

Phenotypic stability in cassava estimated by the AMMI analysis with supplementary genotypes

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Abstract: *Adaptability is the yield response of cassava genotypes to environmental improvement, and stability is the predictability of this response to variations. The objective of this study was to evaluate the yield stability and adaptability of cassava varieties and clones using the AMMI (Additive Main Effects and Multiplicative Interaction) methodology and auxiliary tools as supplementary genotype and environment. The responses of 24 cassava genotypes were evaluated in three environments located in the state of Alagoas, Brazil, in randomized blocks design, with 24 treatments and three replications. The effects of genotype and genotype x environment interactions significantly influenced yield. Genotypes differed regarding the adaptability and stability of yield response, and the varieties Cria Menino and Preta do Araripe were considered ideal genotypes due to high adaptability and yield stability. Among the three tested environments, Limoeiro de Anadia was the best since it presented high yield, phenotypically stable genotypes for cassava breeding programs.*

Key words: *Manihot esculenta, ideal genotype, ideal environment, genotype x environment interaction.*

INTRODUCTION

Cassava is an economically and socially important root vegetable since it generates labor and income for small farmers in large areas of the developing world. The use of Cassava co-products, such as the aerial parts and roots, has grown in recent years, being used to produce tapiocas, special flours, appetizers, and animal feeding. Brazil is currently the world's second largest cassava producer, although the mean yield (14.82 ton ha⁻¹) is 59% lower than that of India (35.65 ton ha⁻¹) (FAO 2016). Cassava is the sixth most widely planted crop in Brazil. Cassava yield accounted for 22.53 million tons in the year of 2016, most of which (61%) was produced in the North, Northeast and South-Central regions (IBGE 2016). In the Northeast, in the 2015/2016 harvest, the largest planted areas occurred in the states of Bahia (290 million ha) and Maranhão (158 million ha), and the most productive states were Alagoas (14.17 ton ha⁻¹) and Sergipe (15.39 ton ha⁻¹) (IBGE 2016).

In Alagoas, as in many other states, yield is limited due to the lack of data on the growth of cassava cultivars in specific environments (Santiago et al. 2015). Indeed, the lack of information on high yielding cassava cultivars for the several Brazilian environmental conditions is one of the main limiting factors. Cultivars with high and stable yields over a range of environmental conditions are especially valuable and can be experimentally identified by quantifying

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genotype x environment interactions, by repeating the trials in several regions of interest and testing a large number of genotypes in several different environments (Sagrilo et al. 2008). Phenotypic variation in characteristics such as yield is a result of the joint action of the genotype (G), environment (E) and their interaction (GxE), and reflects differences in the response of genotypes to environmental variation (Allard and Bradshaw 1964). Consequently, selection procedures solely based on the mean yield of genotypes in a given environment are ineffective, and the identification of high yield cultivars should be based on GxE interactions (Morais et al. 2008).

The evaluation of the GxE interactions using the F-test is relatively straightforward. However, this analysis does not generate detailed information on how the genotypes respond to environmental variations. Therefore, the study on both adaptability and stability of genotypes is desirable in order to identify those with the most predictable responses to diverse environments. Among the several methodologies to analyze adaptability and stability (Eberhart and Russel 1966, Lin and Binns 1988, Gauch and Zobel 1996, Toler and Burrows 1998), the multivariate AMMI or Additive Main Effects and Multiplicative Interaction Analysis is the most widely used in Brazil (Carbonell et al. 2004, Pacheco et al. 2005, Kvitschal et al. 2007, Kvitschal et al. 2009, Gonçalves et al. 2009, Gonçalves et al. 2010, Oliveira et al. 2010, Mattos et al. 2013, Barros et al. 2013, Oliveira et al. 2014). This method combines analysis of variance and principal components to adjust the main effects (genotypes and environments) and the effects of GxE interactions (Duarte and Vencovsky 2001, Zobel et al. 1988). The AMMI analysis can assist the identification of high yield genotypes that are adapted to different environmental conditions, and these analyses are ideal to generate recommendations for regions of agronomic zoning.

The purpose of this study was to evaluate the phenotypic stability and adaptability of 24 cassava genotypes in three distinct environments in the state of Alagoas, using the AMMI analysis with a supplementary genotype tool to identify the ideal genotype and the environment for cultivation.

MATERIAL AND METHODS

Yield stability of 24 genotypes of cassava roots was evaluated in three municipalities in the agrestic region of the state of Alagoas, Brazil: Arapiraca (lat 9°45' 9" S, long 36° 39' 39" W, alt 264 m asl), in Lagoa da Canoa (lat 9° 55' 31" S, long 36° 28' 33" W, alt 175 m asl), and Limoeiro de Anadia (lat 9° 45' 9" S, long 36°39' 40" W, alt 264 m asl).

All experiments consisted of randomized block design with three replications. Plots of 40 m² were used and cultivated according to the technical recommendations for cassava (Ferreira Filho et al. 2013). Genotypes were evaluated for root yield (ton ha⁻¹). Data for root yield per plot were acquired 16 months after planting. Initial analysis of variance was performed for each environment to verify the existence of differences between varieties. After these analyses, homogeneity between residual variances was determined using the Hartley test (Ramalho et al. 2005). Joint analysis of variance was used to test genotype effects, environment effects and the magnitude of the GxE interaction. Joint analysis and homogeneity test were performed using the Genes software (Cruz 2013).

In the presence of significant interactions, stability and adaptability phenotypic analyses were performed according to the AMMI methodology (Zobel et al. 1988), using the supplementary genotype and environment proposed by Duarte and Vencovsky (1999), Pacheco et al. (2005), which in this study will be called genotype and ideal environment. Multivariate procedures have been studied in cassava by Kvitschal et al. (2006), Carvalho et al. (2008) and Ojulong et al. (2010).

The AMMI analysis (Zobel et al. 1988) combines, in a single model, additive components for the main effects of genotypes (g_i); environments (e_j) and multiplicative components to the effect of the interaction (ge_{ij}). Besides the conventional terms of a joint analysis ($\mu, g_i, e_j, \varepsilon_{ij}$), the model includes terms from the principal component analysis (PCA) applied to the matrix of interactions $GE(m \times n) = [(ge)_{ij}]$, being $(ge)_{ij} = Y_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{..}$. Therefore, the model that describes the mean yield of the genotype i in environment j ($j=1,2,\dots, n$) is:

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^n \lambda_k v_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij},$$

in which Y_{ij} is the mean yield from genotype i in environment j ; μ is the general mean yield; g_i is the effect of genotype i ; e_j is the effect of environment j ; λ_k is the k singular value of GE (scalar) from matrix $GE(m \times n)$; v_{ik} is the genotype i in the singular column vector of matrix $GE(m \times n)$; α_{jk} is the environment j in the singular vector line of matrix $GE(m \times n)$; ρ_{ij} is the residue (noise portion) associated with the term (GE)_{ij} of classic interaction of the genotype i with environment j ; and the ε_{ij} is the mean experimental error.

The AMMI analysis recovers the systematic portion of the sum of squares of the genotype interaction with environments (SS_{GE}), known as standard and noise portion, being the latter considered the portion with mistakes discarded to improve the predictive capacity of the model. In AMMI models, the most appropriate choice in terms of the number of axis to be retained (PCA) and capable of explaining the interaction of the standard portion is determined by the (SS_{GE}) ratio, accumulated until the n^{th} axis ($\sum_{k=1}^n \lambda_k / SS_{GE}$).

The F_R test (Cornelius et al. 1992) was used to evaluate the AMMI residue and the choice of the significant PCA axis. A significant result suggests that at least a multiplicative term must still be added to the already adjusted n axis until the best AMMI model is found. The results of the stability and adaptability analysis can be interpreted with a biplot graph obtained from combinations of the PCA scores capturing the standard portion interaction (stable). A hypothetical genotype and environment were then introduced, referred to as the supplementary genotype and environment (G_s and E_s), according to Pacheco et al. (2005).

This study also included the ideal genotype and environment (G_i and E_i), which were estimated according to Lin and Binns (1988). The quadratic distances were estimated between a point corresponding to an evaluated genotype and $G_i(d_{is}^2)$, and between the environments and the $E_i(d_{js}^2)$. These distances provide measures of similarity between the evaluated genotypes and G_i , and between the tested environments and E_i , and identify the genotypes and the environments with the highest yield stability and adaptability. A quadratic distance can be easily calculated by: $d_{is}^2 = \sum_{k=1}^n (CPA_{ik} - CPA_{sk})^2$ and $d_{js}^2 = (CPA_{jk} - CPA_{sk})^2$, where i and j represent, respectively, the genotype and environment evaluated; s is the genotype or supplementary environment or ideal genotype and ideal environment; and k is the axis or principal component by interaction (PCA) selected by the AMMI model, according to Pacheco et al. (2005). Analyses were carried using a computer routine in the Statistical Analysis System/Interaction Matrix Language (SAS/IML), based on a software made available by Duarte and Vencovsky (1999).

RESULTS AND DISCUSSION

The joint analysis of variance showed significant differences ($p < 0.001$) and ($p < 0.005$) for environments (E), genotypes (G) and the GxE interactions (Table 1). Significant GxE interaction indicates the different performance of genotypes in different environments. The coefficient of variation (CV) of the joint analysis performed with original data was of 16.13%, indicating good experimental precision.

Significant interactions between environment and cassava cultivars have been reported by different authors (Rimoldi et al. 2003, Kvitschal et al. 2007, Kvitschal et al. 2009, Costa et al. 2013, Santiago et al. 2015). The GxE interaction was decomposed by the AMMI into two principal components, but only the first axis was significant based on the F_R test (Cornelius et al. 1992).

An AMMI analysis of cassava root yield in three production environments in the state of Alagoas showed that 72.88% of the sum of squares of the GxE interaction was explained, respectively. PCA1 explained 72.88% of SS_{GE} , indicating that the entire pattern adjacent to the genotype x environment interactions focuses on the first axis (Table 1). The first AMMI axis shows that 72.88% of the SS_{GE} of the interaction corresponds to the standard adjacent to the genotype x

Table 1. Summary of the AMMI analysis for root yield (ton ha^{-1}) and the respective values of F for 24 cassava genotypes (*Manihot esculenta* Crantz) in three environments in the state of Alagoas, and proportion of $SS_{G \times E}$ interaction (sum of squares of the interaction) for each main axis of the analysis

Sources of variation	df	MS	F/ F_R	Proportion/Axis	(%) Accumulated
Genotype (G)	23	165.9108	2.85**		
Environment (E)	2	6995.63	120.34**		
G x E	46	96.5964	1.66*		
PCA1 ²	24	34.9748	0.3692	0.7288	72.8831
Residue 1	22	14.1957	0.1499		
PCA2 ²	22	14.1957	0.1499	0.2712	100.0000
Residue 2	0	0.0000	0.0000		
Mean error	79	94.7207			

² PCA=Principal Component Analysis; *, ** Significant at 5 and 1% by the F_R test (Cornelius et al. 1992).

environment interaction related to the characteristics of agronomic importance, and 27.12% represents the noise, i.e., the random variation resulting from the influence of micro environmental factors with no agronomic importance.

Comparison of AMMI analysis applied to several different cultures indicates that this analysis typically explains 60% of the total variation by the SSGE interaction (Carbonell et al. 2004, Kvitschal et al. 2007, Gonçalves et al. 2009, Gonçalves et al. 2010, Barros et al. 2013, Costa et al. 2013, Mattos et al. 2013, Oliveira et al. 2014).

According to Barros et al. (2013) and Duarte and Vencovsky (1999), to estimate and remove noises, the AMMI analysis is a powerful tool to remove the random variation process, which improves the accuracy of the Gx E interactions estimates, and consequently improves the genotypes means. Thus, in this study, genotypes response was estimated based on the AMMI1 model, and the graphical interpretation of adaptability and stability was performed using PCA1 by in the AMMI1 biplot graph (Figure 1).

Figure 1 shows the biplot graph resulting from the AMMI1 method, in which the abscissa represents the mean of genotypes and environments, and the ordinate represents the first principal component axis (PCA1).

The cassava variety Platina (G7) had the highest root yield (39.84 ton ha⁻¹). Varieties Preta do Araripe (G1), Cria Menino (G8), Sergipe (G20), and the Clone 1986-86-24 (G21) were characterized by means between 34 and 40 ton ha⁻¹. Limoeiro de Anadia (E1), Arapiraca (E2) and Lagoa da Canoa (E3) have mean root yield of 35.58 ton ha⁻¹, 20.14 ton ha⁻¹, and 35 ton ha⁻¹, respectively (Tables 1 and 2). Kvitschal et al. (2007) evaluated the adaptability and yield stability of cassava in the state of Paraná, and concluded that the analysis was efficient in evaluating yield stability and adaptability, indicating the clone IAC 190 as the most promising variety.

According to Duarte and Vencovsky (1999), stability is evaluated by genotypes with PCA values close to zero. In this study, the genotypes BRS Poti Branca (G3), Iará (G12), and BRS Tapioqueira (G7) presented this characteristic and the most productive were Iará (33.40 ton ha⁻¹) and BRS Tapioqueira (32.17 ton ha⁻¹).

Becker (1981) explains that stability in the biological context refers to a stable genotype that has minimal variance under different environmental conditions, while stability in the agronomic context refers to stable genotype that exhibits minimal interaction with environments and generates predictable yields to the level of the environmental yield. The genotypes described in the current study fit the agronomic stability concept. As such, they should be characterized by a desirable performance, or desirable agronomic characteristics, such as greater height, resistance to damping off, resistance to disease, and high yield (Carbonell et al. 2004, Morais et al. 2008). Selection by stable and yielding varieties is an indirect way to identify such characteristics.

Based on the AMMI analysis, varieties Poti Branca, BRS Tapioqueira, and Iará have the highest stability indicated by values near the origin of the PCA1 axis, which is indicative of a smaller contribution to the Gx E interaction (Figure 1). In contrast, varieties Preta do Araripe (G1), Caravela do Olho Roxo (G6) Platina (G7) and Cria Menino (8), as well as the Clone 1998-1-006 (G2) (Table 2, Figure 1) were the least stable, despite being characterized as high root yields. These genotypes also show high adaptability, which, according to the concept of Allard and Bradshaw (1964) about individual homeostasis, is the potential ability of genotypes to advantageously assimilate environmental stimulus.

The greatest environmental contribution to the Gx E interaction was observed in Limoeiro de Anadia (E1) and Lagoa

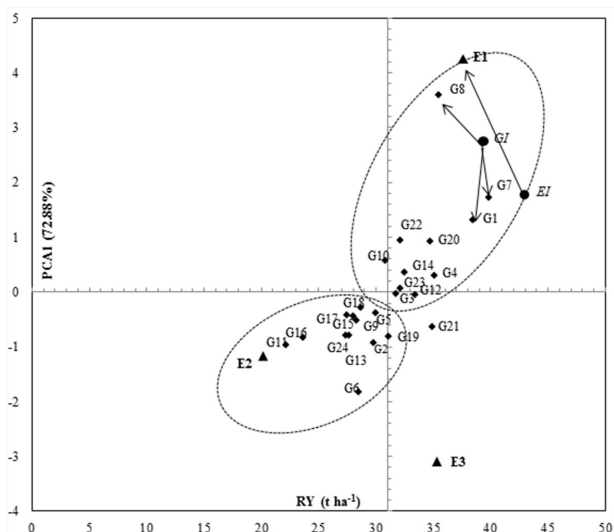


Figure 1. AMMI1 biplot for additive effects PCA1 component in 24 varieties of cassava root yield from three environments in the state of Alagoas, Brazil. RY = Root yield (ton ha⁻¹), G1 = Ideal genotype, E1 = Ideal environment, G1 = Preta do Araripe, G2 = Clone 1998-1-006, G3 = BRS Poti Branca, G4 = Clone 1997-83-13, G5 = BRS Aramaris, G6 = Caravela, G7 = Platina, G8 = Cria Menino, G9 = BRS Verdinha, G10 = Clone 1997-71-2, G11 = Cigana, G12 = Iará, G13 = Mani Branca, G14 = Clone 1997-71-2, G15 = Caravela do olho roxo; G16 = Campinas, G17 = Jalé, G18 = Palmeira, G19 = Valença, G20 = Sergipe, G21 = Clone 1986-86-24, G22 = Branquinha, G23 = Tapioqueira, G24 = Isabel de Souza, E1 = Limoeiro de Anadia, E2 = Arapiraca, E3 = Lagoa da Canoa.

Table 2. Mean yield predicted by the AMMI1 model for genotype x environment interaction captured by the first principal component of the interaction, general means, ideal genotype (G_i), ideal environment (E_i) scores, and quadratic distances between genotype and environments of the 24 cassava genotypes (*Manihot esculenta* Crantz) in three environments of the state of Alagoas

Code	Genotypes	Predicted mean yield			General means	PCA1	Quadratic distance
		E1	E2	E3			
G1	Preta do Araripe	50.6	26.0	38.7	38.4	1.3110	2.0686
G2	Clone 1998-1-006	30.5	17.4	34.1	27.3	-0.7988	12.5888
G3	BRS Poti Branca	38.2 ¹	20.9	36.1	31.7	-0.0315	7.7327
G4	Clone 1997-83-13	42.9	23.9	38.5	35.1	0.2951	6.0228
G5	BRS Aramaris	32.2	17.0	33.0	27.4	-0.4275	10.0922
G6	Caravela	27.3	19.7	38.4	28.5	-1.8283	20.9543
G7	Platina	53.7	26.9	38.8	39.8	1.7215	1.0562
G8	Cria Menino	57.3	20.4	28.6	35.4	3.5935	0.7128
G9	BRS Verdinha	32.7	18.0	34.2	28.3	-0.5151	10.6565
G10	Clone 1996-96-24	39.8	19.3	33.3	30.8	0.5759	4.7233
G11	Cigana	24.5	12.4	29.4	22.1	-0.9719	13.8471
G12	Irará	39.7	22.5	37.8	33.4	-0.0513	7.8433
G13	Mani Branca	32.3	19.9	36.9	29.7	-0.9373	13.5915
G14	Clone 1997-71-2	40.5	21.2	35.7	32.5	0.3502	5.7555
G15	Caravela do Olho Roxo	34.9	19.5	35.4	29.9	-0.3918	9.8668
G16	Campinas	30.8	17.6	34.3	27.6	-0.7903	12.5290
G17	Jalé	26.6	13.7	30.5	23.6	-0.8362	12.8560
G18	Palmeira	34.0	18.1	33.8	28.6	-0.2864	9.2158
G19	Valença	34.2	21.2	37.9	31.1	-0.8226	12.7583
G20	Sergipe	45.2	22.8	36.2	34.7	0.9164	3.3591
G21	Clone 1986-86-24	38.8	24.7	41.1	34.9	-0.6299	11.4189
G22	Branquinha	42.7	20.1	33.5	32.1	0.9486	3.2423
G23	BRS Tapioqueira	39.0	21.2	36.2	32.1	0.0570	7.2481
G24	Isabel de Souza	32.7	17.7	33.7	28.0	-0.4501	10.2365
E1	Limoeiro de Anadia				37.5	4.2522	6.1122
E2	Arapiraca				20.1	-1.1645	8.6702
E3	Lagoa da Canoa				35.3	-3.0877	23.6948
E_i	Environment Ideal				42.9	1.7799	
G_i	Genotype Ideal				39.3	2.7492	

da Canoa (E3) environments, which were also characterized by the highest mean yield. Limoeiro de Anadia environment had the highest contribution to the GxE interaction, reflected in a high PCA score (Table 2, Figure 1). The genotypes in this environment had the highest mean predictive yields (37.58 ton ha⁻¹), while genotypes in Arapiraca (E2) had the lowest mean predictive yields (20.13 ton ha⁻¹). This fact is probably due to factors such as geographical location, soil type, rainfall distribution, etc., suggesting that the group of genotypes studied, with positive interaction in Arapiraca (Figure 1), could be adapted to specific conditions. Arapiraca presented instability associated with low yield, while Limoeiro de Anadia had instability associated with high yield. The predicted values obtained by the AMMI analysis (Table 1) indicate the true performance of genotypes where the noise was discarded and the predictive ability of the model was improved, enabling accurate identification of the interaction, and facilitating the identification of adapted genotypes.

Genotypes and environments in close proximity on the biplot graph have positive associations, which facilitate the identification of zones of similar agronomic performance. For instance, varieties Cria Menino (G8), Platina (G7), Preta do Araripe (G1), Sergipe (G20), Branquinha (G22), BRS Tapioqueira (G23), and Clones 1996-96-24 (G10), 1997-71-2 (G14) and 1997-83-13 (G4) were specifically adapted to Limoeiro de Anadia, which is associated with intermediate and high yields (Figure 1). In conclusion, Limoeiro de Anadia is the most promising environment to grow cassava.

Clones 1998-1-006 (G2) and 1986-86-24 (G21), as well as the varieties Palmeira (G18), Caravela (G6), BRS Aramaris (G5), Campinas (G16), Cigana (G11), BRS Verdinha (G9), Valença (G19), and Caravela do Olho Roxo (G15) were characterized

by low yields (Table 1, Figure 1). Palmeira (G18), Caravela (G6), BRS Aramaris (G5), Campinas (G16), Cigana (G11), BRS Verdinha (G9), and Valença (G19) were better adapted to Arapiraca environment (E2), which should be considered the most suitable for the development of these genotypes. A recent evaluation of BRS Tapioqueira, Irara, BRS Poti Branca, Mestiça, and Clone 1997-83-13 by the AMMI analysis indicated that these genotypes are highly unstable, but have high mean yield (Costa et al. 2013). However, in contrast to Costa et al (2013), in the present study, the varieties BRS Poti Branca, Irara and BRS Tapioqueira had high mean yields, coupled with high phenotypic stability (Table 2 and Figure 1).

Close proximity (quadratic distance) was observed between varieties Platina (G7) (39.84 ton ha⁻¹) and Cria Menino (G8)(35.48 ton ha⁻¹) and the ideal genotype (G1) (39.34 ton ha⁻¹) (Figure 1), here conceived as the one that reached the highest mean yield in each location sampled by the experiments (which has positive interactions with the environments). Poti Branca (G3) and Irará (G12) showed high stability and yield above the overall mean (Figure 1); these genotypes also had high stability, with PCA1 score close to zero. Values close to zero characterize genotypes and environments as stable, with low contribution to the interaction. According to Pacheco et al. (2005), genotypes stability is an indication of adaptation to the tested environments. However, for agricultural purposes, stable genotypes should also have other desirable characteristics, such as high yield.

Table 2 presents the quadratic distances between the projections of each genotype evaluated and the ideal genotype on the first principal components axes. In this study, the genotypes Platina and Cria Menino were closer to the ideal genotype than Preta do Araripe, and are therefore the genotypes that show the most positive interactions with environments in the experimental conditions. Conversely, BRS Poti Branca, Irara and BRS Tapioqueira have high mean yield, high phenotypic stability, but showed high quadratic distance to the ideal genotype (G1). Barros et al. (2013), in their study with cowpeas (*Vigna unguiculata*), also used the ideal genotype and environment in the AMMI analysis; these authors found that the genotypes most distant from the G1 had high dissimilarity, and consequently low phenotypic stability (unstable) and adaptability to the tested environments. The most distant genotypes from the ideal genotypes in the present study were Caravela (G6), Cigana (G11), and Mani Branca (G13), indicating low stability and adaptability to the environments tested (Table 2 and Figure 1).

According to Pacheco et al. (2005), the most favorable agronomical environment presents the lowest quadratic distance estimates between real distance of the environments with the ideal environment (EI) created by the methodology (Table 2, Figure 1). Arapiraca (E2) and Lagoa da Canoa (E3) have higher quadratic distance from EI, indicating poorer conditions for cassava cultivation. Unfavorable environments are characterized by the occurrence of uncontrollable environmental factors, such as low distribution of rainfall, which leads to drought, insect attack, and diseases with difficult control, etc. Such conditions limit the capacity of genotypes to express their yield potential. Nevertheless, varieties Cigana (G11) and Campinas (G16) had specific adaptations to Arapiraca (E2) (Figure 1). Lagoa da Canoa (E3) was the most unfavorable environment for the evaluation of the adaptability and phenotypic stability of cassava varieties, as indicated by the highest quadratic distance from EI (Table 2).

The graphical interpretation of the AMMI analysis has the capacity to identify different varieties of cassava with high and stable yield. Varieties BRS Poti Branca, Irará, and BRS Tapioqueira show high yield and phenotypic stability. Varieties Cria Menino, Platina, Preta do Araripe, Sergipe, Branquinha, BRS Tapioqueira, and Clones 1996-96-24, 1997-71-2, 1997-83-13 are adapted to Limoeiro de Anadia environment; Varieties Campinas and Cigana are adapted to Arapiraca environment. Among the three test environments, Limoeiro de Anadia was the best for the identification of high yield, phenotypically stable genotypes for cassava breeding programs. Varieties Cria Menino and Preta do Araripe have genes for adaptability and stability, and thus are considered as ideal genotypes.

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