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CROP PRODUCTION

Agronomic and biochemical evaluation of cassava clones with roots that have pink pulp

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ABSTRACT: *Sweet cassava breeding programs are focused on the development of bio fortified cultivars that combine significant amounts of carotenoids in their reserve roots with desirable agronomic. The objective of this research was to evaluate agronomic and biochemical traits in sweet cassava clones with roots that have pink pulp. The nine genotypes were evaluated in two seasons in a randomized block design with three replications. Among the evaluated clones, the following stood out: i) for the height of the first branch (390/08, 345/08 and the control IAC 576-70); ii) for plant height (390/08, 345/08 e 378/08); iii) for shoot weight without original steam cutting (390/08, 406/08, 378/08 e 341/08); iv) for the percentage of starch in roots (378/08, 413/08, 390/08 and the control IAC 576-70); and v) for the root yield (the control IAC 576-70 and 341/08, 390/08, 406/08 e 387/08). In the 2011/2012 season, all clones cooked within 30 minutes, indicating that they all have good culinary qualities. Regarding the total carotenoid content in the roots, the clones that stood out were 406/08 and 341/08. All clones evaluated had HCN content below 100 mg kg^{-1}. Clones 341/08 and 406/08 have agronomic and biochemical potential for direct cultivation by producers in the Cerrado region of Central Brazil and / or for use as stock in sweet cassava breeding programs.*
Key words: *Manihot esculenta Crantz, breeding, genetic resources, lycopene.*

Avaliação agronômica e bioquímica de clones de mandioca com polpa rosada

RESUMO: *Os programas de melhoramento genético de mandioca de mesa estão focados no desenvolvimento de variedades biofortificadas que aliem aos caracteres agronômicos a presença de carotenoides nas raízes de reserva. Neste trabalho, objetivou-se avaliar características agronômicas e bioquímicas em clones de mandioca de mesa com polpa rosada. Os nove genótipos foram avaliados por duas safras em delineamento experimental de blocos casualizados com três repetições. Dentre os clones avaliados se destacaram: i) para altura da primeira ramificação (390/08, 345/08 e a testemunha IAC 576-70); ii) para altura da planta (390/08, 345/08 e 378/08); iii) para peso da parte aérea sem a cepa (390/08, 406/08, 378/08 e 341/08); iv) para porcentagem de amido nas raízes (378/08, 413/08, 390/08 e a testemunha IAC 576-70); v) para produtividade de raízes (IAC 576-70 e 341/08, 390/08, 406/08 e 387/08). Na safra 2011/2012, todos os clones cozinharam em até 30 minutos, indicando que todos apresentam boas qualidades culinárias. Com relação ao teor de carotenoides totais nas raízes, os clones que se destacaram foram 406/08 e 341/08. Todos os clones avaliados apresentaram teores de HCN nas raízes de reserva de mandioca, inferiores a 100 mg kg^{-1}. Os clones 341/08 e 406/08 apresentam potencial agronômico e bioquímico para cultivo direto pelos produtores na região do Cerrado do Brasil Central e/ou para a utilização como genitores em programas de melhoramento genético de mandioca de mesa.*
Palavras-chave: *Manihot esculenta Crantz, melhoramento genético, recursos genéticos, licopeno.*

INTRODUCTION

Sweet cassava (*Manihot esculenta* Crantz) is one of the most widely produced crops in the green belts of large and medium-sized cities in the Cerrado region of Central Brazil. It is primarily cultivated for its reserve roots, which are edible (boiled, fried, chips, cassava, pre-cooked cassava, among others). Sweet casssava is the favorite among horticulturists because it is highly profitable and adaptats well to rotation and/or succession with greens.

One of the main characteristics of sweet cassava is the low amounts of hydrocyanic acid (HCN) in its reserve roots, less than 100 mg kg^{-1} in fresh roots, which is not enough to cause intoxication (EL-SHARKAWY, 2012). From a nutritional point of view, reserve cassava roots are an excellent

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source of starch, but not of protein and vitamins (CARVALHO et al., 2012). However, the pink root has the genetic potential to become a source of lycopene for human consumption (CARVALHO et al., 2016). Lycopene is a carotenoid with antioxidant properties that protect the body against free radicals, and may prevent carcinogenesis and atherogenesis (AGARWAL & RAO, 2000).

The main source of lycopene in human nutritionis is the tomato and the products derived from it, such as extracts, pulps and sauces (SHAMI & MOREIRA, 2004). In this context, the possibility of turning cassava into a source of lycopene is excting from the nutritional and economical points of view. This would be especially important for populations located in suburb areas of developing countries, due to the rusticity of cassava cultivation, especially when compared with tomato cultivation. However, in order to further expand the productive potential, it is essential that people have access to technologies such as fertilization (FERMONT et al., 2009; MUNYAHALI et al., 2017), cultural practices (FIALHO & VIEIRA, 2013) and genetic (VIEIRA et al., 2015).

Thus, one of the challenges of cassava breeding programs is the aggregation of nutritional qualities to roots, such as the selection of clones with pink reserve roots, the color associated with the presence of lycopene (CARVALHO et al., 2016).

Selected cultivars need to combine pink root pulp and low levels of hydrogen cyanide in their reserve roots (less than 100 ppm): i) high root yield; ii) roots with good sensory qualities (softness and plasticity after cooking, non-sticky mass, aroma and pleasant appearance); iii) roots with good culinary qualities (low in fiber, short cooking time and homogeneous cooked mass); iv) resistance to pests and diseases; v) architecture favorable to cultural treatment (do not branch or branch as high as possible); vi) roots with low post harvest deterioration; vii) fast growth (harvest up to 12 months), among other traits (VIEIRA et al., 2013).

Research has not yet made avilable to producers the cassava cultivars with pink root pulp and agronomic performance that make commercial sense (SILVA et al., 2014). For this reason, it is imporant to generate, select and make available superior pink sweet cassava clones through cassava breeding.

The objective of this research was to evaluate the agronomic and biochemical characteristics in sweet cassava clones that produce roots with pink pulp.

MATERIALS AND METHODS

The field experiments lasted two crop seasons in the Embrapa Cerrados experimental field, located in Planaltina-DF, between October 2010 and October 2011 and between November 2011 and November 2012. Biochemical analyzes were conducted at the Biochemistry Laboratory of the Embrapa Biology Genetic Resources and Biotechnology.

During the experiments (2010/2011 and 2011/2012); respectively, average daily temperature was 21.28 ºC and 21.77 ºC, the relative average humidity was 68.96% and 66.79%, and the accumulated precipitation was 1,367 mm and 1,402 mm. The chemical composition of the soils in the 2010/2011 and 2011/2012 seasons were, respectively, pH in H2O 5.4 and 5.9, Ca^{++} 2.2 and 3.4 cmolc dm^{-3}, Mg^{++} 0.6 and 1.20 cmolcdm^{-3}, phosphorus 18 and 4.9 mg dm-3, potassium 68 and 67 mg $dm^{-3}$and organic matter 23 and 28 g kg^{-1}.

Eight elite cassava clones with pink root pulp were characterized and selected for the conditions of the Central Brazilian Cerrado (341/08, 345/08, 378/08, 387/08, 390/08, 395/08, 406/08 and 413/08).The sweet cassava cultivar with cream root pulp IAC 576-70 was used as control. This cultivar is used in the Federal District region and is identified as BGMC 753 by the Regional Cassava Germplasm Bank of the Cerrado (BGMC).

The experimental design was a randomized block design with three replications, each plot consisting of four rows of 10 plants, space between plants 0.80 m and 1.20 m between lines. The useful area of each plot was represented by the 16 central plants. Selection of propagation of material and cultural practices followed the recommendations for the Cerrado region (FIALHO et al., 2013, FIALHO & VIEIRA, 2013).

Six agronomic characters were evaluated: i) plant height in meters (AP); ii) height of the first branch in meters (APR); iii) shoot weight without original steam cutting in kg ha^{-1}(PPA); iv) root yield in kg ha^{-1}(RY); v) percentage of starch in the roots (AM) by hydrostatic balance method, described by GROSMANN & FREITAS (1950); and vi) cooking time in minutes (TC) according to BORGES et al. (2002).

The root's cyanuric acid content (mg kg^{-1}) was evaluated by the qualitative method described by WILLIAMS & EDWARDS (1980), from five reserve roots taken randomly from each plot.

In order to determine the total carotenoid content in the reserve roots, at the time of harvest, three uniform commercial cassava roots (diameter

between 50 and 100 mm and length between 20 and 45 cm) were selected from each experimental plot, and immediately identified in ice-cold Styrofoam boxes. At the end of the harvest, the samples were sent to Embrapa's Biochemistry and Biology Laboratory Genetic Resources and Biotechnology.

In the laboratory, under low light, the roots were washed in running water, discarding the outer tissues (periderm, cambium and phloem). Then three cylinders, 2 to 3 cm high by 3 to 5 cm in diameter were obtained from each root, one central and two at the root tips, which were divided into four parts by two longitudinal and homogenized cuts. Then samples of about 35 g were obtained, which were washed in deionized water and purified in Milli-q system, dried in paper towels, identified, wrapped in aluminum foil and immediately frozen in liquid nitrogen and stored at -80 °C. Subsequently, the samples were lyophilized to complete dehydration, macerated (under liquid nitrogen) using porcelain crucible and pistil, until a uniform powder was obtained, which was stored at -80 °C until use.

For extraction and quantification of total carotenoids, about 100 mg of reserve root powder was used, which was hydrated with 3 mL of buffer extraction (TEx) composed of 50 mM pH 7.6, NaCl 100 mM, EDTA 5 mM. The carotenoid extraction process followed the methodology described by CARVALHO et al. (2013).

After extraction, total carotenoids were quantified by reading the optical density of the extract under wave length ranging from 300 to 550 ηm, with reading at 450 ηm. Results were used to calculate the total carotenoid content in μg g^{-1} (CT), according to the model proposed by RODRIGUEZ-AMAYA & KIMURA (2004):

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

In which:

TC: total carotenoid content;

OD = sample optical density at λmax,

$A\%^1_{1cm}$= 2592 - β-carotene extinction coefficient in petroleum ether;

V = extraction volume (mL);

DWt = weight of dehydrated reserve root powder.

Data were subjected to analysis of variance according to a randomized block design in a factorial scheme (9 genotypes and 2 crops), where both were considered as fixed effects. To analyze the hypothesis of data normality, the Shapiro-Wilk's test was used.

Character averages were grouped by means of the SCOTT & KNOTT (1974) agglomerative test. Selective accuracy (SA) was estimated using the equation proposed by RESENDE & DUARTE (2007). Statistical analyses were performed using the Software R, versão 3.4.3 (R Core Team, 2017).

RESULTS AND DISCUSSION

The significant interaction among harvest factors and genotypes for the traits measured (Tables 1 and 2) revealed the need to evaluate the clones for more than one agricultural crop season, to refine the estimate of phenotypic expression, corroborating what had been reported for cassava

Table 1 - Summary of the analysis of variance, mean and selective accuracy (SA) of height of first branch (HFB), plant height (PH), shoot weight without original steam cutting (SW), starch content in roots (SC), root productivity (RY) total carotenoid content in the roots (CT) and cooking time (TC) evaluated in nine table cassava genotypes in the crop seasons of 2010/2011 (S1) and 2011/2012 (S2) in Planaltina-DF.

FV	GL	QM						GL	QM
		HFB	PH	SW	SC	RY	CT		TC
Blocks(B)	2	0.001	0.056	2494225	4.06	1502526	0.72	2	4.04
CropSeason(S)	1	0.001	0.172*	779212896*	21.55*	793477000*	182*	-	-
Genotypes(G)	8	0.036*	0.106*	103271180*	14.24*	125881865*	592*	8	20.90*
G x S	8	0.032*	0.093*	43031613*	4.54*	13598544*	57*	-	-
Residue(R)	34	0.001	0.011	5267681	0.80	4313672	0.96	16	0.29
Total	53	-	-	-	-	-	-	-	-
Mean		0.42	1.75	24677	25.84	17543	23.90	-	27.07
p-SW**		0.39	0.10	0.30	0.21	0.73	0.35		0.30
SA (%)		0.98	0.95	0.97	0.98	0.97	0.99	-	0.99

*significant at 5% probability of error by the F test;
**Shapiro Wilk's test error probability.

in the Cerrado biome by VIEIRA et al. (2011); SILVA et al. (2014) and VIEIRA et al. (2015). The Shapiro-Wilk's test revealed that the residuals of the characters evaluated have a normal distribution at 5% probability of error (Table 1). The selective accuracy ranged from 0.95 to 0.99 for the characters measured, indicating high experimental precision (Table 1).

Among the evaluated clones, the ones with higher average of first branch height (APR) in the crop season of 2010/2011 were 390/08 and IAC 576-70, and in the 2011/2012 crop season it was clone 345/08 (Table 3). As for plant height (AP) in the 2010/2011 crop season, the clone with higher average was 390/08 and in the 2011/2012 crop season there were three, 378/08, 390/08 and 345/08 (Table 3). These traits are important in clone selection because they reflect how easy it is to care for the crop using machines for planting and harvesting, and the availability of stem cuttings. The genotypes with higher first branch height and plant height are thus favored (VIEIRA et al., 2013). However, the height of the plant should not exceed 3 m, because plants that are taller than that tend to break under high winds (OTSUBO et al., 2009). Plants this tall were not detected in the present study, where the highest plant height of measured was 2.23 m for clone 390/08 in the 2010/2011 crop season.

Regarding the shoot weight without original steam cutting (PPA) in the 2010/2011 crop, the clone that presented higher average than the others was 390/08 (27493 kg ha^{-1}). In the 2011/2012 crop season, the clones 406/08 (34406 kg ha^{-1}), 390/08 (33445 kg ha^{-1}), 378/08 (32837 kg ha^{-1}) and 341/08 (31944 kg ha^{-1}) were the heaviest on average (Table 3). Shoot weight without the original steam cutting is important when selecting clones because it is related to the possibility of using cassava shoots as a source of protein for animal feed (FERNANDES et al., 2016) and soil cover (erosion control, moisture maintenance in the soil and weed control).

Regarding the percentage of starch in the roots, clone 378/08 (27.33%) stood out in relation to the others in the 2011/2012 crop season. While in the 2011/2012 season the clones 413/08 (28.48%), 390/08 (28.30%) and the control IAC 576-70 (29.35%) (Table 3) were the ones that had greater percentage of starch. This feature, while being more important in the selection for the flour and starch industry, since they determine the potential yield of a given access along with root productivity, is important in selecting table cassava clones when thinking about harnessing the roots for dual purposes (table and industry). In this case, a pink flour would be an innovation in the cassava flour market.

The control IAC 576-70 had higher average root yield (PY) than the other clones in both crop seasons, which was expected since it is a commercial cultivar, adapted to the Cerrado region and has cream pulp color. Clones with good average root yield were identified in the 2010/2011 crop season as 341/08 (17235 kg ha^{-1}), and in the 2011/2012 crop season,

Table 2 - Probability of significance (p) by the F-test for the unfolding of the interaction access x harvest of first branch height (HFB), plant height (PH), weight of shoot without original steam cutting (SW), percentage of starch in roots (AM), root yield (RY) and total root carotenoid (TC) content evaluated in nine table cassava genotypes in the 2010/2011 (S1) and 2011/2012 (S2) crops in Planaltina-DF.

Factor	GL	HFB	PH	SW	RY	SC	CT
Harvest	1	0.47	0	0	0	0	0
Genotypes	8	0	0	0	0	0	0
Genotypes in the crop season of 2010/2011	8	0	0	0	0	0	0
Genotypes in the crop season of 2011/2012	8	0	0	0	0	0	0
Cropseasonin clone 341/08	1	0	0.07	0	0.06	0.02	0
Cropseason in clone 345/08	1	0	0	0	0	0.77	0
Cropseason in clone 378/08	1	0	0	0	0.03	0.16	0
Cropseason in clone 387/08	1	0.11	0.44	0	0	0.02	0.10
Cropseason in clone 390/08	1	0.11	0	0	0	0	0
Cropseason in clone 395/08	1	0.58	0.09	0.02	0	0	0
Cropseason in clone 406/08	1	0.03	0	0	0	0	0.02
Cropseason in clone 413/08	1	0.11	0.02	0	0	0.03	0.60
Crop season in IAC 576-70	1	0	0.34	0.02	0	0	0.08
Residue	34	-	-	-	-	-	-
Total	53	-	-	-	-	-	-

Table 3 - Mean of first branch height in meters (HFB), plant height in meters (PH), shoot weight without original steam cutting in kg ha-1 (SW) and percentage of starch in roots (SC), evaluated in nine table cassava genotypes in the 2010/2011 (S1) and 2011/2012 (S2) harvests in Planaltina-DF.

Genotypes	HFB	HFB	PH	PH	SW	SW	SC	SC
	S1	S2	S1	S2	S1	S2	S1	S2
341/08	0.32 Ac*	0.22 Be	1.67 Ac	1.70 Ab	22863 Bb	31944 Aa	25.50 Ab	23.65 Bc
345/08	0.32 Bc	0.62 Aa	1.57 Bc	1.90 Aa	12766 Bd	22323 Ac	25.23 Ab	25.44 Ab
378/08	0.42 Bb	0.55 Ab	1.57 Bc	2.03 Aa	19563 Bc	32837 Aa	27.33 Aa	26.29 Ab
387/08	0.42 Ab	0.37 Ad	1.67 Ac	1.60 Ab	18993 Bc	27161 Ab	22.93 Bc	24.82 Ac
390/08	0.55 Aa	0.50 Ac	2.23 Aa	1.90 Ba	27493 Ba	33445 Aa	25.78 Bb	28.30 Aa
395/08	0.37 Ac	0.38 Ad	1.60 Ac	1.75 Ab	23823 Bb	28432 Ab	23.28 Bc	26.03 Ab
406/08	0.40 Bb	0.47 Ac	1.50 Bc	1.80 Ab	21035 Bc	34406 Aa	23.33 Bc	25.88 Ab
413/08	0.43 Ab	0.38 Ad	1.58 Bc	1.80 Ab	19910 Bc	28698 Ab	26.88 Ba	28.48 Aa
IAC 576-70	0.58 Aa	0.38 Bd	1.83 Ab	1.75 Ab	21462 Ac	17038 Bd	26.61 Ba	29.35 Aa
Average	0.42 A	0.43 A	1.69 A	1.80 A	20879 B	28476 A	25.21 A	26.47 A

*= averages followed by the same horizontal and lowercase uppercase letters belong to the same group at 5% probability of error by the Scott and Knott grouping test.

406/08 (24764 kg ha^{-1}), 390/08 (23104 kg ha^{-1}), 387/08 (21071 kg ha^{-1}) and 341/08 (20674 kg ha^{-1}) (Table 4). These averages showed a considerable productivity gain in relation to root yields reported by SILVA et al. (2014), in the same region of the Cerrado biome, whose values ranged from 1292 a 12928 kg ha^{-1}.

Cooking time was not considered in the analysis of variance of the 2010/2011 crop season, since no clone presented CT less than 30 minutes, due an infestation of *Vatigailludens* Drake, which contributed to the anticipation of regrowth and difficulties in cooking. However, in the 2011/2012 season, cooking times were less than 30 minutes for all clones (Table 4), an indispensable quality for the commercialization of cassava roots for culinary use. These results reveal increased culinary quality in the clones when compared with the results presented by SILVA et al. (2014), who noted that none of the four cassava accessions evaluated in the same region of the Cerrado biome had cooking times of less than 30 minutes.

In the group of clones evaluated, all had much higher carotenoid content than the control

Table 4 - Comparison of mean root yield in kg ha^{-1} (RY), cooking time in minutes (TC), total root carotenoid content in µg g^{-1}of dry mass (TC), root protein content µg g^{-1} of dry mass (PT) and root hydrocyanic acid content in mg kg^{-1} (HCN) evaluated in nine table cassava genotypes in the 2010/2011 (S1) and 2011/2012 harvests (S2) in Planaltina-DF.

Genotypes	RY	RY	TC	CT	CT	PT	PT	HCN
	S1	S2	S2	S1	S2	S1	S2	S1
341/08	17235 Ab*	20674 Ab	25.67 c	27.93 Bb	42.80 Aa	1.79 Ab	1.39 Bd	25-40
345/08	7655 Bd	17229 Ac	29.33 a	19.92 Be	31.33 Ac	1.61 Ac	1.43 Bd	40-60
378/08	11451 Bc	15389 Ac	27.00 b	21.57 Bd	27.66 Ad	1.73 Ab	1.38 Bd	40-60
387/08	11820 Bc	21071 Ab	25.33 c	22.33 Ad	20.94 Af	1.57 Ac	1.63 Ac	40-60
390/08	12896 Bc	23104 Ab	29.33 a	23.76 Ac	20.55 Bf	1.84 Ab	1.84 Ab	40-60
395/08	11387 Bc	19417 Ac	30.00 a	18.65 Be	23.05 Ae	1.34 Bd	1.57 Ac	25-40
406/08	13889 Bc	24764 Ab	26.67 b	39.19 Ba	41.06 Ab	2.00 Aa	1.83 Bb	25-40
413/08	13844 Bc	17826 Ac	28.67 a	19.40 Ae	19.82 Af	1.51 Bc	1.79 Ab	60-85
IAC 756-70	23215 Ba	32917 Aa	21.67 d	5.80 Af	4.34 Ag	1.85 Bb	2.21 Aa	25-40
Average	13710 B	21377 A	27.07	22.06 B	25.73 A	1.52 A	1.48 A	

*= averages followed by the same horizontal and lowercase uppercase letters belong to the same group at 5% probability of error by the Scott and Knott grouping test.

(Table 4), with the 2010/2011 crop season highlighting clone 406/08 (39.19 μg g^{-1} dry mass) and the 2011 / 2012 crop season the clone 341/08 (42.80 μg $g^{-1}$of dry mass) (Table 4). The higher absolute values of total carotenoids in clones with pink root color compared to cream and yellow are probably due to the fact that they accumulate lycopene and β-carotene in their reserve roots (CARVALHO et al., 2012; SILVA et al., 2014).

Regarding the hydrocyanic acid (HCN) content in the cassava stock roots, all clones presented amounts below 100 mg kg^{-1} (Table 3); and are therefore, suitable for to being commercialized fresh. Recommendation that clones have low levels of hydrocyanic acid in the raw root pulp is fundamental for consumers' food safety (BORGES et al., 2002).

Based on the results obtained, it is possible assert that, in the group of clones evaluated, some have better agronomic and biochemical performance, which allows their commercial cultivation in the Cerrado region of Brazil. In this sense, the clones that stand out are those with high carotenoid content combined with good productivity, as 341/08 and 406/08. These clones also have great potential for use as parents in breeding, both in crosses with each other and with other clones adapted to the conditions of the Central Brazilian Cerrado.

CONCLUSION

Clones 341/08 and 406/08 have agronomic and biochemical potential for direct cultivation by producers in the Cerrado region of Central Brazil and / or for use as stock in sweet cassava breeding programs.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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