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Influence of sucrose and medium consistency on *in vitro* multiplication and photosynthetic pigment profile of *Eucalyptus saligna*

Influencia de la sacarosa y la consistencia del medio de cultivo en la multiplicación in vitro y el perfil pigmentario fotosintético de *Eucalyptus saligna*

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SUMMARY

In southern Brazil, *Eucalyptus saligna* is one of the most cultivated species within the *Eucalyptus* genus for forest plantations, thanks to genetic improvement programs. Among the primary vegetative propagation methods for recalcitrant genotypes is micropropagation. This study aimed to investigate the effects of two variables in the culture medium on the micropropagation of *E. saligna*. Nodal segments of 2 cm were introduced into a Murashige and Skoog medium supplemented with 0, 15, 30, and 45 g L^{-1} of sucrose, considering two different medium consistencies: semi-solid (7.5 g L^{-1} of agar), and liquid with a spherical solid support. The experiment followed a completely randomized design, with eight treatments in a factorial scheme and four replicates, each containing 10 explants. After 30 days, several parameters were measured, including the mortality rate, number of shoots per explant, average shoot length, chlorophyll *a*, *b*, and total chlorophyll content. The results indicated that the survival and multiplication rates of explants were higher in the semi-solid medium. In contrast, the liquid medium with solid support proved inefficient, as it failed to establish adequate contact between the explants and the culture medium, compromising the nutrient supply. Generally, survival rates were higher in treatments with increased sucrose concentrations, as higher sucrose levels provide metabolic energy and carbon skeletons essential for the biosynthesis of amino acids, proteins, polysaccharides, and other organic compounds required for growth. Furthermore, the higher sucrose concentration promoted shoot formation, although there was no statistically significant difference among the treatments supplemented with sucrose concentrations of 15, 30, and 45 g L^{-1}. The higher sucrose concentration resulted in a significantly reduced chlorophyll content in the semi-solid medium.

Keywords: eucalyptus, micropropagation, culture medium.

RESUMEN

En el sur de Brasil, una de las especies del género *Eucalyptus* más cultivadas para plantaciones forestales es la especie *Eucalyptus saligna,* como resultado de programas de mejoramiento genético. Entre los principales métodos de propagación vegetativa para genotipos recalcitrantes se encuentra la micropropagación. El objetivo del trabajo fue probar dos variables para el medio de cultivo para micropropagar *E. saligna*. Usamos segmentos nodales de 2 cm introducidos en medio Murashige y Skoog suplementado con 0, 15, 30, y 45 g L^{-1} de sacarosa en dos consistencias de medio diferentes: semisólido (7,5 g L^{-1} de agar) y líquido con un soporte sólido esférico. El experimento fue completamente aleatorio con ocho tratamientos en esquema factorial y cuatro repeticiones de 10 explantes cada una. Después de 30 días, fueran medidos la tasa de mortalidad, el número de brotes por explante, la longitud promedio de los brotes, las clorofilas *a*, *b* y el contenido total de clorofila. Las tasas de supervivencia y multiplicación de los explantes fueron mayores en el medio semisólido, mientras que el medio líquido con soporte fue ineficiente, una vez que no proporcionó contacto suficiente entre el explante y el medio de cultivo, comprometiendo el suministro de nutrientes. Generalmente, la supervivencia fue mayor en los tratamientos con mayor cantidad de sacarosa, ya que proporciona energía metabólica y esqueletos de carbono para la biosíntesis de aminoácidos, proteínas, polisacáridos y otros compuestos orgánicos necesarios para el crecimiento. El aumento de la concentración de sacarosa favoreció la formación de brotes, pero sin diferencia estadística entre los tratamientos suplementados con concentraciones de sacarosa de 15, 30 y 45 g L^{-1}. La mayor concentración de sacarosa promovió un contenido de clorofila significativamente menor en el medio semisólido.

Palabras clave: eucalipto, micropropagación, medio de cultivo.

INTRODUCTION

In southern Brazil, *Eucalyptus saligna* Smith is one of the most widely cultivated tree species. It is known for producing high-density, light red wood of high quality suitable for various applications, including veneers, furniture, construction, boxes, poles, fence posts, cellulose, and charcoal (IPEF 2020). The *Eucalyptus* genus is extensively cultivated in Brazil due to genetic improvements, especially through use of clones. Clonal forests have significantly increased production, improved material quality, and ensured material homogeneity for various industries. Cloning has allowed for the transfer of genetic variance, leading to enhanced volumetric productivity, more desirable wood properties (Furlan *et al.* 2020), and increased resistance to both biotic and abiotic factors (Chagas *et al.* 2018, Sá and Tambarussi 2023).

Due to the increasing demand for *Eucalyptus* clones and the challenges associated with propagating certain genotypes through cuttings and mini-cuttings, micropropagation has gained significant importance for several species, including *E. saligna* (Nakhooda *et al.* 2011), *E. benthamii* Miden & Cambage (Brondani *et al.* 2012) and *E. urophylla* S.T. Blake (Mendonça *et al.* 2020). This technique facilitates the cloning of species that exhibit resistance to adventitious rooting. Additionally, it offers the possibility of rescuing and rejuvenating adult trees through successive repotting cycles in a culture medium. In the case of certain genotypes, *in vitro* rejuvenation is employed to attain juvenility, leading to increased rooting rates (Pijut *et al.* 2011).

Brazil is globally recognized as one a leader in the application of innovations in genetics, improvement and propagation of *Eucalyptus* and continues to pursue technological development to enhance and expand its competitiveness (Grattapaglia 2021). The forest sector in Brazil manages approximately nine million hectares of planted forest areas, with *Eucalyptus* species representing 77 % of this total (IBÁ 2020). Starting in the 1980s, companies began utilizing cloning techniques in their breeding programs, allowing for the replication of favorable genetic combinations. However, addressing the challenges related to rejuvenation and enhancing rooting in the cloning process led to the optimization of techniques like micropropagation for eucalyptus trees. This represents a significant biotechnological advancement that has proven advantageous for large-scale clonal production in terms of both quality and quantity. In Brazil, research in tissue culture has been ongoing since the 1950s, and it now delivers commercial outcomes, including seedling production. Notably, this includes the propagation of ornamental and fruit-bearing species, with 111 active records in the National Registry of Seeds and Seedlings (MAPA 2021).

While micropropagation offers several advantages, the effective application of this technique in the production of *Eucalyptus* clones has been limited. Therefore, it is important to establish optimal micropropagation conditions, including the adjustment of culture medium components such as sucrose and agar (Sahu and Sahu 2013). The concentration of sucrose has a significant impact on culture multiplication and growth. Typically, concentrations between 2 % and 4 % are employed for *in vitro* multiplication. However, reducing sucrose content may lead to improved growth and enhanced photosynthetic competence. Moreover, this reduction can help minimize the risk of microbial contamination and enhance the physiological characteristics of the seedlings (Afreen *et al.* 2002). Similarly, the reduction of agar in the culture medium can optimize the production of micropropagated plantlets, particularly when using liquid culture mediums and/or bioreactors (Penchel *et al.* 2007). Assessing the photosynthetic state during the multiplication process is vital for determining plant survival rates. Analyzing chlorophyll content in *in vitro* plants provides valuable insights into the photosynthetic data throughout the micropropagation process (Souza *et al.* 2020).

Investigating limiting factors within the micropropagation process is essential for a comprehensive understanding of how to design experiments aimed at enhancing plantlet production. Consequently, the knowledge gained from our research can potentially facilitate the development of additional protocols that consider culture medium variables for *E. saligna*. Thus, the main objective of this study is to evaluate the survival and multiplication of *E. saligna* under different culture medium conditions with a specific focus on consistency and sucrose concentration.

METHODS

Experiment conditions. The *in vitro* experiments were carried out at the Laboratory of Forestry Biotechnology, Department of Forest Sciences, and the chlorophyll analyses were carried out at the Laboratory of Plant Ecophysiology of the Phytotechnics and Phytosanitary Department, both located at the Federal University of Paraná, Agrarian Sciences Sector, in Curitiba, Paraná, Brazil.

Material collected. The explants were derived from established *in vitro* cultures of *E. saligna,* originating from selected plants within a clonal garden that was maintained in a greenhouse. Each explant, typically consisting of four to six shoots, was individually placed in a test tube containing 4 mL of Murashige and Skoog (1962) culture medium, 4 µM 6-benzylaminopurine, and 2 µM naphthaleneacetic acid. The different treatments, involving the addition of 0, 15, 30, and 45 g L^{-1} of sucrose, were applied.

Culture medium. In the semi-solid culture medium, 7.5 g L^{-1} agar was incorporated, while for the liquid medium, approximately 7 g of inert spheres (2 mm) per tube were used as solid support. The pH of the culture media was adjusted to 5.8 and sterilized for 20 minutes at 1 atm and at 120 ºC. All cultures were maintained in a growth room under whi-

te light conditions at an intensity of 40 µmol m^{-2} s^{-1}, with a 16-hour photoperiod, and temperatures were kept at 25 ± 2 ºC. After a 30-day incubation period, an evaluation was conducted to determine the survival rate, presence of fungal and bacterial contamination, number of shoots per explant, average shoot length, and chlorophyll *a*, *b*, and total chlorophyll content.

Experimental design. The experimental design followed a completely randomized arrangement with a 4 x 2 factorial scheme (sucrose concentrations and semi-solid or liquid culture medium). Each treatment was replicated four times, and each replicate consisted of 10 explants, yielding a total of 320 explants included in the study.

Chlorophyll analysis. During the evaluation period, the explants were weighed, macerated in a mortar with 15 mL of 80 % acetone, and transferred to test tubes. Subsequently, the tubes underwent centrifugation for 10 minutes at 12,000 rpm and were stored at 4°C. The supernatant of each sample was then measured in a spectrophotometer at wavelengths of 645 nm and 663 nm, using 80 % acetone as control treatment. These analyses were conducted in triplicate. Chlorophylls *a* [1], *b* [2], and total chlorophyll [3] content were determined using the following equations (Arnon 1949):

$$Chlorophyll\ a = \left(\frac{12.7 * abs663 - 2.69 * abs645}{1000 * FM}\right) * V \quad [1]$$

$$Chlorophyll\ b = \left(\frac{22.9 * abs645 - 4.68 * abs663}{1000 * FM}\right) * V \quad [2]$$

$$Total\ chlorophyll = \left(\frac{8.02 * abs663 + 20.2 * abs645}{1000 * FM}\right) * V \quad [3]$$

where:
Chlorophyll *a* = chlorophyll *a* content (mg g^{-1}) of fresh matter
Chlorophyll *b* = chlorophyll *b* content (mg g^{-1}) of fresh matter
Total chlorophyll = total chlorophyll content (mg g^{-1}) of fresh matt
abs645 = absorbance at 645 nm
abs663 = absorbance at 663 nm
V = extract final volume (mL)
FM = fresh mass of material used in the extract (g)

Statistical analysis. The chlorophyll values were subjected to a *log10* transformation, and the data related to sucrose concentrations, semi-solid or liquid culture medium were tested for homogeneity or variances using Bartlett's test. Subsequently, an ANOVA was performed, and means were compared using Tukey's test ($P > 0.05$). Data processing was carried out using ASSISTAT program version 7.7 (Silva 2014).

RESULTS

The results of the analysis of variance (ANOVA) for fungal and bacterial contamination, survival, number and length of shoots per explant, and chlorophyll analysis are presented in table 1.

The material was already established with low rates of microbial development. There was 2.5 % of fungal growth in the semi-solid medium treatments with 0 and 15 mg L^{-1} of sucrose, and 2.5 % of bacterial growth in the treatment with 30 mg L^{-1} sucrose (table 2). The survival rate of explants in the liquid culture medium with solid support was low (between 30.0 % and 37.5 %), except for the treatment with the highest concentration of sucrose (45 mg L^{-1}), which had a 77.5 % survival rate. The highest survival rate (100 %) was observed in the semi-solid culture medium with 15 and 45 mg L^{-1} of sucrose.

The highest number of shoots per explant (23.9) was observed in the semi-solid medium using the highest sucrose concentration (45 g L^{-1}) (table 3). However, this number did not significantly differ from the treatments with 15 g L^{-1} (18.3) and 30 g L^{-1} (20.0) sucrose in the semi-solid medium. An average shoot length of 6.1 mm was obtained. The treatments did not significantly differ from each other, but as the sucrose concentration increased, the length of the shoots decreased. Thus, in both medium consistencies, the shortest length was observed in the sucrose concentration of 45 g L^{-1}. The highest values were observed at 15 g L^{-1} sucrose concentration for both the semi-solid medium and the liquid medium with support.

The chlorophyll contents varied according to sucrose concentration. In the semi-solid medium treatments, the highest contents of chlorophyll *a*, *b*, and total chlorophyll occurred in the absence of sucrose (table 4). The treatment with the highest sucrose concentration resulted in the lowest amount of total chlorophyll per explant. In contrast, the results presented for the liquid medium treatments did not follow the same pattern; in general, they showed higher chlorophyll *a*, *b*, and total chlorophyll contents than the semi-solid media, with the highest contents in the absence of sucrose, as well as at the 30 and 45 g L^{-1} sucrose concentrations.

DISCUSSION

In this study, the survival of explants was influenced by both culture medium consistency and sucrose concentration. In the semi-solid medium, the lowest survival percentage occurred in the absence of sucrose (60 %) (table 2). To reduce production costs in large-scale micropropagation, one possible approach is to eliminate sucrose from the culture medium (Kozai and Kubota 2001). However, plants grown *in vitro* require intense light and CO_2 diffusion to promote photosynthesis, transpiration, and dry matter accumulation (Erig and Schuch 2005), and eliminating sucrose entirely may affect the survival percentage.

Table 1. Results of ANOVA for microbial contamination, survival, number of shoots / explant, length of shoots and chlorophyll content (*a*, *b* and total) of *Eucalyptus saligna* explants multiplied *in vitro* under different concentrations of sucrose and culture medium consistencies.

Resultados de ANOVA para contaminación microbiana, supervivencia, de brotes / explantes, longitud de brotes y análisis de clorofila (*a*, *b* y total) de explantes de *Eucalyptus saligna* multiplicados *in vitro* bajo diferentes concentraciones de sacarosa y consistencias del medio de cultivo.

Fungal contamination	**df**	**Mean Square**	**Fc**	**Pr > Fc**
Treatment	7	5.357143	0.818	0.5828
Repetition	3	4.166667	0.636	0.5999
Error	21	6.547619		
Total	31			
CV (%) = 409.41				
Bacterial contamination	**df**	**Mean Square**	**Fc**	**Pr > Fc**
Treatment	7	3.125000	1.000	0.4553
Error	241	3.125000		
Total	31			
CV (%) = 565.69				
Survival	**df**	**Mean Square**	**F**	**Fc**
Treatment	7	3,742.41071	35.5714 **	3.4959
Error	24	105.20833		
Total	31			
CV (%) = 15.41				
Number shoots / explant	**df**	**Mean Square**	**F**	**Fc**
Treatment	7	198.61107	29.5618 **	3.4959
Error	24	6.71851		
Total	31			
CV (%) = 20.77				
Length of shoots (mm)	**df**	**Mean Square**	**F**	**Fc**
Treatment	7	1.92018	1.8872 ^{ns}	2.4226
Error	24	1.01748		
Total	31			
CV (%) = 16.55				
Chlorophyll *a* (mg g^{-1})	**df**	**Mean Square**	**F**	**Fc**
Treatment	8	2.43745	22.7132 **	3.7054
Error	18	0.10731		
Total	26			
CV (%) = 39.08				
Chlorophyll *b* (mg g^{-1})	**df**	**Mean Square**	**F**	**Fc**
Treatment	8	0.93697	10.7483 **	3.7054
Error	18	0.08717		
Total	26			
CV (%) = 56.36				
Total chlorophyll (mg g^{-1})	**df**	**Mean Square**	**F**	**Fc**
Treatment	8	6.06246	20.5585 **	3.7054
Error	18	0.29489		
Total	26			
CV (%) = 39.88				

** significative $P < 0.01$; * significative $0.01 =< P < 0.05$ and ns no significative $P >= 0.05$.

Table 2. Microbial contamination and survival of *Eucalyptus saligna* explants multiplied *in vitro* under different concentrations of sucrose and culture medium consistencies.

Contaminación microbiana y supervivencia de explantes de *Eucalyptus saligna* multiplicados *in vitro* bajo diferentes concentraciones de sacarosa y consistencias del medio de cultivo.

Treatments	Fungal contamination (%)	Bacterial contamination (%)	Survival (%)
T1 (semi-solid medium; 0 g L^{-1} sucrose)	2.5 ^{a}	0.0 ^{a}	60.0 ^{bc}
T2 (semi-solid medium; 15 g L^{-1} sucrose)	2.5 ^{a}	0.0 ^{a}	100.0 ^{a}
T3 (semi-solid medium; 30 g L^{-1} sucrose)	0.0 ^{a}	2.5 ^{a}	95.0 ^{a}
T4 (semi-solid medium; 45 g L^{-1} sucrose)	0.0 ^{a}	0.0 ^{a}	100.0 ^{a}
T5 (liquid medium; 0 g L^{-1} sucrose)	0.0 ^{a}	0.0 ^{a}	32.5 ^{d}
T6 (liquid medium; 15 g L^{-1} sucrose)	0.0 ^{a}	0.0 ^{a}	30.0 ^{d}
T7 (liquid medium; 30 g L^{-1} sucrose)	0.0 ^{a}	0.0 ^{a}	37.5 ^{cd}
T8 (liquid medium; 45 g L^{-1} sucrose)	0.0 ^{a}	0.0 ^{a}	77.5 ^{ab}

Means followed by the same letter are not statistically different from each other according to Tukey's test ($P > 0.05$).

Table 3. Number of shoots / per explant and shoot length of *Eucalyptus saligna* explants multiplied *in vitro* under different sucrose concentrations and culture media.

Número de brotes / explantes y longitud de brotes de *Eucalyptus saligna* multiplicados *in vitro* bajo diferentes concentraciones de sacarosa y consistencia del medio de cultivo.

Treatments	Number of shoots / explant	Length of shoots (mm)
T1 (semi-solid medium; 0 g L^{-1} sucrose)	7.4^{b}	6.0^{a}
T2 (semi-solid medium; 15 g L^{-1} sucrose)	18.3^{a}	6.5^{a}
T3 semi-solid medium; 30 g L^{-1} sucrose)	20.0^{a}	5.9^{a}
T4 (semi-solid medium; 45 g L^{-1} sucrose)	23.9^{a}	4.7^{a}
T5 (liquid medium; 0 g L^{-1} sucrose)	6.2^{b}	6.8^{a}
T6 (liquid medium; 15 g L^{-1} sucrose)	9.0^{b}	6.9^{a}
T7 (liquid medium; 30 g L^{-1} sucrose)	7.7^{b}	6.1^{a}
T8 (liquid medium; 45 g L^{-1} sucrose)	7.4^{b}	5.7^{a}

Means followed by the same letter are not statistically different from each other according to Tukey's test ($P > 0.05$).

Table 4. Chlorophyll *a*, *b,* and total chlorophyll content in *Eucalyptus saligna* explants multiplied *in vitro* under different concentrations of sucrose and culture medium consistencies.

Contenido de clorofila *a*, *b* y contenido total de clorofila en explantes de *Eucalyptus saligna* multiplicados *in vitro* bajo diferentes concentraciones de sacarosa y consistencia del medio de cultivo.

Treatments	Chlorophyll *a* (mg g^{-1})	Chlorophyll *b* (mg g^{-1})	Total chlorophyll (mg g^{-1})
T1 (semi-solid medium; 0 g L^{-1} sucrose)	0.2^{b}	0.1^{b}	0.4^{b}
T2 (semi-solid medium; 15 g L^{-1} sucrose)	0.1^{bc}	0.0^{cd}	0.1^{bc}
T3 (semi-solid medium; 30 g L^{-1} sucrose)	0.1^{b}	0.1^{bc}	0.2^{b}
T4 (semi-solid medium; 45 g L^{-1} sucrose)	0.0^{c}	0.0^{d}	0.1^{c}
T5 (liquid medium; 0 g L^{-1} sucrose)	1.6^{a}	1.2^{a}	2.8^{a}
T6 (liquid medium; 15 g L^{-1} sucrose)	0.1^{bc}	0.0^{bcd}	0.2^{bc}
T7 (liquid medium; 30 g L^{-1} sucrose)	2.5^{a}	1.1^{a}	3.6^{a}
T8 (liquid medium; 45 g L^{-1} sucrose)	1.4^{a}	0.7^{a}	2.1^{a}

Means followed by the same letter are not statistically different from each other according to Tukey's test ($P > 0.05$).

The liquid medium with solid support was not efficient for the survival and multiplication of explants, as it did not provide sufficient contact between the explant and the culture medium, compromising nutrient supply and leading to mortality. The highest survival rate among the liquid medium treatments (77.5 %) (table 2) occurred with the highest sucrose concentration (45 g L^{-1}), indicating that medium consistency affected the availability of sucrose to the explant.

In addition to survival, the multiplication of explants, as observed through the variable "shoots per explant," was also compromised in the liquid medium, with significantly lower averages compared to the semi-solid medium. Generally, survival was higher in treatments with a higher sucrose concentration because it provides metabolic energy and carbon skeletons necessary for the biosynthesis of amino acids, proteins, polysaccharides, and other organic compounds required for growth. This increases the available energy for *in vitro* explants (Mendes *et al.* 2015).

A higher number of shoots per explant was observed at higher sucrose concentrations, ranging from 18.3 to 23.9 (table 3). These results surpass those observed by Oliveira *et al*. (2011), who obtained approximately 10 shoots per explant after 28 days of cultivating *Eucalyptus grandis* W. Hill *ex* Maiden. x *E. urophylla* S. T. Blake clones in semi-solid Murashige and Skoog medium supplemented with 30 g L^{-1} of sucrose, which is half of what we found (20.0) (table 3).

In our study, we found that liquid medium was not efficient, primarily because it failed to establish sufficient contact between the explant and the nutrients present in the culture medium. However, the limited growth performance observed in the liquid medium does not necessarily imply that eucalyptus micropropagation is unviable under such conditions. For example, the use of bioreactors containing liquid media facilitates the rapid uptake of nutrients through submersion in the nutrient-rich medium and may serve as an alternative for cultivating *Eucalyptus* spp. clones (Oliveira *et al*. 2011).

In general, particularly in the semi-solid culture medium, an increase in the multiplication rate correlates with higher sucrose concentrations (Mendes *et al*. 2015, Souza *et al.* 2020). However, in this study, as the sucrose concentration increased, the shoot length decreased, irrespective of the medium consistency. The shortest lengths were observed at the 45 g L^{-1} sucrose concentration, whereas the longest lengths were recorded at the 15 g L^{-1} sucrose concentration (4.7 and 6.9 mm, respectively) (table 3). In a study involving *Miltonia flavescens* Lindl. Cultivated on Murashige and Skoog medium, Besson *et al.* (2010) reported sucrose supplementation at concentrations of 45 and 60 g L^{-1} influenced aerial part length compared to the 30 g L^{-1} concentration. Similarly, for *Handroanthus impetiginosus* Mart. *ex* DC. Mattos, Souza *et al.* (2020) observed longer aerial part lengths at 0 g L^{-1} sucrose concentration compared to 15 and 30 g L^{-1} sucrose concentrations.

Correia *et al.* (2012) observed that the absence of sucrose in the culture medium favored the development of aerial parts and root systems while also promoting a higher survival rate during the acclimatization of *Cattleya labiata* Lindl. Consequently, elevated sucrose levels in the culture medium hinder aerial part development and are associated with reduced photosynthetic capacity. Therefore, modifying the composition of the culture medium can enhance tissue hardening *in vitro*, leading to improvements in photosynthetic competence and water relations, reducing the need for *ex vitro* acclimatization (Penchel *et al.* 2007).

The determination of chlorophyll contents in *in vitro* plants provides important insights into the photosynthetic state during micropropagation, as these levels influence the plants' survival rate during acclimatization (Souza *et al.* 2020). In the semi-solid medium with a higher sucrose concentration (45 g L^{-1}), the explants exhibited lower chlorophyll content, which can potentially inhibit photosynthesis and render autotrophic growth less viable (Thorpe *et al.* 2008).

Cells that grow with sucrose for extended periods may undergo permanent changes, leading to the loss of their ability to synthesize chlorophyll, as plastids are converted to amyloplasts, resulting in alterations to plastid gene expression (Thorpe *et al.* 2008). In a study by Khan *et al.* (2002) on the growth and photosynthetic rates of *E. tereticornis* Smith under photomixotrophic and photoautotrophic micropropagation conditions, it was observed that explants grown on semi-solid Murashige and Skoog medium with 30 g L^{-1} of sucrose exhibited a negative photosynthetic rate, while suppressing sucrose led to a positive photosynthetic rate. Consequently, sucrose can function as both a negative and positive signal, influencing the levels of photosynthetic proteins and photosynthetic capacity of plants.

We observed that higher sucrose concentrations resulted in increased chlorophyll content in the liquid medium. It is possible that the liquid medium and support system prevented the plants from coming into contact with the carbohydrate, leading to similar behavior as the treatment without sucrose. Under such conditions, plantlets must develop their photosynthetic apparatus during *in vitro* cultivation (Afreen *et al.* 2002). Cells containing chlorophyll exhibit a high photosynthetic capacity, which has positive effects on growth and *ex vitro* survival rate (Penchel *et al.* 2007). Enhancing the autotrophic capacity of *in vitro* plantlets through treatments that promote photosynthesis may contribute to an increased survival rate during the greenhouse transfer phase. Consequently, explants originating from liquid medium cultivation might have an advantage during the *ex vitro* period.

CONCLUSIONS

In conclusion, we found that the semi-solid culture medium is more effective than the liquid medium, and increasing sucrose concentration did not significatively affect the number and length of shoots. Therefore, we recommend

the use of a semi-solid culture medium supplemented with 15 g L^{-1} sucrose for multiplication of *E. saligna*. The semi-solid medium promotes better survival and multiplication rates compared to the liquid medium with spherical support, and the increase in sucrose concentration positively influences the survival rate and multiplication of *E. saligna* explants. Additionally, suppressing sucrose in the semi-solid medium resulted in a higher total chlorophyll content, while chlorophyll contents were higher when cultivated in the liquid medium.

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AUTHOR CONTRIBUTIONS

The Professor Giovana Bomfim de Alcantara conceived the study and experimental design, Mariele Reisdorfer Schorr conducted data collection in the laboratory and analyzed the results and Angela Cristina Ikeda contributed to the discussion and interpretation of the results. All three authors prepared the manuscript.

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