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New evidence for *in vitro* conservation of nodal segments of the passion fruit 'UENF Rio Dourado' (*Passiflora edulis* Sims)

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ABSTRACT. The germplasm of *Passiflora* L. is conserved through seed banks and field collections. Dormant seeds, seeds with low viability, and high-cost field collections make it difficult to maintain germplasm banks, and *in vitro* conservation can be a complementary alternative. The aim of this study was to investigate the survival of nodal segments of *Passiflora edulis* Sims 'UENF Rio Dourado' over 180 days of slow growth *in vitro* by reducing the mineral salt and sucrose concentrations and changing the incubation conditions (temperature and light intensity). The experiment was conducted in a completely randomized design with a 2 × 3 × 3 factorial arrangement consisting of two temperatures (20 ± 2°C and 27 ± 2°C), three concentrations of MSM mineral salts (100% MSM, 50% MSM, and 25% MSM), and three sucrose concentrations (10, 20, and 30 g L^{-1}). Evaluations were performed at 60, 90, 120, 150, and 180 days for survival, number of leaves, and plant color (using a color scale where 1 = dark green, 2 = light green, and 3 = yellow). After 180 days of culture, mineral salt, sucrose concentration, and incubation temperature affected plant survival, regeneration, and acclimatization. During the 180 days, it was possible to slow the growth of nodal segments of passion fruit 'UENF Rio Dourado' in culture medium with 25% MSM mineral salts plus 10 g L^{-1} sucrose, at an average temperature of 20°C without compromising plant survival, regeneration, and acclimatization.

Keywords: slow growth; mineral salts; sucrose; temperature; passion fruit.

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Introduction

Passiflora edulis Sims is the main species of passion fruit marketed and cultivated in Brazil, where it is present in over 90% of commercial plantations destined for the juice and fresh-fruit industries (Faleiro et al., 2019). The species is a member of the family Passifloraceae, which consists of approximately 18 genera, with *Passiflora L.* as the most abundant and diversified (Bernacci et al., 2015). In Brazil, representatives of this genus are readily found in all biomes, with 147 species that render the country an important center of diversity for this genus (Bernacci et al., 2015; Flora do Brasil, 2020).

Although *in situ* conservation is essential for the preservation of *Passiflora* species and the maintenance of genetic variability, it poses risks, such as invasion by pests and diseases, and natural disasters. Thus, *ex situ* conservation methods are indicated for the conservation of *Passiflora* germplasm (Cerqueira-Silva, Faleiro, Jesus, Santos, & Souza, 2016).

Ex situ conservation of *Passiflora* is achieved mainly through seed banks, which are a relatively low-cost method for preserving the genetic diversity of many individuals. However, some species suffer from the rapid loss of seed viability, while others produce dormant seeds (Delanoy, Van Damme, Scheldeman, & Beltran, 2006; Gurung, Swamy, Sarkar, Bhutiaand, & Bhutia, 2014; Aguacía, Miranda, & Carranza, 2015; Santos, Cruz Neto, Junghans, Jesus, & Girardi, 2016; Ghosh, Dey, Bauri, & Dey, 2017). Factors such as seed dormancy of some species of *Passiflora* (Delanoy et al., 2006) and the decreased germination of *Passiflora* seeds with increasing seed storage time (Gurung et al., 2014) hinder the maintenance of *Passiflora* species seed banks. Field collections are also used for *ex situ* conservation; however, because of the impacts of pests and diseases and climate change, passion fruit collections must be replanted every two years, thereby increasing labor and maintenance costs in the field (Cerqueira-Silva et al., 2016). Thus, strategies for the *in vitro* conservation of *Passiflora* germplasm can be used to complement other forms of conservation.

In vitro conservation, through slow growth or cryopreservation, allows for reduced labor costs by optimizing the use of physical space and facilitating the exchange of plant material to maintain collections free from the effects of climate change, pests, and diseases for medium and long durations (Engelmann, 2011; Pacheco et al., 2016).

Previous studies developed by our group indicate the possibility of the cryopreservation of *P. edulis* seeds efficiently by reducing the water content to values close to 10% (Generoso et al., 2019).

The advantage of slow growth is the maintenance of many accessions in a small physical space at reduced costs that are free from the risks found in the field (Sharma et al., 2012). In addition, germplasm is more readily available for acclimatization than that of cryopreserved material.

Although slow growth is considered a promising method for the conservation of germplasm in *Passiflora*, difficulties in maintaining seed banks and field collections relate to few available studies in the literature focused on developing protocols for passion fruit species (Faria, Costa, Junghans, Ledo, & Souza, 2006; Garcia, Pacheco, Vianna, & Mansur, 2011; Pacheco et al., 2016; Faria et al., 2017).

To investigate the slow *in vitro* growth of nodal segments of passion fruit, the following questions are presented: i) How do mineral salt, sucrose concentration, temperature, and luminosity influence the survival of shoots from nodal segments during 180 days of slow growth? ii) Is it possible to regenerate plants from this material after this period? and iii) Is it possible to obtain acclimatized plants after 180 days of slow growth?

The passion fruit 'UENF Rio Dourado' was developed by a breeding program at the State University of Northern Rio de Janeiro. The cultivar is the result of three recurrent cycles of intrapopulation selection for increased yield in the northern and northwestern regions of the state of Rio de Janeiro, as well as in other regions producing this fruit in Brazil (Viana et al., 2016).

In view of the above descriptions, this study proposes to investigate the survival of nodal segments of *Passiflora edulis* Sims 'UENF Rio Dourado' over 180 days of slow growth *in vitro* by reducing the mineral salt and sucrose concentrations and using different incubation conditions (temperature and light intensity). It also aimed to obtain acclimatized plants at the end of the process.

Material and methods

Genotypes and seed preparation

Seeds of the passion fruit *P. edulis* 'UENF Rio Dourado' were used. The seeds were removed from the fruits, washed in running water on a steel sieve to remove the aryl, and then dried at room temperature (± 25°C) for four days. Subsequently, the seed coat was removed using a bench vise. In a laminar flow cabinet, the seeds were disinfected in 70% alcohol for 30 s, placed in a 0.5% sodium hypochlorite solution (NaClO) with two drops of Tween® 20 for each 100 mL for 15 min., and finally rinsed three times with autoclaved deionized water. The embryos were then excised under a stereomicroscope (Tecnival®) and set to germinate in a culture medium with half the concentration of MSM salts (Monteiro, Higashi, Gonçalves, & Rodriguez, 2000), White's vitamins (Murashige & Skoog, 1962), 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, pH adjusted to 5.7 ± 0.1, and solidification with 6 g L^{-1} Vetec® bacteriological agar; the culture medium was autoclaved for 15 min. at 121°C and 1.1 atm (Generoso et al., 2019). Passion fruit plants with 60 days of *in vitro* growth were used as a source of nodal segments for slow growth. The explant was a nodal segment with one axillary bud.

Experimental design and culture medium

The experiment was conducted in a completely randomized design with a 2 × 3 × 3 factorial arrangement represented by two incubation temperatures (20 ± 2°C and 27 ± 2°C), three MSM mineral salt concentrations (100% MSM, 50% MSM, and 25% MSM), and three sucrose concentrations (10, 20, and 30 g L^{-1}). Evaluations were performed at 60, 90, 120, 150, and 180 days of slow growth. Each evaluation was performed in triplicate for each treatment. Each replicate consisted of 18 test tubes (25 × 150 mm) containing 10 mL of culture medium and one explant.

The composition of the growth media, according to the treatments, was as follows: three concentrations of MSM mineral salts (100%, 50%, and 25%), White's vitamins, three sucrose concentrations (10, 20, and 30 g L^{-1}), 100 mg L^{-1} myo-inositol, pH adjusted to 5.7 ± 0.1, and solidification with 6 g L^{-1} of Vetec® bacteriological agar. The culture medium was autoclaved for 15 min. at 121°C and 1.1 atm. The culture media tested were as follows: M1 = 100% MSM + 10 g L^{-1} sucrose, M2 = 100% MSM + 20 g L^{-1} sucrose, M3 = 100% MSM + 30 g L^{-1}

sucrose, M4 = 50% MSM + 10 g L^{-1} sucrose, M5 = 50% MSM + 20 g L^{-1} sucrose, M6 = 50% MSM + 30 g L^{-1} sucrose, M7 = 25% MSM + 10 g L^{-1} sucrose, M8 = 25% MSM + 20 g L^{-1} sucrose, and M9 = 25% MSM + 30 g L^{-1} sucrose.

The nodal segments of all treatments were incubated in two environments: a germination chamber at 20 ± 2°C (16h:8h light:dark photoperiod and luminous intensity of 25 µmol m^{-2} s^{-1} provided by OSRAM® daylight lamps), and a growth room at 27 ± 2°C (16:8h light:dark photoperiod and luminous intensity of 54 µmol m^{-2} s^{-1}, provided by OSRAM® daylight lamps).

Slow growth of nodal segments

The nodal segments were incubated in a germination chamber for up to 180 days at 20 ± 2°C with a luminous intensity of 25 µmol m^{-2} s^{-1}, and in a growth room at 27 ± 2°C with a luminous intensity of 54 µmol m^{-2} s^{-1}. Shoots were evaluated after 60, 90, 120, 150, and 180 days for survival, number of leaves, and plant color (using a color scale where 1= dark green, 2= light green, and 3= yellow) (Figure 1).

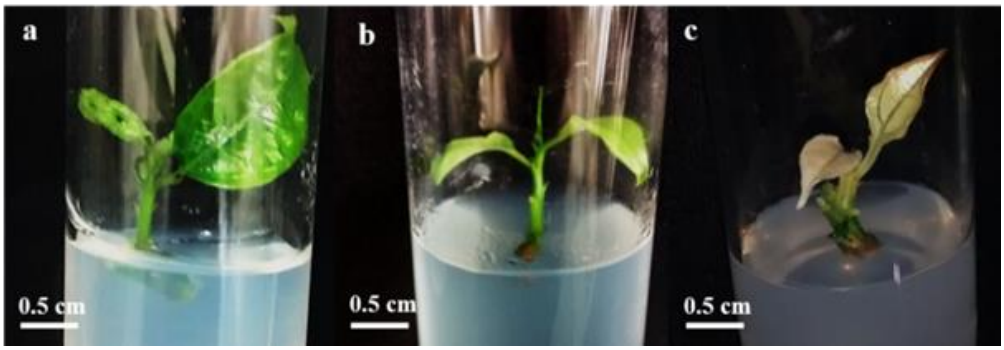

Figure 1. Color evaluation scale of shoots of *Passiflora edulis* 'UENF Rio Dourado'. a) 1 - dark green, b) 2 - light green; c) 3 - yellow.

At the end of 180 days, the surviving shoots were transferred to the regeneration medium constituted by MSM mineral salts, White's vitamins, 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, 2.89 µmol L^{-1} GA_3, 4.92 µmol L^{-1} IBA (Trevisan & Mendes, 2005), pH adjusted to 5.7, and 6 g L^{-1} of Vetec® bacteriological agar. Forty milliliters of culture medium were distributed per culture flask (125 mm × 60 mm). The flasks were autoclaved for 20 min. at 121°C and 1.1 atm. Three shoots were transferred per culture flask and kept in the regeneration medium for 30 days under the same conditions as previously described, in the germination chamber (20 ± 2°C) or the growth room (27 ± 2°C).

Acclimatization

The surviving plants were acclimatized in a greenhouse in a randomized block design with three replicates. Each plot consisted of three plants. The plants were acclimatized in a greenhouse covered with 150-µm-thick agricultural film and a 35% shade net. The plants were washed in running water to remove the culture medium from the roots and transferred to plastic trays with a capacity of 200 mL per cell, containing the commercial substrate Basaplant Hortaliças®, and irrigated twice daily. After 30 days, the plants were evaluated for survival, number of leaves, plant height, root volume, and shoot, root, and total dry matter.

Statistical analysis

The variables evaluated during *in vitro* culture (survival, number of leaves, plant color) and acclimatization (survival, number of leaves, plant height, root volume, and shoot, root, and total dry matter) were initially checked for homogeneity and normality using the Bartlett and Shapiro-Wilk tests, respectively. An analysis of variance was then performed. The variables of survival in slow growth and plant color were evaluated using regression analysis, while the number of leaves during slow growth, regeneration, and acclimatization were analyzed by Tukey's test at 5% probability. SISVAR software was used for the analyses (Ferreira, 2011).

Results

Slow growth for 180 days

There was a significant effect ($p \leqslant 0.05$) of time, salt concentration, sucrose concentration, and temperature on all analyzed variables. The survival rate of passion fruit shoots over the 180-day period

of evaluation decreased in both studied environments (Figure 2a-d). The use of the 25% concentration of MSM salts provided the highest survival means from 60 to 180 days in both environments, whereas the lowest means were observed with the use of 100% MSM (Figure 2a and b). At the end of the evaluation, the lowest survival rates were attained using 100% MSM and 50% MSM mineral salt concentrations, both in the germination chamber, with 37% and 41% respectively and in growth room, with 4% and 15% respectively of survival in comparison with the results obtained with 25% MSM (56% in the germination chamber and 41% in the growth room). There was a considerable decrease in the survival of explants cultivated in the germination chamber from 90 days of slow growth for 100% MSM and from 120 days for 25% and 50% MSM media (Figure 2a). In the growth room, the decrease in survival was from 60 days of culture for 100% MSM and 50% MSM and from 90 days for the 25% MSM medium (Figure 2b).

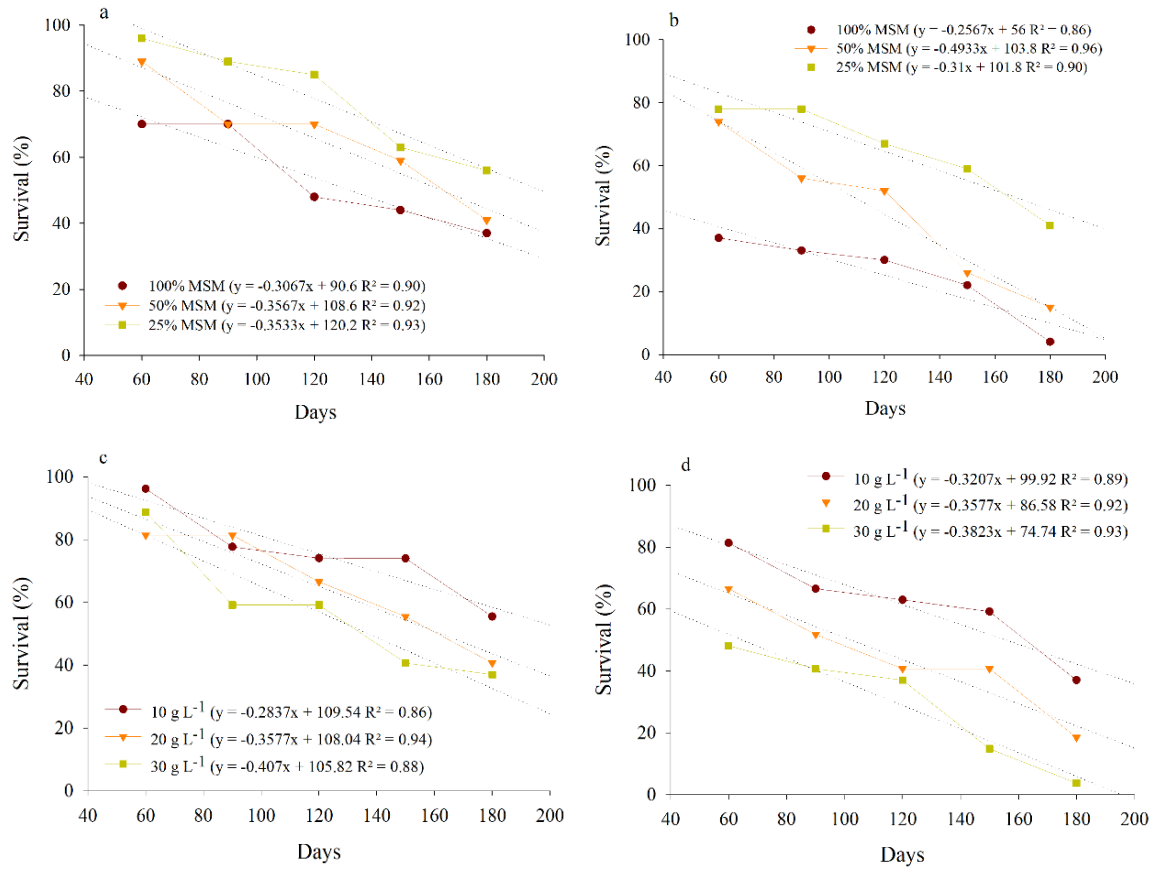

Figure 2. Survival of shoots of *Passiflora edulis* 'UENF Rio Dourado' during 60 to 180 days of *in vitro* culture. (a) Media at 100% MSM, 50% MSM, and 25% MSM incubated in a germination chamber at 20 ± 2 °C and luminous intensity of 25 µmol m^{-2} s^{-1}; (b) Media at 100% MSM, 50% MSM and 25% MSM incubated in a growth room with 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1}; (c) Sucrose concentrations of 10 , 20, and 30 g L^{-1} incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1}; and (d) Sucrose concentrations of 10, 20 and 30 g L^{-1} incubated 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1}.

The highest sucrose concentration (30 g L^{-1}) resulted in the lowest survival rates over 180 days under both conditions (Figure 2c and d). The highest survival rate at the end of 180 days was observed in media containing 10 g L^{-1} sucrose in the germination chamber. There was a considerable decrease in the survival of explants cultivated in the germination chamber from 60 days of slow growth for media with 30 g L^{-1} of sucrose, from 90 days for media with 20 g L^{-1}, and from 120 days for media with 10 g L^{-1} sucrose. In the growth room, the decrease in survival was observed after 60 days of culture for media with 10 and 20 g L^{-1} of sucrose and after 120 days for media with 30 g L^{-1} of sucrose.

For both the results of mineral salts and sucrose, the reduction in temperature was found to influence the survival percentage of the plants, with a difference of 33 to 37% between the treatments in the growth room (Figures 2 and 3). Thus, plants grown in a germination chamber had higher survival rates than those grown at 27°C in a growth room (Figure 3).

Shoot survival over 180 days under incubation in a germination chamber was higher in the culture media with 25% MSM plus 10 (M7) and 20 g L^{-1} of sucrose (M8) and 50% MSM plus 10 g L^{-1} of sucrose (M4), and lower

for the culture media with 100% MSM plus 10 (M1) and 20 g L^{-1} of sucrose (M2) and 50% MSM (M6) or 25% MSM plus 30 g L^{-1} of sucrose (M9) (Figure 4a).

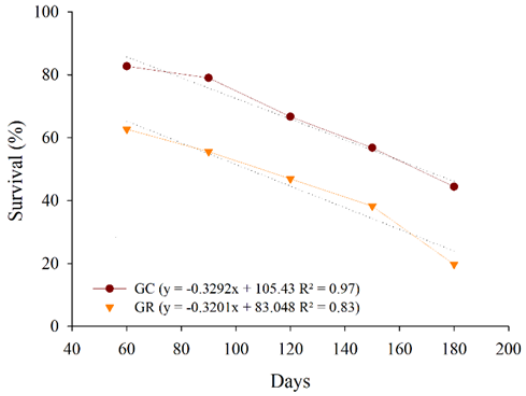

Figure 3. Survival of shoots of *Passiflora edulis* 'UENF Rio Dourado' during 60 to 180 days incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1} and in a growth room at 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1}.

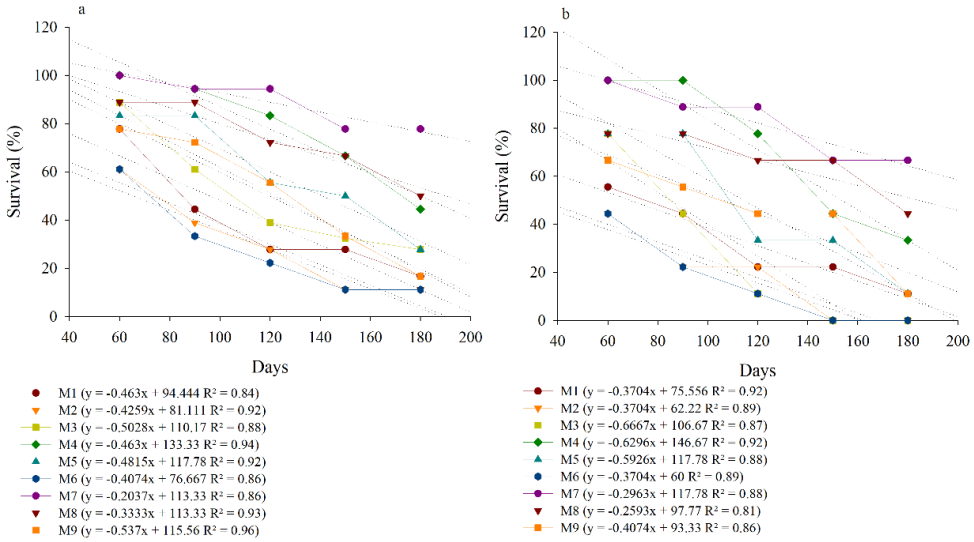

Figure 4. Survival of shoots of *Passiflora edulis* 'UENF Rio Dourado' in 100% MSM, 50% MSM, and 25% MSM media in combination with 10, 20, and 30 g L^{-1} of sucrose during the period of 60 to 180 days incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1} (a) and in a growth room at 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1} (b). M1 = 100% MSM + 10 g L^{-1} sucrose; M2 = 100% MSM + 20 g L^{-1} sucrose; M3 = 100% MSM + 30 g L^{-1} sucrose; M4 = 50% MSM + 10 g L^{-1} sucrose; M5 = 50% MSM + 20 g L^{-1} sucrose; M6 = 50% MSM + 30 g L^{-1} sucrose; M7 = 25% MSM + 10 g L^{-1} sucrose; M8 = 25% MSM + 20 g L^{-1} sucrose; and M9 = 25% MSM + 30 g L^{-1} sucrose.

Between 90 and 150 days, there was an increase in the number of leaves, which was followed by a decline in the last evaluation at 180 days (Table 1). A difference between the mineral salts was observed only in the growth room. In addition, temperature affected the number of leaves from 150 days in the media with higher mineral salt concentrations (100% MSM and 50% MSM) (Table 1).

Table 1. Mean number of leaves per shoot of *Passiflora edulis* 'UENF Rio Dourado' during 60 to 180 days of *in vitro* slow growth in 100% MSM, 50% MSM and 25% MSM media incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1} and in a growth room at 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1}.

	MSM	Days of *in vitro* slow growth				
		60	90	120	150	180
GC	100% MSM	1.3 ± 1.1^{Baa}	3.3 ± 1.8^{Aaa}	2.9 ± 2.8^{ABaa}	2.5 ± 2.1^{ABaa}	1.7 ± 1.6^{ABaa}
	50% MSM	1.1 ± 0.8^{Baa}	3.1 ± 1.5^{Aaa}	2.6 ± 1.7^{ABaa}	2.5 ± 1.7^{ABaa}	1.7 ± 1.9^{ABaa}
	25% MSM	1.7 ± 1.2^{Baa}	4.1 ± 1.0^{Aaa}	3.3 ± 1.6^{ABaa}	2.5 ± 1.6^{ABaa}	2.0 ± 1.5^{Baa}
GR	100% MSM	0.5 ± 0.6^{Aaa}	1.5 ± 1.1^{Aab}	0.9 ± 1.0^{Aab}	0.9 ± 1.1^{Abb}	0.2 ± 0.5^{Aab}
	50% MSM	1.0 ± 0.7^{Baa}	3.0 ± 1.8^{Aaa}	2.0 ± 1.4^{ABaa}	0.8 ± 1.0^{Bbb}	0.4 ± 0.8^{Bab}
	25% MSM	0.5 ± 0.4^{Caa}	2.6 ± 1.4^{ABab}	1.8 ± 1.2^{BCab}	4.2 ± 1.9^{Aab}	1.3 ± 1.2^{BCaa}

Means followed by the same letters do not differ from each other (Tukey's test, $p \leq 0.05$). Upper-case letters refer to the effects of temperature and mineral salts over time (in the row). Lower-case letters refer to the effect of time on each temperature (in the column; see temperatures separately). Underlined lower-case letters refer to the effect of mineral salts and time on each temperature (in the column; see minerals at each temperature). (GC) Germination chamber. (GR) Growth room.

Analysis of the interaction between mineral salts and sucrose revealed a decrease in the number of leaves in the media containing 50% MSM and 25% MSM salts in combination with 30 g L^{-1} sucrose (Table 2). After 150 days of slow growth, many plants began to lose their leaves because of the natural process of leaf abscission. With 10 g L^{-1} of sucrose, the highest number of leaves was observed in media with 50% and 25% MSM, whereas with 20 and 30 g L^{-1} of sucrose, the highest number was obtained with 100% MSM salts (Table 2).

Table 2. Mean number of leaves per shoot of *Passiflora edulis* 'UENF Rio Dourado' in 100% MSM, 50% MSM, and 25% MSM media in combination with 10, 20, and 30 g L^{-1} of sucrose.

MSM	Sucrose (g L^{-1})		
	10	20	30
100% MSM	1.6 ± 2.2^{Ab}	1.2 ± 1.3^{Ab}	1.9 ± 1.8^{Aa}
50% MSM	2.5 ± 1.6^{Aa}	2.0 ± 1.7^{Aab}	0.9 ± 1.2^{Bb}
25% MSM	2.9 ± 1.7^{Aa}	2.5 ± 1.8^{Aa}	1.6 ± 1.6^{Bab}

Means followed by the same letters do not differ from each other (Tukey's test, $p \leq 0.05$). Uppercase letters refer to the effect of sucrose (in the row), and lowercase letters refer to the effect of mineral salts (in the column).

Plant color differed across the tested incubation conditions for both salts and sucrose. At the beginning of the evaluation, the plants grown in the three types of media in a germination chamber showed a dark green color, which changed to light green after 90 and 120 days in the 100% MSM, 50% MSM, and 25% MSM salts (Figure 5a). In the case of shoots grown in a growth room, the color change to light green occurred before 90 days. At 120 days, shoots grown in 100% MSM were yellow and remained so until the end of the evaluation. At 180 days, shoots grown in 50% MSM also showed a yellow color, whereas those in 25% MSM were light green (Figure 5b).

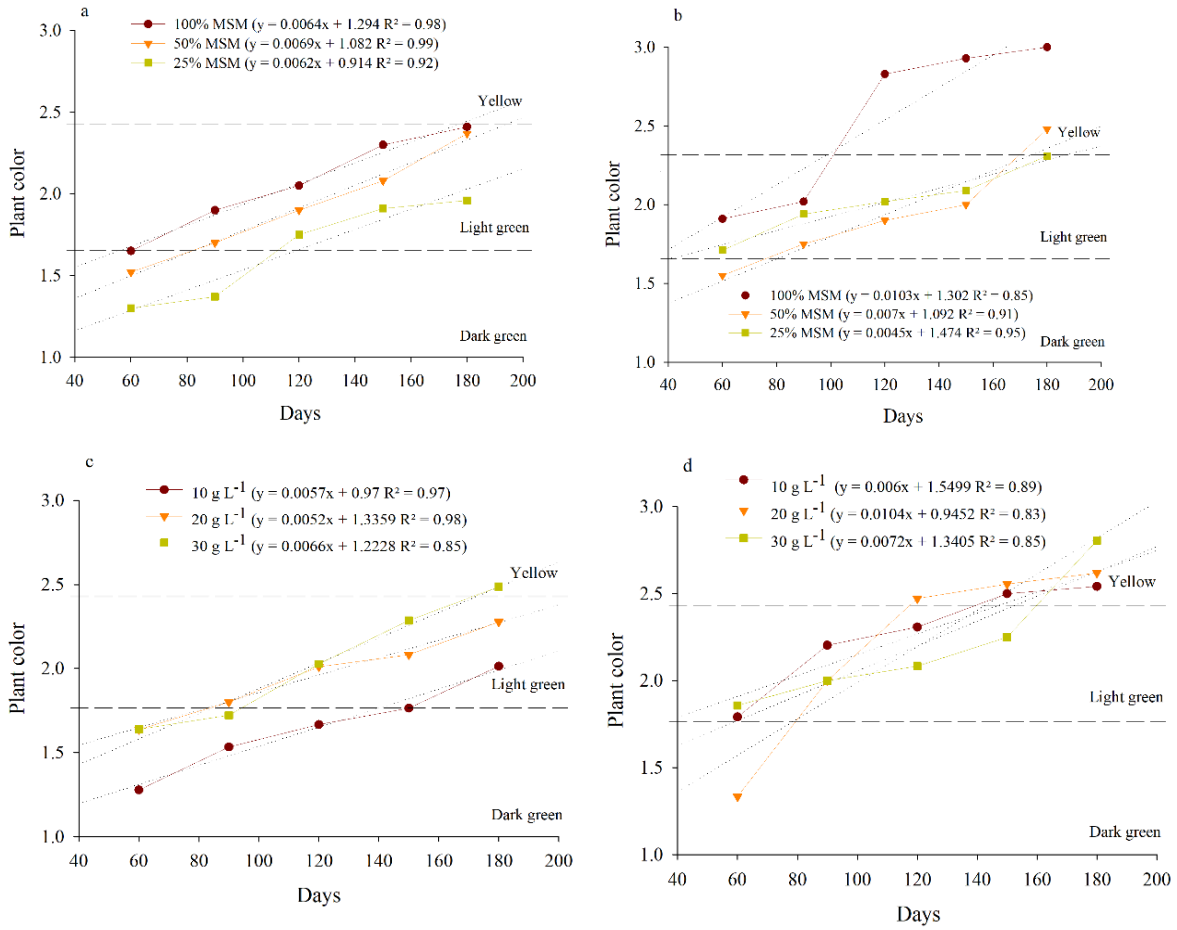

Figure 5. Shoot color of *Passiflora edulis* 'UENF Rio Dourado' during 60 to 180 days of *in vitro* culture: (a) 100% MSM, 50% MSM, and 25% MSM media incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1}; (b) 100% MSM, 50% MSM, and 25% MSM media incubated in a growth room at 27 ± 2 °C and luminous intensity of 54 µmol m^{-2} s^{-1}; (c) Sucrose concentrations of 10, 20 and 30 g L^{-1} incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1}; (d) Sucrose concentrations of 10, 20 and 30 g L^{-1} incubated at 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1}.

Among the sucrose concentrations, the shoots grown in a germination chamber showed a dark green color (Figure 5c), and those in media containing 10 and 20 g L^{-1} sucrose revealed a light green color at the end of the evaluation period. At a sucrose concentration of 30 g L^{-1}, the shoots turned yellow (Figure 5c). Shoots grown in a growth room and in media with 20 g L^{-1} of sucrose at 60 days were dark green, whereas a light green color was observed in shoots in media with 10 and 30 g L^{-1} of sucrose. At the end of 180 days, all shoots were yellow (Figure 5d).

The original medium of the nodal segments influenced survival after 30 days in the regeneration medium. The highest survival means were observed in media with 50% and 25% MSM supplemented with 10 and 20 g L^{-1} of sucrose (Table 3). In addition, the shoots that were incubated in a germination chamber had a higher survival rate than those grown in a growth room (Figure 6).

Table 3. Mean survival of shoots (%) of *Passiflora edulis* 'UENF Rio Dourado' in regeneration medium for 30 days, after a period of 60 to 180 days in 100% MSM, 50% MSM, and 25% MSM media in combination with 10, 20, and 30 g L^{-1} of sucrose.

MSM	Sucrose (g L^{-1})		
	10	20	30
100% MSM	28.8 ± 13.7^{Ab}	18.8 ± 9.7^{Bb}	21.6 ± 22.3^{ABb}
50% MSM	62.5 ± 12.2^{Aa}	47.7 ± 18.6^{Aa}	44.4 ± 13.6^{Ba}
25% MSM	70.0 ± 16.8^{Aa}	58.1 ± 13.9^{Aa}	36.6 ± 9.7^{Bab}

Means followed by the same letters do not differ from each other (Tukey's test, $p \leq 0.05$). Uppercase letters refer to the effect of sucrose (in the row), and lowercase letters refer to the effect of mineral salts (in the column).

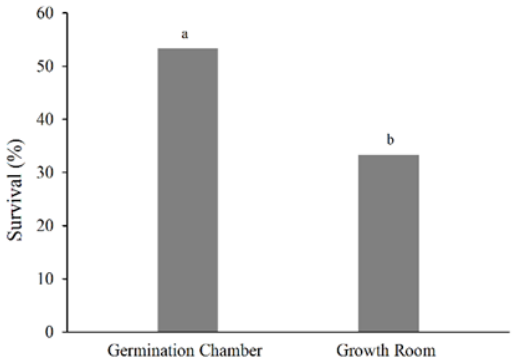

Figure 6. Mean survival of shoots of *Passiflora edulis* 'UENF Rio Dourado' in regeneration medium for 30 days after 60 to 180 days incubated in a germination chamber at 20 ± 2°C and luminous intensity of 25 µmol m^{-2} s^{-1} and in a growth room at 27 ± 2°C and luminous intensity of 54 µmol m^{-2} s^{-1}. Means followed by the same letters did not differ (Tukey's test, $p \leq 0.05$).

Acclimatization

Not all treatments generated plants sufficiently vigorous to acclimatize over 180 days of evaluation. There were a greater number of acclimatized plants from the culture in the germination chamber than from that in the growth room. Among the acclimatized plants, no significant differences were observed for the variables analyzed at the end of acclimatization (Table 4).

Eleven treatments were used for acclimatization after 180 days of slow growth. Among them, eight were kept in a germination chamber (100% MSM + 10, 20, and 30 g L^{-1} sucrose; 50% MSM + 10, 20, and 30 g L^{-1} sucrose, and 25% MSM + 10 and 20 g L^{-1} sucrose), and three were maintained in a growth room (25% MSM + 10, 20, and 30 g L^{-1} sucrose). No differences were found in the variables analyzed after 30 days of acclimatization in the surviving plants (Tables 4 and 5).

Table 4. Summary of analysis of variance for the variables of plant height (PH), number of leaves (NL), root volume (RV), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) analyzed after 30 days of acclimatization of plants of *Passiflora edulis* 'UENF Rio Dourado' after slow growth for 180 days.

SV	DF	PH (mm)	NL	RV (mL)	SDM (g)	RDM (g)	TDM (g)
Treatment	10	281.26^{ns}	7.755^{ns}	0.4047^{ns}	0.0126^{ns}	0.0036^{ns}	0.0294^{ns}
Block	2	1495.89^{ns}	7.949^{ns}	0.4777^{ns}	0.0016^{ns}	0.0044^{ns}	0.0365^{ns}
Residual	61	459.73	3.106	0.6110	0.0015	0.0048	0.0378
Mean		47.53	6.66	0.80	0.1192	0.0595	0.1787
CV (%)		45.10	26.45	48.07	51.92	59.30	53.46

(SV) Sources of variation. (ns) Non-significant difference, (CV) Coefficient of variation.

Table 5. Means of the variables of plant height (PH), number of leaves (NL), root volume (RV), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) analyzed after 30 days of acclimatization of plants of *Passiflora edulis* 'UENF Rio Dourado' after slow growth for 180 days.

	MSM	Sucrose (g L^{-1})	PH (mm)	NL	RV (mL)	SDM (g)	RDM (g)	TDM (g)
GC	100% MSM	10	40.2	6.3	0.53	0.074	0.039	0.112
		20	38.0	5.5	0.55	0.068	0.037	0.105
		30	45.1	6.4	0.82	0.096	0.056	0.152
	50% MSM	10	46.7	7.0	0.51	0.093	0.037	0.130
		20	49.7	5.6	0.74	0.121	0.056	0.177
		30	47.4	5.7	0.83	0.145	0.061	0.206
	25% MSM	10	49.4	6.6	0.90	0.138	0.067	0.204
		20	51.2	7.0	1.01	0.133	0.068	0.201
GR	25% MSM	10	48.2	6.2	1.01	0.157	0.081	0.238
		20	51.7	6.5	1.08	0.123	0.107	0.230
		30	43.8	5.0	0.58	0.105	0.081	0.186

(GC) Germination chamber. (GR) Growth room.

Discussion

Few studies exist in the literature on the slow growth of *Passiflora* species. Higher survival percentages were observed with the use of the 25% MSM concentration, and lower values were obtained following the use of 100% MSM salts over the 180 days of evaluation under both incubation conditions (Figures 2 and 4). The reduction in salt concentration to 25% MSM was also shown to be efficient in the slow growth of nodal segments of *P. suberosa*, with 100% survival achieved after six months and 83% after one year of growth (Garcia et al., 2011). Reductions in mineral salt concentrations in the culture medium have been widely studied in the slow growth of several other fruit species, such as pineapple (Silva et al., 2016), citrus (Gorshkov et al., 2019), and grape (Tehrim & Sajid, 2011).

The incubation conditions and the concentration of salts and sucrose influenced the speed of yellowing of the shoots, since shoots in MSM media grown in a growth room were yellow at the end of the evaluation, while those incubated in a germination chamber were light green at 180 days (Figure 5). Similarly, shoots grown in a germination chamber with 30 g L^{-1} sucrose and in a growth room at the three sucrose concentrations ended the evaluation with a yellow color.

The survival of passion fruit shoots was also influenced by the amount of sucrose added to the culture medium, with the highest concentration used (30 g L^{-1}) providing the lowest survival means and the lowest concentration (10 g L^{-1}) generating the best results over 180 days of slow growth (Figures 2 and 4). Sucrose modifies the osmolarity of the culture medium; therefore, a greater amount could interfere with the absorption of mineral salts and vitamins available to plants. When added to the culture medium, sucrose significantly affects plant growth *in vitro*, acting both as an energy source and as an osmotic regulator, and depending on the concentration, it can remove excess intracellular water using an osmotic gradient, causing slower growth of the crop (Shibli, Shatnawi, Subaih, Viera, & Ajlouni, 2006).

These combinations of light and temperature also interfered with the survival of passion fruit shoots. The low temperature (20°C) and low light intensity (25 µmol m^{-2} s^{-1}) induced higher survival means during the 180 days of slow growth (Figures 3, 4). Reducing the incubation temperature and light intensity is a strategy used for tropical and subtropical species, which produces a significant decrease in plant metabolism (Normah, Chin, & Reed, 2013; Trejgell, Kamin´ska, & Tretyn, 2015). Light intensity can have an effect on *in vitro* plant growth with significant changes in thylakoid arrangements of root length, leaf stomata count, and chlorophyll content (Macedo, Leal-Costa, Tavares, Lage, & Esquibel, 2011; Mulata, Bordignon, Rossi, Ambrosano, & Rodrigues, 2013). Withers (1985) suggested reducing the temperature for tropical climate species to between 15°C and 25°C for slow growth *in vitro*. The reduction in cultivation temperature can lower plant metabolism through changes in the content and action of enzymes and in the functioning of cell membranes (Lemos, Ferreira, Alencar, Neto, & Albuquerque, 2002; Lédo, Cunha, Aragão, & Tupinambá, 2007).

During slow growth, there was an increase in the number of leaves between 90 and 150 days, followed by a reduction in the last evaluation at 180 days (Table 1), mainly for the 100% MSM and 50% MSM media. This is because after evaluation at 150 days of slow growth, many plants began to lose their leaves through the natural process of leaf abscission. In addition, passion fruit plants are known to produce higher concentrations of ethylene when grown *in vitro* (Trevisan & Mendes, 2005; Faria et al., 2017). Ethylene is a

gaseous hormone that can accumulate inside flasks under *in vitro* culture conditions, causing leaf abscission and accelerating plant senescence (Nepomuceno et al., 2007).

Incubation conditions are one of the main factors to consider when optimizing conservation factors by slow growth, since each species has an ideal growing temperature and light intensity. In general, tropical and subtropical plants survive if they are kept at temperatures between 15 and 20°C (Yaacob et al., 2014). The results obtained in this study for passion fruit showed that low temperature (20°C) and low light intensity (25 µmol m^{-2} s^{-1}) were satisfactory and did not compromise the survival of plants conserved for 180 days *in vitro*, thereby ensuring that numerous shoots regenerated (Figure 6) into new plants, allowing higher survival in the acclimatization stage in a greenhouse (Tables 4 and 5). The results of shoot survival after 180 days of slow growth were equivalent to those observed in the *in vitro* regeneration of shoots and in the acclimatization of plants in a greenhouse (Figure 6; Tables 3 and 5).

There are various advantages to establishing an *in vitro* germplasm collection, such as maintaining a large number of accessions in a small physical space that is free of the hazards that occur in the field, and the ability to exchange germplasm safely (Silva et al., 2016). It is possible to maintain a germplasm collection of *P. edulis* in slow growth, allowing the expansion of investigations to evaluate the effects of temperature, mineral salts, and sucrose on other species of the genus *Passiflora*.

Conclusion

After 180 days, it was possible to slow the growth of nodal segments of *P. edulis* Sims 'UENF Rio Dourado' in culture medium with 25% MSM mineral salts plus 10 g L^{-1} sucrose, at an average temperature of 20°C and luminous intensity of 25 µmol m^{-2} s^{-1} without compromising plant survival, regeneration, and acclimatization.

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