CULTIVAR RELEASE

BRS 438: Sugary cassava cultivar for diversification of the use of storage roots

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Abstract: *The sugary cassava cultivar BRS 438 has high roots yield (with expressive concentration of free sugars), adaptation to mechanized planting, and moderate resistance to bacterial diseases. These characteristics make it an option for production of sugars, alcohol, and animal feed (additive in forage grass silage), among other possibilities.*

Keywords: Manihot esculenta *Crantz, plant breeding, silage additive, ethanol, glucose syrup*

INTRODUCTION

Embrapa Cerrados coordinates the Regional Bank of Cassava Germplasm of the Cerrado with the aim of conserving the genetic variability of cassava present in the Brazilian Cerrado (Albuquerque et al. 2016). The expectation is that among the conserved accessions there are sources of genes for adaptation to the climate conditions of the region, genes for resistance to the main diseases, and sources of genes for possible new uses of cassava (Vieira et al. 2008). Prominent among these accessions are those that store not only starch in their storage roots (as usually occurs in the cassava crop) but also free sugars (Carvalho et al. 2004).

The incorporation of cassava accessions known as sugary cassava or *mandiocaba* in the production system is one of the big challenges of the cassava production and industrialization system in Brazil and perhaps the challenge with the greatest innovative potential (Carvalho et al. 2004, Carvalho et al. 2011). Research focused on development of bioproducts from the sweet roots may impact various sectors of the industry, such as in: i) production of fuel alcohol and/or alcohol for fine chemical production for perfumes and cosmetics; ii) production of natural glucose and fructose syrups; iii) production of fermented beverages, such as beer; iv) production of spirits; v) production of additives for improving the nutritional value and the fermentation pattern of forage grass silage; and vi) other possibilities to be discovered in research (Carvalho et al. 2004, Vieira et al. 2008, Carvalho et al. 2011).

After discovery of the possibilities of application of this asset, Embrapa began breeding efforts aiming at incorporation of these genes to genotypes adapted to the cassava production system (Carvalho et al. 2011). Such a step

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was necessary since the free-sugars accessions had low root yield, low adaptation to mechanized planting, and sensitivity to the main cassava diseases in the Cerrado region (Vieira et al. 2008).

In this scenario, Embrapa is making the sugary cassava cultivar BRS 438 available to the production and industrialization sectors; it has high root yield (with expressive concentration of free sugars), adaptation to mechanized planting, and moderate resistance to bacterial diseases.

BREEDING METHOD

To give rise to the segregating population from which the cassava cultivar BRS 438 was selected, the sweet sugary cassava accession BGMC 1213 and the sweet cassava cultivar IAC 576-70 (BGMC 753) were chosen as parents (full-sib progeny); both are conserved in the Regional Bank of Cassava Germplasm of the Cerrado (*Banco Regional de Germoplasma de Mandioca do Cerrado* - BGMC).

In the year 2009, after manual pollination, the still unripe fruit was wrapped in voile bags, and seeds were systematically collected after their natural dispersal. Dormancy was broken by maintaining the seeds for seven days in a laboratory oven at 60 ºC (Mezzalira at al. 2013). After that, the seeds were placed to germinate in a greenhouse and 45 days after emergence, the seedlings were transplanted in the field.

The selection process of the cultivar BRS 438 was conducted in an experimental area of Embrapa Cerrados in Planaltina (lat 15º 35.467' S, long 47º 42.617' W, alt 1,007 m asl), DF, Brazil, according to the breeding method used in Embrapa Cerrados (Vieira et al. 2018, Vieira et al. 2019, Vieira et al. 2020, Rangel et al. 2022, Vieira et al. 2022). Agronomic management practices followed the recommendations for cassava production in the Cerrado of the Brazilian Central region (Fialho et al. 2017).

In the first three selection cycles (2009/2010, 2010/2011, and 2011/2012), both planting and harvesting occurred in November, the harvest at 12 months after planting. In these crop seasons, phenotypic mass selection, was applied with the following considerations: i) the presence of free sugars in the roots, evaluated by means of specific clinical sticks for detection of glucose (®Clinistix); ii) plant architecture (total height and first branch height); iii) resistance to bacterial diseases, based on the grade scale described by Ramos and Takatsu (1987); iv) storage root yield; and v) root uniformity.

VALUE FOR CULTIVATION AND USE TRIALS

The value for cultivation and use trails (VCUs) were conducted for 17 months, from October to March, in the 2012/2014, 2013/2015, 2014/2016, and 2015/2017 crop seasons at Embrapa Cerrados in Planaltina, DF. The climate of the location, according to the Köppen classification, is of the megathermal or tropical humid type (A) of the savanna climate subtype, with dry winter and maximum rainfall in the summer (w), and mean temperature of the coldest month greater than 18 °C. The soil is a *Latossolo Vermelho distrófico* of clayey texture.

A randomized block experimental design was used with three replications, and each plot consisted of four rows of 10 plants, with spacing of 0.80 m between plants and 1.20 m between rows. The area of the plot used for data collection was composed of the 16 central plants. Selection of the propagation material and the crop treatments followed the recommendations of the cassava production system for the Cerrado region (Fialho et al. 2017). The following agronomic traits were evaluated at the time of harvest: i) first branch height in meters (FBH); ii) plant height in meters (PH), iii) root yield in t ha^{-1} (RY); iv) percentage of starch in the roots by means of the hydrostatic balance method (ST); and v) starch yield in t ha^{-1} (SY), estimated as based on root yield and percentage of starch in the roots.

Combined analysis of the experiments was carried out, and each experiment was represented by one of the four crop seasons: 2012/2014 (S1), 2013/2015 (S2), 2014/2016 (S3), and 2015/2017 (S4). Seven treatments consisted of the genotypes IAC 12-829 (starchy check cultivar), IAC 573-70 (starchy check cultivar), sugary Clone 600-8, sugary Clone 580-8, sugary Clone 601-8, sugary Clone 608-8, and BRS 438. Both the effects of crop seasons and of genotypes were considered as fixed.

Shapiro-Wilk's test showed that the residues for the evaluated traits presented normal distribution (Table 1). The coefficients of variation of the variance analyses ranged from 3.10% for percentage of starch in the roots (ST) to 13.11%

Table 1. Summary of the joint analysis of variance, significance of Shapiro Wilk's normality test (p-SW), Hartley´s homogeneity of variances test and variation coefficient (CV%) for first branch height (FBH) in m, plant height (PH) in m, root yield (RY) in t ha^{-1}, percentage of starch in the roots (ST) and starch yield (SY) in t ha^{-1} evaluated in five genotypes of sugary cassava and two cultivars of starchy cassava in the 2012/2014(S1), 2013/2015 (S2), 2014/2016 (S3), and 2015/2017 (S4) crop seasons in Planaltina, DF, Brazil

Sources of variation	df	Mean squares				
		FBH	PH	RY	ST	SY
Crop seasons (CS)	3	0.12*	0.31*	84.11*	93.97*	52.29*
Blocks (B)	2	0.00006	0.09	25.11	0.34	2.84
Error a	6	0.0008	0.03	17.10	0.39	1.91
Genotypes (G)	6	0.16*	0.68*	61.31*	381*	69.58*
CS x G	18	0.01*	0.05	78.90*	2.18*	4356462*
Error b	48	0.003	0.03	12.35	0.80	748945
CV (%)		13.11	8.01	7.49	3.71	8.23
p-SW (safra1)	-	0.58	0.38	0.56	0.36	0.06
p-SW (safra2)	-	0.71	0.74	0.79	0.57	0.92
p-SW (safra3)	-	0.39	0.65	0.58	0.66	0.74
p-SW (safra4)	-	0.18	0.09	0.08	0.35	0.68
Hartley`s test		1.55	3.74	3.49	6.76	3.88

* Significant at $p < 0.05$.

for first branch height (FBH), showing good experimental accuracy and Hartley`s test indicated homogeneity in the experimental variances and the possibility of joint data analysis (Table 1). The treatments were checked for statistical significance by joint analysis of variance (ANOVA) and the mean values of the traits were compared by Tukey's test. All the inferences were tested at $p < 0.05$. Statistical analyses were carried out by the R 4.0.3 software (R Core Team 2020).

Available sugar content before and after enzymatic hydrolysis of the sugary cultivar BRS 438 was evaluated in the roots obtained at the time of harvest of the experiment of the 2012/2014 crop season. At that time, 5 kg of cassava roots (diameter greater than 50 mm and length from 20 to 45 cm) were sampled in each one of the experimental plots. The samples were identified and placed in styrofoam boxes with ice while still in the field. At the end of harvest, the samples were sent to the Bioprocesses Laboratory of Embrapa Agroenergia.

In the laboratory, the roots were washed in running water, discarding the outermost tissues (periderm, cambium, and phloem). Then, three cylinders of 2 to 3 cm height by 3 to 5 cm diameter of each root were obtained, one from the center and two from the tips of the roots; they were divided into four parts through two longitudinal cuts and homogenized. Then, 35 g samples were obtained.

The samples were ground using a portable food processor until obtaining a homogeneous sample. After that, water was added to the samples, and they were filtered; the liquid fraction was analyzed by high-performance liquid chromatography (HPLC) for quantification of the initial soluble sugars (glucose, fructose, and maltose). The amount of water differed for each sample since each cultivar has amounts of water and sugar that differ from the others. The samples underwent the enzymatic hydrolysis process with the enzymes Termammyl 2X and AMG 300 in pH 6.0 at 90 °C, and in pH in the range of 4.0-4.5 at 60 °C, respectively. The sugars present in the samples were characterized before and after hydrolysis in a HPLC device. The column used was HPX87H of the BIORAD brand, and each one of the samples was analyzed for 20 minutes with a flow of 0.600 mL min^{-1} and temperature of 45 °C. The standard curve of sugars containing glucose, fructose, and maltose, all of analytical grade, was obtained through an increasing series of solutions at the concentrations of 0.1252 g L^{-1}, 0.3125 g L^{-1}, 0.625 g L^{-1}, 1.25 g L^{-1}, 2.5 g L^{-1}, and 5 g L^{-1}. Analysis of variance was performed on the data obtained, and the mean values were compared by the Scott-Knott means comparison test at 5% probability of error.

The results obtained in field evaluations showed significant differences among the mean values of the genotypes evaluated (starchy check cultivars and sugary clones) for all the traits assessed in the field (Tables 2 and 3). This variation reflects the phenotypic variability in the group of genotypes evaluated. In addition, the occurrence of significant interaction among the crop season and genotype factors for all the traits assessed, except for the PH trait (Tables 2 and 3), indicates the differential response of the genotypes within each crop season for most of the traits assessed.

Among the genotypes evaluated, the starchy check cultivars IAC 12-829 and IAC 576-70 had the highest mean values for the first branch height (FBH) trait in the four crop seasons, and the clone 600/08 and the cultivar BRS 438 had means statistically equal to those of the check cultivars in the 2013/2015 and 2015/2017 crop seasons, respectively (Table 2). For the plant height (PH) trait, the genotypes that had the highest mean values were 601/08, BRS 438, and clone 580/08, and the means of the genotypes BRS 438 and 580/08 did not differ statistically from the means of the clones 600/08 and 608/08 (Table 2). Higher mean values of FBH and PH are important in recommendation of clones for commercial growing since these traits are associated with i) ease of crop treatments (weed control, field inspections, and agricultural chemical applications); ii) greater availability of stem cuttings – seeds; iii) ease of mechanized planting; and iv) the possibility of use of the cassava shoots as a source of protein in animal feed, among other uses (Vieira et al. 2020, Fernandes et al. 2021, Rangel et al. 2022).

The check cultivar IAC 12-829 had a percentage of starch in the roots (ST) higher than the percentages in the other genotypes evaluated in the four crop seasons; and in the 2015/2017 crop season, the mean value of the ST was statistically similar to that of the check cultivar IAC 576-70 (Table 2). This trait is very important when thinking of using roots in the production of cassava flour and starch. A higher mean value of ST in the roots of the check cultivar IAC 12-829 was expected since the cultivar is recommended for growing in the Cerrado of the Central region of Brazil, aiming at production of cassava flour and starch (Vieira et al. 2020). In contrast, lower starch content in the roots of the sugary clones, such as that shown in all crop seasons by the cultivar BRS 438, is an indication that those clones are storing sugars in the tuberous roots (Vieira et al. 2008).

The sugary cassava cultivar BRS 438 had a mean root yield (RY) higher than the means of all the other genotypes evaluated, with expressive mean values of 92,937 kg ha^{-1}, 98,876 kg ha^{-1}, 97,737 kg ha^{-1}, and 99,289 kg ha^{-1} in the four crop seasons, respectively (Table 3). Root yield is one of the most important traits for selection of cassava genotypes, since it is closely related to the profitability of the crop (Vieira et al. 2020). In regard to starch yield (SY), the cultivar BRS 438 stood out, with high mean values in all the crop seasons (Table 3). In the 2012/2014 crop season, it had a mean value statistically higher than, and in the other crop seasons statistically similar to, that of the starchy check cultivar IAC 12-829, which is recommended for growing with the aim of starch production in the Cerrado (Table 3).

The sugary cassava cultivar BRS 438 also stood out from the others in its high percentages of glucose (2.51%), fructose (0.88%), maltose (2.7%), and total sugars in the roots (5.46%) (Table 4). The presence of soluble sugars gives BRS 438 a unique characteristic for the processes that require a hydrolysis step for the purpose of obtaining free sugars, as in the processes for the production of alcohols, ketones, carboxylic acids, and even glucose syrup itself. This high initial quantity of sugars and the percentage of water in the roots *in natura* (77%) facilitate the enzymatic hydrolysis process, both from the processing perspective and from the economic perspective. For the enzymatic hydrolysis of this cultivar,

Table 2. Mean values of the following traits: first branch height (FBH) in m, plant height (PH) in m, and percentage of starch in the roots (ST) evaluated in five genotypes of sugary cassava and two cultivars of starchy cassava in the 2012/2014 (S1), 2013/2015 (S2), 2014/2016 (S3), and 2015/2017 (S4) crop seasons in Planaltina, DF, Brazil

Genotype	FBH	FBH	FBH	FBH	PH	ST	ST	ST	ST
	S1	S2	S3	S4		S1	S2	S3	S4
IAC 12-829	0.46^{Ba}	0.60^{Aa}	0.55^{ABab}	0.56^{ABa}	1.81^{d}	33.95^{Aa}	31.11^{Ba}	30.79^{Ba}	30.78^{Ba}
580/08	0.30^{Ab}	0.35^{Ac}	0.31^{Acd}	0.35^{Ac}	2.33^{ab}	28.48^{Ac}	24.64^{Bc}	23.90^{Bc}	24.09^{Bb}
600/08	0.23^{Cbc}	0.52^{Aab}	0.42^{ABbc}	0.39^{Bbc}	2.25^{b}	29.14^{Ac}	22.24^{Bd}	22.57^{Bcd}	22.43^{Bbc}
601/08	0.18^{Cbc}	0.45^{Abc}	0.26^{BCd}	0.35^{ABc}	2.52^{a}	24.54^{Ad}	21.03^{Bd}	21.34^{Bd}	20.95^{Bc}
608/08	0.19^{Bbc}	0.37^{Ac}	0.33^{Acd}	0.33^{Ac}	2.15^{bc}	24.51^{Ad}	21.33^{Bd}	20.97^{Bd}	22.15^{Bbc}
BRS 438	0.14^{Cc}	0.37^{Bc}	0.42^{ABc}	0.49^{Aab}	2.33^{ab}	18.46^{Ae}	11.60^{Ce}	12.93^{BCe}	13.79^{Bd}
IAC 576-70	0.52^{Ba}	0.53^{Bab}	0.66^{Aa}	0.62^{ABa}	1.99^{cd}	31.62^{Ab}	27.69^{Bb}	28.38^{Bb}	28.97^{Ba}

[*] Mean values followed by the same uppercase letter in the horizontal direction and lowercase letter in the vertical direction do not differ from each other at $p < 0.05$ by Tukey's test.

Table 3. Mean values of the following traits: root yield (RY) in t ha^{-1} and starch yield (SY) in t ha^{-1} evaluated in five genotypes of sugary cassava and two cultivars of starchy cassava in the 2012/2014 (S1), 2013/2015 (S2), 2014/2016 (S3), and 2015/2017 (S4) crop seasons in Planaltina, DF, Brazil

Genotypes	RY	RY	RY	RY	SY	SY	SY	SY
	S1	S2	S3	S4	S1	S2	S3	S4
IAC 12-829	42.19^{Ac}	41.32^{Ab}	41.27^{Abc}	40.56^{Abc}	14.33^{Abc}	12.85^{Aa}	12.71^{Aa}	12.48^{Aab}
580/08	45.33^{Ac}	33.28^{Bb}	32.63^{Bcd}	33.24^{Bc}	12.86^{Ac}	8.21^{Bcd}	7.81^{Bbc}	7.99^{Bd}
600/08	32.68^{Ad}	33.82^{Ab}	33.04^{Acd}	36.84^{Abc}	9.50^{Ad}	7.52^{Ad}	7.46^{Abc}	8.27^{Ad}
601/08	30.30^{Ad}	37.62^{Ab}	30.24^{Ad}	37.68^{Abc}	7.40^{Ad}	7.91^{Ad}	6.45^{Ac}	7.89^{Ad}
608/08	61.73^{Ab}	37.85^{Bb}	42.70^{Bb}	43.58^{Bb}	15.11^{Aab}	8.10^{Bcd}	8.96^{Bb}	9.66^{Bcd}
BRS 438	92.94^{Aa}	98.88^{Aa}	97.74^{Aa}	99.29^{Aa}	17.15^{Aa}	11.46^{Cab}	12.65^{BCa}	13.68^{Ba}
IAC 576-70	42.91^{Ac}	36.51^{Ab}	39.96^{Abc}	38.38^{Abc}	13.54^{Abc}	10.10^{Bbc}	11.35^{Ba}	11.11^{Bbc}

* Mean values followed by the same uppercase letter in the horizontal direction and lowercase letter in the vertical direction do not differ from each other at $p < 0.05$ by Tukey's test.

Table 4. Comparison of mean values of the following traits: initial glucose percentage (%GI), initial fructose percentage (%FI), initial maltose percentage (%MI), percentage of initial sugars (%AÇI), initial sugars in kg ha^{-1} (AÇI), percentage of sugars after hydrolysis (%AÇF), sugars after hydrolysis in kg ha^{-1} (AÇF), percentage of hydrolyzed starch (%AH), and root yield (RY) in kg ha^{-1} evaluated in five sugary cassava genotypes and two cultivars of starchy cassava in the 2012/2014 crop season in Planaltina, DF, Brazil

Genotypes	Traits								
	%GI	%FI	%MI	%AÇI	AÇI	%AÇF	AÇF	%AH	RY
IAC 12-829	0.20^{D*}	0.06^{D}	0.56^{D}	0.82^{D}	336^{D}	23.35^{B}	9835^{B}	22.31^{A}	42187^{C}
IAC 576-70	0.06^{D}	0.00^{D}	0.21^{D}	0.27^{D}	125^{D}	26.29^{A}	11209^{B}	25.26^{A}	42910^{C}
580/08	1.18^{C}	0.67^{B}	1.56^{B}	3.40^{B}	1534^{C}	21.80^{B}	9790^{B}	18.65^{B}	45334^{C}
600/08	0.20^{D}	0.1^{D}	0.26^{D}	0.56^{D}	182^{D}	14.47^{C}	4682^{C}	13.57^{C}	32681^{D}
601/08	1.05^{C}	0.48^{C}	1.03^{C}	2.56^{C}	756^{D}	17.98^{C}	5384^{C}	14.64^{C}	30299^{D}
608/08	1.60^{B}	0.85^{A}	1.13^{C}	3.58^{B}	2212^{D}	14.21^{C}	8838^{B}	9.25^{D}	61733^{B}
BRS 438	2.51^{A}	0.88^{A}	2.07^{A}	5.46^{A}	5077^{A}	14.43^{C}	13413^{A}	10.09^{D}	92937^{A}

* Mean values followed by the same letter in the vertical direction do not differ from each other at 5 $p < 0.05$ by the Scott-Knott test for grouping means.

the amount of enzyme required for conversion of free sugar is considerably less than that necessary for starchy species, since part of the sugar is already in the monomer form. In the same way, the moisture content in the roots is also an important variable; for conversion of the starch into sugars, large amounts of water are required for the starchy cultivars, as the process occurs in an aqueous medium.

The data obtained show that BRS 438 has superior traits, compared to the check cultivars and to the other clones studied, for use in the processes of production of glucose syrup, ethanol, acetone, lactic acid, acetic acid, and citric acid, among other compounds. BRS 438 can also be used in the beer-brewing industry as, in that industry, most of the sugar must come from hydrolysis of barley (50%). However, since it is a good source of sugars, BRS 438 can be used like other inputs that serve to add flavor, aroma, texture, and other characteristics necessary for a good beer.

Fernandes et al. (2021) evaluated the chemical composition and fermentation characteristics of elephant grass (cv. BRS Capiaçu) silages enriched with different levels of inclusion of sugary cassava roots (BRS 438) and of starchy roots (IAC 12-829) *in natura*. The study showed that the inclusion of 20% and 40% of free-sugars cassava roots in the grass silage led to an increase in the in vitro digestibility of dry matter and reduction in the content of lignin and neutral detergent fiber and acid in the silage. In other words, the inclusion of sugary cassava roots improves the nutrient value and maintains a suitable standard of fermentation in the elephant grass (cv. BRS Capiaçu) silage harvested at 120 days of regrowth (Fernandes et al. 2021). This shows the potential of BRS 438 as an additive in forage grass silage.

Evaluations showed that the sugary cultivar BRS 438 has characteristics that justify its recommendation as the first sugary cassava cultivar for growing in the Cerrado of the Central region of Brazil. Its agronomic characteristics stand out, such as root yield, starch yield, and plant height compatible with mechanized planting. Its root composition characteristics are also prominent, with expressive storage of free sugars, such as glucose, fructose, and maltose, which are important additives/substrates in the enzymatic processes for production of alcohols, ketones, carboxylic acids, and glucose syrup, and in beer brewing and animal feed (forage grass silage).

REGISTRATION, PROTECTION, BASIC PLANT, AND LICENSING OF STEM CUTTING – SEED PRODUCERS

The sugary cassava cultivar BRS 438 was registered under number 51525 with the National Cultivar Registry (Registro Nacional de Cultivares - RNC) of the Brazilian Ministry of Agriculture (Ministério de Agricultura e Pecuária - MAPA) on 25 Oct. 2022. The production of basic plants and licensing of stem cutting – seed producers are under the responsibility of the Plant Genetics Innovation Center (Centro de Inovação em Genética Vegetal - CIGV) of Embrapa Cerrados, Rodovia DF 001, Km 69, Riacho Fundo I, CEP 71805-970, Brasília, DF, Brazil. Telephone (61) 3333-0417, E-mail: cpac.cigv@embrapa.br.

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