

Xanthan gum and sodium hypochlorite *in vitro* rooting of *Gerbera hybrida* cv.

Essandre

Goma xantana e hipoclorito de sódio no enraizamento *in vitro* de *Gerbera hybrida* cv. Essandre

Goma xantano e hipoclorito de sodio en el enraizamiento *in vitro* de *Gerbera hybrida* cv. Essandre

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Abstract

The aim of this study was to check the use of the following gelling agents: the xanthan gum “Adicel®” and the stabilizer “Super Liga Neutra®” to replace agar in the *in vitro* rooting phase of *Gerbera hybrida* cv. Essandre. Additionally, the possibility of using chemical sterilization of both culture media and glassware with sodium hypochlorite to replace autoclaving was analyzed. The gelling agents, xanthan gum “Adicel®” and the stabilizer “Super Liga Neutra®” were tested at the following concentrations (g L⁻¹): 7; 9; 11; 13; 15; 17; 19; and 21. No concentrations of Super Liga Neutra® provided effective solidification. Concentrations of 17 and 21 g of Adicel® provided a good gelling of the culture medium, which was compared to the medium containing agar (control) with two types of sterilization: autoclaving (for 20 and 40 minutes) and chemical sterilization. Autoclaving for 20 minutes did not provide effective elimination of contamination in the culture medium containing xanthan gum; this only occurred when autoclaving time increased to 40 minutes. Plant development in culture media containing 17 and 21 g of xanthan gum, either sterilized by autoclaving for 40 minutes or at a concentration of 17 g xanthan gum using sodium hypochlorite, was statistically the same as the control that contained agar. However, plant development at a concentration of 21g of xanthan gum in a sterilized medium using sodium hypochlorite was lower than that observed in media containing agar.

Keywords: Culture media; Cost reduction; Micropropagation; Replacing reagents for analysis.

Resumo

O objetivo desta pesquisa foi verificar a utilização dos agentes geleificantes goma xantana e liga neutra, em substituição ao ágar, na fase de enraizamento *in vitro*, de *Gerbera hybrida* cv. Essandre. Também foi avaliada a possibilidade de se utilizar a esterilização química do meio de cultura e vidrarias com hipoclorito de sódio, em substituição à autoclavagem. Os agentes geleificantes goma xantana e liga neutra foram testados nas concentrações (g L⁻¹): 7; 9; 11; 13; 15; 17; 19; 21. A liga neutra não foi eficiente na solidificação em nenhuma das concentrações testadas. As concentrações de 17 e 21 g de goma xantana proporcionaram boa geleificação do meio de cultura que foi avaliado em comparação com o meio contendo ágar (controle) em dois tipos de esterilização: autoclavagem (por 20 e 40 minutos) e esterilização química. A autoclavagem por 20 minutos não foi eficiente na eliminação da contaminação no meio de cultura contendo goma xantana o que só ocorreu quando o tempo de autoclavagem aumentou para 40 minutos. O desenvolvimento das plantas em meios de cultura contendo 17 e 21 g de goma xantana, esterilizado por autoclavagem durante 40 minutos, e na concentração de 17 g de goma xantana esterilizado com hipoclorito de sódio, foi estatisticamente igual ao observado no controle contendo ágar. Entretanto, o desenvolvimento das plantas na concentração de 21 g de goma xantana em meio esterilizado com hipoclorito de sódio foi inferior àquele observado nos meios contendo ágar.

Palavras-chave: Meio de cultura; Redução de custos; Micropropagação; Substituição de reagentes PA.

Resumen

El objetivo de esta investigación fue utilizar agentes gelificantes goma xantano y liga neutra, en sustitución del agar, en la fase de enraizamiento *in vitro* de *Gerbera hybrida* cv. Essandre. Se evaluó la esterilización química del medio de cultivo con hipoclorito de sodio, en lugar de autoclave. Los agentes gelificantes goma xantano y liga neutra se ensayaron a concentraciones (g L⁻¹): 7; 9; 11; 13; 15; 17; 19; 21. La liga neutra no fue eficaz para solidificar en ninguna de las concentraciones. Las concentraciones de 17 y 21 g de goma xantano proporcionaron una buena gelificación del medio de cultivo, el cual se evaluó en comparación con el medio que contiene agar (control) en dos tipos de esterilización: autoclave (durante 20 y 40 minutos) y esterilización química. La esterilización en autoclave durante 20 minutos no fue eficaz para eliminar la contaminación en el medio de cultivo que contenía goma xantano, lo que solo ocurrió cuando el tiempo de esterilización en autoclave aumentó a 40 minutos. El crecimiento de las plantas en medios de cultivo que contenían 17 y 21 g de goma xantano, esterilizados en autoclave durante 40 minutos, y en una concentración de 17 g de goma xantano esterilizada con hipoclorito de sodio, fue estadísticamente igual al observado en el control que contenía agar. Sin embargo, el desarrollo de la planta a una concentración de 21 g de goma xantano en un medio esterilizado con hipoclorito de sodio fue menor que el observado en un medio que contenía agar.

Palabras clave: Medio de cultivo; Reducción de costos; Micropropagación; Reemplazo de reactivo PA.

1. Introduction

Gerbera (*Gerbera jamesonii* Adlam), a species that belongs to the Asteraceae family, is marketed as a potted plant as well as a cut flower and stands out economically in agribusiness of national and international floriculture. Gerberas might be propagated by seeds, clump division, and micropropagation (Deng *et al.*, 2018; Tripathi *et al.*, 2021). Although gerbera cultivation in the northeastern region of Brazil is still emerging, it can be an alternative to crop diversification (de Oliveira Silva *et al.*, 2020)

In commercial production, micropropagation enables the large-scale production of gerbera seedlings with limited space, and it allows obtaining uniform plants, which are free of pests and diseases (Gautam *et al.*, 2021). However, micropropagated seedlings are more costly than traditional seedlings due to the cost of the reagents and equipment used to produce them. Owing to the economic importance of gerbera and micropropagation in the production of quality seedlings, new alternatives to reduce the costs of *in vitro* seedling production have been analyzed, aiming to render this propagation method more affordable.

Regarding cost reduction in tissue cultures, the replacement of costly reagents such as agar considered one of the most expensive chemical components used in culture media (Phillips & Garda, 2019; Dhawale *et al.*, 2021) has been addressed in several studies (Conceição *et al.*, 2021). Agar is the most frequently gelling component used as it has characteristics that provide good support for explants, e.g., stability, high clarity, its non-toxic nature, and resistance to culture metabolites (Dhawale *et al.*, 2021). When choosing low-cost agents to replace agar, the use of starch from cassava, barley, corn, potato,

rice, and wheat or plant-based gums has shown to be effective in medium gelling, which is associated with good plant development (Espinosa-Leal *et al.*, 2018; Naik *et al.*, 2020).

The xanthan gum “Adicel®” and the stabilizer “Super Liga Neutra®” are two gelling agents that are commonly used in the food, agricultural, pharmaceutical, and oil industries (Costa *et al.*, 2019). Xanthan gum is a polysaccharide produced by bacterial species of the genus *Xanthomonas* (Ferraz *et al.*, 2021) which are capable of forming viscous solutions and hydrosoluble gels with unique rheological properties (de Castro, 2019). Super Liga Neutra® is commonly used to manufacture ice creams, as it provides better emulsification and consistency (Almeida *et al.*, 2016).

The use of autoclaving for the sterilization of culture media, glassware, and utensils used in micropropagation is another factor that helps increase costs, due to electric energy consumption and equipment maintenance expenses, along with the fact that it requires more work hours (Pais *et al.*, 2016; Cardoso *et al.*, 2018; da Costa Urtiga, 2019; Lu *et al.*, 2021). Several studies have proven the efficacy of sodium hypochlorite (NaClO) as an alternative to replace autoclaving in the micropropagation of several plant species so far, as it is a product with biocidal activity, low cost, and easy to acquire (Pais *et al.*, 2016; Cardoso & Imthurn, 2018; Siekierzyńska & Litwińczuk, 2018).

Therefore, the use of alternative substances other than agar along with the chemical sterilization of culture media and glassware might lead to benefits such as increased speed, higher efficacy, and reduced costs in *in vitro* plant propagation. Therefore, the present study aimed to evaluate gelling agents such as xanthan gum “Adicel®” and the stabilizer “Super Liga Neutra®” in culture media as well as chemical sterilization of culture media and glassware using sodium hypochlorite in the *in vitro* rooting phase of *Gerbera hybrida* cv. Essandre.

2. Methodology

The experiments described below were conducted in Laboratório de Biotecnologia of Departamento de Tecnologia e Ciências Sociais of Universidade do Estado da Bahia, DTCS Campus III- UNEB, in Juazeiro-BA.

2.1 Preparation of gelling agents

The gelling potential of the xanthan gum Adicel® and the stabilizer Super Liga Neutra® was evaluated to determine which amount would solidify the culture medium. The following concentrations of Adicel® and Super Liga Neutra® were tested: 7g L⁻¹; 9g L⁻¹; 11g L⁻¹; 13g L⁻¹; 15g L⁻¹; 17g L⁻¹; 19g L⁻¹; and 21g L⁻¹. The agents were mixed and homogenized in 1000 mL of deionized, distilled, and autoclaved water. The pH was adjusted to 5.7 ± 1, and subsequently, 20 mL of the solution were distributed in glass flasks with capacity for 250 mL, which were sealed and maintained in a dark room. After 48 hours, evaluations were performed to compare the gelling capacity of Adicel® and Super Liga Neutra® to the medium with 7g L⁻¹ of agar of the commercial brand “ÁgarGel” (control).

Once the concentration and gelling agent were defined as ideal for culture medium solidification, the second stage of the study started, in which three experiments were performed to evaluate plant development in the culture medium with the alternative gelling agent. Different concentrations of alternative gelling agents were tested against agar (control) and two sterilization methods were tested (thermal sterilization by autoclaving and chemical sterilization using sodium hypochlorite).

Experiment I consisted of evaluating different concentrations of the gelling agent in media sterilized by autoclaving (121 °C, 1.1 kg cm⁻² of pressure) for 20 minutes. The following treatments were performed: T1: autoclaved control, solidified with 7g L⁻¹ of agar; T2: culture medium solidified with 17g L⁻¹ of xanthan gum; and T3: culture medium solidified with 21g L⁻¹ of xanthan gum.

Experiment II was like an experiment I, evaluating different concentrations of gelling agents in media sterilized by autoclaving (121 °C, 1.1 kg cm⁻² of pressure); however, it used a period of autoclaving of 40 minutes. The following treatments were performed: T1: autoclaved control, solidified with 7g L⁻¹ of agar; T2: culture medium solidified with 17g L⁻¹ of xanthan gum; and T3: culture medium solidified with 21g L⁻¹ of xanthan gum.

Experiment III consisted of evaluating different concentrations of the gelling agents in media sterilized with sodium hypochlorite, following the protocol developed by Teixeira *et al.* (2006). The following treatments were performed: T1: autoclaved control for 20 minutes, solidified with 7g L⁻¹ of agar. T2: culture medium solidified with 17g L⁻¹ of xanthan gum; and T3: culture medium solidified with 21g L⁻¹ of xanthan gum.

In all experiments mentioned above, the gelling agents were homogenized in a blender with 200 mL of distilled, deionized, and autoclaved water (for experiments I and II) and in distilled and deionized water containing 0.0005% of sodium hypochlorite (for experiment III), and then placed in a microwave until their dissolution.

The medium used for plant rooting in all experiments was comprised of inorganic MS salts (Murashige & Skoog, 1962), White's vitamins (White, 1943), 30g L⁻¹ sucrose, 100 mg L⁻¹ inositol, and had pH adjusted to 5.7 ± 1. After preparation, the culture medium was taken to the laminar flow cabinet and 20 mL were poured into glass flasks with a capacity of 250 mL. In the end, the flasks were sealed and placed in a growth room.

2.2 Plant culture

Gerbera hybrida cv. Essandre explants with three leaves derived from the stock culture were inserted in the culture medium 24 hours after preparation, and after that, placed in a growth room at a temperature of 25 ± 2 °C, 16 hours of photoperiod, and radiance of 19 mol.m⁻².s⁻¹ for a period of 40 days.

2.3 Experimental design

The experiments were conducted in a completely randomized design, with three treatments, eight replicates, and plot consisting of a flask with one plant. All experiment was repeated twice. At the end of the *in vitro* rooting of all the experiments, percentage of survival, mean number of leaves, mean number of roots, mean plant length, mean plant biomass, mean biomass of aerial part, and mean root mass were evaluated. Count data were transformed into $(\sqrt{x + 0.5})$ and mean values were compared using Tukey's test at 5% of error probability, using the statistical program Sisvar (Ferreira, 2008).

3. Results and Discussion

3.1 Gelling agents used in thermal sterilization

None of the concentrations of the stabilizer “Super Liga Neutra®” evaluated was effective in culture medium gelling agents. In addition, lumps that were difficult to dissolve were formed, which provided the medium with an uneven appearance; therefore, no experiments were performed with this agent. The xanthan gum “Adicel®” was an effective culture medium solidifying agent at concentrations of 17 and 21g L⁻¹, and both were evaluated in the experiments. The medium was visually transparent and homogeneous.

In the culture medium containing xanthan gum and autoclaved for 20 minutes, there was 100% contamination after 24 hours, thus hampering explant development. When the medium was autoclaved for 40 minutes, the percentage of explant survival after 40 days of *in vitro* cultivation was 100% in all treatments.

No significant differences were observed between the gelling agents' agar and xanthan gum at both concentrations, 17 and 21g, regarding all variables analyzed (mean number of leaves, mean number of roots, mean plant length, mean plant biomass, and mean mass of root and aerial parts) (Table 1).

Table 1- Mean number of leaves (MNL), mean number of roots (MNR), mean plant length (MPL), mean plant biomass (MPB), mean biomass of aerial part (MBAP), mean root biomass (MRB) of *Gerbera hybrida* cv. Essandre, maintained for 40 days in an *in vitro* rooting medium, according to gelling agent, in a medium sterilized by autoclaving for 40 minutes.

Gelling agents' concentrations	MNL	MNR	MPL (mm)	MPB (g)	MBAP (g)	MRB (g)
Agar 7g	3.33a	1.81a	91.75a	0.68a	0.46a	0.19a
17g xanthan gum	3.22a	1.74a	85.32a	0.65a	0.46a	0.16a
21g xanthan gum	3.02a	1.57a	79.27a	0.61a	0.45a	0.13a
Mean value	3.19	1.70	85.44	0.64	0.46	0.16
CV%	15.06	19.60	31.50	45.31	47.70	57.53

Means followed by the same letters in the column did not differ from each other, according to Tukey's tests, at 5% of significance.
Source: Authors (2022).

Hegele *et al* (2021) checked the possibility of total or partial replacement of agar by 17 alternative gelling agents in nutritious media sterilized by autoclaving for the cultivation of two Africa plantain cultivars, which results showed that media with mung bean could be used as a single substitute for expensive gelling agents such as agar, with a cost reduction by more than 80%. Although our results did not show an increase in growth plants variables when subjected to different gelling agents autoclaved for 40 min, on the contrary, studies evidenced that the type of gelling agent used can influence tissues' growth *in vitro* (Raina, 2017). According to Ozel *et al* (2018) studies showed that tragacanth gum offered positive effects on growth of *Nicotiana tabacum* cv. Samsun Canik plants. Some reports suggest that gelling agents used in tissue culture to solidify medium can contain many mineral nutrients that affect plant growth (Al-Mayahi & Ali, 2021). Soares *et al* (2014) who studied *in vitro* cultivation of *Dendrobium nobile* Lindl., reported that replacing 50% of agar for maize starch provided higher plant growth in culture media sterilized by autoclaving. Studies conducted with pure maize starch or cassava starch combined with agar in culture media sterilized by autoclaving showed that they were effective in culture media solidifying agents in the *in vitro* rooting of 'Gold' pineapples (Oliveira *et al.*, 2015). Vieira (2018) analyzed the hydrogel to replace agar *in vitro* showing potential to be used in the *in vitro* cultivation of *A. thaliana*.

Culture media autoclaved for 40 minutes had a dark coloration, most likely due to sucrose degradation. Additionally, 40 minutes is a very long period for culture medium and glassware sterilization, and what is more, it generates surplus expenses related to electric energy consumption. Therefore, this is not a feasible option to be adopted in seedling production via tissue culture.

3.2 Gelling agents used in chemical sterilization

Aside from analyzing the replacement of reagents for analysis by lower-cost options to reduce final seedling cost, the present study analyzed the efficacy of sterilization using sodium hypochlorite, as it has been reported to be effective in the

chemical sterilization of nutritious media instead of thermal sterilization by autoclaving (Pais *et al.*, 2016; Cardoso & Imthurn, 2018; Conceição *et al.*, 2021). Along with its efficacy, chemical sterilization has also proved to be cost-effective compared to autoclaving, as it represents the easiest and most viable technique for microbial decontamination (RESENDE *et al.*, 2021).

Regarding the evaluation of plants developed in culture media gelled with 17g of xanthan gum and sterilized with sodium hypochlorite, the percentage of explant survival was 100%, and there was no significant difference in all variables analyzed (Table 2) regarding plants cultivated in the medium gelled with 7g of agar and sterilized by autoclaving. Conceição *et al.* (2021) managed the reduction of costs in the micropropagation using chemical sterilization of the culture medium with sodium hypochlorite and the replacement of agar with corn starch with positive results in the plant stages of establishment, multiplication, and rooting of cherry tomato.

The use of NaOCl sterilization of culture medium is also related to incomplete sterilization and/or phytotoxicity for plantlet cultivation reducing its efficiency on micropropagation (Vargas *et al.*, 2016), but our results corroborate with data obtained by other authors, who observe that chemically sterilized tissue culture proved to be effective in the micropropagation with any signals of phytotoxicity (Pais *et al.*, 2016; Cardoso & Imthurn, 2018). However, when 21 g of xanthan gum were added to the medium sterilized with sodium hypochlorite, explant responses regarding most variables analyzed were significantly lower than those of the control treatment. Thus, this concentration had a negative effect on explant development, likely due to an interaction of hypochlorite with increased xanthan gum concentration. Therefore, the treatment gelled with 17g and sterilized with sodium hypochlorite can be used in the *in vitro* rooting of *Gerbera hybrida* cv. Essandre, as it provided the same results as the control treatment.

Table 2- Mean number of leaves (MNL), mean number of roots (MNR), mean plant length (MPL), mean plant biomass (MPB), mean biomass of aerial part (MBAP), mean root biomass (MRB) of *Gerbera hybrida* cv. Essandre explants, maintained for 40 days in an *in vitro* rooting medium, according to gelling agent, sterilized with sodium hypochlorite (NaClO).

Gelling agents' concentrations	MNL	MNR	MPL (mm)	MPB (g)	MBAP (g)	MRB (g)
Agar 7g	3.75a	1.75a	106.41a	0.68a	0.52a	0.15a
17g xanthan gum	3.21a	1.68ab	105.74a	0.62a	0.45a	0.13ab
21g xanthan gum	2.41b	1.29b	39.81b	0.29b	0.23b	0.05b
Mean value	3.12	1.57	83.98	0.53	0.40	0.11
CV%	17.41	20.20	33.79	41.13	39.26	59.25

Means followed by the same letters in the column did not differ from each other, according to Tukey's tests, at 5% of significance.
 Source: Authors (2022).

4. Conclusions

Thermal sterilization by autoclaving for 40 minutes provides effective sterilization of culture media containing xanthan gum but autoclaving for 20 minutes does not perform efficiently in the same conditions. However, chemical sterilization with sodium hypochlorite can be used in culture media for *Gerbera hybrida* cv. Essandre rooting instead of thermal sterilization. Regarding stabilizers, it is possible to use the xanthan gum “Adicel®” as an alternative culture medium

gelling agent instead of agar for *Gerbera hybrida* cv. Essandre rooting. The stabilizer “Super Liga Neutra®” is not effective as a gelling agent due to low geleificant capabilities. The concentration of 17 grams of xanthan gum per liter of culture medium promotes a satisfactory growth of *Gerbera hybrida* cv. Essandre during its *in vitro* rooting phase. These results indicate that further investigations are necessary to identify suitable alternative gelling agents from various sources to reduce high costs in plant cell culture.

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