



# Genetic parameters and prediction of genotypic values for root quality traits in cassava using REML/BLUP

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**ABSTRACT.** The aim of this study was to estimate the genetic parameters and predict the genotypic values of root quality traits in cassava (*Manihot esculenta* Crantz) using restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP). A total of 471 cassava accessions were evaluated over two years of cultivation. The evaluated traits included amylose content (AML), root dry matter (DMC), cyanogenic compounds (CyC), and starch yield (StYi). Estimates of the individual broad-sense heritability of AML were low ( $h_g^2 = 0.07 \pm 0.02$ ), medium for StYi and DMC, and high for CyC. The heritability of AML was substantially improved based on mean of accessions ( $h_m^2 = 0.28$ ), indicating that some strategies such as increasing the number of repetitions can be used to increase the selective efficiency. In general, the observed genotypic values were very close to the predicted average of the improved population, most likely due to the high accuracy (>0.90), especially for DMC, CyC, and StYi. Gains via selection of the 30 best genotypes for each trait were 4.8 and 3.2% for an increase and decrease for AML, respectively, an

increase of 10.75 and 74.62% for DMC for StYi, respectively, and a decrease of 89.60% for CyC in relation to the overall mean of the genotypic values. Genotypic correlations between the quality traits of the cassava roots collected were generally favorable, although they were low in magnitude. The REML/BLUP method was adequate for estimating genetic parameters and predicting the genotypic values, making it useful for cassava breeding.

**Key words:** Breeding; Germplasm; *Manihot esculenta* Crantz; Selection; Genotypic value; Mixed models

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in Africa, Asia, and Latin America (after rice and maize) and is characterized as a food security crop in these regions, where most of the cultivation occurs on marginal lands by small-scale farmers. According to the Food and Agriculture Organization of the United Nations (FAO, 2013), cassava has the potential to become the raw material base for a number of processed products that may increase the demand for cassava and contribute to agricultural transformation and economic growth in developing countries.

The genetic breeding of cassava has made significant progress, especially in increasing the crop yield potential through the development of new varieties (Kawano, 2003; Akinwale et al., 2010). Moreover, in addition to the yield and quality of the root, the variety component plays an important role in the diversification of the market due to cassava's starch-specific characteristics that allow for its broad use (Zhang et al., 2010). These characteristics include the contents of root starch, amylose (AML), cyanogenic compounds (CyC), soluble solids, and starch opacity.

Starch is a major component of cassava and has important food and industrial uses (Ceballos et al., 2007). In addition to its widespread application in the food industry, starch is also used in the textile, paper, metallurgy, pharmacy, and plastic industries (Aryee et al., 2006; Emmanuel et al., 2012). In general, cassava in Brazil is used primarily for the production of flour and starch. Thus, it is necessary for developing new varieties with differing starch characteristics that provide a competitive advantage over those that have been traditionally used in the production system.

Even at the international level, there are only a few cassava breeding programs; therefore, much knowledge is needed to optimize genetic gains for this crop. Although the main economic traits in cassava are quantitatively inherited and, therefore, highly influenced by environmental conditions, there have been few studies that have explored genetic variability in selecting the best genotypes for hybridization by using appropriate breeding methods that consider the inheritance of specific traits. In contrast, breeding progress is mainly determined by the size, nature, and inter-relationship of the genotypic and phenotypic variations of the various traits. Therefore, the separation of total variation into its inheritable and non-inheritable components is fundamental to define the progress and best strategies for selection. Accurate estimates of variance components and determinants for selection are of great importance in cassava breeding. These estimates can be obtained using optimal procedures of estimation/

prediction, thus enabling maximization of gains via selection (Furlani et al., 2005).

The prediction of breeding values for superior materials is the main problem in plant breeding because it requires the true values of the variance components; thus, it becomes necessary to use the BLUP (best linear unbiased prediction) and REML (restricted maximum likelihood) methods. BLUP assumes the knowledge of the variance component values, and estimates of these components by REML have been used. Both methods are associated with a linear mixed model. The use of mixed models in order to obtain predictors of breeding values (BLUP) is a method that has been used in several perennial (Resende and Barbosa, 2006; Hiraoka et al., 2011) and annual species (de Carvalho et al., 2008; Borges et al., 2010; Viana et al., 2010).

This study aimed to estimate the genetic parameters and select cassava genotypes using mixed model methodology. BLUP values and REML compounds were used to obtain the variance components for traits that are related to the yield and quality of cassava starch.

## MATERIAL AND METHODS

### Plant material

Four-hundred and seventy-one accessions from various ecosystems in Brazil, Colombia, Venezuela, and Nigeria were evaluated from the Cassava Germplasm Bank from Embrapa Mandioca e Fruticultura (Cruz das Almas, Brazil). This germplasm bank consists of landraces and improved varieties resulting from conventional breeding procedures such as crossing and selection and a selection of landraces with high-yield potential, as identified by farmers and research institutions.

### Experimental design

Two field experiments were performed in 2011 and 2012 in the Experimental Fields Facilities at Embrapa Mandioca e Fruticultura, Cruz das Almas (Bahia, Brazil). In 2011, a randomized block design with three replications and 10 plants per plot was used. In 2012, an augmented block design with the number of accessions distributed homogeneously over 10 blocks with 10 plants per plot was used. Improved clones (9624-09 and 98150-06), landraces (Cigana and Eucalpto), and recommended varieties (BRS Aipim Brasil, BRS Dourada, BRS Verdinha, BRS Tapioqueira, BRS Caipira, and BRS Gema de Ovo) were used as the controls.

Planting was performed at the beginning of the rainy season (May to July) using 15-20-cm stem cuttings in single rows. Spacing between rows and plants was 0.90 and 0.80 m between, respectively. All of the recommended cultivation practices for cassava were followed. The plants were harvested 11 months after planting.

### Sample preparation

The root sampling was performed according to Rodriguez-Amaya and Kimura (2004). For each replicate, the roots of 10 plants from the plot were collected and mixed, and a random sub-sample of 5-6 roots was taken to represent the batch average. The roots selected were washed in tap water, cut into pieces, peeled (removing the bark), and cut into quarters. Two opposite sides of the roots were used for starch extraction. The other two opposing sides of the

root quarters were chopped and crushed in a multiprocessor and mixer to form a homogeneous paste, which was used in the CyC and moisture analyses.

For starch extraction, cassava roots (500 g) were cut into pieces and ground in a blender, with non-cutting helix, helices thick per minute in a 1:1 ratio (500 g cassava/500 mL cold water). Subsequently, this solution was filtered through a 150-mesh sieve covered with “voile” and then washed with cold water. Then, the recovered starch suspension was kept in a cold chamber at 5°C for 12 h to settle the starch. The supernatant was then discarded, and the starch was decanted, washed with approximately 20 mL 95% ethanol, and dried in an oven with forced air circulation at 40°C for 48 h. The dried starch was ground to a fine-grained powder.

### Physicochemical analyses

The determination of CyC, especially cyanide,  $\alpha$ -hydroxynitrile lyase, and cyanogenic glycosides, present in the samples was performed according to Essers (1994) and consisted of extracting these compounds using a subsequent reaction with chloramine T and 1,3-isonicotinate-dimethyl barbiturate followed by spectrophotometric determination at 605 nm. The linamarase enzyme, which was extracted from the bark of the cassava root, was used to release glycosidic cyanide according to Cooke (1979).

The root dry matter analysis (DMC) was performed in an oven with forced air circulation at 60°C for 48 h until a constant weight was obtained. The starch content was obtained by subtracting the DMC per 4.65%, which is related to the ash, protein, lipid, and fiber contents. Therefore, the starch content was multiplied by the average fresh root yield, generating the starch yield (StYi) in t/ha.

The dried starch was analyzed in relation to the AML content according to ISO (1987). The starch sample gelatinized with sodium hydroxide and acidified with acetic acid was dispersed in 95% ethanol (pure for analysis). After the addition of the iodine solution, the blue color complex was quantified by spectrophotometry at 620 nm using a Biospectro model SP 220. For the AML analysis, only 445 accessions were analyzed according to the amount of available starch.

### Linear mixed model

A combined analysis of different experimental designs was performed using models to incomplete the block designs. In this case, all of the blocks were set as random effects, while the design effects were treated as fixed by adjusting the experiments in a randomized complete block design and, at another level, for the experiments in an incomplete block design. In this case, the model set the block effects within each type of design.

The linear mixed model that was used to describe the data, which was according to Resende (2002a) as follows:

$$y = Xb + Zg + Wp + e \quad (\text{Equation 1})$$

where  $y$  is the data vector;  $b$  is the vector of fixed effects associated with the overall average and block effect;  $g$  is the vector of random genetic effects;  $p$  is the vector of random effects of

the plots;  $e$  is the vector of random errors; and  $X$ ,  $Z$ , and  $W$  are incidence matrices that associate the unknown parameters  $b$ ,  $g$ , and  $p$ , respectively, to the  $y$  data vector.

The distributions and structures of means and variances were calculated by the following:

$$\begin{aligned} y|b, V &\sim N(Xb, V) \\ g|A, \sigma_g^2 &\sim N(0, A\sigma_g^2) \\ p|\sigma_p^2 &\sim N(0, I\sigma_p^2) \\ e|\sigma_e^2 &\sim N(0, I\sigma_e^2) \end{aligned} \quad (\text{Equation 2})$$

The covariance between all of the random effect models was calculated by the following:

$$\text{Cov}(g, p') = 0; \text{Cov}(g, e') = 0 \text{ and } \text{Cov}(p, e') = 0 \quad (\text{Equation 3})$$

where

$$E \begin{bmatrix} y \\ g \\ p \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad (\text{Equation 4})$$

and

$$\text{Var} \begin{bmatrix} y \\ g \\ p \\ e \end{bmatrix} = \begin{bmatrix} V & ZG & WC & R \\ GZ' & G & 0 & 0 \\ CW' & 0 & C & 0 \\ R & 0 & 0 & R \end{bmatrix} \quad (\text{Equation 5})$$

where in

$$G = A\hat{\sigma}_g^2, R = I\hat{\sigma}_e^2, C = I\hat{\sigma}_p^2 \quad (\text{Equation 6})$$

and

$$V = ZA\hat{\sigma}_g^2Z' + WI\hat{\sigma}_p^2W + I\hat{\sigma}_e^2 = ZGZ' + WCW' + R \quad (\text{Equation 7})$$

The mixed model methodology allows for the estimation of  $b$  using a generalized least squares procedure, and  $g$  and  $p$  using BLUP. The system of linear equations [mixed model equations (MMEs)] that were used to obtain the solutions of the model were:

$$\begin{bmatrix} \hat{b} \\ \hat{g} \\ \hat{p} \end{bmatrix} = \begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + A^{-1}\lambda_1 & Z'W \\ W'X & W'Z & W'W + I\lambda_2 \end{bmatrix}^{-1} \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix} \quad (\text{Equation 8})$$

where in

$$\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{1 - h^2 - c^2}{h^2} \quad (\text{Equation 9})$$

and

$$\lambda_2 = \frac{\sigma_e^2}{\sigma_p^2} = \frac{1 - h^2 - c^2}{c^2} \quad (\text{Equation 10})$$

are the shrinkage factors of the random effects of the MMEs, in which  $\sigma_g^2$  is the additive genetic variance,  $\sigma_p^2$  is the variance of the effect of the plot,  $\sigma_e^2$  is the residual variance (environment + non-additive),  $A$  is the matrix of additive genetic correlation between individuals, and  $I$  is the identity matrix.

REML estimates of the variance components were obtained using the expectation and maximization algorithm according to the following expressions:

$$\hat{\sigma}_e^2 = \frac{y'y - \hat{b}'X'y - \hat{g}'Z'y - \hat{p}'W'y}{N - r(x)} \quad (\text{Equation 11})$$

$$\hat{\sigma}_g^2 = \frac{\hat{g}'A^{-1}\hat{g} + \hat{\sigma}_e^2 \text{tr}(A^{-1}C^{22})}{q} \quad (\text{Equation 12})$$

$$\hat{\sigma}_p^2 = \frac{\hat{p}'p + \hat{\sigma}_e^2 \text{tr}C^{33}}{s} \quad (\text{Equation 13})$$

where  $C^{22}$  and  $C^{33}$  were derived from:

$$C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix}^{-1} = \begin{bmatrix} C^{11} & C^{12} & C^{13} \\ C^{21} & C^{22} & C^{23} \\ C^{31} & C^{32} & C^{33} \end{bmatrix} \quad (\text{Equation 14})$$

which is the generalized inverse of the coefficient MME matrix,  $tr$  is the trace of a matrix,  $r(x)$  is the rank of the X matrix,  $N - r(x)$  is the number of degrees of freedom of the error,  $q$  is the number individuals,  $s$  is the number of plots, and  $N$  is the total number of observations.

The coefficients of the individual broad-sense heritability of the block were estimated according to

$$\hat{h}_g^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_p^2 + \hat{\sigma}_e^2} \quad (\text{Equation 15})$$

The standard deviation of the heritability coefficients was obtained from the information matrix of the MMEs. The other estimates of variances and genetic parameters were calculated by

$$\hat{\sigma}_f^2 = \hat{\sigma}_g^2 + \hat{\sigma}_p^2 + \hat{\sigma}_e^2 \quad (\text{phenotypic variance individual}) \quad (\text{Equation 16})$$

and

$$\hat{h}_m^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \frac{\hat{\sigma}_e^2}{b}} \quad (\text{heritability adjusted in average of accessions}) \quad (\text{Equation 17})$$

where  $b$  is the number of blocks. The accuracy of the selection of clones was given by

$$Ac = \sqrt{\hat{h}_m^2} \quad (\text{Equation 18})$$

## Genetic gain

The prediction estimates of REML/BLUP were performed using the Selegen software (Resende, 2002b). The genetic gain was calculated as the average of the breeding values of the selected individuals. The selection was performed using the breeding values of the 30 best genotypes for each trait.

The genotypic values of each clone were obtained by adding each genotypic effect to the overall mean. The genetic gain was equal to the average of the vectors of the predicted genetic effects for the selected accession. The overall mean plus the genetic gain resulted in the improved average population. The relative performance of each accession was determined using the relationship between the average of the improved population for each accession and the genetic value of the better accession, depending on the direction of selection.

## RESULTS AND DISCUSSION

In the mixed model analysis using unbalanced data, the random effect models were

tested by the likelihood ratio test, called the deviance analysis. The deviance values are shown in Table 1 and demonstrate the existence of significant differences among the accessions for all of the traits analyzed ( $P < 0.05$ ), indicating the existence of high levels of genetic variation among cassava accessions.

**Table 1.** Deviance estimates for nine traits related to root quality of cassava germplasm accessions.

Effect	AML		DMC		CyC		StYi	
	Deviance	LRT	Deviance	LRT	Deviance	LRT	Deviance	LRT
Genotypes	3401.71	146.25**	4852.32	489.72**	9504.33	899.16**	5561.5	411.80**
Complete model	3547.96		5342.04		10403.49		5973.3	

AML= amylose content; DMC = root dry matter; CyC = cyanogenic compounds; StYi = starch yield; LRT = likelihood ratio test. \*\*Significant at 5% of probability.

### Estimates of genetic parameters

The prediction of breeding values was dependent on the knowledge of the genetic control of the trait and, in particular, the parameters of individual heritability and repeatability. Individual estimates of genetic parameters such as individual heritability are scarce in cassava and should therefore be investigated.

Variance components, heritability estimates, the coefficient of variation, and accuracy are presented in Table 2. Although the AML trait was significantly different between the cassava accessions, the estimated individual broad-sense heritability was low ( $h^2 = 0.07 \pm 0.02$ ). However, the estimates of  $h^2$  were medium magnitude for StYi ( $0.50 \pm 0.05$ ) and DMC ( $0.56 \pm 0.06$ ), and high magnitude for CyC ( $0.74 \pm 0.06$ ). The  $h^2$  contains the additive and dominance variances between units of selection effectively used for the selection of better plants, especially considering that cassava genetic variance is transmitted by cloning.

The standard deviation for  $h^2$  was always <20% of the estimated heritability for all traits, except AML, ensuring a good prediction of breeding values according to Resende (2002a). Thus, there is good genetic control of the expression of these traits related to root quality; therefore, they show great potential for selection because the progress expected from selection depends directly on the heritability and the intensity of selection, which is inversely related to the phenotypic standard deviation (Dudley and Moll, 1969).

Individual heritability estimates were of median magnitudes for DMC and StYi, and of high magnitude for CyC; however, there was high heritability for the average of the accessions ( $h_m^2$  of 0.80, 0.85, and 0.93 for StYi, DMC, and CyC, respectively). The main difference observed between  $h_g^2$  and  $h_m^2$  was attributed to lower phenotypic variation in the calculation of inheritance due to the reduction in residual variance and the absence of plot variance.

Different estimates of broad-sense heritability for DMC have been reported in the literature, such as 0.42 (Balyejusa et al., 2007), 0.56 (Akinwale et al., 2010), and 0.80 (Aina et al., 2007), which show that the results presented in this study are consistent with previous reports. However, according to Kawano et al. (1987), DMC is strongly influenced by the age of the plant, variety, and location and time of harvest. However, different values of heritability can be found for certain traits of the same species, mainly due to differences in the methods that are used for its determination, the genetic materials, the growth location, and the age of evaluation.



Furthermore, Balyejusa et al. (2007) found estimates of broad-sense heritability for CyC to be of a lower magnitude than those observed in the present study (0.43), but the authors mention that the observed moderate heritability for CyC and DMC indicate that the phenotypic variations in these traits have genetic components that are important for QTL mapping. As a result, they identified two QTLs with additive effects for CyC as well as six QTLs, where one had an additive effect and the others had dominance/overdominance effects.

**Table 2.** Estimates of variance components, heritability, accuracy, and coefficients of variation for amylose (AML), dry matter content (DMC), cyanogenic compounds (CyC), and starch yield (StYi).

Parameter	AML	DMC	CyC	StYi
$\sigma_g^2$	0.37	8.46	438.97	11.27
$\sigma_b^2$	0.88	0.66	16.26	0.56
$\sigma_e^2$	3.85	6.07	139.34	10.93
$\sigma_f^2$	5.10	15.19	594.58	22.76
$h_g^2$	0.07 ± 0.02	0.56 ± 0.06	0.74 ± 0.06	0.50 ± 0.05
$c_b^2$	0.17	0.04	0.03	0.02
$h_m^2$	0.28	0.85	0.93	0.80
Ac	0.53	0.92	0.96	0.90
CVg	3.53	7.67	71.88	39.45
CVe	11.33	6.50	40.50	38.84
CVr	0.31	1.18	1.77	1.02
$\bar{X}_f$	17.32	37.93	29.15	8.51

$\sigma_g^2$  = genotypic variance;  $\sigma_b^2$  = environmental variance between blocks;  $\sigma_e^2$  = residual variance;  $\sigma_f^2$  = individual phenotypic variance;  $h_g^2$  = individual broad-sense heritability of total genotypic effects;  $c_b^2$  = intraclass correlation coefficient;  $h_m^2$  = adjusted heritability in average of accessions; Ac = accuracy of genotype selection; CVg = genotypic coefficient of variation; CVe = residual coefficient of variation; CVr = relative coefficient of variation (CVg / CVe);  $\bar{X}_f$  = overall mean.

The importance of these results is that the high  $h_g^2$  estimates for DMC, StYi, and CyC can contribute to rapid selection, even with the use of simple selection methods, such as phenotypic recurrent selection. In contrast, the lowest  $h_g^2$  for AML indicates that the expression of this trait has great environmental influences and direct selection may be ineffective. Special attention should be given to AML, whose heritability was substantially improved when it was calculated using the average of the accessions ( $h_m^2 = 0.28$ ) (Table 2). This procedure most likely attenuated the variation in repeated measurements and proved to be a method for increasing the efficiency of selection for this trait, which is of great importance in cassava breeding. Thus, it can be concluded that the results observed in this study indicate low heritability for AML, and strategies to increase the selection efficiency are required, such as an increase in the number of repetitions in the field.

Heritability estimates subsidizing the choice of the best breeding strategy reveal the potential for genetic progress with breeding and are essential for the prediction of breeding values. For example, da Costa et al. (2007) demonstrated using clonal tests in which 100 plants per clone led to accuracies >90%, regardless of broad-sense heritability, and that the appropriate number of teak (*Tectona grandis* L.f.) trees varies greatly depending on the individual

broad-sense heritability. In contrast, for traits with a heritability of 0.20, it is possible to obtain an accuracy of approximately 95% using 40 plants per clone and an accuracy of 90% using approximately 18 plants per clone. By using this knowledge for cassava breeding, it appears that fewer plants per genotype can be used for DMC, CyC, and StYi traits.

The REML allows for the estimation of the variance components in an unbalanced situation of experiments. This possibility is an interesting aspect in the context of cassava genetic breeding, whereas the implementation of trials with large numbers of accessions in balanced experiments is challenging because of the difficulties related to the low availability of propagation material for use in all repetitions and differential survival rates of genotypes, which is related to the quality of the stakes used for planting.

High values of the coefficient of determination due to the common environment of the plot ( $c_b^2$ ) indicated greater environmental variability between plots, as this parameter quantifies the variability within blocks. In our study, the  $c_b^2$  values ranged from 0.02 to 0.04 for DMC, CyC, and StYi, and 0.17 for AML (Table 2). According to Resende (2002a), the  $c_b^2$  value should be  $<0.10$  for experiments of good quality.

Low genotypic coefficients of variation (CVg) were observed for AML and DMC (3.53 and 7.67%, respectively). In contrast, CVg showed high genotypic variability in CyC and StYi (71.88 and 39.45%, respectively) among cassava accessions. This genetic variability is a prerequisite for selecting the best accessions. For these same traits (CyC and StYi), the highest values of the coefficient of residual variation were observed (40.50 and 38.84% for CyC and StYi, respectively). However, the sole observation of this coefficient does not allow for a proper evaluation of experimental quality, which is necessary to consider the coefficient of relative variation ( $CVr = CVg / CVe$ ). Therefore, CyC was the trait with the highest CVg / CVe ( $CVr = 1.77$ ), indicating a high possibility of selection gains for this trait. A similar situation was observed for DMC ( $CVr = 1.18$ ) and StYi ( $CVr = 1.02$ ).

Experimental coefficients of variation  $>50\%$  were observed for the shoot weight and noncommercial root weight of the sweet potato (*Ipomoea batatas* L.) (Borges et al., 2010), as well as the shoot weight, yield per plant, and total yield per area of cassava (Aina et al., 2007). According to Borges et al. (2010), this magnitude of coefficient of variation, as well as other statistical parameters, is common when evaluating crops traits with underground structures, where environmental control is difficult. However, the maximum CVe observed in this experiment for cassava was 40.50% for CyC.

The accuracy values or correlation between the predicted and true genotypic values were  $>0.90$  for DMC, CyC, and StYi. However, the accuracy for AML was 0.53 (Table 2). According to Resende and Duarte (2007), these accuracies are medium (0.53) for AML and very high (above 0.90) for DMC, CyC, and StYi.

High CVe values were observed for AML, which contributed to the achievement of low CVr estimates (0.31) and selective accuracy. However, this accuracy can be increased in future experiments using more adequate trials while maintaining the same size experiment but reducing the plot size and increasing the number of repetitions, as recommended by da Costa et al. (2007). These changes could increase the individual broad-sense heritability ( $h_g^2$ ) due to the smaller size and greater homogeneity of the block, which certainly contributed to the increase in selective accuracy. According to Resende and Duarte (2007), even with CVr values below unity, it is possible to achieve a selective accuracy  $\geq 0.70$  if an adequate number of repetitions is used.

### Prediction of genotypic values

In the mixed model theory, assuming that the effects of the treatments are random, the multiple comparison tests that are usually implemented in the analysis of variance approach are not performed because these are derivatives of the supposition of treatment as fixed effects, and are applied and produce inferences about average phenotypic and not genotypic effects (Resende, 2002a). Considering the treatment effects to be random is essential for selecting and making inferences about the average values. Thus, the effects and predicted genotypic values, phenotypic values, genetic gains, and improved population mean and phenotypic values of the top 30 cassava accessions that were evaluated are presented in Tables 3, 4, and 5. For AML, the genotypic values ranged from 16.39 to 18.92%, with an average of 17.32%. Table 3 presents the 30 genotypes with the highest genotypic values for AML. The two improved varieties (BRS Dourada and BRS Gema de Ovo) showed higher genotypic AML values, while the improved clones were classified above the 133rd position.

**Table 3.** Rank (Rk) of cassava accessions and their genotypic values ( $\mu + g$ ), new averages (NA), and selective accuracies (Ac) for higher and lower amylose contents.

Higher amylose content					Lower amylose content				
Rk	Accession	$\mu + g$	NA	Ac	Rk	Accession	$\mu + g$	NA	Ac
1	BRS Dourada	18.92	18.92	0.76	445	BGM0678	16.39	17.32	0.53
2	BRS Gema de Ovo	18.26	18.59	0.76	444	BGM0776	16.53	17.32	0.47
3	BGM1183	18.18	18.45	0.47	443	BGM1186	16.56	17.32	0.47
4	BGM1184	18.15	18.38	0.53	442	BGM0818	16.57	17.32	0.47
5	BGM0624	18.13	18.33	0.53	441	BGM0834	16.59	17.32	0.47
6	BGM0631	18.09	18.29	0.53	440	BGM1692	16.66	17.32	0.47
7	BGM0248	17.98	18.25	0.47	439	BGM0847	16.66	17.33	0.53
8	BGM0367	17.97	18.21	0.53	438	BGM0677	16.70	17.33	0.47
9	BGM1552	17.95	18.18	0.53	437	BGM0184	16.71	17.33	0.53
10	BGM0885	17.95	18.16	0.53	436	BGM0928	16.74	17.33	0.47
11	BGM0041	17.94	18.14	0.53	435	BGM0956	16.76	17.33	0.40
12	BGM1333	17.94	18.12	0.53	434	BGM0303	16.78	17.33	0.53
13	BGM1236	17.92	18.11	0.53	433	Cigana	16.80	17.33	0.76
14	BGM0540	17.90	18.09	0.53	432	BGM0800	16.81	17.34	0.53
15	BGM0360	17.86	18.08	0.53	431	BGM0089	16.81	17.34	0.47
16	BGM0331	17.86	18.06	0.53	430	BGM1123	16.81	17.34	0.53
17	BGM1100	17.85	18.05	0.47	429	BGM1085	16.82	17.34	0.47
18	BGM0103	17.84	18.04	0.53	428	BGM0273	16.83	17.34	0.53
19	BGM1190	17.81	18.03	0.53	427	BGM0499	16.84	17.34	0.47
20	BGM0204	17.80	18.02	0.47	426	BGM0341	16.85	17.34	0.53
21	BGM0823	17.79	18.00	0.53	425	9624-09	16.85	17.34	0.76
22	BGM0901	17.79	17.99	0.53	424	BGM0455	16.86	17.34	0.47
23	BGM0057	17.79	17.99	0.53	423	BGM0434	16.87	17.35	0.47
24	BGM0166	17.78	17.98	0.53	422	BGM0390	16.88	17.35	0.53
25	BGM0547	17.78	17.97	0.53	421	BGM0093	16.89	17.35	0.53
26	BGM1491	17.78	17.96	0.40	420	BGM1361	16.89	17.35	0.53
27	BGM1623	17.78	17.96	0.53	419	BGM0649	16.90	17.35	0.47
28	BGM1495	17.77	17.95	0.47	418	BGM0767	16.90	17.35	0.53
29	BGM1124	17.76	17.94	0.47	417	BGM1440	16.92	17.35	0.47
30	BGM0295	17.75	17.94	0.40	416	98150-06	16.94	17.35	0.47

The breeder should implement strategies to predict the individual genetic value and plan how to use individuals with the highest breeding values. In cassava, it is possible to operate using two basic strategies. The first one involves the generation of populations formed by descendants of individuals selected, where the selection will act by altering the allele frequencies in the loci controlling the traits under selection to alter the genotypic mean of the popula-

tion in the desired direction. In this case, for segregating populations derived from the crossing of superior genotypes, it is important to estimate the additive genetic values, considering that only these additive effects are transmitted to offspring via sexual reproduction. In the second strategy, the goal is to select individuals with the highest predicted values, as it is possible to use clonal propagation. In this situation, it is interesting to estimate genotypic values that capitalize the additive and dominance effects, which are fully transmitted to the next generation through vegetative propagation.

**Table 4.** Rank (Rk) of cassava accessions and their genotypic values ( $\mu + g$ ), new averages (NA), and selective accuracies (Ac) for dry matter content (DMC) and cyanogenic compounds (CyC).

Rk	Accession	DMC			Rk	Accession	CyC		
		$\mu + g$	NA	Ac			$\mu + g$	NA	Ac
1	BGM1202	43.46	43.46	0.92	471	BGM0341	0.00	29.15	0.96
2	BGM0443	43.17	43.32	0.92	470	BGM1195	0.00	29.22	0.95
3	BGM0027	43.08	43.24	0.92	469	BGM0376	0.00	29.28	0.96
4	BGM0656	43.02	43.18	0.86	468	BGM0375	0.00	29.34	0.95
5	BGM2043	42.44	43.03	0.92	467	BGM0868	0.17	29.40	0.96
6	BGM0425	42.38	42.93	0.92	466	BGM1491	0.93	29.47	0.93
7	BGM0144	42.36	42.85	0.92	465	BGM0656	0.98	29.53	0.93
8	BGM1116	42.33	42.78	0.92	464	BGM1526	1.00	29.59	0.93
9	BGM1311	42.22	42.72	0.92	463	BGM0367	1.00	29.65	0.96
10	BGM0341	42.17	42.66	0.92	462	BGM1100	1.29	29.71	0.95
11	BGM0542	42.14	42.62	0.92	461	BGM0433	1.34	29.77	0.93
12	BGM0940	42.03	42.57	0.90	460	BGM0800	1.75	29.84	0.95
13	BGM0544	42.02	42.52	0.92	459	BGM0041	2.02	29.90	0.96
14	BGM0620	41.98	42.49	0.90	458	BGM0254	2.92	29.96	0.96
15	BGM1450	41.91	42.45	0.76	457	BGM0330	3.14	30.02	0.96
16	BGM1171	41.85	42.41	0.92	456	BGM0542	3.45	30.08	0.96
17	BGM0783	41.80	42.37	0.90	455	BGM0443	3.52	30.13	0.96
18	BGM1692	41.77	42.34	0.90	454	BGM0624	3.65	30.19	0.96
19	BGM0845	41.75	42.31	0.92	453	BGM0631	3.85	30.25	0.96
20	BGM1495	41.74	42.28	0.92	452	BGM0276	3.99	30.31	0.95
21	BGM0818	41.61	42.25	0.90	451	BGM0149	4.10	30.37	0.96
22	BGM0143	41.60	42.22	0.76	450	BGM0642	5.00	30.43	0.96
23	BGM1956	41.51	42.19	0.76	449	BGM0501	5.24	30.48	0.96
24	BGM0196	41.51	42.16	0.90	448	BGM0434	5.30	30.54	0.95
25	BGM0587	41.49	42.13	0.90	447	BGM0307	5.39	30.60	0.95
26	BGM0455	41.49	42.11	0.90	446	BGM0464	5.87	30.65	0.93
27	BGM0428	41.43	42.08	0.92	445	BGM0211	5.97	30.71	0.96
28	BGM0800	41.38	42.06	0.92	444	BGM1517	6.15	30.76	0.96
29	BGM1362	41.38	42.04	0.76	443	BGM0908	6.24	30.82	0.95
30	BGM0821	41.30	42.01	0.92	442	BGM0356	6.69	30.88	0.96

Considering the generation of segregating populations from crosses of the 30 best genotypes, the new average of the population would be 18.14% for AML, which corresponds to a gain of 4.8 and 2.1% in comparison to the averages of the experiment and controls, respectively. In contrast, if the strategy uses the genotypes with higher average predicted values for commercial use, the varieties BRS Dourada and BRS Gema de Ovo would be optimal for obtaining higher AML contents (Table 3). Other literature reports AML phenotypic values ranging from 17.9 to 23.6% (Defloor et al., 1998), 18.0 to 25.0% (Moorthy, 2004), 13.6 to 23.8% (Rickard et al., 1991), and 0.0% AML, known as the waxy variety (Ceballos et al., 2007). In the last case, the functional properties of waxy starch are different from those of normal starches, including a high viscosity, high swelling index, and low solubility. Other products such as glucose, fructose syrups, and other beverages, are being developed, depending on the starch and amylose contents from cassava varieties (Aryee et al., 2006).

**Table 5.** Rank (Rk) of cassava accessions and their genotypic values ( $\mu + g$ ), new averages (NA), and selective accuracies (Ac) for starch yield (StYi).

Rk	Accession	$\mu + g$	NA	Ac	Rk	Accession	$\mu + g$	NA	Ac
1	BGM1626	19.05	19.05	0.90	16	BGM0279	14.25	15.66	0.89
2	BGM0120	17.93	18.49	0.89	17	BGM2044	14.11	15.57	0.90
3	BGM2020	16.71	17.90	0.71	18	BGM1598	14.09	15.48	0.90
4	BGM1200	16.47	17.54	0.89	19	BGM0165	14.07	15.41	0.87
5	BRS Caipira	15.88	17.21	0.97	20	BGM0937	14.05	15.34	0.89
6	Filha da Preta	15.81	16.97	0.71	21	9624-09	14.03	15.28	0.97
7	BGM0892	15.48	16.76	0.89	22	BGM0011	14.02	15.22	0.87
8	BGM0163	15.39	16.59	0.89	23	BGM0337	14.01	15.17	0.89
9	BGM0425	15.25	16.44	0.89	24	BGM0049	13.98	15.12	0.89
10	BGM0823	15.09	16.30	0.89	25	BGM1165	13.94	15.07	0.89
11	BGM0010	14.92	16.18	0.89	26	BGM0303	13.88	15.03	0.89
12	BGM1085	14.86	16.07	0.87	27	BGM0598	13.80	14.98	0.89
13	BGM0394	14.61	15.96	0.89	28	BGM0332	13.79	14.94	0.89
14	BGM0847	14.47	15.85	0.89	29	BGM0276	13.78	14.90	0.87
15	BGM1282	14.35	15.75	0.87	30	BGM0039	13.62	14.86	0.89

The reduction in AML is an interesting attribute for the starch industry; thus, we selected 30 accessions with lower AML contents (Table 3). In this case, the genotypic values ranged from 16.39 to 16.94%, with an average of 16.76%. In addition to 27 other germplasm accessions, two clones (9624-09 and 98150-06) and a local variety (Cigana) were selected based on this criterion. The genetic gain (reduced amylose) with the selection of these genotypes was 3.2 and 5.6% in comparison to the overall average of the experiments and controls, respectively. The two accessions with the lowest AMLs were BGM0678 and BGM0776, with predicted averages of 16.39 and 16.53%, respectively (Table 3).

Among the various prediction methods, BLUP has the ability to maximize selective accuracy (Resende, 2002a). However, the selective accuracies in both accessions with high and low AMLs were relatively low (ranging from 0.40 to 0.76), which compromises the accurate selection of the best genotypes for an increase or decrease in this trait based on the current data. However, as mentioned earlier, the accuracy of this trait can be improved by reducing the plot size and increasing the number of experimental repetitions.

For DMC, genotypic values for all cassava accessions ranged from 24.93 to 43.46%. Low variation in the phenotypic data was observed by Raji et al. (2007) (33.2-39.2%) and can be explained by the lower number of cassava accessions evaluated, thus restricting the genetic variability to be sampled. In relation to the DMC trait, the 30 top clones showed genotypic values ranging from 41.30 to 43.46%, with an average of 42.01%. The genetic gain from selecting those 30 accessions was 10.75% and 5.5% in comparison to the overall average of the experiments and controls, respectively. The selective accuracy for this trait was quite high (0.76-0.92) (Table 4), which ensures greater precision in selecting the optimal cassava accessions for expressing this trait. Moreover, Akinwale et al. (2010) reported median estimates of genetic heritability and low gains (8.7%) for DMC, which may be due to lower variability in the varieties that were examined by these authors.

The DMC increase is important because, according to Rickard et al. (1991), approximately 74-85% of the dry root weight is starch. Therefore, the DMC directly affects the amount of starch that is extracted from the cassava roots. In addition, the starch producers in Brazil generally pay different prices for fresh roots depending on the DMC.

The predicted values for CyC showed a wide variation, ranging from 0.00 to 115.53

mg/kg (considering all genotypes). When evaluating 11 cassava varieties from Nigeria, Raji et al. (2007) observed phenotypic variation similar to that of CyC (40.0 mg/kg from landrace I sunikankiyan to 128.6 mg/kg from cultivar TMS30572).

According to Jansz and Uluwaduge (1997), based on the contents of the CyCs, the cassava roots can be classified into three classes, including low toxicity or sweet cassava (<50 mg/kg), medium toxicity (between 50 and 100 mg/kg), and high toxicity or bitter cassava (>100 mg/kg). In general, the sweet cassava market demands varieties with good cooking quality and low levels of CyCs (linamarin and lotaustralin), which produce hydrogen cyanide. The identification of cassava varieties with low *in natura* CyC levels in the roots is necessary to reduce the risk of poisoning consumers (Jansz and Uluwaduge, 1997). Moreover, the processing steps of the roots (fermentation, pressing, washing, drying, and exposure to high temperatures) used in the manufacture of flour and starch greatly reduce the CyC levels (Pi-yachomkwan et al., 2005). Thus, considering that most of the production of cassava roots is destined for industrial flour and starch, the CyC content is not a major trait in the selection of varieties for this type of market, wherein high productivity and starch content are the most valued traits.

The wide variation in the CyC content found in this study certainly contributed to a suitable choice of the best genotypes for diverse uses, with a special focus on the fresh market (*in natura*). Thus, the selection of the 30 best clones with lower CyC values showed genotypic variation from 0.00 to 6.69%, averaging 3.03%. The genetic gain (in the sense of reduction) to select these 30 accessions was 89.60 and 88.07% in comparison to the overall average of the experiments and controls, respectively. Furthermore, the selective accuracy of this trait was also quite high (0.93-0.96) (Table 4).

Mainly for the flour and starch industries, the amount of starch is crucial in choosing which variety to use in a large-scale production system where it is necessary to maximize the efficiency of production per hectare. In this sense, it is important to consider the productive potential of the variety and its starch content, which, in the current study, was StYi (Table 5). Similar to other traits, the predicted genotypic values were quite variable among the accessions (3.24-19.05 t/ha), making this population a good choice for selecting the best individuals and use as a parental population to generate segregating populations. In these instances, there would be accurate selection of these accessions, considering that the magnitude of selective accuracy ranged from high (0.71-0.89 in 25 accessions) to very high (>0.90 in five accessions) (Table 5).

For StYi, the top 30 clones showed genotypic values ranging from 13.62 to 19.05%, with an average of 8.51%. The genetic gains by selecting those accessions were 74.62 and 49.95% in comparison to the overall average of the experiments and controls, respectively (Table 5). Among the controls, one clone (9624-09), one recommended variety (BRS Caipira), and one local variety (Filha da Preta) stood out as the most promising accessions for StYi.

Generally, for all of the root quality traits of cassava, we observed that the genotypic values ( $\mu + g$ ) were very close to the new average because REML/BLUP was the best estimator of genotypic values (Resende, 2002a; Klapšć et al., 2007; de Carvalho et al., 2008; Viana et al., 2012). Furthermore, this methodology has the following main advantages, including that it (a) has applications in experiments with unbalanced data, (b) does not require data from rigid experimental structures, (c) allows for the simultaneous use of a large amount of information from various experiments, thus generating more accurate estimates, and (d) corrects



the data for environmental effects accurately and, without bias, predicts the genotypic values, thus maximizing the selective genetic gain (Resende, 2002a).

One point of concern is the ability of breeders to select the best genotypes to be used for crossing to generate segregating populations or *per se* use in the production system. If the goal of the program is to promote the recombination of the best genotypes, then selection should be made based on the additive genetic values of individuals. In contrast, if the goal is to clone the genotype, selection should be made using genotypic values, which are necessary to obtain non-additive variance. In the latter case, making inferences about the genetic materials in field experiments in order to rank those candidates to be released as cultivars is no easy task, as these inferences must be based on true genotypic values. For example, inferring genotypes at any stage of a breeding program should be based on the genetic and not phenotypic means because the genotypic averages indicate future averages when the cultivars are planted in commercial fields (Borges et al., 2010). In this context, the use of improved prediction methods such as BLUP provides higher selection accuracy and, thus, dramatically increases accuracy in the evaluation and selection of superior genotypes.

The high level of diversity observed for root quality traits can be used to aid in the development of cassava segregating populations with specific traits for use in food, feed, or for industrial purposes. This study also revealed new sources for low CyCs and high starch productivity in addition to providing useful information regarding the inheritance of these traits to better plan strategies for selection and recombination in cassava breeding programs.

### Genetic correlations

The genetic correlation ( $r_g$ ) for AML was greater in magnitude than that of the phenotypic correlation. For other combinations, the phenotypic correlations were greater (Table 6). The phenotypic correlations may be smaller than the genetic correlations when associated with low and positive environmental correlations and when controlled by similar genes.

**Table 6.** Estimates of genetic (above the diagonal) and phenotypic (below the diagonal) correlation coefficients in cassava accessions for amylose content (AML), dry matter content (DMC), cyanogenic compounds (CyC), and starch yield (StYi).

Traits	AML	DMC	CyC	StYi
AML	-	0.25	-0.10	0.46
DMC	0.25	-	0.18	0.36
CyC	0.05	0.29	-	0.01
StYi	0.26	0.42	0.05	-

The  $r_g$  among the root quality traits of cassava that was obtained in this study was generally favorable, although low in magnitude. Favorable  $r_g$  between AML x DMC (0.25), AML x StYi (0.46), and DMC x StYi (0.36) (Table 6) indicates that the increase in the DMC may elevate the amylose content and starch yields of cassava roots. On the other hand, no significant  $r_g$  was observed for CyC. Moreover, Dixon et al. (1994) reported that the values of the phenotypic correlation between DMC and CyC are negatively correlated, with variation

ranging from -0.73 to -0.55, depending on the genotype or location.

Although genetic correlations can quantify the possibility of indirect gains by selection on correlated traits, especially for those with low heritability, we found that traits of the cassava roots cannot be efficiently and indirectly selected using other correlations because of the low genetic correlations.

### **Perspectives for use in cassava breeding**

In cassava, variation plays an important role in producing diverse foods due to the inherent characteristics that vary with genotype (Ceballos et al., 2007; Zhang et al., 2010). Such traits include amylose and starch contents, CyCs, carotenoids, and others. These traits are used in breeding programs without neglecting yield, earliness, resistance to pests and diseases, or adaptability to different production regions. Thus, improvements in new methodologies for genetic analysis aimed at increasing yield levels and the quality of cassava starch is increasingly important for ensuring the competitiveness and sustainability of cassava in Brazil. According to Balyejusa et al. (2007), until recently, cassava breeding programs conducted their work without much knowledge of the genetic architecture of the main traits under selection, which certainly tends to reduce the efficiency of breeding and selection gains.

In addition, there are other important problems, such as the long breeding cycle of cassava, which lasts at least five years when calculating a year for each of the following steps: evaluation of seedlings; and the clonal, preliminary, and advanced trials and crossings of the best individuals. This long period is necessary because of the low multiplication rates of cassava (1:6, on average), which means there would be enough propagation material to repeat trials only in the later stages. Therefore, the high cost of extensive and time-consuming progeny evaluations under field conditions requires the accurate estimation of variance components for the prediction of breeding values and maximizing the accuracy of selection in cassava breeding programs.

Throughout this process, selecting the wrong genotypes for crossing or those used in regional trials can pose a serious problem. In this context, the individual BLUP predicts the breeding values of the random effects of the statistical model associated with phenotypic observations, fitting the data to the fixed effects and the unequal amount of information in the plots by mixed model methodology (Resende, 2002a). Thus, it is expected that the use of improved prediction methods such as BLUP provides greater accuracy in selection and drastically reduces the period of genotypic evaluation, as the estimation of genotypic values can be initiated during the seedling stage using familial information.

A seedling stage produces thousands of unique heterozygote individuals originating from crosses between parents selected. Thus, the selection performed at this stage for traits with low individual heritability, such as AML, would not be indicated. However, the selection would be more effective if based on familial information in which most of the phenotypic variation can be explained by genetic components. In contrast, according to Latter (1964), when the heritability exceeds 0.50, selection based on individuals tends to be higher than that based on families. Therefore, the  $h_g^2$  estimates for StYi, DMC, and CyC when applied individually during the early stages of cassava breeding may contribute to the identification of promising new families for these traits and yield a greater number of genotypes for potential use as new cultivars.



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