

## Scientific Notes

### In vitro conservation of Cape gooseberry through slow-growth nodal segment cultures

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**Abstract** – The objective of this work was to evaluate the effects of temperature and osmotic agents on the in vitro conservation of Cape gooseberry (*Physalis peruviana*). Temperatures at 18 and 25°C, as well as the osmotic agents sucrose, mannitol, and sorbitol were tested. A short-term in vitro conservation of Cape gooseberry can be achieved at 18°C, using 30 g L<sup>-1</sup> sucrose.

**Index terms:** *Physalis peruviana*, ex situ conservation, tissue culture.

### Conservação in vitro de fisalis por meio do cultivo de segmentos nodais em crescimento lento

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos da temperatura e de agentes osmóticos na conservação in vitro de fisalis (*Physalis peruviana*). Foram testadas as temperaturas de 18 e 25°C, bem como os agentes osmóticos sacarose, manitol e sorbitol. A conservação de fisalis in vitro, em curto prazo, pode ser conseguida a 18°C, em meio com adição de 30 g L<sup>-1</sup> de sacarose.

**Termos para indexação:** *Physalis peruviana*, conservação ex situ, cultura de tecidos.

Cape gooseberry (*Physalis peruviana* L.) is a Solanacea plant with an important medicinal value because of its curative properties for inflammatory and cancerous diseases (Olivares-Tenorio et al., 2016). Its propagation is mainly carried out by seed. However, in this type of propagation, many growers are faced with seedlings of low sanity, while at the same time encountering germination problems that prolong seedling production time (Singh et al., 2016). In this way, plant tissue culture (PTC) emerges as an efficient approach for rapid multiplication and conservation of Cape gooseberry (Ramar & Ayyadurai, 2014). Shahzad et al. (2017) reported that this technique has been routinely used for the conservation of germplasm, in order to improve the production of secondary metabolites and agronomic characters that aim to increase productivity.

In vitro conservation can be performed by slow growth or cryopreservation. The slow growth technique reduces the metabolism, contributing to the minimum growth of plants (Shahzad et al., 2017).

Many studies on the in vitro propagation of Cape gooseberry have been already conducted successfully (Singh et al., 2016); however, researches aiming at in vitro conservation of this species is still incipient. There are no studies on the addition of osmotic agents on the conservation of Cape gooseberry.

As research concerning the conservation of Cape gooseberry is still scarce, the objective of this work was to evaluate the effects of temperature and osmotic agents on the in vitro conservation of Cape gooseberry by slow growth.

Seed of *P. peruviana* were germinated in vitro, and plants were subcultured every 30 days in MS medium (Murashige & Skoog, 1962) containing 30 g L<sup>-1</sup> sucrose and solidified with 5.5 g L<sup>-1</sup> agar. The pH was adjusted to 5.7 by addition of 0.05 N NaOH. After multiplication, plants were used as explant donors. The cultures were maintained in a growth room under a 16-hour photoperiod and a fluorescent light intensity of 35 μmol m<sup>-2</sup> s<sup>-1</sup>, at 25±2°C.



Equally sized nodal segments (1.0 cm long), containing a single axillary bud, were used as explants. The segments were put individually in 25x150 mm test tubes containing 15 mL of MS medium with salts and vitamins (Murashige & Skoog, 1962), supplemented with osmotic agents as follows: 30 g L<sup>-1</sup> sucrose; 15 g L<sup>-1</sup> sucrose + 7.5 g L<sup>-1</sup> mannitol; and 15 g L<sup>-1</sup> sucrose + 7.5 g L<sup>-1</sup> sorbitol. All media were solidified with 5.5 g L<sup>-1</sup> agar, and pH was adjusted to 5.7 before autoclaving. Plants were maintained at 18 and 25°C inside biological oxygen demand (BOD) chambers, which ensured both the tested temperatures and the stipulated photoperiod (16-hour light) and fluorescent light intensity (35 µmol m<sup>-2</sup> s<sup>-1</sup>). After 30, 60, and 90 days, we recorded the number of leaves, leaf abscission (%), number of buds, and explant viability, according to the following rating scale proposed by Santos et al. (2012), in which: 1, completely green leaves; 2, start of desiccation and initial death of leaves; 3, between 30 and 50% dead leaves; 4, more than 50% dead leaves; and 5, 100% dead leaves. At 90 days, we also evaluated the shoot length (cm), fresh and dry matter (g) of shoots, and fresh and dry matter (g) of roots. Dry matter was assessed after drying the material in a forced-draught oven until a constant mass was achieved.

The experiment was carried out in a completely randomized design, in a 2x3 factorial arrangement (two temperatures x 3 media with osmotic agents), in a split-plot design. The factorial 2x3 was settled in the plots, and the evaluation times (30, 60, 90 days), in the subplots. Each treatment comprised 16 replicate-test tubes, containing one explant each. Data of the evaluated variables were analyzed at 90 days as a factorial 2x3 with 16 replicates. Data were subjected to the analysis of variance, and the means were compared by Tukey's test, at 5% probability, using the Sisvar software (Ferreira, 2011).

At the end of the 90 days of *in vitro* conservation, plants were removed from the slow-growth conditions, and their nodal segments were put in an optimal culture medium for their growth. The medium consisted of salts and vitamins of MS added with 30 g L<sup>-1</sup> sucrose and solidified with 5.5 g L<sup>-1</sup> agar. The material was maintained in a growth room under 16-hour photoperiod and 35 µmol m<sup>-2</sup> s<sup>-1</sup> fluorescent light intensity, at 25±2°C. After 30 days, shoot length (cm), number of leaves, and leaf abscission (%) were

evaluated. A completely randomized experimental design was applied in a 2x3 factorial arrangement (two temperatures x 3 osmotic agents, used in the conservation stage). Data were subjected to the analysis of variance, and the means were compared by Tukey's test, at 5% probability.

A significant interaction was observed between temperature, osmotic agents, and evaluation times for viability (Vi), number of leaves (NL), number of buds (NB), and leaf abscission (LA) (Table 1). All characteristics increased over the evaluation times. All variables showed a very similar behavior during the *in vitro* conservation.

At 18°C, the Vi, NL, and NB, at 30 days, remained the same irrespectively of the osmotic agent used. However, at 60 and 90 days, the highest Vi (lower scores), and the lowest NL and NB were observed in the treatment with sucrose. By contrast, the analysis at 25°C showed that, at 30 days, the highest Vi and the lowest NL and NB were found in the treatment with mannitol, whereas, at 60 and 90 days, the media with mannitol and sorbitol provided better values for NL and NB. The analysis of the temperature inside each level of medium/evaluation time showed differences between temperatures, mostly in the medium with sucrose, and 18°C was the most effective temperature for the conservation of these characteristics.

The interaction between temperatures and osmotic agents evidences the importance of studying physical and chemical factors together for the *in vitro* conservation of plants. The cultivation at 18°C, in the medium containing sucrose only, provided a greater conservation of physalis. Furthermore, the treatment with sucrose has contributed only to the reduction of the evaluated variables. This fact can be explained by the increase of sucrose concentration in a culture medium, which reduces the water potential in such a way that the absorption of water and nutrients becomes highly committed (Lédo et al., 2007). At 25°C, we observed lower values of the variables in the treatments with mannitol, or sorbitol. This probably happened due to the osmoregulatory action of these sugar-alcohols. However, as these agents are poorly metabolized by plants, they can be harmful, depending on the amount in the environment, as occurred in the work of Santos et al. (2011), who observed a high-leaf abscission due to the use of a high-concentration of sorbitol (40 g L<sup>-1</sup>). In the present study, no deleterious effect of osmotic agents was observed.

At 18°C, throughout the evaluation times, LA remained statistically equal in the media with sucrose or sucrose + mannitol, while in the medium with

**Table 1.** Viability (scores), number of leaves, number of buds and leaf abscission of Cape gooseberry (*Physalis peruviana*), at different temperatures and osmotic regulator treatments, at 30, 60, and 90 days<sup>(1)</sup>.

Treatment (days)	Culture media with osmotic regulators		
	Sucrose	Sucrose + mannitol	Sucrose + sorbitol
Viability (scores)			
-----18°C-----			
30	2.40aAα	3.07aAα	3.33aAα
60	3.97bAα	5.43bBα	5.07bBα
90	5.53cAα	6.87cBα	7.93cBα
-----25°C-----			
30	6.03aCβ	2.63aAα	4.33aBβ
60	7.77bBβ	5.07bAα	5.30bAα
90	12.23cBβ	7.03cAα	7.43cAα
CV (%)	21.6		
Number of leaves			
-----18°C-----			
30	2.40aAα	3.07aAα	3.33aAα
60	3.97bAα	5.43bBα	5.07bBα
90	5.53cAα	6.87cBα	7.93cBα
-----25°C-----			
30	6.03aCβ	2.63aAα	4.33aBβ
60	7.77bBβ	5.07bAα	5.30bAα
90	12.23cBβ	7.03cAα	7.43cAα
CV (%)	21.6		
Number of buds			
-----18°C-----			
30	2.23aAα	2.37aAα	2.90aAα
60	3.93aAα	5.20bBα	4.43bABα
90	5.40bAα	6.40cBα	7.30cBα
-----25°C-----			
30	4.73aCβ	2.43aAα	3.63aBα
60	7.00bBβ	5.40bAα	6.10bABβ
90	11.37cBβ	6.60cBα	7.00cAα
CV (%)	24.6		
Leaf abscission (%)			
-----18°C-----			
30	0.00aAα	0.00aAα	0.00aAα
60	1.39aAα	0.48aAα	11.53bBα
90	6.30aAα	2.00aAα	20.22cBα
-----25°C-----			
30	17.18aBβ	0.00aAα	16.06aBβ
60	45.00bCβ	8.10bAα	22.90bBβ
90	51.23cBβ	8.80bAα	26.40bBα
CV (%)	82.1		

<sup>(1)</sup>Means followed by equal lower case letters, in the columns of the same temperature, with upper case letters in rows, and Greek letters between temperatures in the columns, in the same evaluations, showed no significant differences, by the Tukey's test, at 5% probability.

sorbitol, it increased from 0 to 20.22%. At 30 days (at 18°C) LA did not change. However, at 60 and 90 days, we found lower values for LA in treatments with sucrose or sucrose + mannitol. The analysis at 25°C showed that the lowest LA occurred in the medium with mannitol, and the highest LA occurred in the medium with sucrose only. This probably happened because of the plants maintained in the medium with available sucrose, as carbon source showed a greater metabolism and, consequently, a greater ethylene accumulation, a growth regulator responsible for leaf abscission (Santos et al., 2011). The temperature of 18°C was more effective in the maintenance of LA, especially in the treatments with sucrose, or sucrose + sorbitol. In the mannitol treatment, no significant difference for LA was observed, when comparing the temperatures in each evaluation.

At 90 days, there was a significant interaction between temperature and osmotic agents for shoot length (SL), fresh (FWS) and dry weight (DRS) of shootd, and fresh (FWR) and dry weight (DWR)

**Table 2.** Shoot length, fresh and dry weight of shoots, and fresh and dry weight of roots of Cape gooseberry (*Physalis peruviana*), as a function of temperature and osmotic regulators, at 90 days<sup>(1)</sup>.

Temperature (°C)	Culture media with osmotic regulators		
	Sucrose	Sucrose + mannitol	Sucrose + sorbitol
Shoot length (cm)			
18	12.27aB	5.52aA	18.47bC
25	15.68bB	7.43aA	16.25aB
CV (%)	18.9		
Fresh weight of shoots (g)			
18	0.1974aA	0.1368aA	0.3585aB
25	1.1983bC	0.1725aA	0.4142aB
CV (%)	30.9		
Dry weight of shoots (g)			
18	0.0140aA	0.0135aA	0.0238aB
25	0.0729bB	0.0153aA	0.0235aA
CV (%)	35.0		
Fresh weight of roots (g)			
18	0.0186aA	0.0054aA	0.0617aB
25	0.2433bB	0.0114aA	0.0417aA
CV (%)	68.3		
Dry weight of roots (g)			
18	0.0006aA	0.0014aA	0.0042aA
25	0.0231bB	0.0020aA	0.0035aA
CV (%)	71.1		

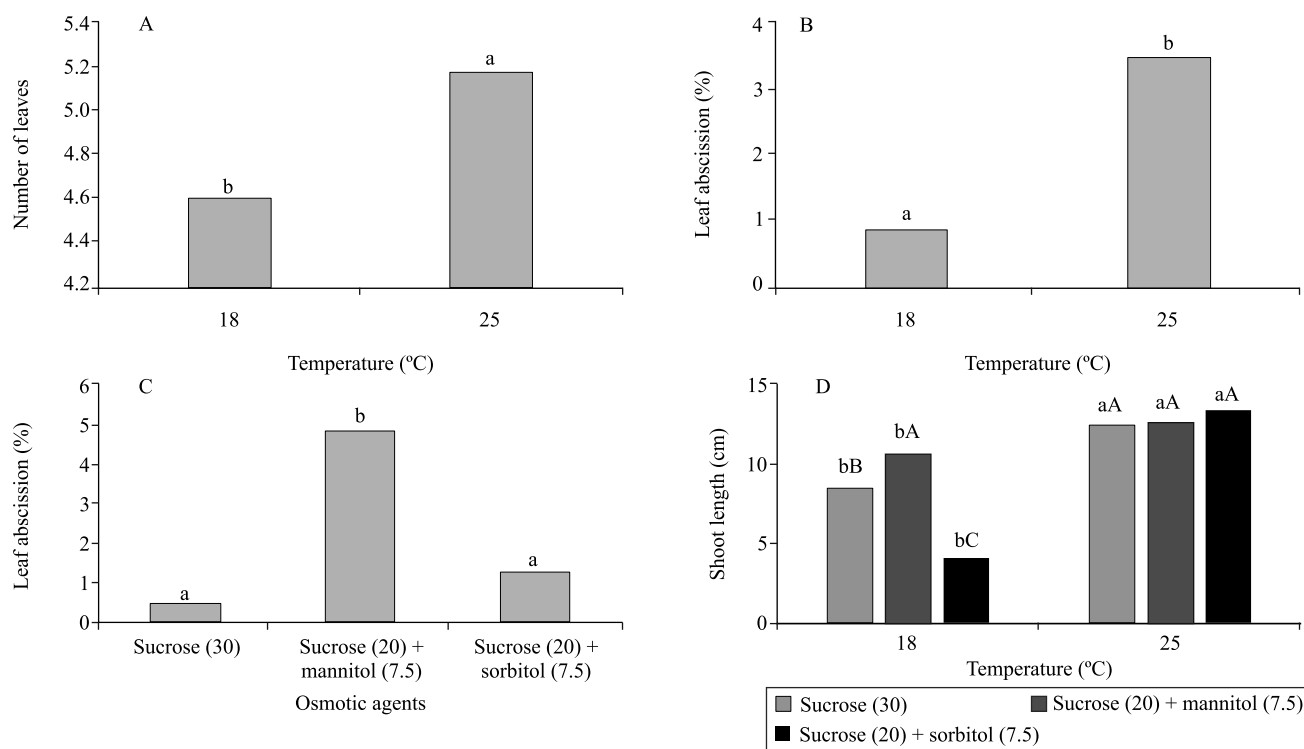
<sup>(1)</sup>Means followed by equal lower case letters in the columns, and upper case letters in the rows, showed no significant differences, by the Tukey's test, at 5% probability.

of roots (Table 2). A lower SL was found in the medium with sucrose + mannitol, irrespectively of the temperature used. This behavior clearly shows the action of osmotic agents as growth retardants; in this case, osmotic agents acted more than temperature. In general, there were no significant effects between temperatures for each culture medium, except for the treatment with sucrose. In the analysis within each temperature, we observed that, at 18°C, the lowest weights were obtained with sucrose, or sucrose + mannitol, while at 25°C the lowest values were found in the media with mannitol, or sorbitol. In addition, the low metabolism of mannitol and sorbitol by plants may have contributed to the low-biomass production.

In an overview, the in vitro conservation of Cape gooseberry was more efficient at 18°C using a medium with sucrose, or at 25°C using mannitol, or sorbitol. However, the lower values of all evaluated characteristics were observed at 18°C. In comparison

to our study, similar results were reported by Arrigoni-Blank et al. (2014) on in vitro-grown sweet potato plants. The authors state that the conservation of that species was successful under the conditions of temperature of 18°C + 30 g L<sup>-1</sup> sucrose. The in vitro conservation of sugarcane was successfully performed using 15°C (compared to 12 and 25°C), and adding 30 g L<sup>-1</sup> sucrose, in comparison to combinations of sucrose with mannitol, or sorbitol (Lemos et al., 2002).

In the growth recovery phase, plants grown at 25°C showed NL 12.4% greater than those stored at 18°C. The lowest values for LA were found in the plants conserved at 18°C (0.87%), in comparison to 25°C (3.46%) (Figure 1). The parameter LA was also lower in plants cultivated in medium with sucrose, or sucrose + sorbitol. The SL analysis showed that the temperature of 25°C favored the higher shoot growth, regardless of the medium used (Figure 1 D). During the conservation period, the plants cultivated with mannitol showed the



**Figure 1.** Number of leaves, leaf abscission, and shoot length of Cape gooseberry (*Physalis peruviana*), at the growth recovery phase, after in vitro conservation at different temperatures and osmotic agents. Means with different letters indicate significant differences between treatments, by Tukey's test, at 5% probability. Lowercase letters in figures A, B, and C. Figure D: uppercase letters indicate differences between osmotic agents, at each temperature, and lowercase letters indicate differences between temperatures.

lowest SL. However, in the recovery step, such plants grew normally.

Cape gooseberry can be conserved in vitro at 18°C, using 30 g L<sup>-1</sup> sucrose. These plants show a satisfactory growth during the growth recovery stage.

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