ISSN: 1984-5529 Científica Jaboticabal v.47, n.1, p.77–82, 2019

http://dx.doi.org/10.15361/1984-5529.2019v47n1p77-82

Agronomic performance and biochemical attributes of yellow-pulped elite sweet cassava clones

Desempenho agronômico e atributos bioquímicos de clones elite de mandioca de mesa com polpa amarela

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Recebido em: 14-08-2018; Aceito em: 29-11-2018

Abstract

Cassava storage root is the staple food of most of the Brazilian population. In this study, 13 cassava clones were evaluated for agronomic and biochemical traits compared to the control variety IAC 576-70. The experiments were conducted at Embrapa Cerrados during two harvest seasons. A randomized complete block design was used with three replicates, each plot consisting of 4 rows of 10 plants. Trait means were grouped by the Scott-Knott clustering test at 5% probability. The results highlighted clones 273/08 and 259/08, based on first branch height; 90/08, 272/08, 273/08, 497/08, 259/08, and 450/08, based on plant height; 94/08 and 272/08, based on shoot weight without the original stem cutting; 26/08, 272/08, 259/08, and 450/08, based on root starch percentage; and 215/08, based on root yield. In the 2011/2012 season, all clones showed cooking time less than 30 minutes. Regarding root protein content, clones 26/08, 90/08, and 91/08 were highlighted. HCN levels in cassava roots were below 100 mg kg^{-1} in all clones evaluated. We found elite clones with high levels of root carotenoids, especially clones 91/08, 94/08, 215/08, 246/08, 272/08, and 497/08. These clones have great potential for direct use by producers, and can be used as promising parents in genetic breeding programs of cassava.

Additional keywords: agronomic biofortification; genetic breeding; *Manihot esculenta* Crantz.

Resumo

A raiz de reserva da mandioca é alimento básico de grande parte da população brasileira. Neste trabalho, avaliaram-se por meio de caracteres agronômicos e bioquímicos, 13 clones de mandioca em comparação com a variedade testemunha IAC 576-70. Os experimentos foram conduzidos na Embrapa Cerrados, por duas safras. Foi utilizado o delineamento experimental de blocos casualizados, com três repetições, cada parcela composta por 4 linhas de 10 plantas. As médias dos caracteres foram agrupadas por meio do teste aglomerativo de Scott & Knott, a 5% de probabilidade de erro. Os resultados destacaram os clones 273/08 e 259/08, com base na altura da primeira ramificação, 90/08, 272/08, 273/08, 497/08, 259/08 e 450/08, segundo a altura da planta, 94/08 e 272/08 para peso da parte aérea sem a cepa, 26/08, 272/08, 259/08 e 450/08, de acordo com porcentagem de amido nas raízes e 215/08 para produtividade de raízes. Na safra de 2011/2012, todos os clones apresentaram tempo de cocção inferior a 30 minutos. Em relação ao teor de proteínas nas raízes, destacaram-se os clones 26/08, 90/08 e 91/08. Os teores de HCN nas raízes de mandioca foram inferiores a 100 mg kg^{-1}, em todos os clones avaliados. Foi possível identificar clones elite com alto teor de carotenoides totais nas raízes, com destaque para os clones 91/08, 94/08, 215/08, 246/08, 272/08 e 497/08. Estes clones têm grande potencial para utilização direta pelos produtores e sua utilização como genitores em programas de melhoramento genético de mandioca de mesa.

Palavras-chave adicionais: biofortificação agronômica; *Manihot esculenta* Crantz; melhorameto genético.

Introduction

Cassava (*Manihot esculenta* Crantz) is a plant of the family Euforbiaceae, cultivated mainly because of its tuberous roots rich in starch. The species shows considerable drought tolerance and adapts to the most varied climatic and soil conditions.

Genetic breeding of cassava has focused on

the development of specific cultivars, aiming at the production of tuberous roots for culinary use (cooked, fried, chips, cassava stick, precooked, pasta, among others). The selected cultivars must have storage roots with hydrocyanic acid contents less than 100 mg kg^{-1} fresh roots; high root yield; roots with good sensory properties (softness and plasticity after cooking, nonsticky mass, pleasant aroma and appearance) and cooking qualities (low fiber, low cooking time, and homogeneous mass after cooking); resistance to pests and diseases; architecture favorable to cultural traits (not branching or branching as high as possible); roots with low postharvest deterioration; earliness (harvest up to 11 months), among other characteristics (Vieira et al., 2013).

In addition to carbohydrates, cassava has genetic potential to be a source of carotenoids for human consumption, especially *β*-carotene, a precursor of vitamin A, present in genotypes with yellow roots (Chávez et al., 2005; Mezzete et al., 2009; Silva et al., 2014). Besides being a source of calories for the world's poorest populations, the possibility of cassava being a source of vitamin A would improve the nutrition of these people and add value to cultivars intended for human consumption (Carvalho et al., 2016).

Tuberous cassava roots usually have low amounts of protein. However, Carvalho et al. (2013), when analyzing 29 cassava accessions, verified a significant and positive correlation (r = 0.68) between protein and carotenoid contents. Thus, it would be possible to increase root protein content by selecting clones richer in carotenoids.

Genetic breeding programs of cassava are currently focused on the development of biofortified varieties, which bind to the desired agronomic traits the presence of carotenoids such as *β*-carotene in yellow tuberous roots (Mezzete et al., 2009; Vieira at al., 2013). Studies on the genetic resources available in Brazil have shown that there is variability in cassava germplasm for this purpose (Carvalho et al., 2012; Carvalho et al., 2016; Silva et al., 2014; Vieira et al., 2011a) .

This study evaluates agronomic and biochemical characteristics in storage roots of yellow-pulped elite cassava clones.

Materials and methods

Field experiments were conducted during two harvest seasons at the experimental field of Embrapa Cerrados, located in Planaltina-DF (15º36'347" S and 47º43'072" W; at 1013 m altitude), between October 2010 and October 2011 and between November 2011 and November 2012.

The soil of the site was classified as Yellow-Red Latosol (Embrapa, 1999). According to the Köppen classification, the climate is type Aw (tropical with dry season). Biochemical analyses were conducted at the Laboratory of Biochemistry and Biology of Embrapa Genetic Resources and Biotechnology.

Thirteen yellow-pulped elite cassava clones were characterized (26/08, 83/08, 90/08, 91/08, 94/08, 215/08, 246/08, 259/08, 272/08, 273/08, 446/08, 450/08, and 497/08), being selected for Cerrado conditions. Cassava cultivar IAC 576-70, indicated for cultivation in the region of the Federal District (Fialho et al., 2009), was used as control. In the Cerrados Cassava Germplasm Regional Bank (BGMC), this cultivar is identified as BGMC 753.

The experimental design was a randomized block with three replicates, each plot consisting of 4 rows of 10 plants, with spacing of 0.80 m between plants and 1.20 m between rows. The useful area of each plot was represented by the 16 central plants. The selection of propagating material and cultural traits followed the recommendations for cassava cultivation in the Cerrado region (Fialho et al., 2013; Fialho & Vieira, 2013).

Six agronomic traits were evaluated: i) plant height (PH), in meters; ii) first branch height (FBH), in meters; iii) shoot weight without the original stem cutting (SW), in kg ha^{-1}; iv) root yield (RY), in kg ha^{-1}; v) root starch percentage (RSC), by the hydrostatic balance method described by Grosmann & Freitas (1950), expressed as a percentage; and vi) cooking time (CT), in minutes, according to the method described by Borges et al. (2002). The content (mg kg^{-1}) of hydrocyanic acid in roots was evaluated using the qualitative method described by Williams & Edwards (1980), from five storage roots taken at random per plot.

To determine carotenoid content in storage roots, at the time of harvesting, three uniform roots of commercial cassava (diameter greater than 50 mm and length between 20 and 45 cm) were selected in each experimental plot, being identified and immediately placed in styrofoam boxes with ice. At the end of harvest, the samples were sent to the Laboratory of Biochemistry and Biology of Embrapa Genetic Resources and Biotechnology.

In the laboratory, under low light conditions, the roots were washed in running water, discarding the most external tissues (periderm, cambium, and phloem). Three cylinders with 2 to 3 cm height by 3 to 5 cm diameter were obtained from each root, one from the center and two from the ends of roots, which were divided into four parts by means of two longitudinal and homogenized cuts. Thus, samples of about 35 g were obtained, which were washed in deionized water and purified in a Milli-Q system, being then dried on paper towel, identified, wrapped in aluminum foil, and immediately frozen in liquid nitrogen and stored at -80 °C. Subsequently, the samples were lyophilized until complete dehydration, and macerated (in liquid nitrogen medium) with a porcelain mortar and pestle until a uniform powder was obtained, which was stored at -80 °C until use.

For the extraction and quantification of total carotenoids, about 100 mg of storage root powder was used, which was hydrated with 3 mL extraction buffer

(TEX buffer) (50 mM Tris, pH 7.6, 100 mM NaCl, 5 mM EDTA). Carotenoid extraction followed the method described by Carvalho et al. (2013).

After extraction, total carotenoids were quantified by reading the optical density of the extract, at a wavelength ranging from 300 to 550 ηm (reading at 450 ηm). The results of the evaluations were used to calculate total carotenoids in μg g^{-1} (TC), according to the mathematical model proposed by Rodriguez--Amaya & Kimura (2004):

$$TC = (OD \times 10^4 \times V)/(A\%^1_{1cm} \times DWt)$$

In which: TC: total carotenoids; OD = optical density of the sample, in λ_{max}; A^1_{1cm} = 2592 – extinction coefficient of β-carotene in petroleum ether; V = extraction volume (mL); DWt = dehydrated storage root powder weight.

For the quantification of total proteins, the protein fraction contained in the precipitate of the carotenoid extraction was used, as described by Carvalho et al. (2013). Optical density reading values were used to estimate protein content in mg g^{-1} dry weight.

The data were subjected to analysis of variance, and trait means were grouped by the Scott-Knott clustering test at 5% probability. Statistical analyses were performed using the statistical program Genes (Cruz, 2013).

Results and discussion

Joint analysis of variance showed a significant interaction at 5% probability between the factors harvest and genotype for all the traits measured, except for root yield (Table 1). This significant interaction highlights the need for evaluation of the clones for more than one harvest season aiming at a reliable estimation of the phenotypic expression of these traits and consequent hierarchy of the genotypes, as already reported for cassava in the Cerrado biome by Fialho et al. (2009); Silva et al. (2014); Vieira et al. (2011b); Vieira et al. (2015). However, as mentioned before, no significant interaction was detected between the factors harvest and genotype for root yield, which was not expected. According to the aforementioned authors, this interaction is usually observed under Cerrado conditions. The coefficients of variation of the analyses of variance ranged from 3.66% for starch percentage to 9.77% for root yield, indicating a high experimental precision (Table 1).

Table 1 - Summary of the joint analysis of variance for first branch height (FBH, m), plant height (PH, m), shoot weight without the original stem cutting (SW, kg ha^{-1}), root starch percentage (RSC, %) root yield (RY, kg ha^{-1}), carotenoid content in roots (TC, μg g^{-1} dry mass), protein content in roots (PCR, μg g^{-1} dry mass) e cooking time (CT, min) of thirteen genotypes of cassava at the harvests 2010/2011 (H1) and 2011/2012 (H2).

Causes of variation	FD	Average squares							FD	Average squares
		FBH	PH	SW	RSC	RY	TC	PCR		CT**
Block(B)/Harvest (H)	4	0.008	0.028	17843315	1.26	27412659	0.05	0.04	-	-
Genotypes (G)	12	0.073*	0.19*	46057143*	23.15*	192373312*	39.23*	0.45*	12	32.75*
H	1	0.39*	0.66*	9224017	115*	1281080355*	40.68*	0.08	-	-
G x H	12	0.03*	0.109*	35464982*	4.06*	10595180	4.49*	0.33*	-	-
Residue (R)	48	0.002	0.011	2851011	0.89	6613659	0.32	0.01	24	0.62
Total	77									
Average		0.59	1.74	19332	25.72	26310	10.21	2.04	-	26.21
CV (%)		8.32	6.12	8.73	3.66	9.77	5.52	5.34	-	3.13

* Significant by F test ($p < 0.05$); ** Result of the 2011/2012 (H2) harvest evaluation

The highest values for first branch height (FBH) in the 2010/2011 season were observed for clones 259/08 and 273/08; in the 2011/2012 season, clone 259/08 stood out (Table 2). For plant height (PH), in the 2010/2011 season, the clones that presented higher averages were 90/08, 272/08, 273/08, and 497/08; in the 2011/2012 season, in turn, clones 259/08 and 450/08 stood out (Table 2). These variables are important for the selection of clones because they are linked to the ease of realization of cultural traits, further relating to stem cutting availability for new plantings, ease of mechanized planting, and ease of harvesting. It is noteworthy that the preferred clones are those with higher first branch height or those that do not branch at all, in addition to those with high plant height (Fukuda et al., 2002; Vieira et al., 2011b).

For shoot weight (SW), four clones had averages higher than the others, namely: 94/08 and 272/08 in the 2010/2011 season, with 25,602 and 26,059 kg ha^{-1}, respectively; and clones 259/08 and 450/08 in the 2011/2012 season, with 25,656 and 25,750 kg ha^{-1}, respectively (Table 2). This trait is important because it relates to stem cutting supply for new plantings and the use of shoots as a source of protein in animal feed (Fernandes et al., 2016).

As for root starch percentage (RSC), in the 2010/2011 season, the clones that presented higher averages were 26/08 and 450/08, with 27.02% and 28.37%, respectively. In the 2011/2012 season, clone 259/08 and the control IAC 576-70 showed the highest averages, with 31.08% and 30.67%, respectively (Table 2). This trait, despite being more important in the selection of materials for the industry, is important in cassava breeding when considering the use of roots in the production of yellow flour.

The means clustering test revealed that clone 215/08 showed higher mean value for root yield (RY) in

both harvests, with a yield of 32,795 t ha^{-1} and 36,587 t ha^{-1}, respectively. In the 2011/2012 season, clones 272/08, 446/08, 497/08, 215/08, and the control IAC 576-70 had their means grouped together (Table 3). Root yield is one of the most important traits for the selection of cassava genotypes, since it is closely related to crop profitability.

Table 2 - First branch height (FBH, m), plant height (PH, m), shoot weight without the original stem cutting (SW, kg ha^{-1}), root starch percentage (RSC, %) of thirteen genotypes of cassava at the harvests 2010/2011 (H1) and 2011/2012 (H2).

Genotypes	FBH H1	FBH H2	PH H1	PH H2	SW H1	SW H2	RSC H1	RSC H2
26/08	0.48 Ad*	0.47 Ad	1.43 Ad	1.53 Ab	16.689 Ad	15.726 Ad	27.02 Aa	28.56 Ab
90/08	0.68 Ab	0.57 Bc	2.00 Aa	1.40 Bc	20.840 Ab	23.028 Ab	22.37 Bd	24.97 Ac
91/08	0.70 Ab	0.48 Bd	1.67 Ac	1.47 Bc	14.907 Ad	14.198 Ad	23.88 Bc	26.42 Ac
94/08	0.53 Ad	0.47 Ad	1.87 Ab	1.62 Bb	25.602 Aa	17.493 Bc	21.00 Bd	24.88 Ac
215/08	0.58 Ac	0.43 Bd	1.60 Ac	1.60 Ab	16.834 Ad	18.201 Ac	24.48 Ac	26.00 Ac
246/08	0.58 Ac	0.63 Ab	1.73 Ab	1.63 Ab	18.542 Ac	14.521 Bd	21.87 Bd	26.76 Ac
259/08	0.82 Ba	0.90 Aa	1.80 Ab	1.93 Aa	16.944 Bd	25.656 Aa	26.00 Bb	31.08 Aa
272/08	0.60 Ac	0.42 Be	2.13 Aa	1.53 Bb	26.059 Aa	18.017 Bc	25.37 Ab	25.81 Ac
273/08	0.85 Aa	0.48 Bd	2.20 Aa	1.90 Ba	21.256 Ab	20.444 Ab	23.74 Bc	26.35 Ac
446/08	0.72 Ab	0.37 Be	1.67 Ac	1.58 Ab	16.523 Bd	20.694 Ab	24.76 Ac	25.72 Ac
450/08	0.67 Ab	0.47 Bd	1.67 Bc	1.90 Aa	21.679 Bb	25.750 Aa	28.37 Aa	28.14 Ab
497/08	0.73 Ab	0.68 Ab	2.20 Aa	1.97 Ba	20.260 Ab	17.757 Ac	22.96 Bd	24.83 Ac
IAC 576-70	0.60 Ac	0.33 Be	1.90 Ab	1.40 Bc	19.645 Ac	15.354 Bd	26.82 Ba	30.67 Aa
Ideotype**	0.85	0.90	2.20	1.97	26.059	25.750	28.37	31.08
Average	0.66 A	0.52 B	1.84 A	1.65 B	19.675 A	18.988 A	24.51 B	26.94 A
Amplitude#	0.37	0.57	0.77	0.57	11.152	11.552	7.38	6.25

* Means followed by the same uppercase letters in the rows and lowercase letters in the columns do not differ by Scott e Knott test ($p > 0.05$); ** The largest means for FBH, PH, SW and RSC characters. # Difference between the highest and lowest means.

Table 3 - Root yield (RY, kg ha^{-1}), cooking time (~~T~~CT, min), carotenoid content in roots (TC, µg g^{-1} dry mass), protein content in roots (PC, µg g^{-1} dry mass) and hydrocyanic acid content in roots (HC, mg kg^{-1}) evaluated in thirteen genotypes of cassava in the harvests 2010/2011 (H1) and 2011/2012 (H2).

Genotypes	RY H1	RY H2	CT H2	TC H1	TC H2	PC H1	PC H2	HC H1
26/08	13,947 Be*	18,833 Ad	21.33 d	7.62 Bh	8.98 Ad	1.88 Bd	2.54 Aa	25-40
90/08	17,419 Bd	29,726 Ab	30.00 a	10.89 Ae	9.60 Bd	2.61 Aa	2.43 Aa	40-60
91/08	19,103 Bd	27,191 Ac	29.67 a	13.85 Ac	10.41 Bc	2.60 Aa	1.37 Bd	25-40
94/08	13,405 Be	23,114 Ac	29.33 a	16.95 Aa	12.13 Ba	2.40 Ab	1.90 Bc	25-40
215/08	32,795 Aa	36,587 Aa	25.33 b	12.05 Ad	11.35 Ab	2.35 Ab	2.26 Ab	25-40
246/08	17,451 Bd	25,434 Ac	25.67 b	15.13 Ab	11.13 Bb	2.40 Ab	2.19 Bb	40-60
259/08	21,382 Bd	26,906 Ac	23.67 c	6.47 Ai	5.47 Bf	1.50 Af	1.49 Ad	40-60
272/08	25,324 Bc	35,510 Aa	26.33 b	12.17 Ad	10.89 Bb	1.73 Ae	1.75 Ac	25-40
273/08	23,310 Bc	32,958 Ab	29.00 a	10.96 Ae	10.43 Ac	1.57 Bf	1.87 Ac	25-40
446/08	28,692 Bb	37,253 Aa	25.67 b	8.18 Ag	7.28 Ae	1.83 Bd	2.22 Ab	25-40
450/08	25,926 Bc	30,590 Ab	25.33 b	9.26 Af	9.64 Ad	1.91 Bd	2.24 Ab	40-60
497/08	27,506 Bb	36,316 Aa	29.67 a	11.86 Ad	9.89 Bd	2.01 Ac	1.97 Ac	25-40
IAC 576-70	23,085 Bc	34,295 Aa	19.67 e	6.71 Ai	6.10 Af	2.12 Ac	1.83 Bc	25-40
Average	22,257 B	30,363 A	26.21	10.93 A	9.49 B	2.07 A	2.00 A	
Amplitude#	19,390	18,420	10.33	10.48	6.67	1.11	1.17	

*= Means followed by the same uppercase letters in the rows and lowercase letters in the columns do not differ by Scott e Knott test ($p > 0.05$); #Difference between the highest and lowest means.

Cooking time was not considered in the analysis of variance of the 2010/2011 season, since no clone showed CT less than 30 minutes. This occurred due to the incidence of lace bug (*Vatiga illudens* Drake), which contributed to the anticipation of regrowth of the materials, having as a consequence the difficulty of cooking them. However, in the 2011/2012 season, all the clones showed cooking time less than 30 minutes, which is an indispensable factor for the commercialization of cassava roots for culinary use (Fukuda et al., 2002; Rinaldi et al., 2017).

Among the clones evaluated, only clone 94/08 had an average higher than the others for root carotenoid content in both harvests, which resulted in

twice the number of total carotenoids compared to the control. Among the clones studied in the first harvest season, those with yellow root pulp color (246/08, 91/08, 272/08, 215/08, and 497/08) showed the highest mean values of root carotenoid content, with 15.13, 13.85, 12.17, 12.05, and 11.86 μg g^{-1} dry weight, respectively. In the second harvest season, the clones with the highest mean values were 215/08, 246/08, and 272/08, with 11.35, 11.13, and 10.89 μg g^{-1} dry weight, respectively (Table 3). A similar result was reported by Silva et al. (2014) after analyzing 13 cassava accessions of the Cerrados Cassava Germplasm Regional Bank (BGMC), in which the mean values of root carotenoid content were higher than 10 μg g^{-1}. Mezette et al. (2009), when analyzing 13 cassava clones of the IAC Cassava Genetic Breeding Program, reported carotenoid values ranging from 3.30 to 11.08 μg g^{-1}. Total carotenoid content can be considered a good indicator of *β*-carotene content in cassava storage roots, since studies have shown that, on average, 70% of the total carotenoid content correspond to this pigment (Carvalho et al., 2012; Mezette et al., 2009).

For root protein content (RPC), in the 2010/2011 season, clones 90/08 and 91/08 had averages above the others, with 2.61 and 2.60 μg g^{-1} dry weight, respectively. In the 2011/2012 season, clones 26/08 and 90/08 stood out with 2.54 and 2.43 μg g^{-1} dry weight, respectively (Table 3). Carvalho et al. (2013), after analyzing 29 local cassava varieties, observed protein levels ranging from 0.27 to 8.0 mg g^{1}. In India, clones showed crude protein variation from 1.11% to 10.40% and from 0.37% to 2.74%, on a dry and fresh basis, respectively (Sheela et al., 2008).

Hydrocyanic acid (HCN) content in cassava storage roots showed quantities below 100 mg kg^{-1} (Table 3) in all clones; therefore, these clones are suitable for *in natura* commercialization. Silva et al. (2014) highlighted 5 out of 13 accessions (BGMC 1221, BGMC 1223, BGMC 1224, BGMC 1226, and BGMC 1227) with root hydrocyanic acid contents greater than 100 mg kg^{-1}. The identification of cassava cultivars with low levels of hydrocyanic acid in the raw root pulp is necessary to increase food safety and reduce the risk of intoxication of consumers (Borges et al., 2002).

Based on the results obtained, it is possible to state that among the clones evaluated, there are promising clones with agronomic and biochemical performance that allow commercial cultivation in the Cerrado region of Brazil. In this sense, clones with high yield and high carotenoid content (215/08, 446/08, and 497/08) stand out as possible alternatives for the commercial planting of cassava. However, prior to the recommendation of any of the genotypes evaluated for commercial planting in the region, it is necessary to validate their performance in a greater number of locations, for more than one harvest season.

Conclusions

Promising clones were identified, which stood out in the agronomic performance based on first branch height (273/08 and 259/08), plant height (90/08, 272/08, 273/08, 497/08, 259/08, and 450/08), shoot weight without the original stem cutting (94/08 and 272/08), root starch percentage (26/08, 272/08, 259/08, and 450/08), and root yield (215/08).

Regarding cooking time, in the 2011/2012 season, all clones had cooking time less than 30 minutes.

Regarding root carotenoid content, the clones that stood out were 91/08, 94/08, 215/08, 246/08, 272/08, and 497/08.

Regarding root protein content, clones 26/08, 90/08, and 91/08 were the best.

The HCN content in cassava storage roots was less than 100 mg kg^{-1} in all clones evaluated.

Acknowledgements

The authors would like to thank the Brazilian Agricultural Research Corporation (Embrapa), the Banco do Brasil Foundation (FBB), the University of Brasília (UnB), the National Council for Scientific and Technological Development (CNPq), and the Coordination for the Improvement of Higher Level Personnel (CAPES) for financial support.

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