.87	579	0.70	466
BRS Guariba	435	1.11	733	1.00	662
Patativa	458	1.38	915	1.05	697
Vita-7	279	1.11	737	0.71	470

The genotypes MNC99-542F-5 and MNC99-537F-4 can be grown in various environments, as they exhibit high grain yield, adaptability, and stability. There was agreement between $\hat{\mu} + \hat{g}$, HMGV, and RPGV in identifying the most productive genotypes with high adaptability and stability, indicating that they can be used as selection criteria in cowpea breeding programs.

Conflicts of Interest

The authors declare no conflicts of interest.

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