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Research article

Disminución de la oxidación e inducción de estructuras proembriogénicas en *Sideroxylon capiri* (A. DC.) Pittier

Reduction of oxidation and induction of proembryogenic structures in *Sideroxylon capiri* (A. DC.) Pittier

Víctor Rubén López Santos[1], Federico Antonio Gutiérrez Miceli[1], María del Carmen Silverio Gómez[2], Carlos Alberto Lecona Guzmán[1]*

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[1]Laboratorio de Biotecnología Vegetal. Tecnológico Nacional de México, Campus Tuxtla Gutiérrez. México.
[2]Campo Experimental Huimanguillo. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. México.

*Autor para correspondencia;correo-e: leconaguzmancarlos@gmail.com.
*Corresponding author; e-mail: leconaguzmancarlos@gmail.com.

Resumen

Sideroxylon capiri (tempisque) es un árbol tropical de la familia Sapotaceae, considerado de suma importancia debido a su papel biológico, uso tradicional y alto contenido de metabolitos secundarios de interés. No obstante, debido a factores tanto naturales como antropogénicos, en México se ha catalogado como una especie amenazada de acuerdo con la NOM-059-SEMARNAT-2010. Por lo anterior, es crucial el desarrollo de un protocolo de propagación *in vitro* para su conservación. En este contexto, se evaluó el efecto de los reguladores de crecimiento 6-Bencilaminopurina (BAP) y Picloram en la formación, proliferación y desarrollo de callo embriogénico y estructuras proembriogénicas, así como el efecto de la L-Cisteína en la reducción de la oxidación en callos, los cuales fueron expuestos a irradiación UV-B. Los resultados mostraron una respuesta positiva ante la aplicación de la combinación de BAP y Picloram en concentraciones de 4 y 2 mg L^{-1}, respectivamente sobre el crecimiento de los callos. Además, se observó la generación de callos proembriogénicos en concentraciones de 0.5 y 1 mg L^{-1} de BAP y 1.5 y 2 mg L^{-1} de Picloram. Adicionalmente, se logró estandarizar un medio de cultivo que disminuyó los procesos de oxidación y pardeamiento en los callos mediante el uso de L-Cisteína en concentraciones de 100 a 200 mg L^{-1}.

Palabra clave: Embriogénesis somática, fenoles totales, L-Cisteína, recalcitrancia, regeneración, tempisque.

Abstract

Sideroxylon capiri (*Tempisque*) is a tropical tree of the Sapotaceae family, considered of great importance from its biological role, traditional use and high content of secondary metabolites of interest. However, both natural and anthropogenic factors have led to its classification as a threatened species in Mexico according to the NOM-059-SEMARNAT-2010 regulation. Therefore, the development of an *in vitro* propagation protocol for the conservation of this species is crucial. Within this context, the effect of the growth regulators 6-Benzylaminopurine (BAP) and Picloram on the formation, proliferation and development of embryogenic callus and/or pro-embryogenic structures, as well as the effect of L-Cysteine on the reduction of oxidation in callus

which were exposed to UV-B irradiation, was evaluated. Results show a positive response to the application of the combination of BAP and Picloram at concentrations of 4 and 2 mg L^{-1} respectively, on growth callus. Additionally, the generation of pro-embryogenic callus was observed at concentrations of 0.5 and 1 mg L^{-1} of BAP and 1.5 and 2 mg L^{-1} of Picloram. Furthermore, a culture medium was standardized that reduced callus oxidation and browning by using 100 to 200 mg L^{-1} of L-Cysteine.

Key words: Somatic embryogenesis, total phenols, L-Cysteine, recalcitrance, regeneration, *Tempisque*.

Introduction

Sideroxylon capiri (A. DC.) Pittier, better known by its common name as "*tempisque*", is a timber tree that belongs to the Sapotaceae family. Its distinctive morphological characteristics include a cylindrical stem and elongated green leaves; the fruits of *tempisque* are ovoid in shape and contain a single seed with a hard seed (Newman, 2008). *Tempisque* is distributed from Mexico to Panama, except for Belize; its presence is fundamental at an environmental and ecological level in the ecosystems where it lives (García and Di Stefano, 2005).

The *S. capiri* ecosystem involves complex interactions between humans and animals. The leaves and fruits of this species are used as a food source by a wide variety of animals (Hiramatsu *et al.*, 2009; Almazán-Núñez *et al.*, 2021). On the other hand, in the regions where it grows, residents use its fruits in the preparation of condiments for gastronomy; furthermore, its wood is widely used not only as firewood, but also as material for the construction of homes and fences (Díaz *et al.*, 2011; Lazos-Monterrosa *et al.*, 2015). Another use that stands out in some regions of Central America is its medicinal application, since the bark is used to treat kidney infections (Martínez-Silvestre *et al.*, 2022), which confirms its multifaceted function in these societies.

In recent years, interest in *tempisque* has grown due to its potential use as a source of secondary metabolites. Robles-García *et al.* (2016) reported the presence of phenols and flavonoids in methanolic extracts of the leaves. Recently, Martinez-Silvestre *et al.* (2022), demonstrated that the stimulation of *tempisque* callus with UV-B light and laser significantly increases the concentration of phenols and flavonoids in callus, which distinguishes the potential of this species as a natural source of these compounds for their application in biotechnology. These compounds are highly valued in various industries such as pharmaceutical, food and cosmetics, for their antioxidant, anti-inflammatory and anti-cancer activity (Mierziak *et al.*, 2014; Martínez-Silvestre *et al.*, 2022). Many of them are produced in response to stress, although their presence *in vivo* can vary significantly (Li *et al.*, 2020).

However, promoting its use requires rethinking the management of natural populations and plantations, since in recent decades a worrying reduction has been observed in the population of *tempisque* trees in their natural habitat, to the extent that it has been classified in Mexico as a threatened species in NOM-059-SEMARNAT-2010 (Semarnat, 2010). This is attributed to various anthropogenic causes, among which excessive logging, the invasion of its ecosystem and the poor management of the species stand out, combined with the pressures exerted by climate change (Cordero *et al.*, 2003; Antúnez, 2022). Due to the above, the search for conventional propagation strategies to promote seed germination has intensified, such as mechanical and chemical scarification and imbibition, which have not managed to significantly increase germination rates, since they are reported below 30 % (Lazos-Monterrosa *et al.*, 2015).

The application of biotechnological techniques to propagate woody species, such as plant tissue culture, presents challenges due to the low response of tissues to *in vitro* manipulations and the high rate of phenolization and oxidation of explants (Mihai *et al.*, 2023). The high oxidation rate of the explants used (leaf and seeds) in the induction process in the *tempisque* prevents them from generating reactions that

trigger the morphogenic response *in vitro*, therefore the establishment of an efficient regeneration system in which obtaining complete plants has not been possible (Martínez-Silvestre *et al.*, 2022). To date there is no record of *in vitro* regeneration of *S. capiri*, Martinez-Silvestri *et al.* (2022) described obtaining non-friable and compact calli without being able to regenerate shoots and/or somatic embryos due to the high rate of phenolization and necrosis that the calli presented after 30 days of culture. In this same work, the callus obtained were stimulated by UV-B radiation with the aim of obtaining secondary metabolites of interest in *in vitro* systems. An option to solve or reduce the problem of oxidation in *in vitro* systems is the use of antioxidants such as L-Cysteine added to the culture medium, which represents an alternative for the control of oxidative processes in explants thanks to the effect of this amino acid as a reducing agent (Vásquez-Hernández *et al.*, 2021).

The objective of this research was to evaluate the effect of 6-Benzylaminopurine and Picloram on the formation, proliferation and development of embryogenic callus and/or pro-embryogenic structures, as well as the effect of L-Cysteine on the reduction of callus oxidation of *tempisque* previously stimulated with UV-B radiation.

Materials and Methods

Vegetal material

Non-irradiated and UV-B light-irradiated callus from *tempisque* leaf explants obtained by Martínez-Silvestre *et al.* (2022) were used in a previous study. The irradiated calli had been exposed to UV-B light for 0, 5, 10 and 15 min week^{-1}, for 2 weeks. Subsequently, they were maintained in solid Murashige and Skoog (MS) medium supplemented with 4 mg L^{-1} of 6-Benzylaminopurine (BAP) (Sigma Aldrich®, Germany), 30 g L^{-1} of sucrose, 2.5 g L^{-1} of Phytagel (Sigma Aldrich®, Germany) at a pH of 5.7 for 2 months, and replanting was carried out every 15 days. All the material was preserved in a culture room at 25±2 °C, with a 16 h light-8 h dark photoperiod and a light intensity of 35 μmol m^2 s^{-1}.

Effect of L-Cysteine on the oxidation of callus irradiated with UV-B light

To assess the effect of L-Cysteine (Sigma Aldrich®, Germany) and UV-B radiation on oxidation in callus, a multifactorial experimental design was used. The first factor was the concentration of L-Cysteine, with 5 levels, which were: 0, 25, 50, 100 and 200 mg L^{-1}. The second factor was the exposure time, with 4 levels that were 0, 5, 10 and 15 min week^{-1}. Each experimental unit contained a callus and all treatments were carried out with four replications each. Calli were cultured in 15×20 mm test tubes (Pyrex®, 9800-15) containing 10 mL of MS culture medium described above, supplemented with the corresponding concentrations of L-Cysteine. The callus were kept for 30 days in the previously mentioned conditions.

The oxidation percentage was calculated by dividing the callus into four parts and the presence of oxidation in each quadrant was taken as a positive value. The data were analyzed using a multifactorial ANOVA and the means were compared

using the Tukey test ($p \leq 0.05$) using the Statgraphics Centurion XVI® statistical software (StatPoint Technologies Inc., 2010).

Obtaining methanolic extracts

To obtain methanolic extracts the methodology of Martínez-Silvestre *et al.* (2022) with some modifications was carried out. To do this, 1 g of fresh callus from each L-Cysteine exposure treatment was macerated with liquid nitrogen in a mortar until obtaining a fine powder. 10 mL of 99 % v/v methanol were added and maceration continued for 1 min and then allowed to rest for 10 min. The extracts were placed in 15 mL Falcon® tubes and allowed to settle for 24 h at a temperature of 25 °C. Subsequently, they were sonicated (CO-Z®, PS-30) for 20 min and centrifuged (Eppendorf®, 5430 R) at 3 500 rpm for 10 min at 4 °C. The supernatant was recovered from each sample and concentrated in a rotary evaporator (BUCHI® R-100) and then resuspended in 2 mL of methanol. The final extract was placed in Eppendorf® tubes and stored at 4 °C until use. To avoid photooxidation, all steps were carried out in the dark or covering the materials in aluminum foil to limit light exposure.

Quantification of total phenols

The determination of total phenols was carried out based on the methodology described by Koufan *et al.* (2020) with some modifications. A standard curve of gallic acid (Fermont®, Mexico) was performed, dilutions of 0.1 mg mL^{-1} were made from a stock solution (1 mg mL^{-1}). For each sample, 2.1 mL of distilled water, 0.250 *µ*L of Folin-Ciocalteu reagent (Sigma Aldrich®, Germany) and 50 *µ*L of extract were used, which were homogenized in a vortex (IKA®, Vortex 2). Subsequently, 0.5 mL of 20 % (w/v) sodium carbonate was added, homogenized again and allowed to react for 2 h at 24 °C in complete darkness to avoid photooxidation. Finally, the absorbance was measured at 765 nm in a UV-VIS spectrophotometer (Beckman Coulter®, DU 730) using methanol as a blank. The results were expressed as equivalents in mg of gallic acid per gram (mg GAE g^{-1}). Three repetitions were performed per sample and the data obtained were analyzed using a simple ANOVA with the Statgraphics Centurion XIV® statistical software (StatPoint Technologies Inc., 2010).

Induction of embryogenic callus

Two treatments were tested for the induction of somatic embryos, and callus previously stimulated with 2 mg L^{-1} of Picloram (Sigma Aldrich®, Germany) were used as explants. The first treatment (IN1) consisted of MS medium supplemented with 0.5 mg L^{-1} of BAP and 1.5 mg L^{-1} of Picloram. The second treatment (IN2) was MS medium supplemented with 1 mg L^{-1} of BAP and 2 mg L^{-1} of Picloram. For both treatments, 25 mg L^{-1} of L-Cysteine, 30 g L^{-1} of sucrose and 2.5 g L^{-1} of Phytagel were added as a gelling agent. The evaluation period was 30 days and replanting was done 15 days after starting the crop. The response variables were the number of pro-embryos g^{-1} of callus. The pro-embryos were considered as

friable, smooth structures, with defined and semicircular edges, white-yellow in color. The data were analyzed by a simple ANOVA and comparison of means using the Tukey test ($p \leq 0.05$) using the Statgraphics Centurion XIV® statistical software (StatPoint Technologies Inc., 2010).

Results

Antioxidant effect of L-Cysteine in callus irradiated with UV-B light

The addition of L-Cysteine to the culture medium resulted in a reduction in callus oxidation and promoted callus development. The statistical analysis showed that there are significant differences between the concentrations of L-Cysteine supplied to the culture medium at 15 days (p=0.0009) and at 30 days (p=0.0001). Furthermore, a multivariate ANOVA demonstrated that both factors and their interaction have significant effects on oxidation, in particular the effect of L-Cysteine concentrations (p=0.0002) and UV-B light exposure times (p=0.0043) in contrast to the effect of the interaction between factors (p=0.076). Table 1 shows that the highest percentage of oxidation was verified in the callus without the addition of L-Cysteine to the culture medium and exposed to a time of 5 min week^{-1} of radiation. In contrast, the lowest percentages of oxidation at 15 days were observed in calli exposed to 10 and 15 min week^{-1} of radiation, which were developed in MS culture medium supplemented with

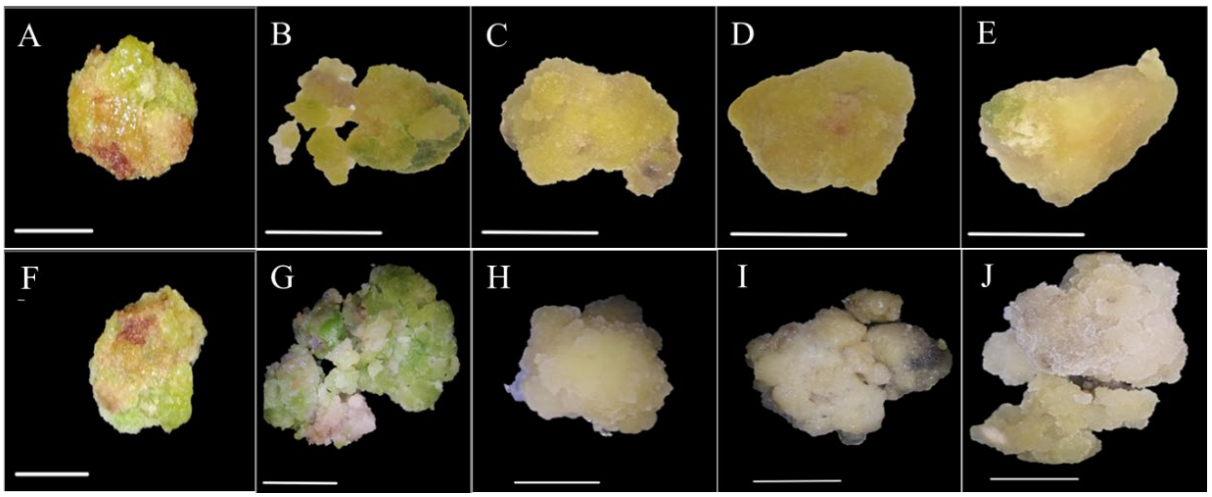
100 and 200 mg L^{-1} of L-Cysteine (Table 1). However, after 30 days, 100 % reduction in oxidation was obtained for all exposure times to UV-B light with the addition of 200 mg L^{-1} of L-Cysteine, which was the best treatment.

Table 1. Effect of L-Cysteine on the oxidation of *Sideroxylon capiri* (A. DC.) Pittier callus after 15 and 30 days of culture.

L-Cysteine (mg L^{-1})	Radiation frequency (min $week^{-1}$)	% oxidation	
		15 days	30 days
0	0	50±20.41^{ab}	50±0.00^{b}
	5	75±14.43^{b}	100±0.00^{c}
	10	25±14.43^{ab}	50±20.41^{b}
	15	25±14.43^{ab}	25±0.00^{ab}
25	0	25±14.43^{ab}	25±14.43^{ab}
	5	50±20.41^{ab}	25±17.68^{ab}
	10	25±0.00^{ab}	25±0.00^{ab}
	15	25±0.00^{ab}	0±0.00^{a}
50	0	50±17.68^{ab}	50±17.68^{b}
	5	25±0.00^{ab}	25±0.00^{ab}
	10	25±0.00^{ab}	0±0.00^{a}
	15	25±10.21^{ab}	0±0.00^{a}
100	0	25±10.21^{ab}	25±10.21^{ab}
	5	0±0.00^{a}	0±0.00^{a}
	10	0±0.00^{a}	0±0.00^{a}
	15	25±0.00^{ab}	0±0.00^{a}
200	0	25±0.00^{ab}	0±0.00^{a}
	5	25±0.00^{ab}	0±0.00^{a}
	10	0±0.00^{a}	0±0.00^{a}
	15	0±0.00^{a}	0±0.00^{a}

Different letters indicate a significant difference between treatments according to the Tukey test ($p \leq 0.05$). Statistical analyzes were performed separately for each evaluation time.

Variations in the color and consistency of the callus were observed throughout the cultivation process, which are related to the concentration of L-Cysteine. Specifically, it was detected that an increase in the concentration of L-Cysteine resulted in a slight loss of pigmentation in the callus, in addition to causing changes in their texture, from a compact to a friable consistency (Figure 1).

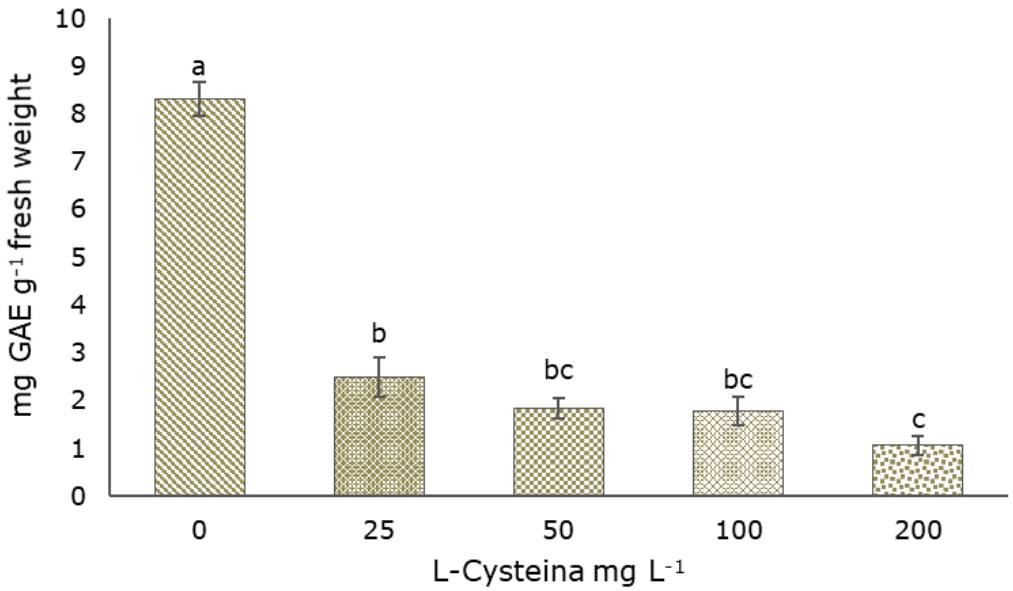


A-E = Callus 15 days after culture establishment in MS medium; F-J = Callus 30 days after culture establishment on MS medium; A and F = Control callus; B and G = 25 mg L^{-1}; C and H = 50 mg L^{-1}; D and I = 100 mg L^{-1}; E and J = 200 mg L^{-1}. The bar is equivalent to 1 cm.

Figure 1. Effect of L-Cysteine on *Sideroxylon capiri* (A. DC.) Pittier callus.

Effect of L-Cysteine on the synthesis of phenols

Regarding the content of total phenols in the *tempisque* callus, significant differences are recognized with respect to the control treatment (Figure 2). The treatment with 200 mg L^{-1} of L-Cysteine recorded the lowest content of total phenols (1.06 mg GAE g^{-1}), and the control treatment the highest (8.312 mg GAE g^{-1}). The rest of the treatments did not show a significant difference between themselves, but a significant decrease can be observed for all treatments and an inversely proportional behavior depending on the amount of L-Cysteine to which the callus were subjected.

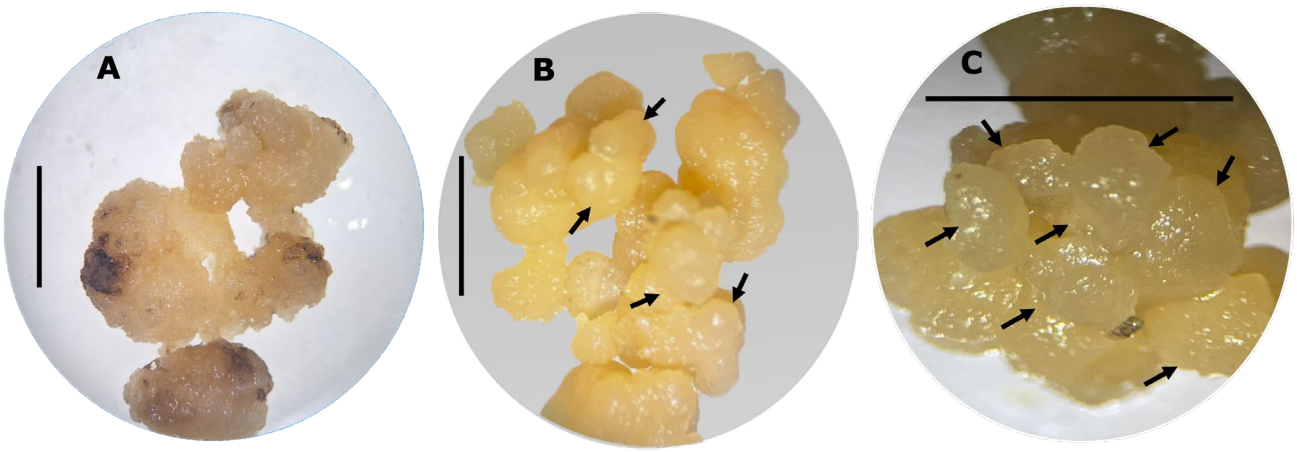


Different letters indicate a significant difference according to the Tukey test ($p \leq 0.05$).

Figure 2. Effect of L-Cysteine on the phenol content in *Sideroxylon capiri* (A. DC.) Pittier callus.

Effect of Picloram on the formation of pro-embryogenic structures

Callus development in media treated with 4 mg^{-1} of BAP and different concentrations of Picloram did not result in the formation of somatic organs or embryos; instead, callus growth and the formation of friable, smooth-textured, semicircular, white-yellow structures were promoted (Figure 3).



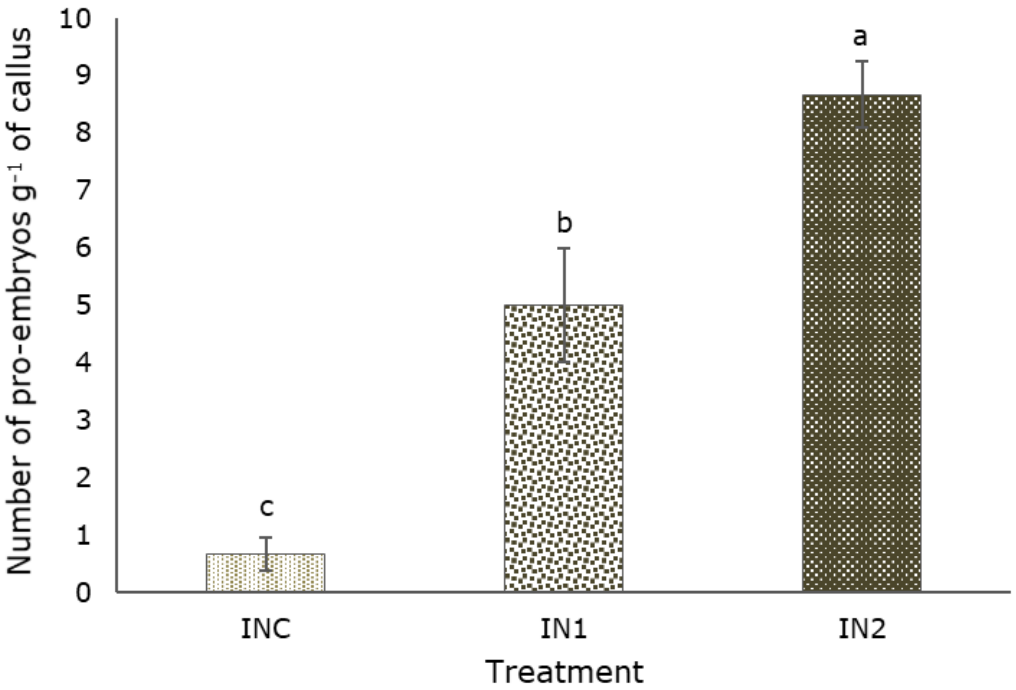
A = Control callus; B = Pro-embryogenic masses from the IN1 treatment seeded in MS medium supplemented with 0.5 mg L^{-1} BAP+1.5 mg L^{-1} Picloram; C = Pro-embryogenic masses from the IN2 treatment in MS medium supplemented with 1 mg L^{-1} BAP+2 mg L^{-1} Picloram. The bar is equivalent to 2 cm.

Figure 3. Pro-embryogenic masses formed in *Sideroxylon capiri* (A. DC.) Pittier calli after 21 days of induction.

In the treatments where the concentration of BAP was reduced and that of Picloram was increased, small semicircular structures formed in the callus, with defined edges that disintegrated compared to the callus of the control treatment (Figure 3C); however, these structures did not progress to more advanced stages of

development, and had characteristics of globular embryos in a primary stage of somatic embryogenesis, with a white-yellow coloration (pro-embryogenic masses).

The IN2 treatment exhibited the best response in terms of the number of pro-embryogenic structures formed, with an average of 9 g^{-1} callus, followed by the IN1 treatment that showed an average of 5 g^{-1} callus; both treatments recorded a significantly greater formation of pro-embryogenic structures compared to the control treatment, in which an average of 1 g^{-1} callus was counted (Figure 4).



INC = Regulator-free control treatment; IN1 = MS medium supplemented with 0.5 mg L^{-1} BAP+1.5 mg L^{-1} Picloram; IN2 = MS medium supplemented with 1 mg L^{-1} BAP+2 mg L^{-1} Picloram. Different letters indicate a significant difference according to the Tukey test ($p \leq 0.05$).

Figure 4. Effect of different concentrations of regulators on the formation of pro-embryos of *Sideroxylon capiri* (A. DC.) Pittier at 21 days of induction.

Discussion

A fundamental challenge for the *in vitro* culture of *S. capiri* is to achieve control of callus oxidation and *de novo* induction of somatic embryos or adventitious shoots. Callus oxidation is observed as a darkening/browning of the tissue. It is a common phenomenon in the *in vitro* culture of tropical species (Pancaningtyas, 2015) and is attributed to the accumulation of phenolic compounds, which when released into the culture medium, induce the activation of enzymes involved in their degradation, such as polyphenol oxidases and peroxidases, which generate quinones, highly reactive compounds that often cause cellular stress (Azofeifa, 2009; Lezcano *et al.*, 2020). These can interfere with the culture from its establishment, its growth and the ability to form pro-embryogenic structures or somatic embryos.

In this work, it was observed that L-Cysteine effectively decreases oxidation in callus cultures treated with UV-B light of *S. capiri* and the subsequent induction of pro-embryos. The positive effect of using L-Cysteine to control phenol synthesis has been recorded in bud cultures of *Pimenta dioica* L. Merr. during the *in vitro* establishment phase with concentrations of 100 and 200 mg L^{-1} L-Cysteine (Vásquez-Hernández *et al.*, 2021), as well as in callus cultures, at a concentration of 25 mg L^{-1}, in which, in addition, they generated callus with a compact texture and green color (Rajput *et al.*, 2023).

The decrease in the content of total phenols in the callus reaffirms the inhibitory effect of L-Cysteine on the synthesis of phenols. This effect is attributed to the thiol group present in L-Cysteine, which traps free radicals and quinones (Cruz-Gutiérrez

et al., 2020), and thus inhibits the action of the polyphenol oxidase enzyme (Richard-Forget *et al.*, 1992). Likewise, the amino acid L-Cysteine acts as a precursor of glutathione, a powerful reducing agent that plays a crucial role in processes of assimilation of reactive oxygen species (ROS) in plant cells (Iqbal *et al.*, 2021; Koramutla *et al.*, 2021; Vásquez-Hernández *et al.*, 2021).

This inhibitory effect of L-Cysteine on the production of phenols, in addition to reducing the oxidation of *tempisque* callus, caused their discoloration, according to the increase in the concentration used and the exposure time (Figure 1). This decrease in coloration is attributed to the alteration of various enzymatic processes linked to secondary metabolism, synthesis of phenols and pigments, as a response to oxidative stress (Azofeifa, 2009).

It is important to note that the cultivation of *tempisque* callus is an option for the production of phenols and other compounds with pharmacological and biotechnological potential (Martínez-Silvestre *et al.*, 2022); however, the application of antioxidants such as L-Cysteine directly affects the accumulation of these compounds, in favor of the development of callus and pro-embryogenic structures, so it may be counterproductive for this purpose. Therefore, the application of these antioxidant agents will depend on the objective pursued, whether micropropagation or the production of secondary metabolites *in vitro*.

On the other hand, the combination of growth regulators can affect the characteristics of the crop, in addition to the oxidation itself (Kamarul *et al.*, 2020; Cabañas-García *et al.*, 2021). For *tempisque*, the joint use of auxins and cytokinins is necessary for the induction and proliferation of callus (Martínez-Silvestre *et al.*, 2022); the results of the present work show that they are also necessary for the induction of pro-embryogenic structures. In particular, combinations of BAP and Picloram are recorded for the growth of callus and for the production of masses or structures with characteristics of globular embryos, in an early phase, of white-

yellow color, in *Polyalthia bullata* King (Kamarul *et al.*, 2020) and in *Euterpe precatori*a Mart. (Barbosa *et al.*, 2022).

Picloram, at concentrations of 2-4 mg L^{-1} in the culture medium, stimulates the growth of callus masses and embryo formation in woody species (Chukwunalu *et al.*, 2018; Kamarul *et al.*, 2020; Barbosa *et al.*, 2022), although effective concentrations may vary depending on the species (Manoharan *et al.*, 2016). This regulator increases the endogenous concentrations of auxin, which allows the formation of the first embryonic stages, by effectively promoting cell elongation and division (Rodríguez *et al.*, 2014), although subsequently it is necessary to decrease the concentration of auxin for it to be allow the transition of the different phases of histodifferentiation (Liu *et al.*, 2021).

In addition to auxins, cytokinins also play a positive role in the acquisition of embryonic capacity by somatic cells, which is why the presence of both regulators is required (Rodríguez *et al.*, 2014; Liu *et al.*, 2021). Cytokinins actively participate in the regulation of plant growth and development through the signal transduction pathway, they help cell differentiation, tissue differentiation, the formation of lateral shoots and help mitigate stress generated by abiotic factors (Li *et al.*, 2021). During the induction stage of somatic embryogenesis, the incorporation of cytokinins, together with auxins, is essential for some woody species such as *Picea abies* (L.) H. Karst., in which these structures are formed after of the elimination of both regulators (Larsson *et al.*, 2008). Likewise, Sánchez *et al.* (2019) report that in the case of coffee (*Coffea arabica* L.), the formation of pro-embryogenic masses is related to the reduction of the concentration of BAP in the culture medium. In contrast, the suppression of cytokinin activity, as a consequence of the overexpression of auxin response regulator (ARR) genes, reduces the formation of somatic embryos or induces their abnormal development since they lack a root

meristem (Su *et al.*, 2015). This demonstrates that cytokinins act as an important factor for auxin-induced somatic embryo formation.

The lack of organogenic or embryogenic response during the cultivation of woody species may be a result of the limited receptivity of plant tissue as a consequence of *in vitro* manipulations (Lazos-Monterrosa *et al.*, 2015). However, the inhibitory effect on development should not be ruled out, which may be linked to the production of phenolic compounds, the concentration and time of exposure to growth regulators that could affect the synthesis of proteins involved in the formation and development of organs and embryos (George *et al.*, 2008; Quiñones *et al.*, 2020). Therefore, the incorporation of compounds such as L-Cysteine represents an alternative to control its excessive accumulation.

Conclusions

In this study, it was demonstrated that for the *in vitro* culture of *S. capiri* callus, the incorporation of L-Cysteine in concentrations of 100 and 200 mg L^{-1} to the medium was efficient to manage oxidation since the synthesis of phenols totals is considerably reduced. On the other hand, the combination of 0.5 mg L^{-1} of BAP with levels between 1.5 and 2 mg L^{-1} of Picloram promotes the formation of pro-embryogenic structures in *tempisque* callus.

These findings offer valuable information that can be used in the formulation of future regeneration protocols, whether through embryogenic or organogenic pathways. This contribution will potentially boost conservation programs, allowing

better use of this species, which is a significant source of secondary metabolites with relevance in the biotechnological field.

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Conflict of interests

The authors declare that they have no conflict of interest.

Contribution by author

Víctor Rubén López Santos, Federico Antonio Guitérrez Miceli and María del Carmen Silverio Gómez: statistical analysis and interpretation of results; Carlos Alberto Lecona Guzmán and Federico Antonio Guitérrez Miceli: conception and design of the study; Carlos Alberto Lecona Guzmán and María del Carmen Silverio Gómez: writing and correction the manuscript. All authors reviewed the results and approved the final version of this paper.

References

Almazán-Núñez, R. C., E. A. Alvarez-Alvarez, P. Sierra-Morales and R. Rodríguez-Godínez. 2021. Fruit size and structure of zoochorous trees: Identifying drivers for the foraging preferences of fruit-eating birds in a Mexican successional dry forest. Animals 11(12):3343. Doi: 10.3390/ani11123343.

Antúnez, P. 2022. Main environmental variables influencing the abundance of plant species under risk category. Journal of Forestry Research 33:1209-1217. Doi: 10.1007/s11676-021-01425-6.

Azofeifa, Á. 2009. Problemas de oxidación y oscurecimiento de explantes cultivados *in vitro*. Agronomía Mesoamericana 20(1):153-175. Doi: 10.15517/am.v20i1.4990.

Barbosa F., J. C., I. M. de A. Silva-Cardoso, R. de Oliveira M. and J. E. Scherwinski-Pereira. 2022. Somatic embryogenesis and plant regeneration from zygotic embryos of the palm tree *Euterpe precatoria* Mart. Plant Cell, Tissue and Organ Culture 148:667-686. Doi: 10.1007/s11240-022-02227-2.

Cabañas-García, E., C. Areche, Y. A. Gómez-Aguirre, J. Borquez, … and E. Pérez-Malphe Balch. 2021. Biomass production and secondary metabolite identification in callus cultures of *Coryphantha macromeris* (Engelm.) Britton & Rose (Cactaceae), a traditional medicinal plant. South African Journal of Botany 137:1-9. Doi: 10.1016/j.sajb.2020.10.002.

Chukwunalu, O., M. Balogun, N. Maroya and R. Asiedu. 2018. Development of micropropagation system for yam (*Dioscorea* spp.) using somatic embryogenesis. International Institute of Tropical Agriculture (IITA). Ibadan, OY, Nigeria. 8 p. Doi: 10.13140/RG.2.2.17246.38726.

Cordero, J., F. Mesén, M. Montero, J. Stewart, … y G. Detlefsen. 2003. Descripciones de especies de árboles nativos de América Central. *In*: Cordero, J. y D. H. Boshier (Edits.). Arboles de Centroamérica, un Manual para el Extensionista. Centro Agronómico Tropical de Investigación y Enseñanza (CATIE). Turrialba, C, Costa Rica. pp. 311-959. https://books.google.com.mx/books?id=q-

0NAQAAIAAJ&printsec=frontcover&hl=es&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false. (21 de agosto de 2023).

Cruz-Gutiérrez, E. J., L. M. Hernández-Fuentes and L. A. Gómez-Reyes. 2020. Uso de L-Cisteina para el control de oxidación *in vitro* de *Annona muricata* L. Revista Multidisciplinaria de Avances de Investigación 6(3):1-6. https://www.remai.ipn.mx/index.php/REMAI/article/view/75. (16 de mayo de 2023).

Díaz L., M. I., J. M. Rodas R., L. R. González S. y M. Vera de O. 2020. Establecimiento *in vitro* de segmentos nodales de *Handroanthus heptaphyllus* de flores blancas. Biotecnología Vegetal 20(3):203-210. https://revista.ibp.co.cu/index.php/BV/article/view/672. (21 de agosto de 2023).

Díaz M., M. G., Ó. Farrera S. y M. A. Isidro V. 2011. Estudio etnobotánico de los principales mercados de Tuxtla Gutiérrez, Chiapas, México. Lacandonia 5(2):21-42. https://repositorio.unicach.mx/handle/20.500.12753/1759. (13 de octubre de 2023).

García, E. G. y J. F. Di Stefano. 2005. Fenología del árbol *Sideroxylon capiri* (Sapotaceae) en el Bosque Seco Tropical de Costa Rica. Revista de Biología Tropical 53(1-2):5-14. Doi: 10.15517/rbt.v53i1-2.14292.

George, E. F., M. A. Hall and G.-J. De Klerk. 2008. Plant tissue culture procedure-Background. *In*: George, E. F., M. A. Hall and G.-J. De Klerk. (Edits.). Plant propagation by tissue culture. Springer. Dordrecht, ZH, The Netherlands. pp. 1-28. Doi: 10.1007/978-1-4020-5005-3_1.

Hiramatsu, C., A. D. Melin, F. Aureli, C. M. Schaffner, M. Vorobyev and S. Kawamura. 2009. Interplay of olfaction and vision in fruit foraging of spider monkeys. Animal Behaviour 77(6):1421-1426. Doi: 10.1016/j.anbehav.2009.02.012.

Iqbal, N., S. Umar, N. A. Khan and F. J. Corpas. 2021. Nitric oxide and hydrogen sulfide coordinately reduce glucose sensitivity and decrease oxidative stress via ascorbate-glutathione cycle in heat-stressed wheat (*Triticum aestivum L.*) plants. Antioxidants 10(1):108. Doi: 10.3390/antiox10010108.

Kamarul Z., M. A., A. M. Azzeme, I. K. Ramle, N. Normanshah, … and S. N. Akmar A. 2020. Induction, multiplication, and evaluation of antioxidant activity of *Polyalthia bullata* callus, a woody medicinal plant. Plants 9(12):1772. Doi: 10.3390/plants9121772.

Koramutla, M. K., M. Negi and B. T. Ayele. 2021. Roles of glutathione in mediating abscisic acid signaling and its regulation of seed dormancy and drought tolerance. Genes 12(10):1620. Doi: 10.3390/genes12101620.

Koufan, M., I. Belkoura, M. A. Mazri, A. Amarraque, … and T. Alaoui. 2020. Determination of antioxidant activity, total phenolics and fatty acids in essential oils and other extracts from callus culture, seeds and leaves of *Argania spinosa* (L.) Skeels. Plant Cell, Tissue and Organ Culture 141:217-227. Doi: 10.1007/s11240-020-01782-w.

Larsson, E., F. Sitbon, K. Ljung and S. von Arnold. 2008. Inhibited polar auxin transport results in aberrant embryo development in Norway spruce. New Phytologist 177(2):356-366. Doi: 10.1111/j.1469-8137.2007.02289.x.

Lazos-Monterrosa, F. A., C. Orantes-García, O. Farrera-Sarmiento, A. G. Verdugo-Valdez, M. S. Sánchez-Cortés y L. E. Ruiz-Meza. 2015. Evaluación de la viabilidad y germinación de tempisque [*Sideroxylon capiri* (A. DC.) Pittier Sapotaceae]. Phyton Revista Internacional de Botánica Experimental 84(1):138-143. Doi: 10.32604/phyton.2015.84.138.

Li, S. M., H. X. Zheng, X. S. Zhang and N. Sui. 2021. Cytokinins as central regulators during plant growth and stress response. Plant Cell Reports 40:271-282. Doi: 10.1007/s00299-020-02612-1.

Li, Y., D. Kong, Y. Fu, M. R. Sussman and H. Wu. 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry 148:80-89. Doi: 10.1016/j.plaphy.2020.01.006.

Liu, Y., C. Wei, H. Wang, X. Ma, H. Shen and L. Yang. 2021. Indirect somatic embryogenesis and regeneration of *Fraxinus mandshurica* plants via callus tissue. Journal of Forestry Research 32(4):1613-1625. Doi: 10.1007/s11676-020-01199-3.

Manoharan, R., J. N. Tripathi and L. Tripathi. 2016. Plant regeneration from axillary bud derived callus in white yam (*Dioscorea rotundata*). Plant Cell, Tissue and Organ Culture 126:481-497. Doi: 10.1007/s11240-016-1017-2.

Martínez-Silvestre, K. E., J. A. Santiz-Gómez, M. C. Luján-Hidalgo, N. Ruiz-Lau, Y. Sánchez-Roque and F. A. Gutiérrez-Miceli. 2022. Effect of UV-B radiation on flavonoids and phenols accumulation in Tempisque (*Sideroxylon capiri* Pittier) Callus. Plants 11(4):473. Doi: 10.3390/plants11040473.

Mierziak, J., K. Kostyn and A. Kulma. 2014. Flavonoids as important molecules of plant interactions with the environment. Molecules 19(10):16240-16265. Doi: 10.3390/molecules191016240.

Mihai, R. A., E. J. Melo H., E. A. Pinto V., I. A. Espinoza C., … and L. I. Florescu. 2023. Somatic embryogenesis of representative medicinal trees in South America—Current status. Forests 14(10):2066. Doi: 10.3390/f14102066.

Newman, M. F. 2008. Flora del Valle de Tehuacán-Cuicatlán, Fascículo 57. Sapotaceae Juss. Instituto de Biología de la Universidad Nacional Autónoma de México. Coyoacán, D. F., México. 20 p.

Pancaningtyas, S. 2015. Study on the presence and influence of phenolic compounds in callogenesis and somatic embryo development of cocoa (*Theobroma cacao* L.). Pelita Perkebunan 31(1):14-20. Doi: 10.22302/iccri.jur.pelitaperkebunan.v31i1.81.

Quiñones A., M., N. Yafac, R. Garavito-Salini y L. Príncipe. 2020. Desdiferenciación celular *in vitro* de *Coffea arabica* L. "Café" var. *caturra* a partir de explantes foliares. Scientia 22(22):141-148. Doi: 10.31381/scientia.v22i22.3573.

Rajput, D. S., T. S. Rathore, S. A. Ansari, M. Faisal, … and A. Shahzad. 2023. Biochemical changes in embryogenic and non-embryogenic callus of *Bambusa*

nutans Wall. during somatic embryogenesis. Plant Cell, Tissue and Organ Culture 155(1):127-135. Doi: 10.1007/s11240-023-02559-7.

Richard-Forget, F. C., P. M. Goupy and J. J. Nicolas. 1992. Cysteine as an inhibitor of enzymic browning. 2. Kinetic studies. Journal of Agricultural and Food Chemistry 40(11):2108-2113. Doi: 10.1021/jf00023a014.

Robles-García, M. A., J. Aguilar A., M. Gutiérrez-Lomelí, F. Rodríguez-Félix, … y C. L. Del-Toro-Sánchez. 2016. Identificación cualitativa de metabolitos secundarios y determinación de la citotoxicidad de extractos de tempisque (*Sideroxylom capiri* Pittier). Biotecnia 18(3):3-8. Doi: 10.18633/biotecnia.v18i3.328.

Rodríguez B., M. M., M. I. Latsague V., M. A. Chacón F. y P. K. Astorga B. 2014. Inducción *in vitro* de callogénesis y organogénesis indirecta a partir de explantes de cotiledón, hipocótilo y hoja en *Ugni molinae*. Bosque 35(1):111-118. Doi: 10.4067/S0717-92002014000100011.

Sánchez J., K., R. Cabrera P. y J. Jiménez D. 2019. Inducción de embriogénesis somática a partir de explantes foliares en tres variedades de café. Scientia Agropecuaria 10(2):259-264. Doi: 10.17268/sci.agropecu.2019.02.11.

Secretaría de Medio Ambiente y Recursos Naturales (Semarnat). 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010 Protección Ambiental-Especies Nativas de México de Flora y Fauna Silvestres-Categorías de Riesgo y Especificaciones Para Su Inclusión, Exclusión o Cambio-Lista de Especies en Riesgo. Diario Oficial de la Federación. 30 de diciembre de 2010. México, D. F., Mexico. 78 p.

StatPoint Technologies Inc. 2010. Statgraphics Centurion XVI.II. Warrenton, VA, United States of America. StatPoint Technologies, Inc.

Su, Y. H., Y. B. Liu, B. Bai and X. S. Zhang. 2015. Establishment of embryonic shoot–root axis is involved in auxin and cytokinin response during *Arabidopsis* somatic embryogenesis. Frontiers in Plant Science 5:792. Doi: 10.3389/fpls.2014.00792.

Vásquez-Hernández, S., C. A. Cruz-Cruz, M. Santiago-Santiago and J. J. Bello-Bello. 2021. Evaluation of different antioxidants during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill): a recalcitrant species. Agro Productividad 14(11):149-157. Doi: 10.32854/agrop.v14i11.2167.