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Performance of sweet potato clones for bioethanol production in different cultivation periods

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ABSTRACT

Sweet potato is a perennial plant but cultivated as an annual crop. Thus, identifying the best period of cultivation is important for optimizing production, aiming human and animal consumption, as well as ethanol production. The aim of this work was to evaluate the performance of sweet potato clones grown in different cultivation periods for the production of bioethanol. Plots consisted of three cultivation periods (3, 5 and 7 months), and the subplots of five sweet potato clones (accessions IBP-007, IBP-038, IBP-075, IBP-079 and IBP-149) and the cultivar Brazlândia Rosada, considering a split plot scheme. We studied the root dry mass, starch and amylose content and starch and ethanol yield. Clone IPB-007 showed high values for starch yield (6.63 t/ha) and ethanol yield (4,379 L/ha). For root dry mass (38.32%) and starch (26.70%) and etha,nol yield (176.26 L/t), clone IPB-149 stood out. There was no significant difference for amylose content. Starch content (13.94%) and starch (1.64 t/ha) and ethanol yield (1,034 L/ha and 92.04 L/t) were lower for most of the clones in the three-month period of cultivation. We recommend the five-month period of cultivation and clones IPB-007 and IPB-149.

Keywords: *Ipomoea batatas*, plant science, bioenergy, ethanol.

RESUMO

Desempenho de clones de batata doce para a produção de bioetanol em função de períodos de cultivo

A batata doce é tecnicamente uma planta perene, plantada e explorada como anual. A identificação do período de cultivo e de clones é importante para melhorar o aproveitamento dessa cultura, tanto na alimentação humana e animal, como para produção de etanol. Objetivou-se avaliar o desempenho de clones de batata doce cultivados em diferentes períodos de cultivo para a produção de bioetanol. Foram testados nas parcelas três períodos de cultivo (3, 5 e 7 meses), e nas subparcelas, cinco clones de batata doce (acessos IBP-007, IBP-038, IBP-075, IBP-079 e IBP-149), além da cultivar Brazlândia Rosada. Foram analisados o teor de matéria seca de raiz, amido e amilose e rendimento de amido e etanol. O clone IPB-007 apresentou elevados valores para rendimento de amido (6,63 t/ha) e etanol (4.379 L/ha). Já para teor de matéria seca de raiz (38,32%) e amido (26,70%) e rendimento de etanol (176,26 L/t), o clone IPB-149 se destacou. Não houve diferença significativa para o teor de amilose. O teor de amido (13,94%) e rendimento de amido (1,64 t/ha), e etanol (1.034 L/ha e 92,04 L/t) foram menores para a maioria dos clones avaliados nos períodos de cultivo de três meses. Recomenda-se adotar o período de cultivo de cinco meses e os clones IPB-007 e IPB-149.

Palavra-chave: *Ipomoea batatas*, fitotecnia, bioenergia, etanol.

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Sweet potato (*Ipomoea batatas*) belongs to Convolvulaceae family. This species shows many agronomic privileges regarding planting on marginal lands, such as resistance to drought and tolerance to saline soils (Li & Chan-Halbrendt, 2009).

Despite being cultivated annually, sweet potato is a perennial plant of continuous tuberization, natural death of the plant only occurs under severe weather conditions, such as frosts and a very long dry season. In optimal growing conditions, harvest can begin at 90 days after planting (DAP), but in general, harvest is carried out between 120 and 150 DAP (Silva *et al*., 2004). Determining harvest season has great influence on vegetative growth and quality and productivity of tuberous roots and it may vary according to the cultivar, environmental conditions and destination of the production (Queiroga *et al*., 2007).

Some studies on potential resources of carbohydrates for ethanol production, in Alabama and Maryland (USA), show the sweet potato crop as a source for ethanol production, reaching, in an experimental condition, 8,839 L/ha, against 6,195 L/ha of sugarcane, in that country (Ziska *et al*., 2009). Brazil, as a world leader in biofuels (ethanol), has enough conditions to develop sustainable programs for biofuels production, in which sweet potato fits as a promising resource mainly due to its short production cycle as well as for the average yields of ethanol production ranging from 76.30 to 178.70 liters of ethanol per ton of root (Alves *et al*., 2014).

Since sweet potato has a high starch content and a great genetic variability, implementation of a breeding program targeting the market of ethanol production is possible, highlighting its use as an alternative for plants and farmers in later crop seasons in relation to the planting of sugarcane (Pavlak *et al*., 2011). In Palmas, Tocantins State, Brazil, the potential of sweet potato as a biofuel option is being evaluated in the experimental plant at Fundação Universidade do Tocantins (Tocantins University Foundation), while researches are selecting sweet potato cultivars for the alcohol production process, more productive and adapted to different producing regions (Pavlak *et al*., 2011).

Generally, starch content in sweet potato roots is 20 and 30% in fresh weight, making the roots an optimal source of glucose for several applications. Recently, studies show growing interest in using sweet potato as a substitute source of energy as a replacement for maize grains to produce bioethanol (Ziska *et al*., 2009).

Structurally, starch is a homopolysaccharide composed of chains of amylose and amylopectin. Amylose consists of glucose units connected by α-1,4 glycosidic bonds forming a linear chain. The amylopectin consists of glucose units connected by α-1,4 and α-1,6, forming a branched structure. The proportions in which these structures appear differ with respect to botanical sources, varieties of the same species and even in the same botanical variety according to the degree of plant maturity (Tester & Karkalas, 2004).

Degrees between 15% and 25% of amylose are typical in most grains; however some cereals, namely the waxy-cereals, such as maize, rice and barley, are virtually amylose-free whereas mutants with high amylose levels are also known. The maize amylose extender (*ae*) mutants have amylose contents ranging from 50% to 85%. The rice (*ae*) mutants show amylose contents ranging from 35% to 40% (Vandeputte & Delcour, 2004).

Amylose plays an important role in the conversion of starch to glucose, since it is a water-soluble polymer (Aehle, 2007), thereby reducing the energy cost, which is the second largest cost for ethanol production after the cost of raw materials (Bai *et al*., 2008), considering that the major amount of energy is consumed for converting starch into fermentable sugars, particularly in the liquefaction process.

Thus, this work aimed to evaluate different periods of cultivation effects on biochemical characteristics of sweet potato clones for the production of bioethanol.

MATERIAL AND METHODS

Clones IBP-007, IPB-038, IPB-075, IBP-079 and IBP-149, besides the cultivar Brazlândia Rosada, from the Active Germplasm Bank of Universidade Federal de Sergipe (Sergipe Federal University, Brazil) were evaluated in three periods of cultivation (3, 5 and 7 months). Planting was carried out on May 29, 2012. The local climate is tropical semiarid, mild summer and humid winter, hot and dry, and according to Instituto Nacional de Meteorologia (National Institute of Meteorology) (www.inmet.gov.br/projetos/rede/pesquisa/) the average rainfall and the lowest and highest temperature were 144.6 mm, 23.4°C, 29.9°C for May; 118.0 mm, 22.7°C, 28.9°C for June; 100.0 mm, 22.3°C, 28.3°C for July; 77.1 mm, 22.2°C, 28.1°C for August; 60.8 mm, 22.4°C, 28.8°C for September; 69.5 mm, 22.7°C, 28.7°C for October; 5.6 mm, 24.2°C, 30.0°C for November; 2.0 mm, 24.5°C, 30.7°C for December, 2012, respectively. The experimental design was randomized blocks, split-plot scheme, with three replications and ten plants per replication, the following traits being evaluated: root dry mass; starch content and yield; amylose content and ethanol yield.

In order to evaluate root dry mass, about 200 g of roots of each clone was sampled, grated and homogenized. The authors used 10 g to determine the root dry mass content which was dried in an oven with forced air circulation at 100°C, until they reached constant weight. The content was calculated by the relation dry mass/fresh mass multiplied by 100 and the results were expressed in percentage. To determine the starch content, the authors used the Lane-Eynon method, which is based on the reduction of a known volume of an alkaline copper reagent (Fehling) to cuprous oxide, and the end point is indicated by methylene blue, which is reduced to its leuco form by a small excess of reducing sugar (Instituto Adolfo Lutz, 2005), being calculated in relation to the sweet potato fresh matter, that means, the starch content was exposed taking into account the sweet potato overall composition. Starch yield is obtained by multiplying starch content on basis of fresh mass by total root yield and it is expressed in t/ha. The amylose content was determined according to the methodology proposed by Megazyme (Amylose/Amylopectin, 2006), using the amylose/amylopectin kit, and the results were expressed in percentage in relation to dry mass. Ethanol yield was obtained by multiplying starch yield by the conversion factor 0.662 (Pavlak *et al*., 2011), expressed in L/ha. Ethanol yield per ton of roots was obtained by dividing ethanol yield by total yield of roots and it was expressed in L/t.

For the chemical analysis, both for starch and amylose content, the authors used about 200 grams of each sample which were identified, grated and dried in a forced air circulation oven at temperature of 60°C, for 72 hours. After dried, samples were macerated in order to obtain flour and then the analyses were carried out. The experiments were carried out at enzymology and plant science laboratories of UFS.

Results were evaluated statistically through analysis of variance (F test) and the averages were grouped through Scott-Knott test at 5% probability using the software SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Values and significance of the mean squares verified in analysis of variance (F test), including the interaction clone X periods of cultivation, for the traits studied, showed significant differences (α= 5%) for almost all the traits, except for the amylose content.

Root dry mass content

Results obtained showed that the three-month period of cultivation yielded lower root dry mass contents for

IPB-038 (25.01), IPB-149 (30.56) and IPB-007 (21.18) clones, the IPB-007 clone showing the lowest yield among the other clones evaluated in this period of cultivation.

In five-month period of cultivation, clone IPB-149 showed the highest percentage of dry mass in relation to other accessions tested, reaching 38.32%. The highest dry mass content of clone IPB-007 was verified in this period of cultivation, 34.44%.

Clone IPB-149 and cultivar Brazlândia Rosada differed statistically from the other clones with the highest root dry mass contents in the seven-month period of cultivation, 36.68% and 35.48%, respectively (Table 1).

Similar values (31.50%) were found in a work with 36 accessions and three sweet potato cultivars (Gonçalves Neto *et al.*, 2011). Researchers also observed the formation of two groups with low variation among the genotypes, highlighting the genotype BDI2007. PA37 with 37.92%, the highest percentage of dry mass (Santana *et al.*, 2013). Roesler *et al.* (2008) verified a decrease in water content between the first harvest (115 days) and the second harvest (183 days) for the four clones tested, including cultivar Brazlândia Rosada.

Gonçalves Neto *et al.* (2011) identified the aptitudes of sweet potato genotypes for fresh consumption, ethanol production and animal feed, through aptitude indices. They attributed the total production of root dry mass as the factor with the greatest impact in aptitude for ethanol biofuel.

Starch content and yield

The ethanol production process from sweet potato is similar to the processing of sugarcane. The main differences are in the preparation of raw materials and in the fermentation system. Sweet potato, cassava and maize have no sugar but starch; in this case, it is necessary to convert starch into sugars and then ferment it, which is done with cooking and saccharification, using enzymes. The concentration of starch in sweet potato directly influences the final cost of a liter of hydrated ethanol, since the higher the starch content is, the higher will be the ethanol production per ton of raw material and the lower will be the cost of ethanol production (Taborda *et al.*, 2015).

As for starch content, clone IPB-007 showed a lower performance in the three-month period of cultivation (13.94%) and a better result in the five-month period (24.08%). The three-month period of cultivation also affected clones IPB-038 (16.60%), IPB-149 (20.43%) and the cultivar Brazlândia Rosada (21.02%), resulting in a lower starch yield.

Table 1. Average values for dry matter content (%) of sweet potato clones on different cultivation periods. São Cristóvão, UFS, 2013.

Clone	Dry matter content (%)		
	3 months	5 months	7 months
Brazlândia Rosada	31.40 a A	34.77 b A	35.48 a A
IPB-007	21.18 c C	34.44 b A	28.02 b B
IPB-038	25.01 b B	28.47 c A	30.56 b A
IPB-075	25.27 b A	27.60 c A	29.47 b A
IPB-079	28.44 a A	27.66 c A	31.18 b A
IPB-149	30.56 a B	38.32 a A	36.68 a A
CV-a (%)*		7.98	
CV-b (%)**		7.27	

Means followed by the same small letter in column and capital letter in line are not different, Scott-Knott test, 5%; *CV-a = coefficient of variation for period of cultivation; **CV-b = coefficient of variation for clones.

Table 2. Average values for starch content and yield of sweet potato clones on different cultivation periods. São Cristóvão, UFS, 2013.

Clone	Starch content (%)		
	3 months	5 months	7 months
Brazlândia Rosada	21.02 a B	24.64 a A	25.91 a A
IPB-007	13.94 b C	24.08 a A	18.95 b B
IPB-038	16.60 b B	19.30 b A	20.95 b A
IPB-075	16.27 b B	18.37 b B	20.53 b A
IPB-079	18.57 a A	19.63 b A	21.66 b A
IPB-149	20.43 a B	26.70 a A	26.46 a A
CV-a (%)*		7.22	
CV-b (%)**		8.12	
		Starch yield (t/ha)	
Brazlândia Rosada	1.56 a B	4.75 b A	5.48 b A
IPB-007	2.73 a B	6.63 a A	6.18 a A
IPB-038	2.07 a B	4.44 b A	4.81 b A
IPB-075	1.81 a C	5.14 b A	3.78 c B
IPB-079	2.30 a B	4.04 b A	2.93 c B
IPB-149	1.64 a C	5.90 a A	3.46 c B
CV-a (%)*		18.26	
CV-b (%)**		14.99	

Means followed by the same small letter in column and capital letter in line are not different, Scott-Knott test, 5%; *CV-a = coefficient of variation for cultivation periods; **CV-b = coefficient of variation for clones.

Clones IPB-007, IPB-149 and the cultivar Brazlândia Rosada showed statistically values higher in the five-month period of cultivation, of 24.08%, 26.70% and 24.64%, respectively. Clone IPB-149 and the cultivar Brazlândia Rosada also stood out when harvested in the seventh month of cultivation, obtaining starch contents of 26.46% and 25.91%.

The evaluated clones showed lower starch content in the three-month period of cultivation, with exception for IPB-079 which also obtained low starch yield in the seven-month period of cultivation with yield of 2.30 and 2.93 t/ha (Table 2).

For starch yield, no significant difference among the clones for the first period of cultivation was noticed, with productivity from 1.56 to 2.73 t/ha. For five-month period of cultivation, clones IPB-007 and IPB-149 showed the best results (6.63 and 5.90 t/ha). Clone IPB-007 also showed the highest value for seven-month period of cultivation, reaching 6.18 t/ha.

Lower percentages were observed for sweet potato starch yield, 14.72%, in fresh mass basis, using the method of enzymatic hydrolysis. Results were superior for starch yield per hectare, a relevant characteristic for food and biofuels industries (Leonel & Cereda, 2002).

The clones evaluated in the present work obtained starch yields similar to other starchy tuberous as cassava, yam and biri which showed 7.5 t/ha, 6.1 t/ha and 5.5 t/ha, respectively. Yam was harvested after 12 months, biri after nine months and cassava after 10 to 14 months of cultivation (Leonel & Cereda, 2002).

Amylose content

Starch is considered one of the most abundant sources of carbohydrates in nature. However, the yeast *Saccharomyces cerevisiae*, used for ethanol production, cannot convert starch into ethanol directly; two previous steps for the conversion of starch into fermentable sugars are necessary: liquefaction and dextrinization with α-amylase and saccharification with amiloglicosidase (Silva *et al*., 2006).

Neither the period of cultivation, nor the clones, nor the interaction between the two factors were significant by F test at 5% probability (Table 3). Other researchers did not observe any influence of the plant development stage on amylose content, with contents ranging from 19.7 to 23.7% (Noda *et al*., 1995).

This same author reported, in different works, amylose contents with little variation in sweet potato, from 19.15% (CNPH314) to 22.54% (CNPH003), harvested eight months after planting. He also stated averages

Table 3. Average values for amylose content (%) of sweet potato clones on different periods of cultivation. São Cristóvão, UFS, 2013.

Clone	Amylose content (%)			
	3 months	5 months	7 months	Mean
Brazlândia Rosada	29	27	24	27 a
IPB-007	18	28	25	24 a
IPB-038	20	19	13	17 a
IPB-075	19	25	41	28 a
IPB-079	20	27	37	28 a
IPB-149	25	23	22	23 a
Mean	22 A	25 A	27 A	
CV-a (%)*		18.08		
CV-b (%)**		36.91		

Means followed by the same small letter in column and capital letter in line are not different, Scott-Knott test, 5%; *CV-a = coefficient of variation for period of cultivation; **CV-b = coefficient of variation for clones.

Table 4. Average values for ethanol yield (L/ha and L/t) of sweet potato clones on different periods of cultivation. São Cristóvão, UFS, 2013.

Clones	Ethanol yield (L/ha)		
	3 months	5 months	7 months
Brazlândia Rosada	1,034 a B	3,135 b A	3,621 b A
IPB-007	1,803 a B	4,379 a A	4,079 a A
IPB-038	1,368 a B	2,931 b A	3,176 b A
IPB-075	1,200 a C	3,395 b A	2,496 c B
IPB-079	1,519 a B	2,667 b A	1,934 c B
IPB-149	1,087 a C	3,898 a A	2,283 c B
CV-a (%)		18.26	
CV-b (%)		14.99	
		Ethanol yield (L/t)	
Brazlândia Rosada	138.74 a B	162.64 a A	171.01 a A
IPB-007	92.04 b C	158.99 a A	125.12 b B
IPB-038	109.58 b B	127.40 b A	138.32 b A
IPB-075	107.43 b B	121.25 b B	135.52 b A
IPB-079	129.57 a A	122.61 b A	142.98 b A
IPB-149	134.89 a B	176.26 a A	174.65 a A
CV-a (%)*		7.22	
CV-b (%)**		8.12	

Means followed by the same small letter in column and capital letter in line are not different, Scott-Knott test, 5%; *CV-a = coefficient of variation for period of cultivation; **CV-b = coefficient of variation for clones.

for amylose content in other crops: biri (26.12%), taioba (*Xanthosoma* spp.) (16.47%), cassava (16.33%) and sweet potato (19.34%) (Leonel *et al*., 2004).

The sweet potato starch characteristics are much influenced by the soil temperature and period of cultivation (Katayama *et al*., 2002; Ishiguro *et al*., 2003). Cultivation under conditions of higher soil temperature or during the summer was reported to induce higher contents of amylose and amylopectin of shorter chains in sweet potato starch (Noda *et al*., 2001).

Ethanol yield L/ha and L/t

For ethanol yield in liters per hectare, the clones did not differ when harvested in the three-month period, with yields from 1,034 to 1,803 L/ha; nevertheless, the three-month period of cultivation showed the lowest yields among the clones evaluated except for the clone IPB-079, which did not differ from the seven-month period of cultivation (Table 4).

The highest yields, in the five-month period, were obtained by clones IPB-007 (4,379 L/ha) and IPB-149 (3,898 L/ha). In the seven-month period of cultivation, clone IPB-007 also showed the highest yield comparing to the other evaluated clones, with productivity of 4,079 L/ha.

Results of ethanol yield obtained by Santana *et al.* (2013) with genotypes BDI2007.PA37, BDI2007.PA26 and BDI2007.0217 ranged from 8,440.78 L/ha to 6,136.8 L/ha. Cultivar Duda obtained yield 10,007.1 L/ha, a high yield comparing to the results obtained in this work and with sugar cane, which is 6,800 L/ha (Kohlhepp, 2010).

The averages of ethanol yield in liters per ton of roots were lower in the three-month period of cultivation for clones IPB-007, IPB-038, IPB-075, IPB-149 and for cultivar Brazlândia Rosada with values of 92.04; 109.58; 107.43; 134.89 and 138.74 L/t respectively, keeping constant for the clone IPB-079 in all periods of cultivation (129.57; 122.61 and 142.98 L/t).

Clones IPB-007, IPB-149 and cultivar Brazlândia Rosada showed ethanol yield reaching 158.99, 176.26 and 162.64 L/t respectively, higher than the other clones tested in the five-month period of cultivation. In the seven-month period of cultivation, clone IPB-149 (174.65 L/t) and the cultivar Brazlândia Rosada (171.01 L/t) were statistically superior to the other clones evaluated in the same period of cultivation.

The variation in ethanol yield in liters per ton reported in this work is similar to the one found in a previous work, 89.26 L/t of roots for genotype BDI2007.10652 to 181.65 L/t for cultivar Duda (Santana *et al*., 2013). This fact shows the genetic variability of the genotypes studied and the sweet potato potential as raw material for industrial ethanol production.

The results obtained in this work show great genetic variability of the genotypes studied and the sweet potato potential as raw material for industrial ethanol production. The authors recommend the five-month period of cultivation, which showed to be the most advantageous in relation to the evaluated variables, for most of the evaluated clones, occupying the area for less time.

According to the data found in this study, the authors also verified the possibility of selecting superior genotypes for biochemical characters concerning ethanol production. Among these genotypes, clones IPB-007 and IPB-149 and the cultivar Brazlândia Rosada stand out. Despite being a crop less studied than the sugarcane, concerning to its management as to breeding, the sweet potato should be considered as an option for biofuel production.

The authors recommend the five-month period of cultivation for sweet potato and clones IPB-007 and IPB-149.

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