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Cost reduction in the micropropagation of *Solanum lycopersicum* L. var. *cerasiforme*

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Abstract
The aim of this study was to establish a low-cost alternative protocol for micropropagation of *Solanum lycopersicum* L. var. *cerasiforme*, popularly known as cherry tomato. In the *in vitro* establishment, culture media containing Laboratory Reagent-grade (LR) and commercial sucrose and varied concentrations of corn starch and agar were tested. The replacement of thermal sterilization, using autoclave, with chemical sterilization, adding sodium hypochlorite (2%) in the medium, was also evaluated. In the multiplication stage, the medium was supplemented with agar and/or corn starch and commercial sucrose. For rooting, a growth regulator-free medium with commercial sucrose supplemented with agar and/or starch was used. The microplants were then transplanted into plastic containers containing only garden substrate and subsequently acclimatized in a greenhouse. The results make it possible to conclude that the reduction of costs in the micropropagation of cherry tomato can be obtained by replacing LR sucrose with commercial sucrose, and by the use of chemical sterilization of the culture medium with sodium hypochlorite. The replacement of agar with corn starch can be done partially, in the stages of establishment and multiplication, and totally, during rooting.
Keywords: gelling agent; chemical sterilization; carbon source; alternative medium; cherry tomato.

Redução de custos na micropropagação de *Solanum lycopersicum* L. var. cerasiforme

Resumo
O objetivo deste estudo foi estabelecer um protocolo alternativo de baixo custo para micropropagação de *Solanum lycopersicum* L. var. cerasiforme, conhecida popularmente como tomate cereja. No estabelecimento in vitro foram testados meios de cultura contendo sacarose P.A. e comercial e concentrações variadas de amido de milho e ágar. Também avaliou-se a substituição da esterilização física pela esterilização química. Na etapa de multiplicação o meio foi suplementado com ágar e/ou amido de milho e sacarose comercial. Para o enraizamento utilizou-se meio isento de regulador com sacarose comercial suplementado com ágar e/ou amido. As microplantas foram transplantadas para terra vegetal e aclimatizadas em casa de vegetação. Os resultados permitem concluir que a redução de custos na micropropagação do tomate cereja é obtida pela substituição de sacarose P.A. pela sacarose comercial, substituição parcial (estabelecimento e multiplicação) e total (enraizamento) de ágar por amido de milho e pela utilização de esterilização química do meio.
Palavras-chave: agente gelificante, esterilização química, fonte de carbono, meio alternativo, tomate cereja.

1. Introduction

Solanum lycopersicum L. var. *cerasiforme*, popularly known as cherry tomato, is a variety of tomato plants of the Solanaceae family widely cultivated in Brazil, given its economic, food and even medicinal importance. Its fruit has low calorie, is rich in vitamins, lycopene and calcium, and is considered an appetizing adornment. Demand for this species has grown due to the great acceptance by consumers and a growing

interest by farmers, given the good market prices (SILVA *et al.*, 2011; SOLDATELI *et al.*, 2020).

Tomato plants are highly susceptible to pests and diseases, requiring a large amount of chemical pesticides, besides being a plant very sensitive to climate change and its cultivation requires ample spaces (SILVA *et al.*, 2011; CAMPOS MENEZES *et al.*, 2012). The micropropagation of this species can thus be an efficient alternative to meet the commercial demand, being a technique widely used by the agricultural sector, as it offers the possibility of producing seedlings at any time of the year, great multiplication of genotypes with excellent phytosanitary status, thus avoiding the spread of pests and diseases, all in a short time and reduced space (LAMEIRA *et al.*, 2000), besides showing uniformity in the development of seedlings, which allows the standardization of planting and synchronization of harvest.

Micropropagation can be divided into four steps. The first step comprises the *in vitro* establishment, where there is the selection and disinfestation of explants. In step number two, the sprouts are induced through the proliferation of existing buds of somatic organogenesis or embryogenesis. Usually, in this step, plant regulators such as auxins and cytokinin are used. The third step is characterized by the transfer of the sprouts produced to a rooting medium, usually containing auxins. Finally, acclimatization occurs, in which microplants undergo a process that promotes their gradual passage from the *in vitro* to the *ex vitro* environment. Although micropropagation is adopted for several plant species, its commercial use is still limited. Its main disadvantage is the high costs associated with infrastructure, chemical reagents, and equipment such as the autoclave, which is expensive and generates high energy consumption.

The use of low-cost alternative protocols has been shown to be viable for *in vitro* cultivation of plants. Kodym and Zapata-Arias (2001) reported the use of commercial sucrose as a substitute for grade sucrose in tissue culture, as well as the effect of replacing agar with corn starch and potato starch on banana micropropagation. Costa *et al.* (2007) partially and totally replaced agar with cassava starch in the establishment and multiplication of banana and pineapple. The partial replacement of agar with corn starch was also indicated for *in vitro* multiplication of apple cv. Galaxy by Erig *et al.* (2004). In addition to these, Teixeira *et al.* (2006, 2008) and Ribeiro *et al.* (2011) established a protocol for the use of NaClO (sodium hypochlorite) in chemical sterilization as an alternative for reducing costs. Low-cost micropropagation protocols are significant for agronomic development and intensification of *in vitro* production of seedlings, because the difficulty of access to analytical reagents is critical in developing countries such as Brazil, either due to the high cost of the product (intensified by the economic crisis and the increase in the dollar price) or due to the lack of financing for acquisition, especially by small seedling producers (RESENDE *et al.*, 2021).

In vitro propagation of cherry tomatoes was reported by Otroshy *et al.* (2013), who used a conventional protocol of tissue culture with high added value; however, low-cost protocols for *in vitro* cultivation of this species are not found in the literature. Given the high commercial demand, the establishment of economically viable methods can contribute to strengthening the cultivation and commercialization of this fruit. In this context, the present study evaluated the effects of commercial sucrose as a carbon source, corn starch as partial or total substitute for agar in the gelling of the medium and the efficiency of chemical sterilization on the micropropagation of cherry tomatoes.

2. Material and Methods

The following methods were performed in the Plant Tissue Culture Laboratory at the Horto Florestal Experimental Unit of the Department of Biological Sciences of the State University of Feira de Santana in Feira de Santana, Bahia, Brazil.

2.1 Obtaining of seeds and *in vitro* establishment

The seeds obtained from fresh fruits were dried at room temperature for 3 days and, subsequently, in a laminar flow hood, were disinfested in 70% ethanol solution for one minute and then in sodium hypochlorite (2.5%) solution for 15 minutes. After rinsing in distilled water for 3 times, the seeds were introduced into test tubes containing 10 mL of medium composed of the inorganic salts of MS (MURASHIGE; SKOOG, 1962) with half of the concentrations (MS ½) and the vitamins thiamine, pyridoxine HCL, nicotinic acid, myo-inositol and glycine, in addition to pH adjusted to 5.8.

The replacement of Laboratory Reagent-grade (LR) sucrose with commercial sucrose consisted of the following treatments: T1: 30 $g.L^{-1}$ of LR sucrose (control); T2: 30 $g.L^{-1}$ of commercial sucrose.

The replacement of agar with corn starch consisted of the following treatments: T1: 7 $g.L^{-1}$ of agar (control); T2: 15 $g.L^{-1}$ of corn starch + 5.25 $g.L^{-1}$ of agar; T3: 30 $g.L^{-1}$ of corn starch + 3.5 $g.L^{-1}$ of agar; T4: 45 $g.L^{-1}$ of corn starch + 1.75 $g.L^{-1}$ of agar and T5: 60 $g.L^{-1}$ of corn starch.

The replacement of thermal sterilization, using the autoclave, with chemical sterilization of culture medium and glassware, using sodium hypochlorite according to the methodology of Teixeira *et al.* (2006; 2008) consisted of the following treatments: T1: sterilization with autoclave (121 °C, 1.1 $kg.cm^{-2}$ of pressure), for 15 minutes; T2: addition of three drops of 2% NaClO to each liter of distilled water at least one hour before the addition of salts and sucrose. Subsequently 0.15mL of 2% NaClO (0.0003%) was added to the water. After 15 minutes, the volume of water was completed. The culture medium was prepared in a non-sterile environment and the explant was inoculated in a laminar flow hood, according to the methodology of Teixeira *et al.* (2006; 2008).

In all replacements mentioned, the cultures were kept in a growth room with temperature of 25 ± 30 °C, photoperiod of 16 hours and irradiance of 60 µmol m^{-2} s^{-1}.

Germination, characterized by radicle emergence, was monitored daily. The following variables were evaluated: percentage of germinated seeds (%G), mean germination time (MGT) (LABOURIAU, 1983) and germination speed index (GSI) (MAGUIRE, 1962). At the end of 30 days, shoot (SL) and root (RL) lengths (cm) and the number of green leaves (NGL) were evaluated.

2.2 *In vitro* multiplication

Explants of the cotyledonary node obtained from *in vitro* germination of the previous stage were inoculated in MS ½ medium supplemented with commercial sucrose (30 $g.L^{-1}$) and different concentrations of corn starch and agar: 7 $g.L^{-1}$ of agar, 3.5 $g.L^{-1}$ of agar + 30 $g.L^{-1}$ of corn starch, and 60 $g.L^{-1}$ of corn starch. At the end of 30 days, the variables percentage of responsive explants for sprouts, number of sprouts per explant and length of the sprouts were evaluated.

2.3 *In vitro* rooting

The sprouts were subjected to rooting in MS ½ medium free of growth regulator, supplemented with commercial sucrose and different concentrations of corn starch and agar: 7 $g.L^{-1}$ of agar, 3.5 $g.L^{-1}$ of agar + 30 $g.L^{-1}$ of corn starch, and 60 $g.L^{-1}$ of corn starch. At the end of 30 days, the variables percentage of rooted sprouts and root length were evaluated.

2.4 Acclimatization

The microplants were transplanted to garden substrate, covered by transparent disposable cups, kept in a greenhouse with 70% shading and irrigated every 3 days. The containers were arranged in trays with a film of water. On the 15th day, the transparent cover was removed. After 30 days, the survival rate of the plants was observed.

2.5 Experimental design and statistical analysis

The experimental design used was completely randomized, with ten replicates of four samples per treatment. The results were tested for normality by the Shapiro-Wilk test. Parametric data were evaluated by analysis of variance (ANOVA) followed by Tukey test, and nonparametric data were evaluated by Kruskal-Wallis analysis of variance followed by Mann-Whitney test. All analyses were performed at 5% significance in the software programs PAST (HAMMER *et al.*, 2009) and SISVAR (FERREIRA, 2011).

2.6 Production cost

Price quotes were obtained in three establishments in the Region of Feira de Santana (Bahia) in the period of May/June 2019. The value of kWh (R$0.83) was calculated based on the region's electricity price in June 2019. Based on the power of the autoclave, it was estimated how many kWh were spent for 15 minutes and its value was calculated (Table 1).

Table 1. Consumption to produce 1 liter of culture medium.

Item	Amount	Price*
LR sucrose	30 g	R$0,86
Commercial sucrose	30 g	R$0,05
Agar	7 g	R$4,99
Corn starch	60 g	R$1,18
Agar+Corn starch1	Agar: 5.25 g + Corn starch: 15 g	R$4,03
Agar+Corn starch2	Agar: 3.5 g + Corn starch: 30 g	R$3,08
Agar+Corn starch3	Agar: 1.75 g + Corn starch: 45 g	R$2,12
Thermal sterilization	1 hour with the autoclave on	R$1,45
Chemical sterilization	0.8 mL of sodium hypochlorite	R$0,003

*Values estimated based on the quote of October 2019.

3. Results and Discussion

3.1 *In vitro* establishment: Effect of the replacement of LR sucrose with commercial sucrose

The means for germination percentage obtained in the media supplemented with LR sucrose and commercial sucrose did not differ significantly from each other. The mean germination time in the medium with addition of commercial sucrose was shorter than that of the control; consequently, a higher germination speed was obtained in this medium. In addition, plants grown in the medium with commercial sucrose showed better results in the variables shoot length and number of green leaves. Root length showed no significant difference between the two treatments (Table 2).

Table 2. Effect of the replacement of LR sucrose (control) with commercial sucrose (CMS) on the *in vitro* establishment of *S. lycopersicum* var. *cerasiforme*.

Treatments	%G	MGT	GSI	SL (cm)	NGL	RL (cm)*
Control	92.0 a	11.70 b	4.12 b	9.60±3.73 B	7.66±2.74 b	5.28±2.02 a
CMS	92.5 a	7.04 a	9.46 a	14.76±2.73 A	11.50±2.09 a	5.22±0.77 a

Values represent the mean ± standard deviation.
Values followed by equal letters in the same column do not differ from each other by Tukey test (lowercase letters) and Mann-Whitney test (uppercase letters) ($p \leq 0.05$).
* Tukey test performed with means transformed into log.

The replacement of LR sucrose with commercial sucrose promotes a great reduction in costs in the *in vitro cultivation*, since in Brazil the cost of 1 kg of LR sucrose is approximately 17 times greater than that of 1Kg of commercial sucrose. The results of this study confirm the efficiency of this replacement without compromising the formation of cherry tomato plants and corroborate those of Kodym and Zapata-Arias (2001) and Bernardi *et al.* (2004), who used commercial sucrose to micropropagate banana seedlings. Agrawal *et al.* (2010) also demonstrated the success of using commercial sucrose in the culture medium to reduce costs in the *in vitro* conservation of bananas (*Musa* spp.).

3.2 *In vitro* establishment: Effect of partial and total replacement of agar with corn starch

The results showed that germination was significantly affected by gelling agents (Table 3). In general, the media supplemented with corn starch led to the highest germination rates, the lowest values of mean germination time and the highest germination speeds when compared to the control medium.

It was observed that the gelling with corn starch gives a less rigid aspect to the culture medium when compared to agar, suggesting that there is a greater availability of water for seed imbibition. Early germination advances plant growth and can be used as an alternative way to bring the removal of explants forward, which would reduce the period necessary for the micropropagation of cherry tomatoes.

Table 3. Effect of partial and total replacement of agar (AG) with corn starch (ST) on the *in vitro* establishment of *S. lycopersicum* var. *cerasiforme*.

Treatments	%G	MGT	GSI	SL (cm)	NGL	RL (cm)*
Control	92.0 d	11.7 e	4.125 d	9.60±3.73 C	7.66±2.74 cd	5.28±2.02 b
ST+AG1	97.5 b	6.72 c	10.157 b	16.83±0.94 A	10.55±1.90ab	7.09±1.44 a
ST+AG2	100.0 a	5.73 a	10.800 a	17.15±1.23 A	11.55±2.45 a	6.88±1.83 ab
ST+AG3	95.0 c	6.48 b	10.058 b	16.00±1.03 B	8.85±2.99 bc	7.70±1.44 a
ST	95.0 c	7.01 d	9.699 c	16.06±2.35 B	5.65±1.49 d	7.19±1.90 a

Values represent the mean ± standard deviation.
Values followed by equal letters in the same column do not differ from each other by Tukey test (lowercase letters) and Mann-Whitney test (uppercase letters) ($p \leq 0.05$).
* Tukey test performed with means transformed into log.

Regarding the control, the treatments with addition of corn starch showed better results for shoot length, with the highest averages in the treatments with the lowest starch concentrations, ST+AG1 (with 15 $g.L^{-1}$ corn starch + 5.25 $g.L^{-1}$ agar) and ST+AG2 (with 30 $g.L^{-1}$ corn starch + 3.5 $g.L^{-1}$ agar) (Fig. 1A). The ST+AG2 treatment also showed better results compared to the other treatments for number of green leaves, not differing only from the ST+AG1 treatment, confirming the efficiency of the combination of agar and corn starch at low concentrations in the gelling of the culture medium. For root length, the treatments with corn starch showed better results than the control, except for ST+AG2, which that did not differ from the treatment gelled with agar (Table 3). This result is due to the fact that treatments with addition of corn starch have higher availability of water, making nutrients diluted.

Figura 1. Micropropagation of *Solanum lycopersicum* var. *cerasiforme*. A. Initial establishment with agar + corn starch + common sucrose; B. Sprouts from the regeneration of the cotyledonary node; C. Sprout in the process of rooting; D. Acclimatized plants. Bar= 1cm.



The possibility of using corn starch as a gelling agent proved to be viable because of its low cost and ease of obtaining, but it is not possible to use it as a total substitute for agar, as it does not provide the best conditions for germination and plant growth. In treatments with higher proportion of corn starch than agar, a greater number of senescent leaves was observed at the end of 30 days, indicating that the availability of nutrients of the culture medium may have been affected by corn starch. Golle *et al.* (2010) also observed that the use of high concentration of corn starch negatively interfered with the germination of *Pinus taeda* L. According to the authors, highly consistent culture media can limit the diffusion of water and nutrients to the cultivated explants.

3.3 *In vitro establishment*: Effect of the replacement of thermal sterilization with chemical sterilization

The obtained data showed that the germination percentage was not significantly affected by the type of sterilization; however, in the chemically sterilized medium, the mean germination time was shorter than that of the control and there was higher germination speed (Table 4), which is positive for the *in vitro* establishment stage. Moreover, the different types of sterilization did not affect plant growth.

Table 4. Effect of the replacement of thermal sterilization (control) with chemical sterilization (ChemS) in the *in vitro* establishment of *S. lycopersicum* var. *cerasiforme*. %C: percentage of contamination.

Treatments	%G	MGT	GSI	SL (cm)	RL (cm)*	NGL	%C
Control	92 a	11.7b	4.125b	9.60±3.73 a	5.28±2.02ª	7.66±2.74a	0 a
ChemS	92 a	9.3 a	5.415 a	10.15±3.33a	5.00±1.45ª	8.86±3.16a	0 a

Values represent the mean ± standard deviation.
Values followed by equal letters in the same column do not differ from each other by Tukey test (lowercase letters) and Mann-Whitney test (uppercase letters) ($p \leq 0.05$).
* Tukey test performed with means transformed into log.

The use of sodium hypochlorite as a sterilizing agent was efficient for decontaminating the medium (Table 4), as observed in other studies with pineapple (TEIXEIRA *et al.,* 2006, OLIVEIRA *et al.,* 2015), sequoia (RIBEIRO *et al.*, 2011), sugarcane (TIWARI *et al.,* 2012) and the "cabeça-de-frade" cactus (RESENDE *et al.,* 2021). In addition, the use of sodium hypochlorite is a viable and efficient option in the sterilization of the culture medium, since it has a lower toxicity index when compared to other products, such as mercury (TEIXEIRA *et al.,* 2006; 2008). The results suggested that chemical sterilization is an economical and sustainable alternative when compared to the cost for the acquisition of an autoclave and the energy expenditure involved (Table 7).

3.4 Effect of partial and total replacement of agar with corn starch on multiplication

Of the treatments evaluated, only in the one with 60 $g.L^{-1}$ of corn starch there was no formation of sprouts. Considering the treatments that had sprouts, there was no significant difference between the gelling agents tested for the variables analyzed (Table 5).

Table 5. Effect of gelling agents on *in vitro* multiplication of *Solanum lycopersicum* var. *cerasiforme*. AG: agar; ST: corn starch.

Treatments	Responsive explants (%)	Number of sprouts per explant	Length of the sprout (cm)
7$g.L^{-1}$ AG	13.04 a	1.66±0.57 a	0.80±0.20 a
3,5 $g.L^{-1}$ AG + 30 $g.L^{-1}$ ST	12.50 a	1.33±0.57 a	0.76±0.51 a
60$g.L^{-1}$ ST	0.00 b	0.00±0.00 b	0.00±0.00 b

Values represent the mean ± standard deviation.
Values followed by equal letters in the same column do not differ from each other by Tukey test (lowercase letters) and Mann-Whitney test (uppercase letters) ($p \leq 0.05$).

This is the first study describing the regeneration of *S. lycopersicum* var. *cerasiforme* plants without the use of plant regulators (Fig. 1B). However, the use of regulators can increase the multiplication rate since this was observed in the study conducted by Otroshy *et al.* (2013), who obtained better results in the *in vitro* propagation of the same species with the use of BAP and IAA (indole-3-acetic acid).

3.5 Effect of partial and total replacement of agar with corn starch on rooting

In treatments with partial or total replacement of agar with corn starch, the

percentage of sprouts responsive to root formation was significantly higher, being 1.86 times higher than that of the control. This suggests that corn starch in the culture medium favored the development of roots. On the other hand, root length did not show significant differences between the three treatments (Table 6).

Table 6. Effect of gelling agents on *in vitro* rooting of *Solanum lycopersicum* var. *cerasiforme*. AG: agar; ST: corn starch.

Treatments	Rooted sprouts (%)	Root length (cm)
7 $g.L^{-1}$ AG	48.48 B	6.09±2.04 a
3,5 $g.L^{-1}$ AG + 30 $g.L^{-1}$ ST	89.65 A	7.25±1.75 a
60 $g.L^{-1}$ ST	90.90 A	7.25±3.08 a

Values represent the mean ± standard deviation.
Values followed by equal letters in the same column do not differ from each other by Tukey test (lowercase letters) and Mann-Whitney test (uppercase letters) ($p \leq 0.05$).

Costa *et al.* (2007) also reported positive results for root development in media with corn starch in the *in vitro* cultivation of pineapple and banana, corroborating the present study. According to Otroshy *et al.* (2013), cherry tomatoes have enough endogenous auxin for root formation. Despite this, the authors observed that the presence of the growth regulator IAA increased root induction. Therefore, the use of corn starch can be considered an alternative for reducing costs in the rooting stage (Fig. 1C), since plant regulators are expensive.

3.6 Acclimatization

After 30 days of transfer from the *in vitro* to the *ex vitro* environment, it was observed that 100% of the plants survived the acclimatization process (Fig. 1D). The use of different gelling agents did not interfere in the success of acclimatization. The plants grew well and looked healthy.

3.7 Production cost

The medium that had good results and proved more viable in the establishment stage was the one with addition of 30 $g.L^{-1}$ of corn starch and 3.5 $g.L^{-1}$ of agar. One liter of this medium costs R$5.25 and it leads to a 34.1% reduction of costs compared to the control. The medium that had the highest efficiency and great reduction of costs in the multiplication and rooting stages was the agar totally replaced with corn starch, costing R$3.35 (Table 7) and promoting a reduction of 58% compared to the control.

Table 7. Production cost per liter of each culture medium used. AG: agar; ST: corn starch; CMS: commercial sucrose; LRS: LR sucrose; ChemS: chemical sterilization; ThermS: thermal sterilization.

Treatments	Price
AG + LRS (Control) + ThermS	R$ 7,97
AG + CMS + ThermS	R$ 7,16
5,25 $g.L^{-1}$ AG+ 15 $g.L^{-1}$ ST + CMS + ThermS	R$ 6,20
3,5 $g.L^{-1}$ AG + 30 $g.L^{-1}$ ST + CMS + ThermS	R$ 5,25
1,75 $g.L^{-1}$ AG + 45 $g.L^{-1}$ ST + CMS + ThermS	R$ 4,29
60 $g.L^{-1}$ ST + CMS + ThermS	R$ 3,35
AG + LRS + ChemS	R$ 6,523

Bernardi *et al.* (2004) reported in their study a reduction of 50.2% in the cost for producing 1 L of culture medium replacing LR sucrose with commercial sucrose, for multiplication of banana cv. Maçã (*Musa* spp. AAB). Tyagi *et al.* (2007) managed to reduce by 73% the production costs of culture medium in the *in vitro* regeneration of turmeric (*Curcuma longa* L.), using commercial sucrose instead of LR

sucrose and the gelling agent isubgol in place of agar.

4. Conclusions

The use of chemical sterilization with NaOH and the replacements of LR sucrose with commercial sugar and agar with corn starch are efficient for the micropropagation of *Solanum lycopersicum* var. *cerasiforme*, representing a reduction of costs in the production of the species.

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