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# Developing an *in vitro* optimized protocol to sweet potato landraces conservation

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**ABSTRACT.** This study aimed to develop a protocol for the *in vitro* conservation of 30 accessions of sweet potato collected in the northern region of Rio de Janeiro State, Brazil, aiming to extend the period between subcultures for this species. An *in vitro* minimal growth experiment was established with accession UENF 1931 in a factorial arrangement consisting of four concentrations of mineral salts in MS medium and four concentrations of sucrose, at two temperatures. The others accessions were grown in medium containing 100% MS salts and 2% sucrose, which was the medium that allowed the *in vitro* conservation of accession UENF 1931 for a longer time period. For this second experiment, 30 accessions of sweet potato and two temperatures were used. Plant height, number of leaves, and survival rate were measured every 30 days for 12 months, in both experiments. In the last stage, the sweet potato plantlets originating from *in vitro* minimal growth were acclimatized and exhibited 100% survival. The recommended *in vitro* minimal growth conditions for the studied accessions are MS medium with 100% concentration of mineral salts and 2% sucrose, at a temperature of 27±2°C, with subculture performed every 180 days.

**Keywords:** *Ipomoea batatas*, minimal growth, germplasm bank, maintenance of diversity.

## Desenvolvimento de um protocolo otimizado para a conservação *in vitro* de variedades locais de batata-doce

**RESUMO.** Este trabalho objetivou determinar um protocolo para conservação *in vitro* de trinta acessos de batata-doce coletados na região Norte do Estado do Rio de Janeiro, visando aumentar o período entre os subcultivos para esta espécie. Foi montado um experimento de cultivo mínimo *in vitro* com o acesso UENF 1931 em um esquema fatorial com quatro concentrações dos sais minerais do meio MS e quatro concentrações de sacarose em duas temperaturas. Os demais acessos de batata-doce foram cultivados em meio contendo 100% dos sais do MS e 2% de sacarose, sendo o meio que propiciou a conservação do acesso UENF 1931 por maior período de tempo. Neste segundo experimento foram utilizados 30 acessos de batata-doce e duas temperaturas. Altura das plantas, número de folhas e taxa de sobrevivência foram mensurados a cada 30 dias durante 12 meses, em ambos os experimentos. Na última etapa foi feita a aclimatização das mudas de batata-doce provenientes do cultivo mínimo *in vitro*, apresentando sobrevivência de 100%. Para o cultivo mínimo *in vitro* dos acessos estudados recomendou-se a utilização de meio MS com 100% da concentração de sais minerais e 2% de sacarose, temperatura de 27±2°C, com subcultivos a cada 180 dias.

**Palavras-chave:** *Ipomoea batatas*, cultivo mínimo, banco de germoplasma, manutenção da diversidade.

## Introduction

The sweet potato is considered the seventh most important crop in the world and the fifth most important in developing countries (Loebenstein, 2009). In Africa and Asia, thousands of people depend on sweet potato for food safety (Zhang, Wang, Liu, & Wang, 2009). On the Latin American continent, Brazil is the main producer, with an estimated annual production of 505,000 ton. (Reetz, 2014).

This vegetable crop can be used in home cooking or as a raw material in industrial processes for the production of sweets, flour, flakes, and starch, in addition to the production of biomass for biofuel (Ferrari, Guigou, & Lareo, 2013; Wang, Shi, Xia, Li, & Chen, 2013; Zhang et al., 2013).

In several areas of production, the sweet potato is a typical crop for small rural producers who are responsible for the maintenance of high genetic variability through the conservation of local varieties on their farms. However, some factors have

jeopardized this so-called important form of conservation of genetic resources, e.g., the modernization of agriculture, which leads to rural exodus and contributes to the loss of genetic diversity of crops that were traditionally farmed by small farmers, such as the sweet potato. Another factor is the change in the eating habits of populations brought about by the emergence of new consumption options. In addition, landraces have been replaced by commercial varieties (Rodrigues & Santos, 2011). To prevent this loss of diversity, it is essential to preserve the germplasm so that it can be available for future use (Sá, Lédo, & Lédo, 2011).

*In vitro* plant culture is an alternative method for the conservation of germplasm, especially vegetatively propagated species, such as the sweet potato. This technique offers many advantages over the germplasm conservation process in the field. *In vitro* plant material is free from pathogens and weather conditions and risks present in the field such as theft, predation, attacks by pests and diseases, floods and droughts. In addition, the technique allows the maintenance of a large number of accessions in a small physical space, contributing to the reduction of financial costs (Withers & Williams, 1998; Cid, 2001).

One of the ways to preserve plant material *in vitro* is through the slow-growth or minimal-growth technique, in which the plant metabolism is reduced, thereby increasing the interval between subcultures. To this end, alterations are made in the chemical conditions of the growth medium, such as the addition of growth regulators or a reduction in the concentrations of salt and organic components of the growth medium, as well as a reduction in the light intensity or temperature in the growth room. In this way, the labor and space necessary for the maintenance of the germplasm are reduced (Tahtamouni, Shibli, & Ajlouni, 2001; Islam, Leunufna, Dembele, & Keller, 2003; Sarwar & Siddiqui, 2004; Divakaran, Babu, & Peter, 2006; Santos, Lédo, Lédo, Souza, & Junior, 2011). Sweet potato subculture is performed on average every 90 days, and this is one of the most time- and cost-consuming factors in the maintenance of *in vitro* collection (Teixeira & Nascimento, 1999).

Published studies on the preservation of sweet potato by minimal *in vitro* growth report an evaluation period of up to 90 days (Teixeira & Nascimento, 1999). However, for the creation of an *in vitro* sweet potato germplasm bank, several unanswered questions arise in the literature: (1) How will *in vitro* conditions, such as salt and sucrose concentrations and temperature, influence plant growth after these 90 days of minimal growth? (2) How long can I maintain this material *in vitro*? (3) What happens to the plant material after subculture? (4) What is the maximum period between subcultures? (5) How will the *in vitro* conditions affect the *ex vitro* response of plants after preservation?

This study describes a protocol to optimize the *in vitro* conservation of landraces of sweet potato by increasing the period between subcultures for this species.

## Material and methods

### Plant material

Twenty-eight accessions of sweet potato were collected from small farms in the northern region of Rio de Janeiro State (Brazil) and characterized by Moulin et al. (2012a and 2012b), were established in vitro, kept in a growth room at a temperature of 27±2°C and irradiance of 25 µmol $m^{-2}$ $s^{-1}$, provided by fluorescent lamps (OSRAM®, daylight), under a photoperiod of 16:8 hours of light:darkness, for 90 days. Two commercial genotypes developed by Empresa Brasileira de Pesquisa Agropecuária (Embrapa), i.e., 'Brazlândia Rosada' (UENF 1997) and 'Princesa' (UENF 1994), were also used (Table 1).

For the in vitro establishment of the material, nodal segments of branches of sweet potato containing one bud each were utilized. A semi-solid growth medium was used containing mineral salts of MS medium (concentration of 100%) and White's vitamins (Murashige & Skoog, 1962), 100 mg $L^{-1}$ myo-inositol, sucrose at a concentration of 3%, and pH adjusted to 5.7. Next, the medium was solidified with 8.0 $L^{-1}$ bacteriological agar Vetec® and autoclaved for 15 min. at 121ºC and 105 kPa.

The experiments were conducted in the Setor de Horticultura of the Laboratório de Fitotecnia, in the Centro de Ciências e Tecnologias Agropecuárias of Universidade Estadual do Norte Fluminense Darcy Ribeiro, located in Campos dos Goytacazes, Rio de Janeiro State, Brazil.

### Establishment of *in vitro* minimal growth conditions

A preliminary trial was conducted to establish the conditions for *in vitro* minimal growth, randomly using accession UENF 1931 as the model plant. In this experiment, nodal segments containing one bud from accession UENF 1931 obtained from plants established *in vitro* and maintained in a growth room under the afore-described conditions were used as explants. The experimental design was completely randomized,

**Table 1.** Origin and characteristics of roots of 28 accessions of sweet potato collected in Rio de Janeiro State, Brazil, and two commercial genotypes grown *in vitro* (*Adapted from* Moulin et al., 2012 a).

| Accession | Provenance | Skin color | Flesh color | Latitude | Longitude |
|---|---|---|---|---|---|
| UENF 1917 | Campos dos Goytacazes | Dark purple | Cream | 21°54'27.3" | 41°02'30.2" |
| UENF 1920 | Campos dos Goytacazes | Cream | Cream | 21°57'08.5" | 41°08'19.2" |
| UENF 1922 | Campos dos Goytacazes | White | White | 21°37'21.4" | 41°13'12.2" |
| UENF 1923 | Campos dos Goytacazes | Pink | Strongly pigmented with anthocyanins | 21°37'21.4" | 41°13'12.2" |
| UENF 1925 | Campos dos Goytacazes | Cream | White | 21°39'01.7" | 41°11'20.2" |
| UENF 1927 | Espírito Santo | White | Pale yellow | - | - |
| UENF 1928 | Espírito Santo | Cream | White | - | - |
| UENF 1931 | Campos dos Goytacazes | Dark purple | White | 21°46'30.8" | 41°18'35.2" |
| UENF 1932 | São João da Barra | Cream | Intermediate orange | 21°45'41.2" | 41°17'26.7" |
| UENF 1935 | São João da Barra | Dark purple | White | 21°45'29.3" | 41°19'33.6" |
| UENF 1937 | São João da Barra | White | White | 21°46'31.6" | 41°19'11.1" |
| UENF 1939 | Cabo Frio | Dark purple | Cream | 21°45'38.8" | 41°19'41.5" |
| UENF 1940 | Espírito Santo | Dark purple | Cream | 21°44'23.6" | 41°21'04.4" |
| UENF 1941 | São João da Barra | Pink | Cream | 21°44'56.7" | 41°19'34.3" |
| UENF 1942 | Espírito Santo | Cream | Cream | 21°43'25.5" | 41°19'15.8" |
| UENF 1944 | Espírito Santo | Dark purple | Cream | 21°42'58.4" | 41°19'26.9" |
| UENF 1945 | Espírito Santo | Cream | Cream | 21°35'59.4" | 41°19'01.2" |
| UENF 1947 | Espírito Santo | Dark purple | White | 21°45'43.2" | 41°19'34.3" |
| UENF 1949 | Campos dos Goytacazes | Dark purple | Cream | 21°57'05.1" | 41°03'45.7" |
| UENF 1953 | São João da Barra | Cream | Cream | 21°56'03.2" | 40°59'28.0" |
| UENF 1960 | Campos dos Goytacazes | Cream | Cream | 21°36'33.6" | 41°18'59.2" |
| UENF 1962 | Campos dos Goytacazes | Pink | Cream | 21°38'08.6" | 41°16'32.3" |
| UENF 1965 | Campos dos Goytacazes | Cream | Cream | 21°37'07.4" | 41°13'23.4" |
| UENF 1969 | São João da Barra | Cream | Cream | 21°43'48.1" | 41°07'48.8" |
| UENF 1970 | São João da Barra | Pink | Cream | 21°54'28.8" | 41°05'46.6" |
| UENF 1987 | Campos dos Goytacazes | Cream | Pale orange | 21°58'43.6" | 41°29'42.2" |
| UENF 1988 | Campos dos Goytacazes | Cream | Cream | 21°58'43.6" | 41°29'42.2" |
| UENF 1990 | Campos dos Goytacazes | White | White | 21°58'28.8" | 41°29'19.0" |
| UENF 1994 | Brasília | Dark purple | White | - | - |
| UENF 1997 | Brasília | Dark purple | White | - | - |

with a 4 × 4 factorial arrangement, which consisted of four concentrations of the mineral salts from the MS medium (0, 10, 50, and 100%), and four concentrations of sucrose (0, 1, 2, and 3%), at two temperatures (18±2°C and 27±2°C), resulting in a total of 16 treatments with three replicates. Each replicate consisted of three test tubes (25 × 150 mm) with 10 mL of medium containing one explant each.

After the explants were inoculated, test tubes were sealed with PVC film. Half of the tubes were kept in a B.O.D. growth chamber and the other half in a growth room, both under a photoperiod of 16:8 hours of light:darkness and irradiance of 25 μmol $m^{-2}$ $s^{-1}$ provided by fluorescent lamps (OSRAM®) similar to daylight. The temperature in the growth chamber was maintained at 18±2°C and that in the growth room at 27±2°C. Assessments were made every 30 days, for 12 months; plant height (mm), number of leaves, and survival rate were measured.

At 360 days, the plants of accession UENF 1931 in the treatment with 100% MS medium and 2% sucrose at both temperatures were explanted, and nodal segments containing one bud were transferred to a new medium with the same concentrations of salts and sucrose. Plant survival rate was measured after 90 days.

### *In vitro* minimal growth of sweet potato accessions

The combination that allowed the *in vitro* conservation of accession UENF 1931 for the longest period was that which contained mineral salts from the MS medium at 100% concentration and 2% sucrose. This combination was then tested using all other accessions. For this purpose, nodal segments containing one bud each from plants retained *in vitro* under the previously described conditions were used.

The experimental design in this phase of the study was completely randomized, with 30 genotypes of sweet potato plants at two temperatures (18±2°C and 27±2°C), totaling 30 treatments, with three replicates. Each replicate consisted of three test tubes (25 × 150 mm) with 10 mL of medium containing one explant each.

After the inoculation of the explants, the test tubes were sealed with PVC film. Half of the test tubes were maintained in a B.O.D. growth chamber at a temperature of 18±2°C, and the other half in a B.O.D. growth chamber at a temperature of 27±2°C, both under a photoperiod of 16:8 hours of light:darkness and irradiance of 25 μmol $m^{-2}$ $s^{-1}$ provided by fluorescent lamps (OSRAM®) similar to daylight.

Evaluations were carried out every 30 days for 12 months; plant height, number of leaves, and survival

rate were measured to determine the performance of the other sweet potato accessions in the treatment that was previously established with accession UENF 1931.

At 360 days, the plants of the surviving accessions at both temperatures were explanted, and nodal segments containing one bud were transferred to a new medium with the same concentrations of salts and sucrose and the same experimental design. Plant survival rate was measured after 90 days.

### Acclimatization of plants

The experimental design in this phase was completely randomized, consisting of 30 genotypes of sweet potato plants, totaling 30 treatments, with two replicates. Each replicate consisted of three test tubes (25 × 150 mm) with 10 mL of medium containing one explant each, maintained at 27±2°C. The medium contained 100% MS mineral salts and 2% sucrose.

After 180 days of *in vitro* conservation, plants of all 30 accessions tested that were approximately 9.0 cm in height were removed from the test tubes and placed in pots containing 1.5 L of the substrate Basaplant Hortaliças®. Two pots were used for each accession, and each pot contained three plantlets. Next, plantlets were transferred to a greenhouse with a plastic cover (100 µm) and shade net 50% (Sombrite®). The temperature was monitored daily, and it ranged from 21 to 33ºC. Plant survival was assessed every 30 days, for 360 days.

### Statistical analysis

To determine whether the data would meet the assumptions of analysis of variance (ANOVA), they were subjected to preliminary analyses to determine the normality and homogeneity of variances among treatments, for each factor (plant height, number of leaves, and survival rate), based on the Lilliefors (1967) and Bartlett, Bobko, and Mosier (1978) tests. Subsequently, the data were subjected to analysis of variance, and the means were compared according to the F test at 5% probability, utilizing SAEG 9.1 (2007) software. The variation in the parameters analyzed over time was evaluated using regression analysis.

## Results

### Establishment of *in vitro* minimal growth conditions

Overall, the temperature of 27±2°C was more favorable for plant survival (66.29%) and provided the greatest height (2.20 cm) and lowest number of leaves (1.41) when the entire *in vitro* growth period was evaluated (Table 2).

**Table 2.** Mean values for height, number of leaves, and survival of sweet potato accession UENF 1931 conserved *in vitro* for 12 months.

| Temperature | Height (cm) | Leaf number | Survival (%) |
|---|---|---|---|
| 18±2°C | 1.02 b | 1.20 b | 63.14 b |
| 27±2°C | 2.20 a | 1.41 a | 66.29 a |

*Means followed by the same letter within a column do not differ according to the F test at the 5% probability level.

The plants from the treatments with total suppression of mineral salts and sucrose in the growth medium grew less (0.20 cm), produced fewer leaves (0.16) and had one of the lowest survival rates (59.82%), irrespective of the temperature.

The treatments with 100% MS medium and 1%, 2%, and 3% sucrose simultaneously displayed a greater number of leaves, reduced growth, and high survival rate and were thus the most indicative of minimal growth for the accession under study for subculturing every 360 days (Figures 1 and 2).

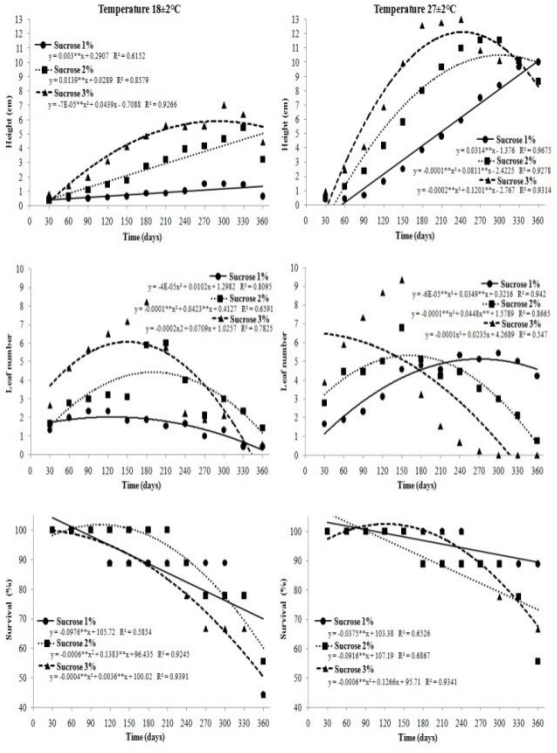


**Figure 1.** Height (cm), number of leaves, and percent survival of sweet potato accession UENF 1931 conserved *in vitro* in 100% MS medium with sucrose at concentrations of 1%, 2% and 3% and temperatures of 18±2°C (left) and 27±2°C (right) as a function of time (every 30 days).

After 360 days, the plants of accession UENF 1931 in the treatment with 100% MS medium and 2% sucrose at both temperatures were explanted, and nodal segments containing one bud were transferred to a new medium with the same

concentrations of salts and sucrose. After 90 days of reculturing, it was observed that the plants at 18±2°C showed only 10% survival, whereas those maintained at 27±2°C had a survival rate of 100%.

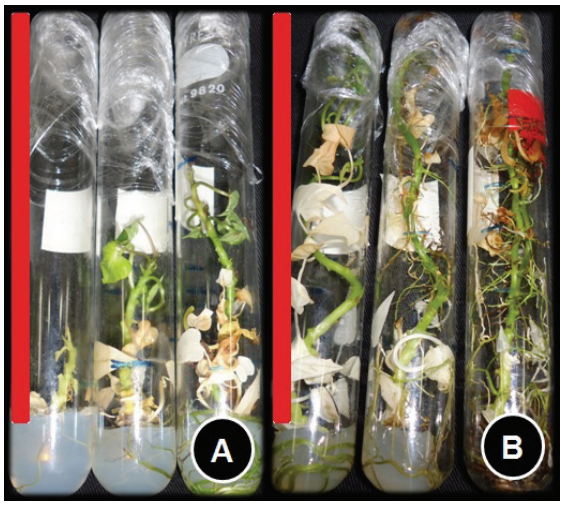


**Figure 2.** Sweet potato plants (accession UENF 1931) conserved *in vitro* after 12 months. (A) Temperature of 18±2°C and 100% MS medium with sucrose at concentrations of 1%, 2% and 3% from left to right; (B) Temperature of 27±2°C and 100% MS medium with sucrose at concentrations of 1%, 2% and 3% from left to right. Bars= 13 cm.

### *In vitro* minimal growth of sweet potato accessions

A temperature of 18±2°C resulted in the highest plant survival rate (77.06%), whereas a temperature of 27±2°C resulted in the tallest plant height (4.88 cm) and the greatest number of leaves (1.77) when the entire *in vitro* growth period was evaluated (Table 3).

**Table 3.** Mean values for height, number of leaves, and survival of 30 accessions of sweet potato conserved *in vitro* for 12 months.

| Temperature | Height (cm) | Leaf number | Survival (%) |
|---|---|---|---|
| 18±2°C | 1.54 b | 1.04 b | 77.06 a |
| 27±2°C | 4.88 a | 1.77 a | 72.40 b |

*Means followed by the same letter within a column do not differ according to the F test at the 5% probability level.

Plants of accession UENF 1937 simultaneously exhibited the greatest height and number of leaves (not differing from UENF 1928) and the highest survival rate (not differing from UENF 1947), whereas accession UENF 1988 displayed the lowest height, lowest number of leaves (not differing from UENF 1920) and lowest survival rate.

For most accessions, the greatest plant height was observed from 150 to 270 days under *in vitro* minimal growth at both temperatures. The most representative accessions at temperatures of 18±2°C and 27±2°C were UENF 1925 and UENF 1953, respectively (Figure 3).

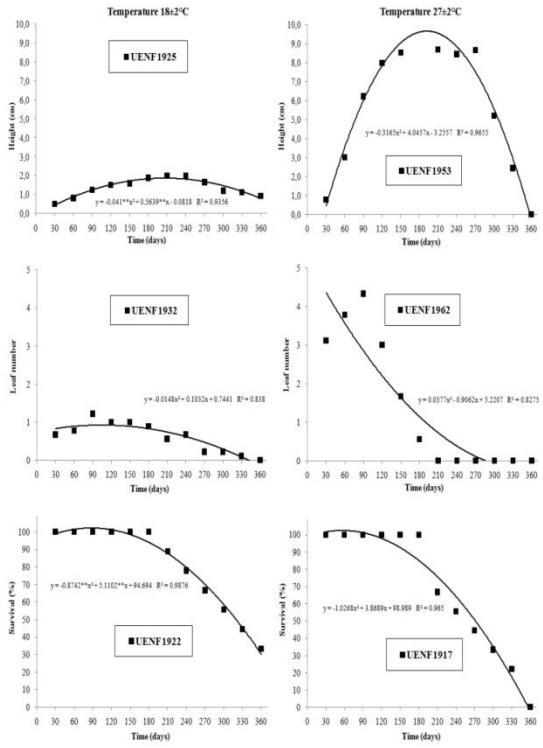


**Figure 3.** Height (cm), number of leaves, and percent survival of sweet potato plants conserved *in vitro* in 100% MS medium with 2% sucrose, at temperatures of 18±2°C (left) and 27±2°C (right) as a function of time (every 30 days).

At both temperatures, the highest number of leaves was observed at 90 days of *in vitro* conservation. Accessions UENF 1932 and UENF 1962 were the most representative at the temperatures of 18±2°C and 27±2°C, respectively (Figure 3).

The percent survival was 100% for all accessions up to 180 days. After 180 days, the percent survival of most accessions started to decline, with accessions UENF 1922 and UENF 1917 being the most representative at the temperatures of 18±2°C and 27±2°C, respectively (Figure 3).

After 360 days of *in vitro* conservation, only some accessions survived: 11 at 18±2°C (UENF 1917, UENF 1922, UENF 1925, UENF 1928, UENF 1931, UENF 1935, UENF 1937, UENF 1944, UENF 1947, UENF 1960, and UENF 1962), six at 27±2°C (UENF 1922, UENF 1928, UENF 1937, UENF 1947, UENF 1987, and UENF 1990), and these were recultured. Four accessions survived at both temperatures (UENF 1922, UENF 1928, UENF 1937, and UENF 1947).

At 90 days of reculture of the accessions that survived, only four of the 11 accessions that were maintained at 18±2°C showed survival rates greater than 50%: UENF 1917, UENF 1935, UENF 1944, and UENF 1947 (Figure 4).

Of the six accessions maintained at 27±2°C, five showed survival equal to or greater than 90%, as follows: UENF 1922, UENF 1937, UENF 1947, UENF 1987, and UENF 1990 (Figure 4).

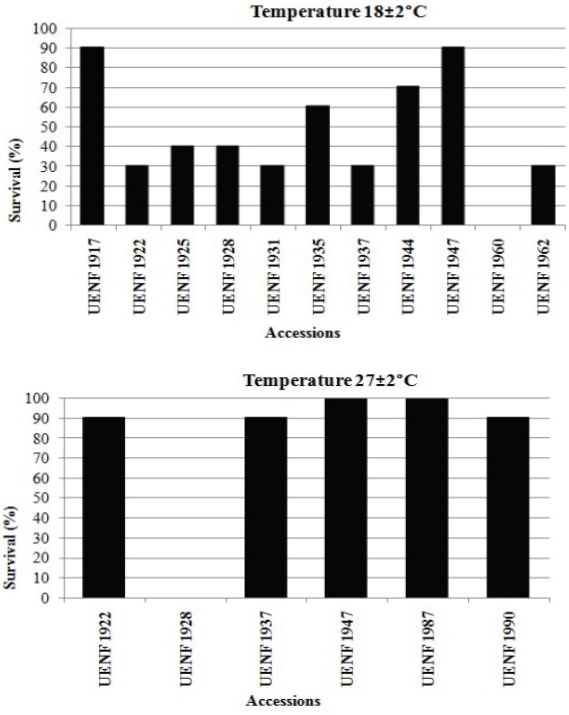


**Figure 4.** Percent survival of *in vitro* sweet potato plants 90 days after the first subculture, in 100% MS medium with 2% sucrose, at temperatures of 18±2°C (left) and 27±2°C (right).

### Plant acclimatization

The survival rate of the plants in the greenhouse was 100% for all accessions maintained at 27±2°C (Figure 5).

## Discussion

In the treatments with total suppression of mineral salts and sucrose in the growth medium, it was observed that the plants grew less, produced fewer leaves, and had the lowest survival rates irrespective of the temperature. Therefore, mineral salts and sucrose are essential components for the growth and survival of sweet potato plants *in vitro* and should not be totally suppressed in the growth medium.

The highest survival rate, the greatest height, and the highest number of leaves were observed in the treatments with MS salts at a concentration of 100%, at both temperatures (except for the treatments with complete suppression of sucrose). Other researchers working with *in vitro* conservation of sweet potato also utilized MS salts at a concentration of 100%, despite not having tested other concentrations, such as 10% and 50% (Jarret, 1997; Jarret & Gawel, 1991a; Jarret & Gawel, 1991b; Hirosse, Creste, Custódio, & Machado-Neto, 2012; among others). The reduction of the concentration of the salts in the present study did not benefit the *in vitro* conservation of the studied accessions.


**Figure 5.** Sweet potato plants originated from *in vitro* minimal growth, in a greenhouse. After 30 (A), 60 (B), 90 (C), 120 (D), 150 (E) and 180 (F) days of acclimatization.

Plants in the treatment with 100% MS medium and 2% sucrose, at both temperatures, showed the highest survival rates and the largest number of leaves. Jarret and Gawel (1991b) analyzed the effect of different levels of sucrose on the minimal growth of sweet potato. Sucrose concentrations reduced to 1.5% and 2% also resulted in higher survival rates, corroborating the results observed in the present study. However, these authors evaluated the development of plants for 90 days, unlike the period evaluated in the present study (360 days).

Although the literature cites sucrose concentrations of 2.0 to 8.0% as being effective for the minimal growth of other crops such as potato (Gopal, Chamail, & Sarkar, 2004), *Drosophyllum lusitanicum* (Gonçalves & Romano, 2007), *Elettaria cardamomum* (Tyagi et al., 2009), *Malus domestica* (Kovalchuk, Lyudvikova, Volgina, & Reed, 2009) and *Manihot esculenta* (Londe, Alves, & Ribeiro, 2012), lower concentrations in the range of 2.0% were more efficient for sweet potato.

Plants from the treatment with 100% MS medium and 2% sucrose showed a higher number of axillary buds; i.e., shorter internodes, which generates a larger number of nodes. A higher number of nodes results in a higher number of explants and consequently, greater efficiency with respect to *in vitro* minimal growth. Until now, no studies have been conducted on *in vitro* minimal growth of sweet potato that have reported shortening of plant internodes.

Some studies on the *in vitro* minimal growth of sweet potato utilized growth retardants such as abscisic acid (Arrigoni-Blank et al., 2014; Jarret & Gawel, 1991a), sorbitol, and mannitol (Jarret & Gawel, 1991b). Growth retardants were not utilized in this study, to reduce costs and to avoid somaclonal variation. Moreover, according to the obtained results, sweet potato requires mineral salts and sucrose in the growth medium for its survival *in vitro*. The reduction of these components and the use of growth retardants can result in increased mortality of sweet potato *in vitro* (Arrigoni-Blank et al., 2014).

The positive effect of the temperature decrease was not observed for the majority of the sweet potato accessions in this study after 360 days of *in vitro* growth. The temperature of 27±2°C was more efficient compared with the temperature of 18±2°C, since 90 days after the subculture of accession UENF 1931, the plants at 27±2°C showed 100% survival, whereas those maintained at 18±2°C showed 10% survival. Although Jarret and Gawel (1991b), who studied the effect of different temperatures on the minimal growth of sweet potato, reported a 50% decrease in plant growth with a temperature decrease from 21.1 to 15.6ºC, the evaluated period was only 90 days, and no subculturing was performed. In the present study, the evaluation period was 360 days, followed by another subculture, evaluated after 90 days.

The recommended temperature regimes differ from culture to culture, with some more tolerant to cold than others. Some plants can well withstand a temperature decrease, e.g., *Asparagus officinalis* (Bekheet, 2000), *Saccharum officinarum* (Lemos, Ferreira, Alencar, Neto, & Albuquerque, 2002), *Vanilla planifolia* (Divakaran et al., 2006), *Piper aduncum* and *P. hispidinervum* (Silva & Scherwinski-Pereira, 2011), and *Vitis labrusca* and *V. vinifera* (Silva, Luis, & Scherwinski-Pereira, 2012), among others. Sweet potato develops better in regions or during times when the average temperature is higher than 24ºC (Silva, Lopes, & Magalhães, 2004), and in its natural environment (*ex vitro* conditions), it does not withstand low temperatures well, maintaining this characteristic when grown *in vitro*.

Plants of accession UENF 1931 maintained at 18±2°C did not acclimatize after 360 days of *in vitro* conservation, resulting in plant death. However, the plants maintained at 27±2°C had 100% survival (data not shown). For the plants of all accessions acclimatized after 180 days of *in vitro* growth in 100% MS medium and 2% sucrose at a temperature of 27±2°C, there was 100% survival. According to Roesler, Gomes, Moro, Kummer, and Cereda (2008), sweet potato is a rustic, easily grown vegetable crop with great adaptation and high tolerance to drought. Moreover, the greenhouse temperatures (21 and 33ºC) did not vary widely in relation to the temperature at which the plants were grown *in vitro* (27±2°C), which is a factor that must have contributed to the high survival rate.

Although there were similar responses among all accessions studied, slight variations were observed among these genotypes. These different responses during the *in vitro* conservation of sweet potato may be related to the genetic traits of each accession. The results of different reactions of genotypes conserved *in vitro* were already reported in previous studies with *Passiflora giberti* (Faria, Costa, Junghans, Ledo, & Souza, 2006), *Aechmea fasciata* and *A. miniata* (Moreira, Costa, Souza, Bastos, & Rocha, 2008), among others. However, few studies have reported such differences in sweet potato (Manrique-Trujillo, Díaz, Reaño, Ghislain, & Kreuze, 2013; Arrigoni-Blank et al., 2014).

The results of the present study indicate it is possible to extend the period between subculturing of sweet potato from 90 (Teixeira & Nascimento, 1999) to 180 days, thereby reducing the working time of the curator and the amount of reagents utilized in the preparation of the growth medium, which can decrease the maintenance costs of the *in vitro* collection. The same growth medium can be utilized for all accessions because all of the accessions studied had a survival rate of 100% up to 180 days. In addition, the need for a temperature reduction may increase the maintenance costs of the collection, as this implies higher energy expenses to cool the environment, especially in tropical countries such as Brazil. For the sweet potato accessions evaluated in the present study, this temperature decrease was detrimental and is not necessary.

The main difference between this and other studies was that the *in vitro* conservation of sweet potato germplasm was evaluated for a longer time period (360 days). In addition, the survival rate after the first subculture of plants was maintained at 18±2°C and 27±2°C was presented. Although all accessions survived at both temperatures, after the subculture, the survival of explants at 18±2°C was only 10%. Thus, a temperature decrease is not recommended for the sweet potato accessions studied here.

In conclusion, it was possible to establish and maintain *in vitro* minimal growth of the sweet potato accessions collected on small farms from the northern region of Rio de Janeiro State. This methodology contributed to the reduction in the loss of genetic diversity of sweet potato in this region.

## Conclusion

The recommended *in vitro* minimal growth conditions for the studied accessions are MS medium with 100% concentration of mineral salts and 2% sucrose, at a temperature of 27±2°C, with subculturing performed every 180 days. The results obtained in this study provide a technical basis for the establishment of a new *in vitro* germplasm bank of *Ipomoea batatas*.

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