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Assessment of minimum growth conditions for *in vitro* conservation of *Passiflora edulis* Sims

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***Passiflora edulis* Sims, besides having economic and social importance, is included in the medicinal plant list that is considered of interest by the Unified Health System (Sistema Único de Saúde – SUS). Due to its high nutritional value, this species is widely used in food, cosmetics and pharmaceutical industries. It also contains carotenoids, as well as passiflorine and maracugine, which have sedative effects. The aim of this research was to assess *P. edulis* minimum growth conditions in different concentrations and combinations of MS culture medium and sucrose for *in vitro* conservation. The following variables were analyzed: Plant height; number of green and senescent leaves; and number of roots. Root height means were highest in MS media containing 30 g L^{-1} sucrose (8.33). With regard to plant height, the best results were obtained in MS/4 media with 15 g L^{-1} or 30 g L^{-1} sucrose, which enabled the maintenance of lower plants (5.35 and 4.91, respectively) with green leaves after 120 days *in vitro* culture. The MS/2 media supplemented with 15 g L^{-1} sucrose was the best alternative for *in vitro* maintenance of plants with appropriate height and number of green leaves (8.22 and 3.86, respectively). Thus, we may conclude that MS/2 with 15 g L^{-1} sucrose represents an efficient strategy for *in vitro* conservation of *P. edulis* plants for a culture period of 120 days and 100% survival after acclimatization.**

Key words: Passion fruit, medicinal plants, *in vitro* culture, germplasm conservation.

INTRODUCTION

The most economically relevant Passifloraceae genus in Brazil is *Passiflora* (Bernacci, 2003). *Passiflora edulis*, *Passiflora alata* and *Passiflora incarnata* are the main species cultivated in the country (Morgani, 2007). Of those, *P. edulis* has the largest commercial production volume due to the quality of its fruits, vigor, productivity and juice yield (Meletti and Bruckner, 2001).

P. edulis is registered in the simplified list of herbal plants published by ANVISA in 2014 (ANVISA, 2014).

This inclusion added to the fact that SUS (Unified Health System) supports of the use of this medicinal plant may increase the interest of the pharmaceutical industry.

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Thus, further research on *in vitro* culture techniques for conservation of plants with adequate genetic and phytosanitary properties is of importance.

Its economic and social importance, and the fact that it is part of the medicinal plant list that is considered of interest by the Unified Health System (SUS) justifies the development of new conservation strategies for *P. edulis,* whose preservation is carried out on the field, where biotic and abiotic factors might increase the vulnerability of the accessions. Thus, *in vitro* conservation of *P. edulis* appears as an efficient preservation strategy due to its good results in terms of tissue culture, which enables the conservation of pathogen-free vegetal material in a controlled and accessible location, so that it can be easily used for multiplication in genetic improvement research (Keller et al., 2013; Cardoso, 2014; Maldaner, 2014).

Traditionally, *P. edulis* propagation is carried out by means of seeds, which is a method that promotes genetic variability and does not ensure the conservation of valuable characteristics of the mother plant, such as high growth ratios and resistance to pests and diseases. To ensure that the characteristics of the mother plant are present in the next generations, micropropagation associated with *in vitro* conservation is the adequate choice, since it allows for large-scale clone production and conservation of characteristics of commercial interest (Contijo et al., 2004; Chiancone and Germanà, 2013).

The main advantages of *in vitro* conservation include avoidance of biotic and abiotic stress, material availability for propagation whenever needed, reduced physical space requirements and easy access for scientists to develop innovative studies on chemical compounds (Cid, 2010).

According to Lemos (2002), *in vitro* genotype conservation in a slow growth system allows the reduction of the number of subcultures due to reduced plant metabolism. Therefore, the assessment of minimum growth conditions for *in vitro* conservation protocols such as culture media and sucrose concentrations have been widely evaluated in various plant species (Cis LPB, 2014). Among the most popular culture media, the MS medium (Murashige and Skoog, 1962) is widespread in tissue culture research and has been used in *in vitro* culture of many species in specific concentrations for each of them. In a study developed by Silva et al. (2020) with *Alcantarea nahoumii* (Bromeliaceae), for example, plants grown in MS medium with half the salt concentration had the lowest growth. According to the authors, this fact indicates a metabolism reduction useful for *in vitro* conservation.

Sucrose, a non-reducible disaccharide, is universally used as a carbon source for explants kept *in vitro*. Its concentration is decisive for *in vitro* plant growth, and different sucrose concentrations in the culture medium may significantly alter the metabolism of plants (Ankita and Animesh, 2013).

The reduction of consecutive subcultures and the development of an appropriate methodology for *in vitro* conservation is mandatory to reduce labor costs and to avoid problems that might arise when trying to preserve genetic material in the field (Lédo et al., 2014).

Thus, the objective of this research was to assess *P. edulis* minimum growth conditions by using different concentrations and combinations of MS medium and sucrose for *in vitro* conservation.

METHODOLOGY

The experiments were conducted at the Faculdade Maria Milza's Laboratory of Biotechnology Applied to Health in the municipality of Governador Mangabeira, Bahia.

Vegetal material

P. edulis Sims plants, purchased from Embrapa Mandioca e Fruticultura, located in Cruz das Almas, Bahia, were used as explant source. Initially, leaf disks (Figure 1A), were immersed in 70% alcohol for 3 min, followed by a 3-min immersion in a solution of commercial sodium hypochlorite containing 2.5% of active chlorine and water (1:1), and a threefold rinse in a laminar flow cabinet with autoclaved distilled water. The explants were cultivated in Petri dishes with MS culture medium (Murashige and Skoog, 1962), supplemented with 1.0 mg L^{-1} of 6-benzilaminopurine (BAP), 30 g L^{-1} sucrose, solidified with 7 g L^{-1} agar and pH adjusted to 5.8. The explants were maintained at 25 ± 2°C for 3 days, under a 16 h photoperiod and 40 µM m^{-2} s^{-1} light intensity. Shoots (Figure 1B) grown from leaf disks were cultured *in vitro* (Figure 1C) during two subcultures in the same culture media described earlier, without growth regulator BAP, for plant generation (Figure 1D and E). Those plants provided the microcuttings that were used for *in vitro* conservation (Figure 1G).

Assessment of minimum growth conditions

P. edulis 1.0 cm microcuttings (Figure 1G) obtained from *in vitro* cultivated plants were inoculated in test tubes containing different concentrations (MS, MS/2 and MS/4) of MS culture medium and sucrose (0, 15 and 30 g L^{-1}) solidified with 7 g L^{-1} de agar, and pH adjusted to 5.8.

The explants were cultivated in a growth chamber under controlled temperature, photoperiod and light intensity. Evaluation periods were 30, 60, 90 and 120 culture days and the following traits were assessed: plant height (PH); number of green leaves (NGL); number of senescent leaves (NSL); and number of roots (NR).

Plants obtained from the selected treatment in the *in vitro* conservation phase were subcultivated for two subcultures in MS culture medium supplemented with 30 g L^{-1} sucrose at a 45 day interval each. Later, plants obtained from the selected treatments for *in vitro* conservation under minimum growth conditions were acclimatized in polyethylene terephthalate (PET) bottles (Figure 2A) filled with autoclaved vegetal soil. The bottle lids were progressively removed (Figure 2B) for 10 min on the first acclimatization day, for 20 min on the second day and so on until the complete removal of the upper half of the bottle (Figure 2C and D) according to the methodology used by Vicente et al. (2009) and Almeida et al. (2020). During acclimatization, the plants were maintained in a

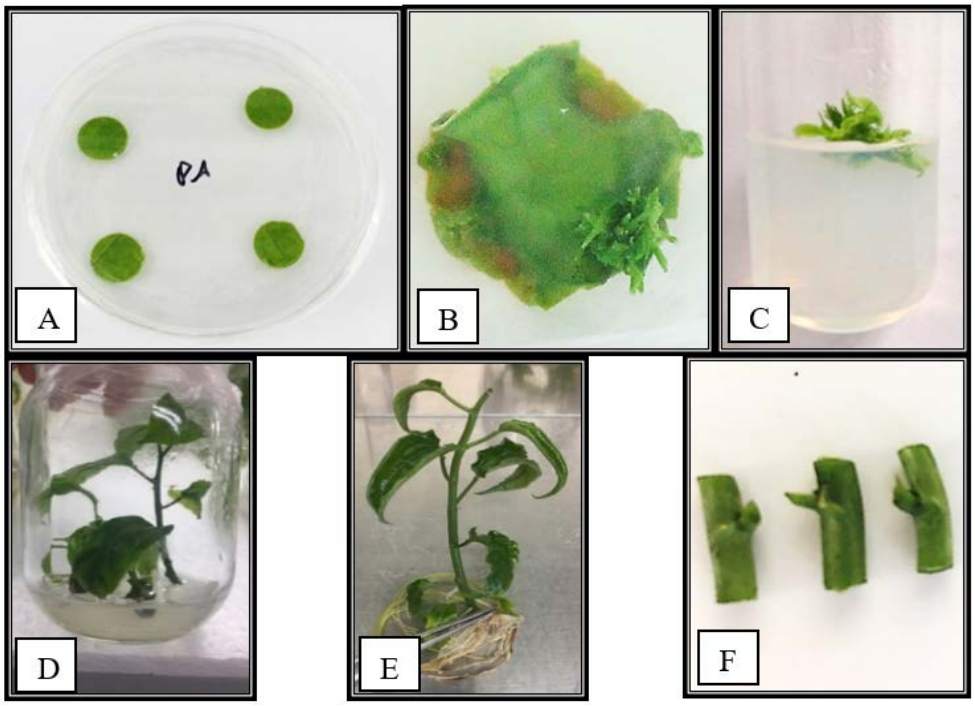

Figure 1. Leaf disks of Passiflora edulis Sims used as explant (A); shoots (B) subcultures (C); in vitro grown plants (D, E); microcuttings (F).
Source: own authorship (2019).

covered area (70% light reduction) and watered with a sprayer. Survival percentage was assessed every 30 days of acclimatization.

Statistical analysis

In this research, a completely randomized design was used. Factorial time plot experiments (3 × 3 × 4) were carried out with three concentrations of MS culture media (MS, MS/2 and MS/4), three sucrose concentrations (0, 15 and 30 g L^{-1}) and four evaluation periods (30, 60, 90 and 120 days).

Fifteen repetitions per treatment were used, and the experimental unit was an explant per test tube. Statistical analysis included ANOVA, and the means related to MS culture media and sucrose concentrations were compared through the Tukey test at 5% probability. Polynomial regression models were adjusted for mean comparison at different evaluation periods. The variables NGL, NSL and NR were transformed according to $\sqrt{x+0.5}$ in order to fulfill ANOVA requirements. Statistical analysis was carried out with the software SAS 9.3 – Statistical Analysis System (SAS Institute, 2004).

During acclimatization, the plants were maintained in autoclaved vegetal soil and the culture conditions were identical for all the plants that originated from the same experimental combination when assessing minimum growth conditions.

RESULTS AND DISCUSSION

According to the variance analysis (Table 1), the isolated effect of different sucrose concentrations and evaluation periods, as well as the combination of evaluation period and sucrose were statistically significant for all the variables. On the other hand, the isolated effect of MS culture media concentration was significant for the variables plant height and number of green leaves as was also the interaction between culture media concentration and sucrose concentration. The interaction between evaluation period and culture medium concentration was not significant for number of green and senescent leaves. However, the interaction among evaluation period, culture medium concentration and sucrose concentration was not significant only for the trait number of green leaves. Besides, as can be observed in Table 1, the coefficients of variation varied between 21.34 and 30.59%, which are similar to the values observed in *in vitro* conservation studies (Jesus et al., 2011).

In terms of pH (Table 2), the lowest means were obtained in *in vitro* grown plants in culture media without sucrose, independently of evaluation period. No significant differences were observed between culture medium concentrations and absence of sucrose. However, many plants in culture media without sucrose grew without leaves, which reduced their survival in the subculture after *in vitro* conservation (Figure 3). Thus, MS/4 with 15 g L^{-1} or 30 g L^{-1} sucrose allows the maintenance of shorter plants with green leaves (Table

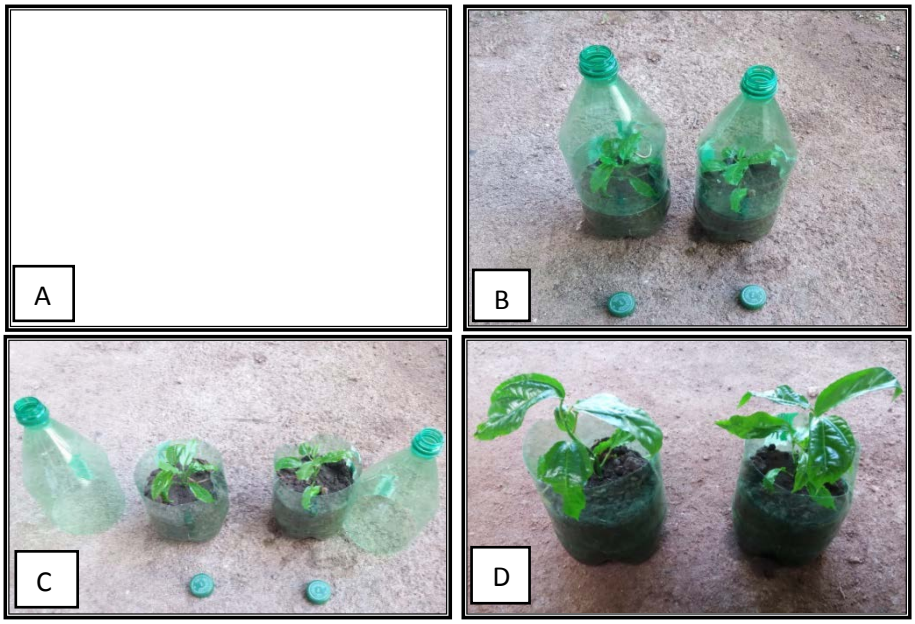

Figure 2. *P. edulis* acclimatized in plastic bottles with autoclaved vegetal soil (A); acclimatization process with lid removal (B); complete removal of the upper half of the bottle (C and D).
Source: own authorship (2019).

Table 1. Variance analysis for plant height (PH) in cm, number of green leaves (NGL), number of senescent leaves (NSL), and number of roots (NR) of *P. edulis* plants grown *in vitro* in different concentrations of MS culture medium (1/1, 1/2 and 1/4) and of sucrose (0, 15 and 30 g L^{-1}) during 30, 60, 90 and 120 days.

Variation source	Mean square				
	DF	PH	NGL	NSL	NR
Medium MS	2	273.10**	21.06**	0.47^{ns}	240^{ns}
Sucrose (Sac)	2	1179.89**	65.65**	8.63**	62.89**
Medium × Sac	4	73.08**	2.03*	0.33^{ns}	1.03^{ns}
Error 1	125	10.44	0.61	0.18	0.81
Time	3	504.88*	5.27**	6.45**	6.20**
Time × Medium	6	16.04**	0.13^{ns}	0.08^{ns}	0.86**
Time × Sac	6	91.61**	0.76**	1.74**	1.65**
Time × medium × Sac	12	5.80**	0.17^{ns}	0.15**	0.30**
Error 2	375	1.46	0.12	0.06	0.11
CV (%)	-	30.59	21.34	26.39	23.16
General mean	-	3.94	2.69	0.60	2.12

**, *Significant at 1% and 5% probability, respectively, according to the F test; ns not significant at 5% de probability. Source: Research Data (2019).

2 and Figure 4).

Similar results were obtained by Galdiano et al., 2013. who reported that the absence of sucrose resulted in the reduction of *Cattleya loddigesii* Lindley height. When the culture medium was supplemented with 21.5 g L^{-1} (2006) sucrose, the mean plant height increased. Faria et al. (2006) analyzed the effect of sucrose and sorbitol on the *in vitro* culture of *Passiflora gibert* N. E. Brown and observed that the development and growth of microplants were affected by sugars. When grown without sucrose but with 20 g L^{-1} or 40 g L^{-1} sorbitol, plant height was lower. The highest values were obtained in media

Table 2. Mean plant height (cm) of *P. edulis* plants grown in different MS culture medium (1/1, 1/2 and 1/4) and sucrose concentrations (0, 15 and 30g L^{-1}) during 30, 60, 90 and 120 days.

MS medium concentration	Sucrose concentraions (g L^{-1})		
	0	15	30
30 days			
1/1	0.74^{aB}	1.98^{aAB}	2.50^{aA}
1/2	0.57^{aB}	1.89^{aA}	2.17^{aA}
1/4	0.67^{aA}	0.98^{aA}	0.73^{bA}
60 days			
1/1	0.99^{aB}	5.71^{aA}	6.54^{aA}
1/2	0.68^{aB}	5.31^{aA}	6.01^{aA}
1/4	0.79^{aB}	2.68^{bA}	3.56^{bA}
90 days			
1/1	1.25^{aC}	8.58^{aB}	10.61^{aA}
1/2	1.20^{aB}	7.35^{aA}	7.69^{bA}
1/4	1.23^{aB}	4.73^{bA}	4.53^{cA}
120 days			
1/1	1.44^{aC}	9.30^{aB}	11.16^{aA}
1/2	1.20^{aB}	8.22^{aA}	7.85^{bA}
1/4	1.35^{aB}	5.35^{bA}	4.91^{cA}

Numbers followed by the same lowercase letters in the columns and uppercase letters in the rows show no statistical difference according to the Tukey test ($P< 0.05$).Source: Research Data (2019).

supplemented with 15 and 30 g L^{-1} sucrose combined with 10 and 20 g L^{-1} sorbitol, respectively. These results show that the presence of sucrose in the culture medium is necessary for plant development, and are similar to those obtained in the present research, in which the lowest means for plant height were observed in culture media without sucrose.

Nicoloso et al. (2003) compared the effect of different carbohydrate sources (sucrose, fructose, lactose, glucose and maltose) on the growth of *Pfaffia glomerata* (Spreng.) Pedersen (Brazilian Ginseng), and reported that the mean height of shoots was higher in high sucrose doses (30, 45 and 60 gL^{-1}) showing that the carbon source directly affects plant growth.

In *in vitro* conservation experiments with black mulberry, Silva (2016) found that the supplementation of MS medium with high concentrations of osmotic agents or their absence reduced plant length. The increased growth caused by sucrose reveals its importance in *in vitro* plant growth (Ankita and Animesh, 2013).

Since *in vitro* grown plant photosynthetic activity is not sufficient to satisfy their energetic needs, they require an additional carbon source added to the culture medium to allow the development of the explant. Consequently, carbohydrates in the culture medium directly affect growth and physiological responses of plants, since they are an energetic source and an osmotic agent in the culture medium (Flores et al., 2013).

According to some studies, supplementing the culture medium with sucrose affects plant growth and development *in vitro* (Farua et al., 2006; Flores et al., 2013). In the current research, it was observed that the addition of sucrose to the culture medium resulted in growth increase. On the other hand, the absence of sucrose slowed the development of the explant. It is interesting to note that *in vitro* germplasm collections housed by research centers such as Centro Internacional de Agricultura Tropical (CIAT) in Colombia or International Institute of Tropical Agriculture (IITA) in Nigeria, are always grown in culture media with sucrose or some other carbon source in order to avoid the reduction of plant viability and the ability to resume growth after *in vitro* conservation. In the CIAT's and IITA's cassava collections, a concentration of 20 and 30 g L^{-1} sucrose, respectively, is used. The subculture intervals vary from 4 to 19 months (Cgiar, 2012). *In vitro* conservation of IITA's yam collection is carried out with 30 g L^{-1} sucrose during a period of 11 to 24 months

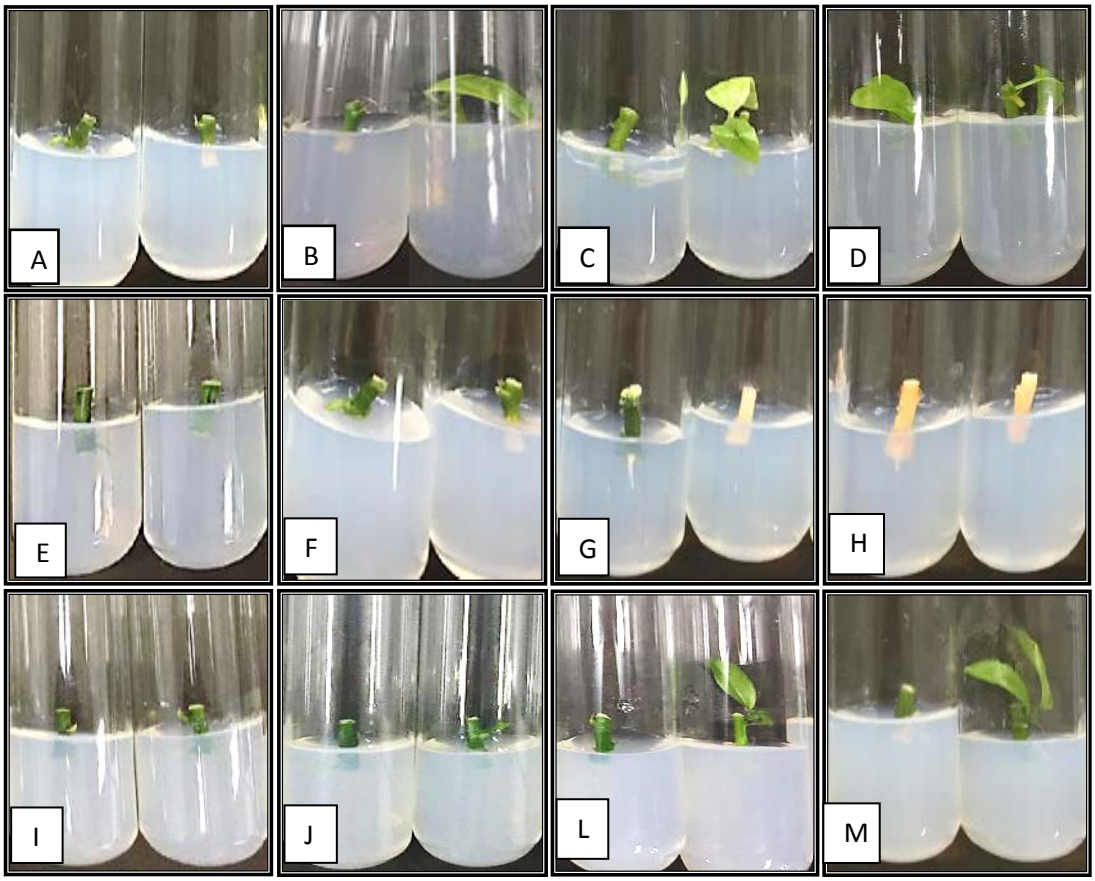

Figure 3. *P. edulis* explants grown *in vitro* in MS culture media without sucrose during 30 days (A), 60 days (B), 90 days (C) and 120 (D); explants in MS/2 medium without sucrose for 30 days (E), 60 days (F), 90 days (G) and 120 days (H); explants in MS/4 medium without sucrose for 30 days (I), 60 days (J), 90 days (L) and 120 days (M).
Source: Author (2019).

Table 3. Mean values of number of green leaves of *P. edulis* plants grown *in vitro* in different MS medium (1/1, 1/2 and 1/4) and sucrose (0, 15 and 30 g L^{-1}) concentrations.

MS medium concentration	Sucrose concentrations (g L^{-1})		
	0	15	30
1/1	1.27^{aB}	5.43^{aA}	5.25^{aA}
½	0.00^{bB}	3.86^{bA}	4.05^{aA}
¼	0.23^{bB}	2.17^{cA}	2.02^{bA}

Means followed by the same lowercase letters in the columns and uppercase letters in the rows show no statistical difference according to the Tukey test (P< 0.05). Source: Research Data (2019).

without subculture (Dumet et al., 2008).

As shown in Table 3, the reduction of MS medium concentration results in a decrease of number of green leaves mean values. On the other hand, the addition of sucrose to the culture medium significantly increases the NGL, whose means when grown in MS culture medium, regardless of concentration, do not differ statistically when 15 g L^{-1} or 30 g L^{-1} sucrose are added. Although MS/4 culture medium supplemented with 15 g L^{-1} or 30 g L^{-1} sucrose resulted in lower plant height mean values and green leaves presence (Table 3 and Figure 4) after 120 days (5.35 and 4.91 cm, respectively) (Table 2), MS/2

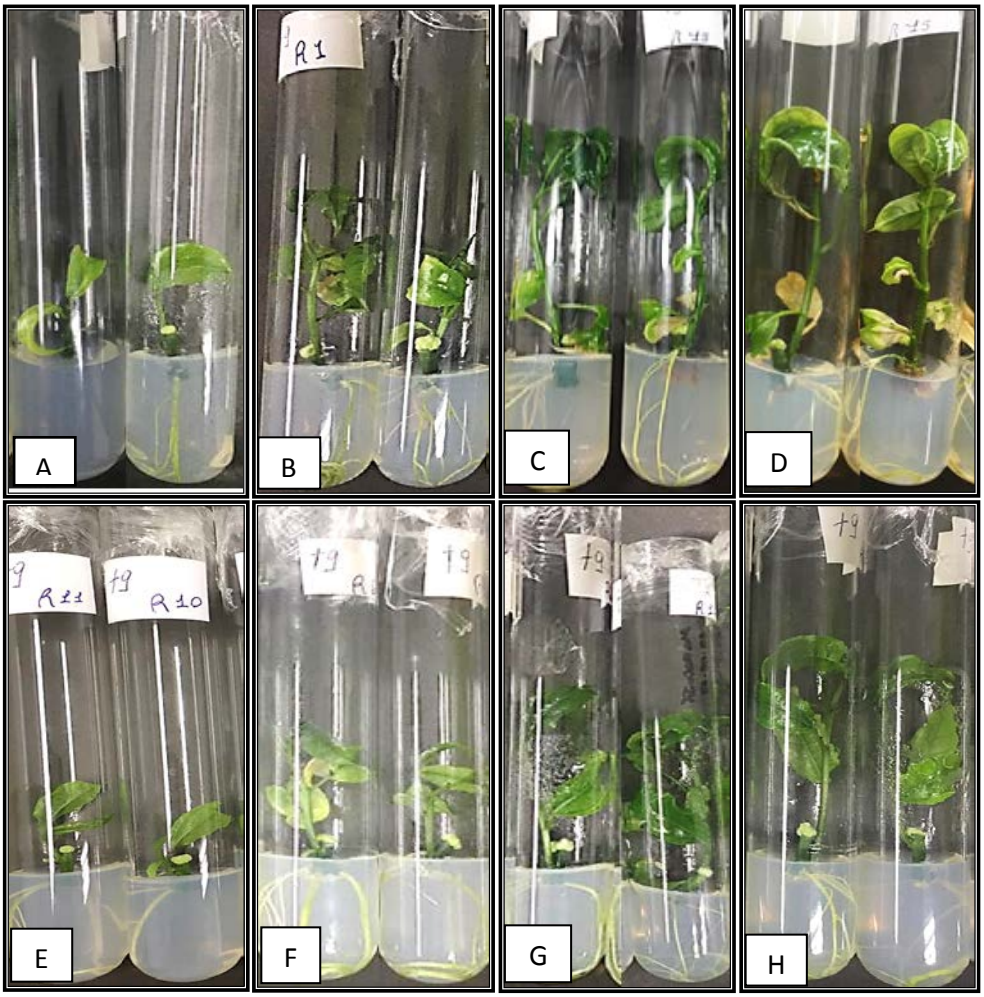

Figure 4. *P. edulis* plants grown *in vitro* in MS/4 culture medium supplemented with 15 g L^{-1} sucrose during 30 days (A), 60 days (B), 90 days (C) and 120 days (D); P. edulis plants grown *in vitro* in MS/4 culture medium supplemented with 30 g L^{-1} sucrose during 30 days (E), 60 days (F), 90 days (G) and 120 days (H).
Source: Author (2019).

medium supplemented with 15 g L^{-1} sucrose seems to be a better option for *in vitro* conservation of plants with an adequate number of green leaves (3.86), because it increases the growth resumption capability of plants after the subculture by the end of the *in vitro* culture stage in minimum growth conditions (Table 3 and Figure 5).

Galdiano Júnior et al. (2012), working with *C. loddigesii* Lindley grown *in vitro,* observed that the highest mean for number of green leaves were obtained in an intermediate concentration (18 g L^{-1}) of sucrose in the culture medium. Their results are similar to those observed in the present study, where intermediate values of sucrose resulted in an appropriate number of green leaves.

Maldaner's (2014) study with *Desmodium incanum* DC showed that MS medium supplemented with 15 g L^{-1} sucrose favored an increase of the number of green leaves, which corroborates the results obtained in our research.

Faria et al. (2006) evaluated the effect of sucrose and sorbitol on *P. giberti* N. E. Brown *in vitro* conservation and observed that regardless of the concentration of sorbitol, the highest number of green leaves was obtained when sucrose was added to the culture medium. The authors also related their results with the quality of the microplant according to the color of the leaves. They reported that culture media with any concentration of sorbitol but without sucrose had no effect on plant quality. On the other hand, when the culture medium was supplemented with 15 and 30 g L^{-1} sucrose, the microplants were stronger. Plant quality increased when the culture medium was supplemented with 10 and 20 g L^{-1} sorbitol. In the present research,

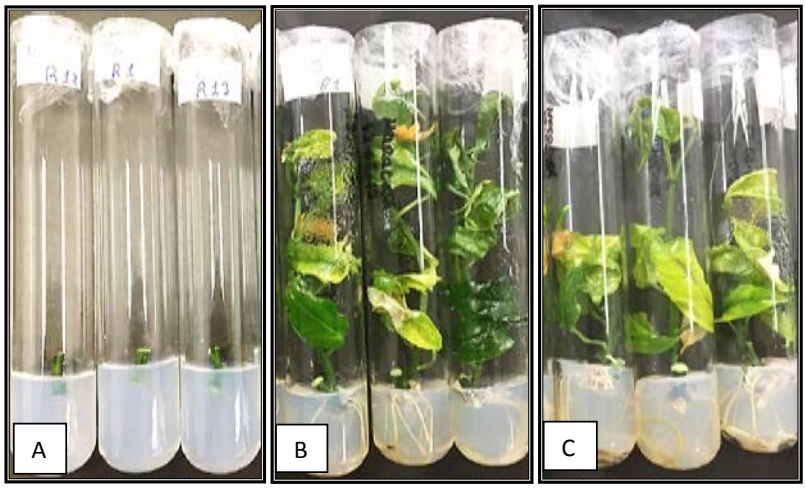

Figure 5. *P. edulis* plants grown *in vitro* in MS/2 culture medium for 120 days: plants grown in MS/2 culture medium without sucrose (A); and in culture medium supplemented with 15 g L^{-1} (B) and 30 g L^{-1} sucrose (C). Source: Author (2019).

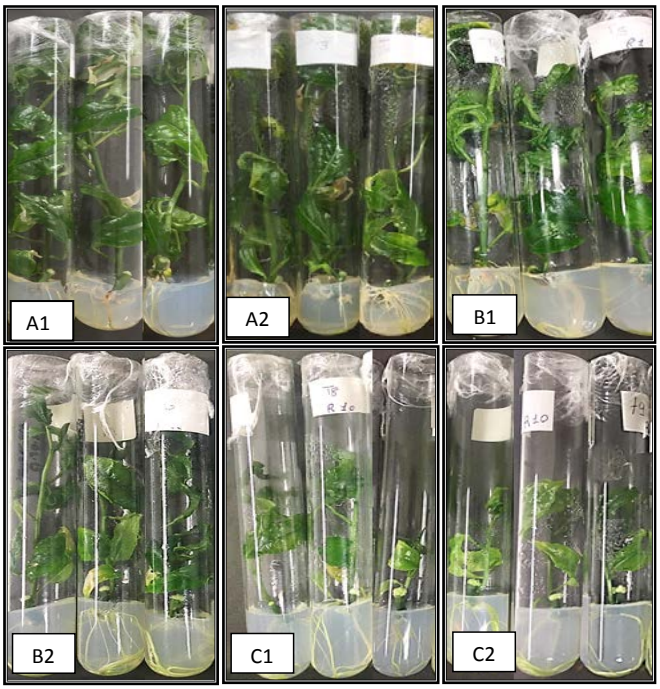

Figure 6. *P. edulis* plants grown *in vitro* in MS medium supplemented with 15 g L^{-1} (A1) and 30 g L^{-1} sucrose (A2); plants in MS/2 medium with 15 g L^{-1} (B1) and 30 g L^{-1} sucrose (B2); plants in MS/4 with 15 g L^{-1} (C1) and 30 g L^{-1} de sucrose (C2) after 120 days of culture Source: Author (2019).

concentrations of 15 and 30 g L^{-1} resulted in more vigorous plants (Figure 6) with intense green color.

Oliveira (2017), working with mangaba trees, observed that culture medium without sorbitol and 15 g L^{-1} sucrose resulted in the highest number of green leaves. Oliveira (2017) findings are consistent with the present study since

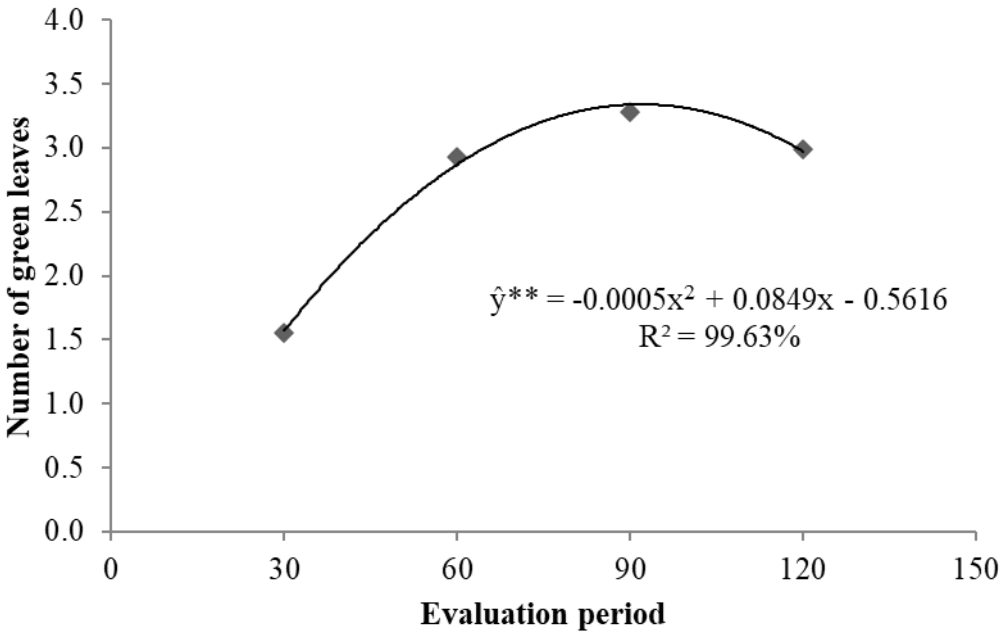

Figure 7. Mean values of number of leaves of *P. edulis* grown *in vitro* during 30, 60, 90 and 120 days.*Source: Research Data.*

the highest mean for NGL (5.43) was obtained in MS medium supplemented with 15 g L^{-1} sucrose (Table 3). Canto et al. (2004) carried out *in vitro* conservation assays of pineapple germplasm treated with the growth inhibitor paclobutrazol (PBZ) and observed that the highest number of green leaves was found in treatments without PBZ and 30 g L^{-1} sucrose, indicating that sucrose improves leaf development.

With regard to the number of green leaves as a function of evaluation period, we adjusted a second degree equation with R^2 = 99.63% which shows that the maximum value of NGL (3.04) occurred after 84.9 days of *in vitro* culture (Figure 7).

With regard to the mean values of NGL as a function of sucrose concentrations and evaluation period (Table 4), the addition of sucrose to the culture medium resulted in an increase of green leaves, but no significant differences among the sucrose concentrations tested (15 and 30 g L^{-1}) were observed. The highest NGL value was found after 90 days of *in vitro* culture with 15 g L^{-1} sucrose (4.80). No statistical difference in NGL mean values was observed in plants grown in culture media supplemented with 30 g L^{-1} sucrose (Table 4 and Figure 8).

To analyze the NGL as a function of evaluation period in each sucrose concentration, regression models of first and second degree equations with R^2 varying between 84.98 and 99.71% were adjusted. As shown in Figure 9, in the absence of sucrose the highest NGL (0.71) was observed after 120 days of *in vitro* culture. However, when the medium was supplemented with 15 and 30 g L^{-1} sucrose, the highest numbers (4.37 and 4.43, respectively) were obtained after 91.83 and 80.88 days, respectively.

Faria et al. (2007) studied different passion fruit species and reported that a MS medium supplemented with 30 gL^{-1} produced leaves with a more intense green color regardless of evaluation period (45, 75 and 105 days). Among the species under study (*P. edulis, P. giberti* and *P. laurifólia*), *P. laurifolia* produced the highest number of green leaves with the most intense green color after an evaluation period of 105 days.

The findings of the current study, namely that the highest number of green leaves was obtained in media supplemented with sucrose (15 and 30 g L^{-1}), are in accordance with those of Lima-Brito et al. (2011), who analyzed the effect of osmotic agents and temperature in the *in vitro* conservation of *Syngonanthus mucugensis* Giul. subsp. *mucugensis.* They found that the best results regarding green leaves were obtained in media containing 15 g L^{-1} sucrose followed by media supplemented with 30 g L^{-1} after 180 days at 18°C. However, at 25°C the mean values of NGL of plants maintained in a culture medium with 15 g L^{-1} sucrose were significantly higher than in the other treatments.

In general, plants showed little leave senescence. Senescence was observed after 60 days of culture in MS culture medium supplemented with sucrose (Table 5). After 120 days in the three concentrations of culture media without sucrose, no senescent leaves were observed. In culture media with sucrose, the lowest NSL

Table 4. Number of green leaves mean values of P. edulis grown *in vitro* with different concentrations of sucrose (0, 15 and 30 g L^{-1}) during 30, 60, 90 and 120 days.

Sucrose concentration (g L^{-1})	Assessmente time (days)			
	30	60	90	120
0	0.22[b]	0.49[b]	0.64[b]	0.64[b]
15	2.14[a]	3.84[a]	4.80[a]	4.50[a]
30	2.31[a]	4.47[a]	4.44[a]	3.87[a]

Means followed by the same letters in the columns do not differ statistically according to the Tukey test ($P< 0.05$).
Source: Research Data (2019).

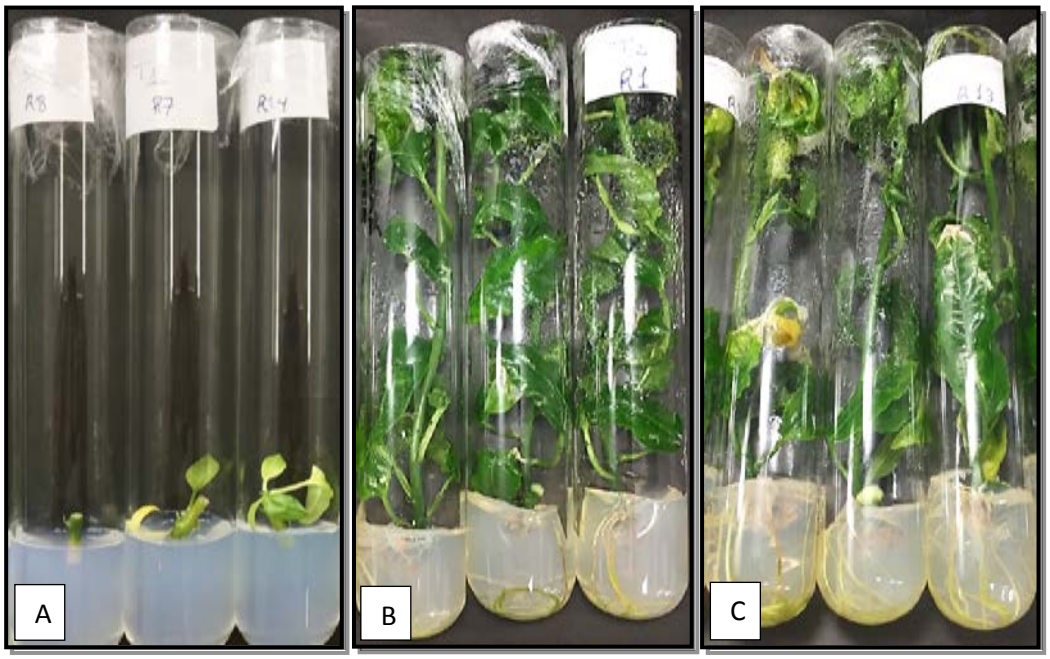

Figure 8. *P. edulis* plants grown *in vitro* in MS culture medium with different sucrose concentrations (0, 15 and 30 g L^{-1}) during 90 days: plants grown in MS medium without sucrose (A); plants grown in MS medium with 15 g L^{-1} (B) and 30 g L^{-1} sucrose (C).
Source: Author (2019).

(1.07) at the same evaluation period was obtained in MS/4 culture medium with 30 g L^{-1} sucrose (Table 5 and Figure 10).

Garcia (2013) carried out a micropropagation and *in vitro* conservation study with *Aechmea blancheteana* comparing different osmotic agents such as sucrose, manitol and sorbitol. He found that the treatment with 15 g L^{-1} sucrose resulted in the highest NSL means, while the lowest means were obtained in media supplemented with 30 g L^{-1} sucrose. However, with regard to the three osmotic agents, the lowest mean was found in media containing 15 g L^{-1} or 30 g L^{-1} mannitol.

In a research carried out by Canto et al. (2004) with pineapple, the authors observed that a culture medium supplemented with 0.5 mg L^{-1} paclobutrazol (PBZ) resulted in a higher number of senescent leaves, indicating that this concentration of PBZ might have stimulated plant growth and, consequently, senescence. They also found that treatments without PBZ containing just MS medium with 30 g L^{-1} sucrose had a lower NSL.

With respect to the number of roots, in all evaluation periods the highest mean values were observed in plants grown in MS culture media containing sucrose (15 or 30 g L^{-1}) (Table 6 and Figure 11).

Furthermore, after 120 days, the highest NR mean values were obtained in MS culture media (total concentration) supplemented with 30 g L^{-1} sucrose (8.33) as well as in full and half concentration of MS culture media with 15 g L^{-1} de sucrose (5.80 and 3.71, respectively).

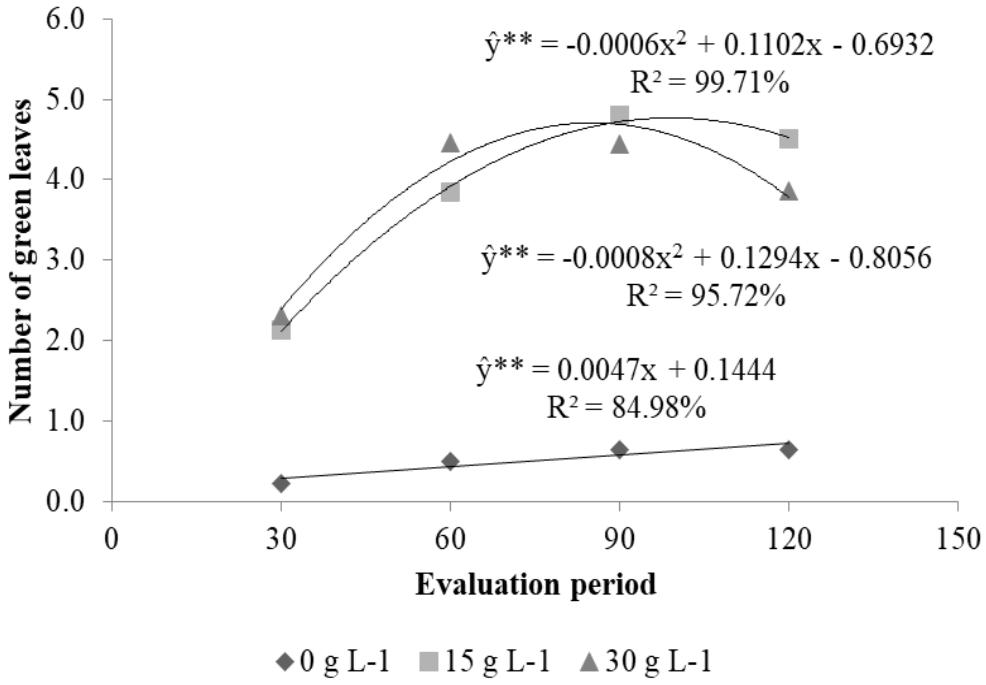

Figure 9. *P. edulis* green leaves mean values in different sucrose concentrations during 30, 60, 90 and 120 days. Source: Research Data (2019).

Table 5. Mean values of number of senescent leaves of *P. edulis* plants grown *in vitro* in different MS medium (1/1, 1/2 and 1/4) and sucrose (0, 15 and 30 g L^{-1}) concentrations during 30, 60, 90 and 120 days.

Ms medium concentration	Sucrose concentrations (g L^{-1})		
	0	15	30
30 days			
1/1	0.00^{aA}	0.00^{aA}	0.00^{aA}
½	0.00^{aA}	0.00^{aA}	0.00^{aA}
¼	0.00^{aA}	0.00^{aA}	0.00^{aA}
60 days			
1/1	0.00^{aB}	0.60^{aA}	0.80^{aA}
½	0.00^{aB}	0.93^{aA}	0.27^{bB}
¼	0.00^{aA}	0.33^{aA}	0.07^{bA}
90 days			
1/1	0.00^{aB}	1.07^{aA}	1.33^{abA}
½	$0.00^{a}C$	1.14^{aB}	1.80^{aA}
¼	0.00^{aB}	1.00^{aA}	1.00^{bA}
120 days			
1/1	$0.00^{a}C$	1.66^{aB}	2.67^{aA}
½	0.00^{aB}	1.86^{aA}	2.20^{aA}
¼	$0.00^{a}C$	2.00^{aA}	1.07^{bB}

Means followed by the same lowercase letters in the columns and uppercase letters in the rows show no statistical difference according to the Tukey test (P< 0.05).Source: Research Data (2019).

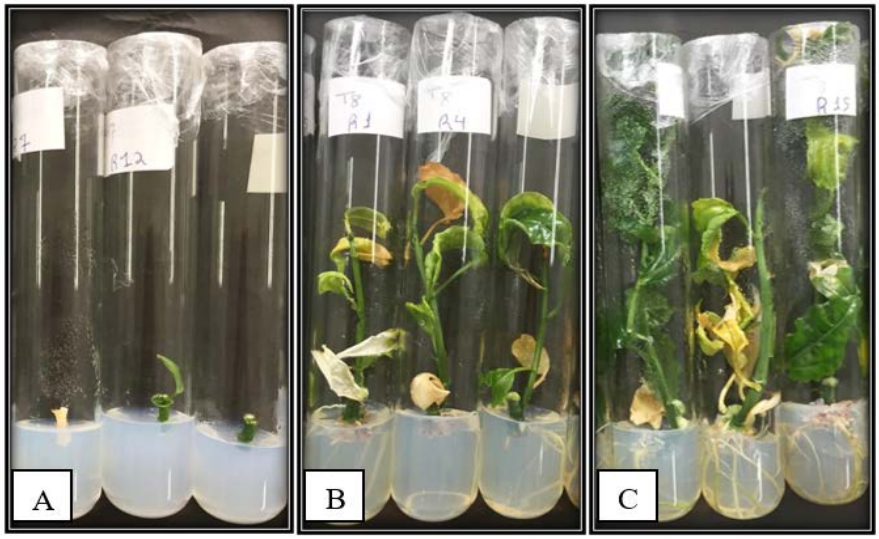

Figure 10. P. edulis grown in vitro in MS/4 culture medium with different sucrose concentrations of (0; 15 and 30 g L-1) during 120 days: plants grown in MS/4 without sucrose (A) and in MS/4 with 15 g L-1 (B) and 30 g L-1 sucrose (C).
Source: own authorship (2019).

Table 6. Mean number of roots of *P. edulis* plants grown *in vitro* in different MS culture medium (1/1, 1/2 and 1/4) and sucrose (0, 15 and 30 g L^{-1}) concentrations during 30, 60, 90 and 120 days.

MS medium concentration	Sucrose concentration (g L^{-1})		
	0	15	30
30 days			
1/1	0.00^{aB}	1.93^{aA}	2.73^{aA}
½	0.27^{aB}	1.57^{aA}	1.27^{bA}
¼	0.00^{aB}	0.47^{bB}	1.60^{abA}
60 days			
1/1	0.00^{aB}	2.93^{aA}	3.13^{aA}
½	0.33^{aB}	3.57^{aA}	3.87^{aA}
¼	0.07^{bA}	2.33^{aA}	3.07^{aA}
90 days			
1/1	0.00^{aB}	2.93^{aA}	3.40^{aA}
1/2	0.33^{aB}	3.57^{aA}	3.87^{aA}
1/4	0.07^{aB}	2.33^{aA}	3.07^{aA}
120 days			
1/1	0.00^{aC}	5.80^{aB}	8.33^{aA}
1/2	0.33^{aB}	3.71^{abA}	3.87^{bA}
1/4	0.07^{aB}	2.60^{bA}	3.47^{bA}

Means followed by the same lowercase letters in the columns and uppercase letters in the rows show no statistical difference according to the Tukey test (P< 0.05).Source: research data (2019).

In the absence of sucrose, there is almost no root development (Table 6), which shows the importance of sucrose in this process. According to George et al. (2008), culture media such as MS have high salt content, which may inhibit root formation. Thus, in order to foster rooting they can be replaced by media with low salt

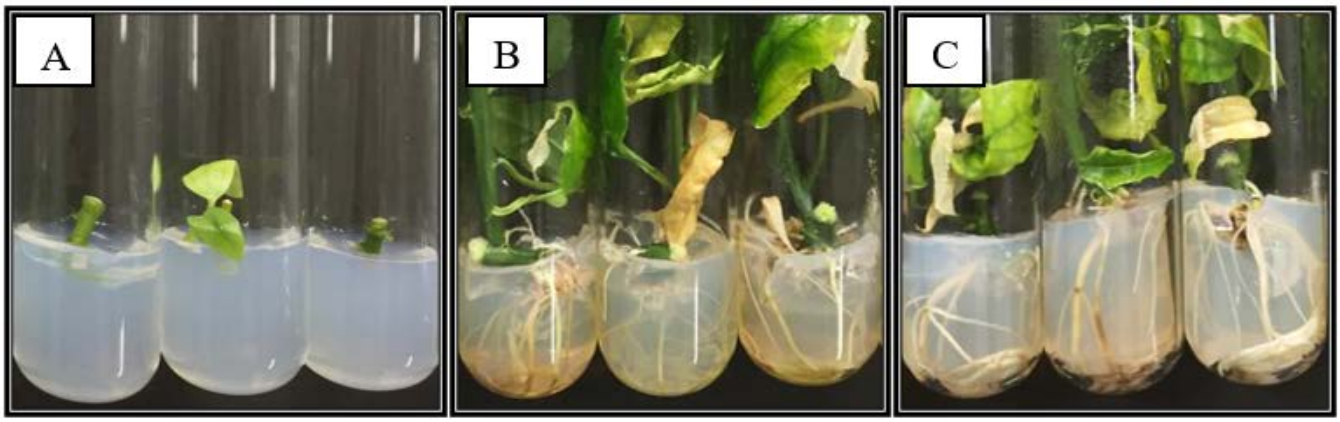

Figure 11. P. edulis plants grown in vitro during 120 days in MS culture medium without sucrose (A) and in MS culture medium supplemented with 15 g L-1 (B) and 30 g L-1 sucrose (C).
Source: own authorship (2019).

content, as for example MS/2. As is known, nitrogen concentrations are required for root formation and this can be obtained with low levels of salt in the culture medium.

Calvete et al. (2002) also reported positive results regarding strawberry root formation when using sucrose as carbon source. Similar results were obtained by Schmildt et al. (2007), who were able to develop roots in papaya plants grown *in vitro* in culture media with sucrose. According to Faria et al. (2006), who studied *P. giberti,* the presence of sucrose in the culture medium is adequate for root formation except when in combination with 40 g L^{-1} sorbitol.

P. edulis f. *Flavicarpa* was studied by Faria and Segura (1997), who obtained high rooting rates in MS media supplemented with 30 g L^{-1} sucrose. However, Veierskov et al. (1982) reported that high carbohydrate concentrations in the culture medium might result in an excess of sugar that surpasses physiological levels causing negative effects on rooting.

In the current study, the culture medium supplementation with sucrose contributed significantly to increase the number of green leaves and roots. According to the results of the present study, the supplementation of the culture media with sucrose resulted in higher leaf and root numbers when compared with plants grown without sucrose. Thus, we may conclude that this sugar source plays an important role in the present research.

After the *in vitro* conservation, the plants were sub cultured twice at 45 days intervals in MS medium supplemented with 30 g L^{-1} sucrose. During this period it was possible to verify that the plants had an adequate number of green leaves and roots (similar to those observed during the assessments), and were viable and conditions (MS culture medium with 15 L^{-1} sucrose) were acclimatized after the subcultures, and 100% survival was observed after 30 days (Figure 12). It is important to note that after the acclimatization period the plants showed vigorous green leaves and adequate root development, which is a necessary condition for their survival in the field.

Although acclimatization is a very sensitive stage in *in vitro* culture of plants, in some passion fruit genotypes, this stage has been successfully carried out (Isutsa, 2004; Shekhawat et al., 2015). According to Tanno and Biasi (2013), grapevines were efficiently acclimatized in MS medium with increasing doses of sucrose. They assessed the experiment after 48 days of *in vitro* culture. They were acclimatized immediately after and evaluated after 35 days. In concentrations of 15 and 30 g L^{-1} sucrose, 100% of the plants developed roots, and their height, mean root length and leaf number were higher than in the absence of sucrose.

It is worth noting that the methodology used in this research enables the *in vitro* conservation of *P. edulis* plants with identical characteristics as the stock plant, which is of interest for the pharmaceutical industry (as input supplier with efficient propagation), for health agents and for the establishment of live pharmacies. Thus, the conservation of species that arouse the interest of the pharmaceutical industry and of the Unified Health System (SUS) becomes relevant.

Conclusions

MS/4 culture medium supplemented with 15 g L^{-1} or 30 g L^{-1} sucrose enables the *in vitro* maintenance of *P. edulis* plants with smaller mean height values. MS/2 medium with 15 g L^{-1} sucrose is an efficient strategy for *in vitro* conservation of *P. edulis* plants with adequate green

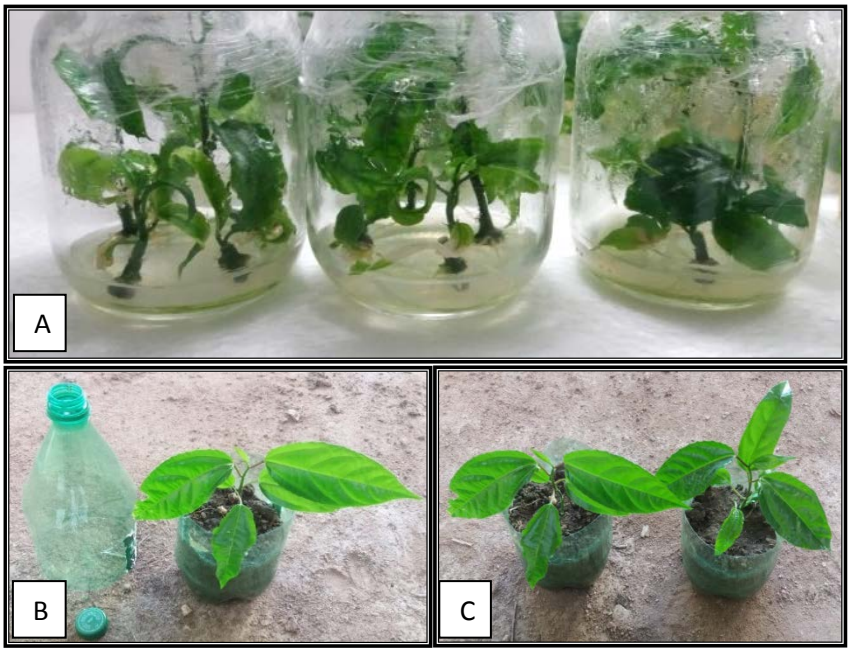

Figure 12. *P. edulis* plants after the *in vitro* conservation period and two subcultures (with a 45 day interval) in MS medium supplemented with 30 g L^{-1} sucrose (A) and seedlings originated from the selected treatment during the *in vitro* conservation (MS/2 culture medium with 15 L^{-1} sucrose) after 30 days acclimatization (B, C).
Source: Author (2019).

leaves and root number after 120 days of culture, enabling 100% survival after acclimatization.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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