In vitro multiplication of lulo (*Solanum quitoense* Lamarck) for preservation purposes

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ABSTRACT
Objective: To evaluate the effect of treatment, temperature, and time on 2-mm long stems of *Solanum quitoense*, using the minimal growth technique, under in vitro conditions.
Design/Methodology/Approach: Eight treatments with different concentrations of mannitol, sucrose, and Murashige and Skoog (1962) (0 5g L^{-1}, 10 g L^{-1}, 15 g L^{-1}, 20 g L^{-1}, 25 g L^{-1}, 30 g L^{-1}, and 30 g L^{-1}) were analyzed. The experiments were placed in two rooms at 25 °C and 21 °C. Stem growth was recorded every fifteen days.
Results: The Generalized Linear Model showed that the treatments with the best results were 20 g L^{-1} and 30 g L^{-1} mannitol, which reduced the *in vitro* growth of *S. quitoense* to a remarkable degree, preserving the subsistence and vigor characteristics, at a temperature of 21 °C. meanwhile the applied concentrations of sucrose promoted a rapid growth of both the stem and shoots.
Findings/Conclusions: *S. quitoense* recorded resistance to 30 g L^{-1} mannitol, enabling a 3-month preservation of seedlings; however, *S. quitoense* could potentially be preserved for longer periods.

Keywords: minimal growth, mannitol, temperature, *Solanum quitoense*.

Citation: Cruz-García, N. I., Castillo-Martínez, C. R., Velasco-Bautista, E. & Cadena-Iñiguez, J. (2023). In vitro multiplication of lulo (Solanum quitoense Lamarck) for preservation purposes. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i9.2619

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: June 28, 2023.
Accepted: August 24, 2023.
Published on-line: November 27, 2023.

Agro Productividad, 16(10). October. 2023. pp: 69-78.

INTRODUCTION

Lulo (*Solanum quitoense* Lam.) (Solanaceae) is native to the Andes and it is mainly grown in Colombia and Ecuador (Gallo *et al.*, 2018). The interior of this round-oval fruit is divided into four parts, each one of which is filled with a green pulp and many small seeds. Its pulp is very fragrant and has a sweet taste; likewise, it has a high vitamin A, C, B1, and B2 content (INIAP, 2010; Silva, 2015). The consumption of lulo has increased in the American, Canadian, German, French, Japan, and Chinese markets, as a result of its nutraceutical value (Gomez-Merino *et al.*, 2014; Cámara de Comercio Bogotá *et al.*, 2015; Alvarez-Duque *et al.*, 2021).

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Nevertheless, the low transportation resistance of lulo limits its exportation as fresh fruit. This situation forces exporters to replace fresh fruit with products made with processed pulp, juices, and preserves (Lago-Burgos, 2011). Arias *et al.* (2014) recorded a deficit in the production of lulo which could be an opportunity for Mexico to develop its own technologies to grow and sale this fruit, both in the domestic and export markets. As a whole, research have shown the need to develop propagation protocols, including grafting, rooting, and the minimal growth technique under *in vitro* conditions. The main objective of the third technique is the preservation and the exchange of multiple phytogenic resources, promoting the potential storage of vegetal germplasm in a limited area and facilitating access to plant material (García-Águila *et al.*, 2007). Likewise, minimal growth extends the time between cultures and subcultures, compared with the 3-5 week regular intervals. The length of the intervals depends on the species. Additionally, minimal growth enables the micropropagation of the plant material in limited spaces and reduces cost. Several studies about minimal growth have been carried out with species such as *Swietenia macrophylla* King and *Tectona grandis* L. (Montiel-Castelán *et al.*, 2016), *Stevia rebaudiana* (Zayova *et al.*, 2017), *Ipomoea batatas* L. (Rayas *et al.*, 2019), *Solanum chilotanum* (Muñoz *et al.*, 2019), and *Arnica montana* L. (Petrova *et al.*, 2021). Since *S. quitoense* is not native to Mexico, no biological variation can be used to develop a selection and genetic improvement program. Consequently, determining *in vitro* propagation and multiplication protocols is important to generate plants that can be subjected to irradiation processes. The purpose of these processes is to induce variation and mutagenesis as soon as possible, as well as to obtain clones that can be exploited as a commercial crop. Therefore, the stems of *Solanum quitoense* were evaluated and multiplied under *in vitro* conditions, with different minimal growth treatments, modifying the culture medium and the temperature to preserve and obtain plants suitable for cultivation.

MATERIALS AND METHODS

Plant material

The experiments were carried out in the Laboratorio de Biotecnología y Germoplasma, CENID-COMEF, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The lulo fruits were collected in Huatusco, Veracruz, Mexico (19° 08' 56" N, 96° 57' 58" W, at 1,344). The local climate is warm humid, with summer rains, and a mean temperature of 19.4 °C (maximum temperature: 26.3 °C; minimum temperature: 12.4 °C) (CONAGUA, 2022). The vegetation is typical of the cloud forest category, with 85% relative humidity and an annual precipitation of 2,250 mm. The soils have abundant nutrients. They are moderately fertile, with a thick texture, volcanic glass fragments, and a slightly acid pH (4.3-6.5); they also have abundant organic matter, with low Ca content and high Fe, Mn, and Zn content (Cadena-Iñiguez *et al.*, 2011).

The initial propagation used seeds of a variety acclimatized to the area since 2014 as a self-fertilization pure line. The seeds were extracted by hand and washed with running water five times to remove mucilage. Afterwards, they were immersed in a 70% alcohol solution for 3 min and then washed again with running water. Finally, they were placed in a 30% chlorine solution for 20 min. Once this process concluded, the seeds were

washed with sterile water in a laminar flow hood; the excess water was removed with a sterile paper.

Establishment of the *in vitro* cultivation

The seeds were placed in test tubes with 6.0 mL of a MS medium (Murashige and Skoog, 1962). The tubes were kept in a room at 25 °C, during a 24/0 (light:dark) photoperiod. The seeds germinated after four weeks (Gutiérrez *et al*., 2019). Subsequently, they were kept for 60 additional days in the culture medium, in order to obtain the tallest plants possible and the said medium was used for the minimal growth technique.

Minimal growth

Table 1 shows the treatments used to evaluate the lulo (*S. quitoense*) stems grown in a MS medium, with different sucrose and mannitol ratios. Stem width and length were measured at 2.0 cm to reduce growth differences. The incubation conditions for the culture were two rooms with different temperatures (25 °C and 21 °C) and a 24/0 photoperiod. Evaluations were carried out every 15 days for three months.

Variables

n=210 experimental units (test tubes) were subjected to eight treatments which, in their turn, were divided into two blocks with different temperatures (25 °C and 21 °C). Each experimental unit recorded stem height seven times, resulting in a total of n=1,470 observations.

Statistical model

Considering the experimental design, the data were analyzed with a Generalized Linear Model. According to Stroup (2014), the model has the following predictor:

$$\log\left(\mu_{jkl}\right) = \mu + \alpha_j + \tau_k + (\alpha\tau)_{jk} + \beta_l$$

Then, $\mu_{jkl} = \exp\left(\mu + \alpha_j + \tau_k + (\alpha\tau)_{jk} + \beta_l\right)$

Table 1. Treatments of the evaluated mediums of *Solanum quitoense* Lamarck.

Treatment	MS (g $L^{-1)}$	Agar (g L^{-1})	Sucrose (g L^{-1})	Mannitol (g L^{-1})
1	4.4	8	30	0
2	4.4	8	0	30
3	4.4	8	0	0
4	4.4	8	25	5
5	4.4	8	20	10
6	4.4	8	15	15
7	4.4	8	10	20
8	4.4	8	5	25

MS=Murashige and Skoog (1962).

Where: $y_{ijkl} \sim Gamma\left(\mu_{jkl}, \phi\mu^2_{jkl}\right)$ is the height of the stem in the *i*-th experimental unit (tube) of the *j*-th treatment in the *k*-th time or the moment when the measurement took place in the *l*-th block (temperature level). μ is the mean value for the reference level. α_j is the log-mean difference for the *j*-th treatment level with the reference level. τ_k is the log-mean difference for the *k*-th time level with the reference level. β_l is the log-mean difference for *j*-th the block level with the reference level.

The y_{ijkl} are considered independent regarding block treatments. Likewise, they have a first-order autoregressive covariance regarding time within the same experimental unit.

RESULTS AND DISCUSSION

A differentiated effect was observed in the treatment and time for the stem height variable. Overall, a higher variation was recorded among the three observations at the end of the evaluation period. In that respect, although treatments 2 and 3 were similar at the start of the period, the latter almost doubled the height of the former by the end of the evaluation (Figure 1). Figure 2 shows a growing linear trend in all the treatments. Treatments 1 and 4 recorded a higher growth rate, while treatment 2 and 7 grew at a slow rate. Treatment 4 achieved an almost 80 mm height; meanwhile, the stems of treatment 2 grew almost 25 mm at the end of the evaluation period.

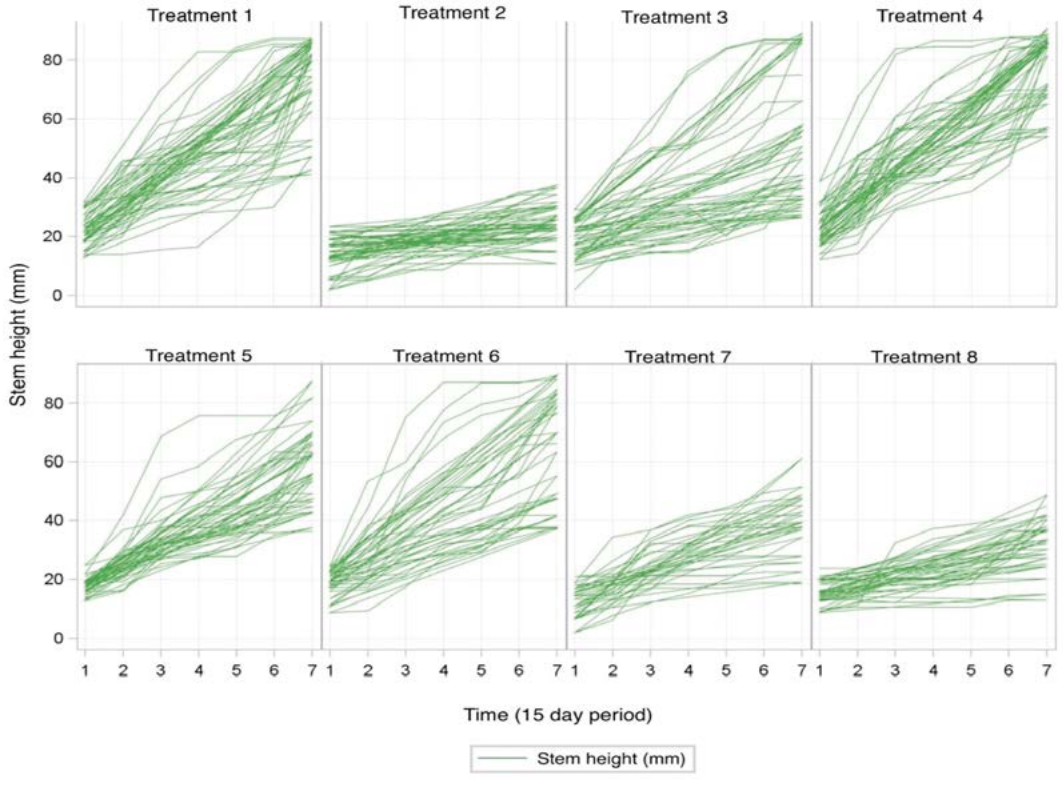

Figure 1. Stem height of *Solanum quitoense* Lamarck per treatment, as a function of time. T1: 30 gL^{-1}, T2: 30 gL^{-1}, T3: 0 gL^{-1}, T4: 25 gL^{-1}, T5: 20 gL^{-1}, T6: 15 gL^{-1}, T7: 10 gL^{-1}, and T8: 5 gL^{-1}. Average values of 1470 observations.

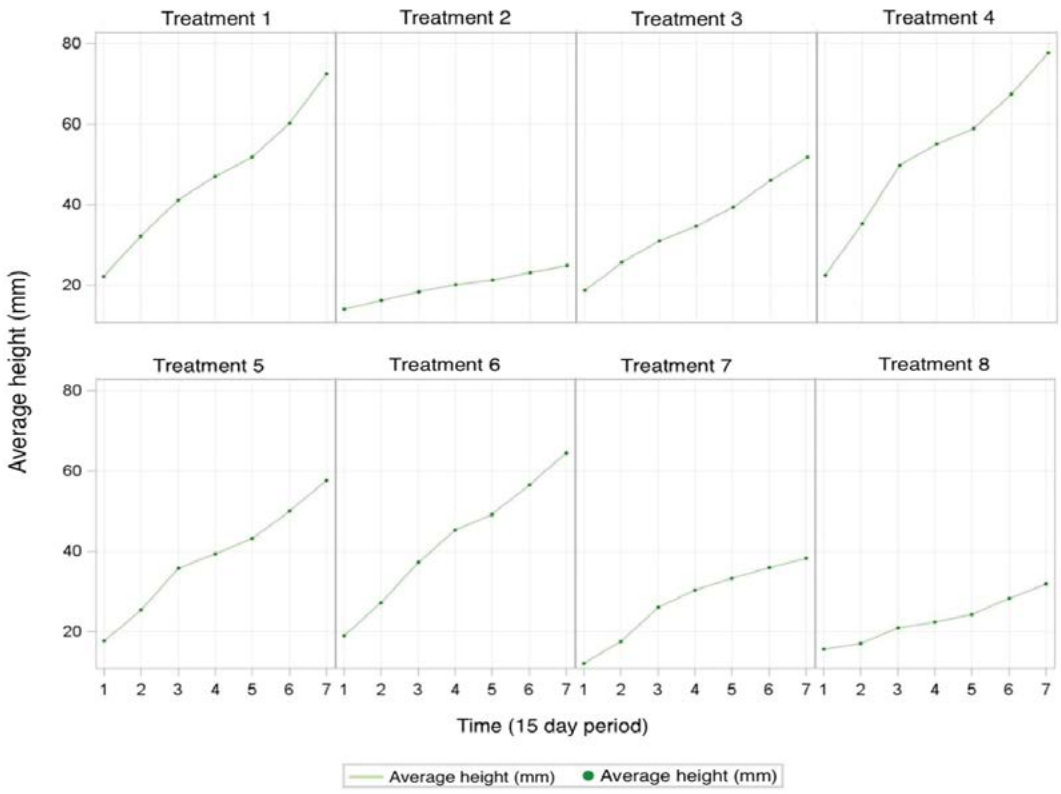

Figure 2. Mean stem height of *Solanum quitoense* Lamarck per treatment, as a function of time. T1:30 gL^{-1}, T2: 30 gL^{-1}, T3: 0 gL^{-1}, T4: 25 gL^{-1}, T5: 20 gL^{-1}, T6: 15 gL^{-1}, T7: 10 gL^{-1}, and T8: 5 gL^{-1}. Average values of 1,470 observations.

Evaluation of the effect of temperature and time evaluation on stem height

Time observations within the same experimental unit were highly correlated in closer measurements; however, as they separated, the temporal dependency notably decreased. In conclusion, the correlation between times 1 and 7 reached 50% regarding the correlation between the two first times (Table 2).

The statistical analysis took into consideration the gamma distribution and the first-order autoregressive covariance. Consequently, it identified the main effects of the treatments and time, as well as their interaction, both of which have a significant

Table 2. Correlation matrix estimated between measurement times for the stem height of *Solanum quitoense* Lamarck, under *in vitro* conditions.

Time	1	2	3	4	5	6	7
1	1.0000	0.8644	0.7472	0.6458	0.5583	0.4826	0.4171
2	0.8644	1.0000	0.8644	0.7472	0.6458	0.5583	0.4826
3	0.7472	0.8644	1.0000	0.8644	0.7472	0.6458	0.5583
4	0.6458	0.7472	0.8644	1.0000	0.8644	0.7472	0.6458
5	0.5583	0.6458	0.7472	0.8644	1.0000	0.8644	0.7472
6	0.4826	0.5583	0.6458	0.7472	0.8644	1.0000	0.8644
7	0.4171	0.4826	0.5583	0.6458	0.7472	0.8644	1.0000

effect on stem length. Therefore, the F value of the interaction —based on the degree of freedom in the numerator (42) and the dominator (1,212)— was 4.27, with a <0.0001 p-value (Table 3).

This situation confirmed that treatments had a significantly different effect on stem length throughout the study. The interaction-based multiple comparison tests showed that the observations of time 7 (treatments 4 and 1) were the highest, while the observations of time 1 (treatments 7, 2, and 8) were the lowest (Figure 3). Overall, the observations of the different times of treatment 2 held the lowest positions among the Tukey groups. Figure 4 shows the remarkable differences in growth between treatment 1 (control) and treatments 2 and 7.

Pineda-Lázaro *et al.* (2021) observed that 0.8% mannitol reduced the stem growth (height) and the number of nodes of the explants of *Solanum tuberosum*, showing that

Table 3. Gamma distribution and first-order autoregressive covariance for temperature, time, and *Solanum quitoense* Lamarck stem treatments, under *in vitro* conditions.

Efect	Numbered degrees of freedom	Denominator of degrees of freedom	Value F	Pr>F
Temperature	1	201	12.90	0.0004
Treatment	7	201	44.02	<.0001
Time	6	1212	218.13	<.0001
Treatment *Time	42	1212	4.27	<.0001

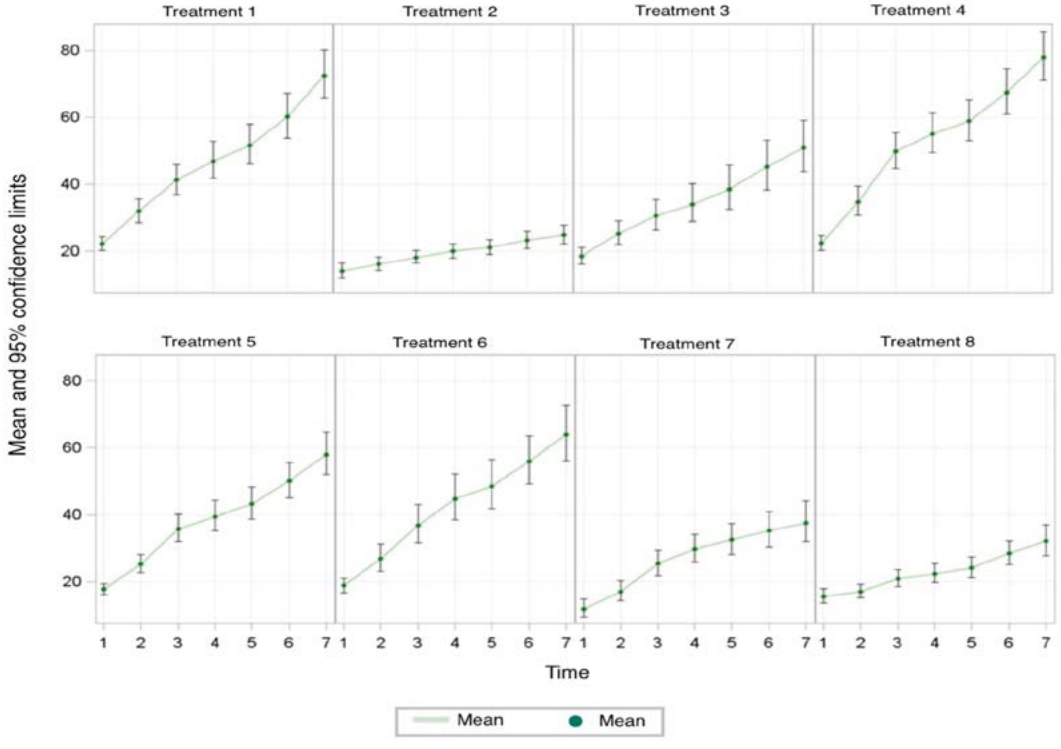

Figure 3. Mean and least-square confidence intervals of *Solanum quitoense* Lamarck stem height, under *in vitro* conditions.

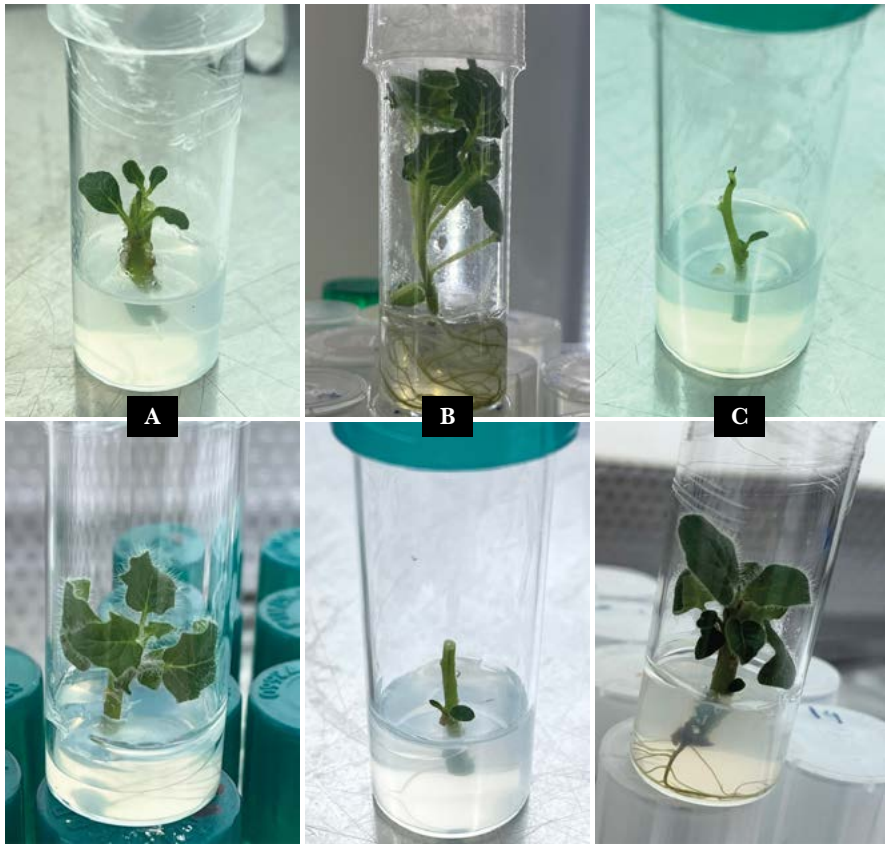

Figura 4. *Solanum quitoense* Lamarck. 1: first measurement. 2: last measurement. Temperature: 21 °C. A (treatment 1), B (treatment 2), and C (treatment 7).

mannitol efficiently preserves a long-term or a minimal growth. The final plant height results were similar in other studies: Pineda-Lázaro *et al.* (2021) recorded 39.1 mm resulting for their treatment C5, while this study recorded 39.8 for treatment 7 (Figure 2). Other preservation studies —which focused on different plant species— were analyzed for comparative purposes. Ventura (2019) studied the use of mannitol and sorbitol for the in vitro preservation of *Physalis peruviana* and concluded that a 20 g L^{-1} mannitol treatment was more efficient than a 20 g L^{-1} sorbitol treatment.

According to Carmona *et al.* (2015), the minimal growth response is linked to a reduction of the water collection required for the growth of the sprouts or the seedlings. The resulting lack of water causes a reduction of turgor pressure, preventing the expansion of cells. For their part, Pérez-Molphe *et al.* (2012) pointed out that a lower nutrient absorption will reduce growth without altering the biochemical balance of the vegetable cells.

According to Rayas Cabrera *et al.* (2019), the in vitro preservation of *Ipomoea batatas* L. should be carried out using 10 g L^{-1} mannitol, because >1.0 g L^{-1} can impact preservation. However, no impacts were recorded in this experiment when that amount of mannitol was used, except for a reduction of the growth. The final height recorded during this experiment was 25 mm (treatment 2). Nevertheless, Rayas Cabrera *et al.* (2019) carried out their last measurement at eight months, while the plants of this experiment

were measured at 3 months. The resulting time difference between the measurements suggest that several results can match the results of this study. However, the amount of mannitol can vary depending on the species studied. Loureiro da Silva *et al.* (2011) and Bello-Bello *et al.* (2015) pointed out that, in some cases, high concentrations of mannitol can be toxic or even deadly for some species. Although a lethal concentration depends on the species, the Solanaceae family can resist an amount of mannitol similar to the one used for the minimal growth technique.

Temperature is an important factor for minimal growth because it can reduce the metabolic activity and, consequently, the growth of the explants (Engelmann, 1991; Sánchez-Chiang *et al.*, 2010; Vásquez *et al.*, 2011; Tandazo, 2015). Jaime-Guerreo (2021) indicated that the Solanaceae family (lulo) are plants from cold weather regions. However, they develop faster in higher temperatures, which encourage earlier harvests, unlike under cold weather conditions. Montiel-Castelán *et al.* (2016) studied the *in vitro* preservation of *Tectona grandis* L. and *Swietenia macrophylla* King, with temperatures of 18 °C, 24 °C, and 28 °C. They proved that a lower temperature, combined with mannitol, is the best preservation alternative, as a result of its direct effect on metabolism.

Meanwhile, Cioloca *et al.* (2021) evaluated temperatures of 16 °C, 20 °C, and 24 °C. Their last evaluation was carried out at day 140, under different conditions. They observed that the most feasible temperatures were 16 °C and 20 °C. These results match the findings of this research. For their part, Espinosa Reyes (2003) recorded null survival under *in vitro* conditions of sweet potato (*I. batata*) plant material at 25 °C; meanwhile, 60% of all clones survived 17 °C. These results confirm that lower temperatures promote minimal growth.

Lima-Brito *et al.* (2011) studied the effect of temperature (18 °C and 25 °C) on the survival and preservation of *Syngonanthus mucugensis* plants. They proved that 18 °C promoted the feasibility of the material for 180 days and recorded a significatively higher plant survival percentage than the plants subjected to a temperature of 25 °C, regardless of the medium (sucrose, mannitol, and sorbitol).

CONCLUSIONS

Unlike other species, *S. quitoense* recorded resistance to 30 g L^{-1} mannitol, enabling a 3-month preservation of seedlings; however, *S. quitoense* could potentially be preserved for longer periods.

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