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Induction and characterization of embryogenic calli of *Libidibia ferrea* under LED lighting for in vitro plant regeneration

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INFO

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ABSTRACT

The present study aimed to develop an efficient protocol for indirect somatic embryogenesis in Libidibia ferrea, an Amazonian species of high ecological and pharmacological value. For callus induction, embryonic axis explants were cultured for 45 days on a medium supplemented with different concentrations of the growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) under three light conditions: red-blue LEDs, white LEDs, and darkness. During the maturation phase, the calli were transferred to a medium containing 10 mg L-1 of abscisic acid (ABA) for 15 days, followed by 30 days on a medium supplemented with combinations of benzylaminopurine (BAP) and indole-3-acetic acid (IAA). Callus formation was observed from the 15th day of culture, with the highest induction rate (93.3%) obtained using 5.0 µM L-1 of 2,4-D under red-blue LED light. During maturation, the formation of pro-embryogenic masses and embryos at the globular and torpedo stages was observed. The combination of 13.31 µM L-1 BAP and 2.85 µM L-1 IAA resulted in the highest number of embryogenic calli, although complete conversion into plantlets was not achieved. In total, 31% of the induced calli exhibited embryogenic potential. The developed protocol proved effective for inducing embryogenic calli, representing a methodological advance for the in vitro propagation of L. ferrea. The results highlight the potential of combining plant growth regulators and LED lighting in somatic embryogenesis; however, further optimization of subsequent stages is required to achieve complete plantlet regeneration, which may support future applications.

Palavras-chaves
calogênese
embriogênese somática indireta
micropropagação
jucá

RESUMO

Indução e caracterização de calos embriogênicos de Libidibia ferrea sob iluminação LED para regeneração de plantas in vitro

O presente estudo teve como objetivo desenvolver um protocolo eficiente de embriogênese somática indireta para Libidibia ferrea, espécie amazônica de elevado valor ecológico e farmacológico. Para a indução de calos, explantes de eixo embrionário foram cultivados durante 45 dias em meio de cultura suplementado com diferentes concentrações do regulador de crescimento ácido 2,4-diclorofenoxiacético (2,4-D) sob três condições de luminosidade: LEDs vermelho-azul, LEDs branco e ausência de luz. Para a fase de maturação, os calos foram transferidos para meio de cultura contendo 10 mg L-1 de ácido abscísico (ABA) durante 15 dias, seguindo de 30 dias em meio suplementado com combinações de benzilaminopurina (BAP) e ácido indolacético (AIA). A formação de calos foi observada a partir do 15º dia de cultivo, com maior taxa de indução (93,3%) na combinação de 5,0 µM L-1 de 2,4-D sob LEDs vermelho-azul. Durante a maturação, foi observado formação de massas pró-embriogênicas e embriões em estágio globular e torpedo. A combinação de 13,31 µM L-1 de BAP e 2,85 µM L-1 de AIA resultou no maior número de calos embriogênicos, embora a conversão completa em plântulas não tenha sido alcançada. No total, 31% dos calos induzidos apresentaram potencial embriogênico. O protocolo desenvolvido foi eficaz para a indução de calos embriogênicos, representando um avanço metodológico para propagação in vitro de L. ferrea. Os resultados reforçam o potencial da combinação de reguladores de crescimento e luzes LED na embriogênese somática, entretanto necessita de aprimoramento das etapas subsequentes para a completa regeneração de plântulas que poderão subsidiar futuras aplicações.

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INTRODUCTION

Libidibia ferrea (Mart. ex Tul.) L. P. Queiroz (Fabaceae), popularly known as "jucá" and "pau-ferro," is an endemic Brazilian tree species (Stehmann et al., 2019). Its medical potential is widely recognized in popular medicine, being utilized in treating various conditions, such as inflammation, wounds, and respiratory and gastrointestinal disorders (Almeida et al., 2021; Oliveira et al., 2022). Several studies have demonstrated the presence of secondary metabolites that own anti-oxidant, anti-microbial, anti-inflammatory, antinociceptive, and anticarcinogenic properties, making it an aimed species in either phytotherapy and biotechnology fields (Falcão et al., 2019; Guerra et al. 2017; Lins et al., 2022; Macedo et al., 2024; Nozaki et al. 2007; Ohira et al. 2013).

Micropropagations emerge as a biotechnological tool to overcome the limitations of conventional propagation of *L. ferrea*, allowing the vast production of genetically uniform plants in a short period (Silva et al., 2018; Silva et al., 2020). Furthermore, in vitro cultures make obtaining secondary metabolites of pharmacological interest possible without directly exploiting the natural populations (Murthy et al., 2023; Silva et al., 2024; Wawrosch and Zotchev, 2021). Among the propagation techniques, somatic embryogenesis (SE) is a distinct and efficient process for plant regeneration from the totipotent cells, which can occur through direct and indirect pathways (Guan et al., 2016; Ramírez-Mosqueda, 2022).

Direct Somatic embryogenesis (DSE) occurs when somatic embryos are formed directly from explant cells without an intermediate callus stage. In contrast, Indirect Somatic Embryogenesis (ISE) involves cellular dedifferentiation and embryogenic calli formation, subsequently giving rise to somatic embryos (Von Arnold et al., 2002). ISE offers advantages for the large-scale multiplication of plants and is widely used in the propagation of various plant species (Liu et al., 2021; Kessel-Domini et al., 2022). The induction of embryogenic calli can be influenced by factors such as the type and concentration of growth regulators, the type of explant, the composition of the culture medium, and environmental conditions (Al-Oqab et al., 2022; Lu et al., 2017; Mondal et al., 2020; Zhang et al., 2021).

Among environmental factors, light quality exerces a fundamental role in regulating cell growth and differentiation (Folta et al., 2005; Kim et al., 2004). The use of light-emitting diodes (LED) in in vitro culture allows precise control of the light spectrum, influencing processes such as cell proliferation, morphogenesis, and the accumulation of secondary metabolites (Almeida et al., 2019; Dutta Gupta and Jatothu, 2013). Combined spectra, such as red and blue, have been reported as practical inducers in the embryogenic callus formation of many plant species (Ferreira et al., 2017; Heringer et al., 2017; Khosravi et al., 2024; Silva et al., 2020).

Thus, this study aimed to establish an efficient and reproducible protocol for embryogenesis callus induction of *L. ferrea*, using LED as a lighting source. The effects of light quality and hormonal supplementation were evaluated in terms of their efficiency in ISE in *L. ferrea*. It is expected that the results obtained will contribute to the propagation of the species, providing support for its sustainable use, as well as expanding knowledge on the influence of artificial lighting on the micropropagation processes of biotechnologically important plants.

MATERIAL AND METHODS

Seeds of *Libidibia ferrea* were obtained from the Seed Bank of the Laboratório de Microbiologia e Fertilidade do Solo at the Instituto Nacional de Pesquisas da Amazônia (INPA), Campus III, Manaus, Brazil. Botanical identification was confirmed by Prof. Dr. Luiz Augusto Gomes de Souza, and a voucher specimen was deposited in the INPA Herbarium under number 228,022.

The Seeds were mechanically scarified using an emery stone to facilitate water absorption, followed by asepsis in a neutral detergent solution and rinsing under running water for 1 minute. They were then immersed in Cabrio® Top fungicide (2.0%) for 1 hour under agitation (100 rpm), washed in 70% ethanol for 1 minute, and disinfected with 0.25% sodium hypochlorite (NaClO) for 30 minutes. After asepsis, the seeds were rinsed four times with autoclaved distilled water and soaked for 20 hours. The seeds were transversely cut to remove the cotyledons to obtain the explants, and the embryonic axes were isolated for use as explants in in vitro culture.

The explants were inoculated into glass jars containing 40 mL of MS culture medium (Murashige and Skoog, 1962), supplemented with different concentrations (0.0, 5.0, 10.0, 20.0, and 30.0 μM L^{-1}) of 2,4-dichlorophenoxyacetic acid (2,4-D), as well as 30 g L^{-1} of sucrose and 7 g L^{-1} of agar. The pH was adjusted to 5.8 ± 0.1 before autoclaving. Incubation was carried out in a growth room under three light conditions: LED with 50% red and 50% blue light (TEC-LAMP, Tecnal®); full white LED (T8-TUBE LAMP, Taschibra®); and complete darkness (absence of light). The cultivation conditions included a 16-hour

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photoperiod, irradiance of 31 ± 1 µmol m^{-2} s^{-1}, and a controlled temperature of 25 ± 2°C. The experimental design was completely randomized in a 3 × 5 factorial scheme (three light conditions × five 2,4-D concentrations), totaling 15 treatments, with five replicates per treatment and six explants per replicate. After 45 days of cultivation, the explants were analyzed for callus induction frequency (%) and callus morphological characteristics (texture and coloration). Callus induction was considered positive when visible cell proliferation was observed on the explant.

Calli that exhibited satisfactory quality (friable texture and absence of oxidation) were transferred to MS culture medium containing 10 g L^{-1} of the growth regulator abscisic acid (ABA) to halt cell proliferation and standardize the maturation of embryogenic calli. After 15 days, the calli were transferred to MS medium supplemented with three concentrations (8.87, 13.31, and 17.75 µM L^{-1}) of benzylaminopurine (BAP), either alone or combined with 2.85 µM L^{-1} of indole-3-acetic acid (IAA). The experimental design was completely randomized, with nine replicates per treatment. After 30 days, the calli were observed under a stereomicroscope (Leica MZ7.5) and classified as embryogenic or non-embryogenic. Callus was considered embryogenic when any embryonic developmental stage was observed.

Data were subjected to analysis of variance (ANOVA), followed by Tukey's multiple comparison test ($p \leq 0.05$) to compare treatments. Statistical analyses were performed using Minitab Statistical Software version 22.2.0.

RESULTS AND DISCUSSION

Combined effects of 2,4-D and light quality on callus induction in *Libidibia ferrea*

Callus formation was first observed from the 15th day of culture, becoming more evident between 20 and 30 days. Analysis of variance (ANOVA) indicated that light conditions ($F = 11.37$; $p < 0.001$), 2,4-D concentration ($F = 12.11$; $p < 0.001$), and the interaction between both factors ($F = 8.12$; $p < 0.001$) significantly influenced callus induction in *Libidibia ferrea*.

The induction of embryogenic calli is the initial step in indirect somatic embryogenesis, during which cellular dedifferentiation occurs to form embryogenic cells necessary for conversion into somatic embryos (Von Arnold et al., 2002). Somatic embryogenesis is affected by multiple factors, such as the type and age of the explant and the growth regulators (Vasconcelos et al., 2024; Zhang et al., 2021). Additionally, light quality plays a significant role in plant morphogenetic processes, and there is evidence that LED-emitted light can modulate various physiological and morphological responses (Dutta Gupta and Jatothu, 2013).

In this study, combinations of five 2,4-D concentrations and three light conditions were tested (Table 1). The highest callus formation percentage was observed in treatment L1-2 (93.3%), which used red-blue LED light supplemented with 5.0 µM L^{-1} of 2,4-D, statistically differing from all other treatments according to Tukey's test. Treatments under white LED light exhibited callus formation rates ranging from 23.3% (L2-5 and L2-4) to 33.3% (L2-2), whereas complete darkness resulted in the lowest callus formation, with a maximum of 10.0% (AL-4).

Table 1 - Callus induction in explants of L. ferrea under different light conditions and 2,4-D concentrations.

Treatment	Light Condition	2,4-D (µM L^{-1})	Callus Induction (%)	Texture and Color
L1-1	Red-blue LED	0.0	0.0 ± 0.0 d	-
L1-2	Red-blue LED	5.0	93.3 ± 4.6 a	Friable, green, white
L1-3	Red-blue LED	10.0	0.0 ± 0.0 d	-
L1-4	Red-blue LED	20.0	13.3 ± 6.3 c	Friable, translucent, White
L1-5	Red-blue LED	30.0	10.0 ± 5.5 c	Friable, green, white
L2-1	White LED	0.0	0.0 ± 0.0 d	-
L2-2	White LED	5.0	33.3 ± 8.7 b	Friable, white
L2-3	White LED	10.0	26.6 ± 8.2 b	Friable, white
L2-4	White LED	20.0	33.3 ± 8.7 b	Friable, translucent, white
L2-5	White LED	30.0	23.3 ± 7.8 b	Friable, translucent, white
AL-1	Darkness	0.0	0.0 ± 0.0 d	-
AL-2	Darkness	5.0	0.0 ± 0.0 d	-
AL-3	Darkness	10.0	3.3 ± 3.3 d	Compact, white
AL-4	Darkness	20.0	10.0 ± 5.5 c	Compact, yellowish, white
AL-5	Darkness	30.0	0.0 ± 0.0 d	-

Values are means of five replications ± standard error.
Means followed by different letters differ significantly according to Tukey's test ($p \leq 0.05$).

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The results indicate that the isolated use of 2,4-D is sufficient to induce callus formation in *L. ferrea*, contrasting with the findings of Silva et al. (2018), who achieved a maximum friable callus induction (90%) in nodal segments of the species when the culture medium was supplemented with 1.0 µM L^{-1} 2,4-D in combination with 5.0 µM L^{-1} of the cytokinin thidiazuron (TDZ). Although this combination is common, 2,4-D has exhibited a dominant role in callus induction protocols, whereas TDZ acts as a negative regulator to some extent (Zhang et al., 2021). Thus, the isolated use of 2,4-D may be a more efficient alternative, reducing growth regulators without compromising callus induction.

Combining blue and red LED has been associated with higher efficiency in producing embryogenic callus and somatic embryos (Khosravi et al., 2024). Silva et al. (2020) successfully induced 100% embryogenic callus in *L. ferrea* on MS and B5 media supplemented with 2.21 and 4.42 mg L^{-1} of 2,4-D, respectively, under red-blue LED light (50% red, 50% blue). Similarly, for sugarcane (*Saccharum* spp.), the highest percentage of responsive explants (70%) was obtained on MS medium supplemented with 9 µM L^{-1} of 2,4-D and 1.1 µM L^{-1} of BAP, maintained under red-blue LED light (82% red, 18% blue), resulting in the formation of whitish calli and somatic embryos (Ferreira et al., 2017).

The use of LED in in vitro culture allows precise control over the light spectrum and intensity, owing to the specificity of the wavelengths emitted. This allows applying spectral regions that directly influence plant physiological responses, affecting their morphology and metabolism (Folta et al., 2005; Kim et al., 2004). The blue-red LED spectrum may positively affect callus induction; however, it does not appear to be as effective in regenerating somatic embryos. Ferreira et al. (2017) attribute the low regeneration rate to the predominant formation of non-embryogenic calli under LED lighting. In addition, they also associate the ability to produce embryogenic calli during SEI with species-specific factors, such as biochemical and genetic characteristics. However, they do not rule out the influence of environmental factors, such as light quality, on the acquisition of embryogenic competence.

On the other hand, Heringer et al. (2017) reported that the combination of red and blue LED contributes to improved results in the maturation and conversion of somatic embryos in sugarcane (*Saccharum* spp.). Six light spectrum combinations were tested, and the combination of medium-blue and deep-red light was essential during maturation. Treatments using white-blue LED (12–14% blue), white-blue-red (12–14% blue; 30–50% deep red), and white-blue-red (12–14% blue; 30–50% deep red; 12% far-red) resulted in the highest numbers of somatic embryos. The highest conversion of somatic embryos into plantlets was observed under white-blue-red LED lighting (12–14% blue; 30–50% deep red; 12% far-red).

The results of the present study confirm that the combination of the growth regulator 2,4-D with red-blue LED illumination was decisive for callus induction in *L. ferrea*, promoting morphogenic responses and cellular dedifferentiation of tissues when compared with treatments conducted in the absence of light or under white LEDs. The proposed protocol showed consistent performance when compared with studies using a similar light spectrum and further demonstrated that the use of 2,4-D alone, combined with an appropriate spectral quality, was sufficient to trigger the callogenic process in the species.

Influence of light quality and photoperiod conditions on callus morphology in *Libidibia ferrea*

In terms of morphological aspect, the calli texture varied between friable and compact, and the color varied from white and translucid to green and yellowish (Figure 1). The results showed that calli were induced with different textures and colors, even under the same combination of 2,4-D concentration and light source (Table 1). Predominantly, friable calli with translucent coloration were observed in treatments under red-blue and white LED lighting. In contrast, calli exhibited a compact texture and yellowish coloration in the absence of light. Non-embryogenic calli were primarily compact, green, and yellowish, whereas embryogenic calli were friable, white, translucent, and green.

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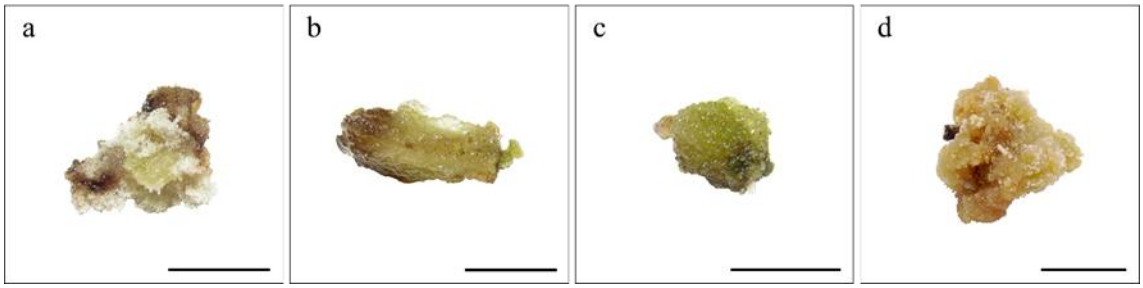

Figure 1 - Texture and coloration of calli induced from Libidibia ferrea explants under different culture conditions. a) friable and white callus; b) friable and translucent callus; c) friable and green callus; d) compact and yellowish callus. Scale bar = 1 cm.

The variation in callus color may be related to the explants response to the concentration and type of growth regulators present in the culture medium (Yunita et al., 2021). Different hormone combinations, types of explants, types of light, and their spectrum can also influence this characteristic, resulting in distinct callus coloration (Afshari et al., 2011; Sari et al., 2018). The white coloration may be associated with the absence of chlorophyll production. In contrast, greenish calli suggest possible partial cellular differentiation and the onset of the photosynthetic pathway, especially under high cytokinin concentrations and light exposure (Streit et al., 2005). Conversely, yellowish calli are associated with high auxin concentration or even with chlorophyll degradation, accumulation of phenolic compounds, and lignification (Sari et al., 2018).

Analyzing the isolated effect of light conditions, we observed that callus induction under LED treatments was higher than observed in the absence of light. However, no significant differences were found between the treatments (Figure 2).

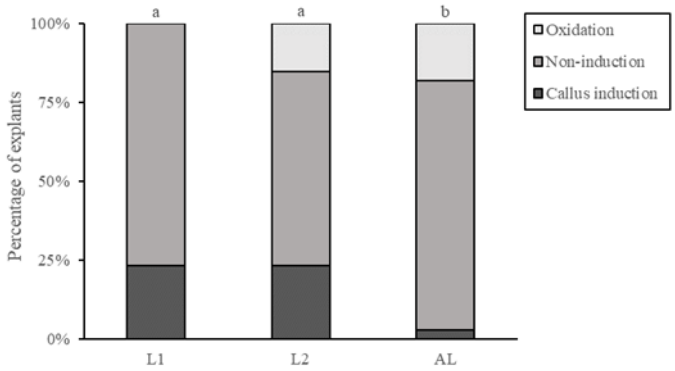

Figure 2 - L. ferrea explants behavior under different light conditions: LED red-blue (L1); LED white (L2); darkness (absence of light) (AL).

The induction averages were 23.3% under both LED conditions, while the explants with no morphogenic response reached 76.6% in L1 and 61.3% in L2, with 15.3% showing oxidation. In the absence of light, callus induction was reduced to 2.6%, with 79.3% of the explants showing no morphogenic response and 18% undergoing oxidation. These results indicate that the presence of light, regardless of the spectrum, favored callus induction, while its absence significantly impaired this response. The predominance of friable and translucent calli under LED illumination indicates that light favors cellular organization and photomorphogenic metabolism, corroborating previous observations by Afshari et al. (2011) and Sari et al. (2018), who also reported a direct influence of light intensity and spectral quality on callus color and texture, suggesting its role as a regulatory factor in in vitro morphogenesis.

Response of calli from *Libidibia ferrea* to the maturation stage and assessment of their embryogenic potential

The number of calli obtained was relatively low compared to the total number of inoculated explants. Therefore, considering the quality of the material, friable calli that showed no signs of oxidation were selected and transferred to the maturation medium. A 27.0% reduction in the induction rate was observed at this stage. After 30

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days, calli that exhibited proembryogenic mass (PEM) or any other formation stage were considered embryogenic (Table 2). The highest percentage of embryogenic calli was observed in the treatment supplemented with 13.31 µM L^{-1} of BAP and 2.85 µM L^{-1} of IAA (55.6%). Distinct embryogenic stages were observed in the calli.

Table 2 - Embriogenic callus and embryonic stages observed in L. ferrea explants after 30 days in MS medium with combinations of three different BAP concentrations with and without IAA.

BAP (µM L^{-1})	IAA (µM L^{-1})	Total callus number	Embryogenic callus number	Embryogenic callus (%)	Embryonic stage
8.87	0.00	9	4	44.4	PEM, globular
8.87	2.85	9	3	33.3	PEM
13.31	0.00	9	4	44.4	PEM
13.31	2.85	9	5	55.6	MPE
17.75	0.00	9	3	33.3	PEM, globular, torpedo
17.75	2.85	9	4	44.4	PEM, torpedo

Formation of PEM occurred at all concentrations and combinations of BAP and IAA tested. The development of embryogenic calli arises from the development of proembryogenic masses, which can appear both on the surface and within the callus. These masses consist of cells with embryogenic potential, capable of reorganizing and giving rise to somatic embryos, either individually or in groups (Halperin, 1966). Auxin plays an essential role in this process, stimulating both callus induction and proliferation and forming PEM (Liu et al., 2021). Furthermore, the concentration and type of auxin used directly influence the embryogenic competence of the cells, determining the efficiency of somatic embryogenesis and the viability of the formed embryos (Kępczyńska and Kępczyńska, 2023; Kumar et al., 2020).

Globular and torpedo-stage embryos were also observed (Figure 3). The results indicate that embryogenic calli and PEM formed during the 30-day maturation period, and globular embryos began to differentiate at the end of this stage. However, the presence of torpedo-stage embryos suggests that a minimal number of globular embryos may have developed over the 30-day cultivation period. The formation of globular embryos is the key process in somatic embryogenesis, as with the development of this stage, differentiation into somatic embryos becomes a natural process without the need for hormonal stimulation (Zhang et al., 2021). However, the predominance of proembryogenic mass indicates that this cultivation period may not have been sufficient to allow for complete embryonic differentiation in *L. ferrea*.

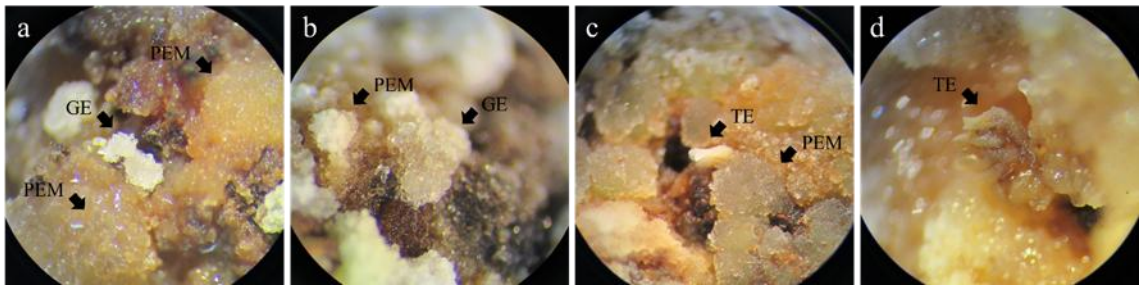

Figure 3 - Observation under a stereomicroscope (32x) of embryogenic calli of L. ferrea and their respective embryonic stages: proembryogenic mass (PEM); globular (GE); torpedo (TE).

Vasconcelos et al. (2024) reported the induction of embryogenic calli of *Himatanthus sucuuba* in the presence and absence of white LED, although the maturation and germination stages did not result in the acquisition of plantlets, the authors observed the formation of PEM in friable calli derived from nodal segments (28.01%) and leaf segments (20.21%) after 30 days in B5 maturation medium supplemented with ABA. However, the percentage of globular embryos was below 25% in both cases. During germination, combining two concentrations of indolebutyric acid (IBA) and BAP (1 + 2.5 µM L^{-1} and 1 + 5 µM L^{-1}) did not favor the conversion into plantlets.

Conversely, Varis et al. (2021) observed a more significant proliferation of embryogenic tissue

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from cryopreserved immature zygotic embryos of *Picea abies* in the absence of light (187%) compared to exposure to blue LED light (131%). During maturation, when cultured in modified Litvay medium (mLM) supplemented with 30 µM L^{-1} ABA for 56 days, the highest embryo production was recorded under green light (73 embryos/g of fresh weight - E/gFW), while far-red light resulted in the lowest productivity (42 E/gFW). Germination was carried out in unsupplemented mLM medium for 14 days, during which the AP67 spectrum (40% red; 30% blue; 15% green; 15% far-red) and far-red light favored root growth, while the most extended shoots were observed under red, green, and far-red light (up to 13 mm).

Although structures with embryogenic characteristics were formed, the maturation process in *L. ferrea* still proved to be limited, indicating that the species depends on specific hormonal and environmental conditions to complete somatic differentiation, as previously reported for other woody species (Kępczyńska & Kępczyńska, 2023; Etienne et al., 1993). Adjustments in culture duration, the composition of growth regulators, and the spectral configuration of light may influence the progression of embryogenic stages and plantlet regeneration (Lu et al., 2017; Mondal et al., 2020).

The employed protocol was effective for callus induction but may not have provided the optimal conditions for subsequent stages, as suggested by Heringer et al. (2017) and Varis et al. (2021), in which wavelengths different from those used in this study favored maturation. Thus, although the red-blue spectrum was effective for callogenic induction, it may not be the most suitable for maturation, suggesting that light quality may also play a decisive role in the advancement of embryogenic development stages in *L. ferrea*.

Constraints and factors affecting somatic embryogenesis in *Libidibia ferrea*

The mechanism of SE varies among different species, exhibiting distinct degrees of hormonal dependency for DSE and ISE (Lu et al., 2017; Mondal et al., 2020; Sharma, 2023). It is suggested that immaturity, low desiccation tolerance, and loss of morphogenetic capacity are limiting factors in converting somatic embryos into plantlets in many woody species (Etienne et al., 1993). In ISE, the induction of embryogenic calli allows for the relatively rapid production of somatic embryos, although most do not reach the globular or heart-shaped stages, with only a few developing to the cotyledonary stage (Kępczyńska and Kępczyńska, 2023). It is generally accepted that SE competence is genetically controlled and mediated by the ontogenetic state of the explants; however, there are reports in which genotype was not a key factor in the efficiency of somatic embryo induction (Wang et al., 2016; Zhang et al., 2021).

In this study, *L. ferrea* embryonic axis explants were cultured for a total of 90 days until they differentiated into somatic embryos through ISE. A 31.0% rate of embryogenic callus formation was observed, along with high explant loss rates. However, the cultivation period was not sufficient for the observed PEM and embryos to reach the cotyledonary stage, and thus, they did not proceed to the germination phase. Other factors also deserve due attention, in addition to embryo immaturity, such as the species hormonal dependency, its response to light quality during the maturation stage, and the influence of genotype on the SE processes. Although somatic embryo induction in model plants is well elucidated, the in vitro application of somatic embryogenesis is limited by the low responsiveness of many species, especially woody plants such as *L. ferrea*.

CONCLUSIONS

The study enabled the establishment of an efficient callus induction protocol for *Libidibia ferrea* using the growth regulator 2,4-D in combination with red-blue LED illumination. The combination of these factors proved suitable for promoting cellular dedifferentiation and callus formation, highlighting the effectiveness of 2,4-D as an inductive agent even in the absence of cytokinins. The response to light indicated that the use of LEDs favored callogenic induction, yielding superior results compared to treatments without illumination, although no significant differences were observed between the tested spectra. During the maturation phase, the combination of ABA and IAA allowed the development of pro-embryogenic masses and embryos at the globular and torpedo stages, although complete conversion into plantlets was not achieved. Embryogenic calli accounted for 31.0% of the total calli induced by the described protocol. The proposed method represents a methodological advance for indirect somatic embryogenesis in *L. ferrea*, and the refinement of subsequent stages may contribute to in vitro plantlet regeneration of the species, providing a foundation for future biotechnological applications.

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