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Crescimento, síntese de ácido ferúlico e histoquímica de calos de *Pouteria caimito* (Ruiz & Pav.) Radlk sob diferentes qualidades de luz

Growth, ferulic acid synthesis, and histochemistry of calli of *Pouteria caimito* (Ruiz & Pav.) Radlk under different light qualities

Crecimiento, síntesis del ácido ferúlico e histoquímica de los callos de *Pouteria caimito* (Ruiz & Pav.) Radlk bajo distintas cualidades de luz

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Resumo

O interesse no desenvolvimento de bioprocessos para a produção de compostos bioativos a partir de fontes naturais tem aumentado consideravelmente. A utilização da qualidade de luz em cultura de calos é apontada como uma das estratégias promissoras para a produção de metabólitos *in vitro*. Nesse contexto, objetivou-se com este estudo investigar a influência da qualidade de luz no crescimento, histoquímica e teor de ácido ferúlico em cultura de calos de *P. caimito*. Para a indução dos calos *in vitro*, utilizaram-se fragmentos foliares ($1cm^2$) em meio MS 50% suplementado com ácido 2,4-diclorofenoxiacético e benzilaminopurina, na presença de luz (branca, azul, verde, amarelo e vermelho) e no escuro. Realizou-se a extração metanólica com partição do extrato e posterior quantificação do ácido ferúlico, executada em cromatógrafo líquido acoplado a espectrômetro de massa. A presença da luz proporcionou crescimento mais expressivo em relação aos calos induzidos na ausência da luz, e na interação entre as qualidades de luz e tempo de cultivo observou-se que houve crescimento linear de biomassa até aos 28 dias nas luzes amarela, vermelha e azul e no escuro. Os valores mais expressivos de biomassa de calos foram observados sob as luzes amarela e vermelha. Nos testes histoquímicos verificou-se presença de compostos fenólicos, alcaloides, flavonoides e terpenos. A exposição dos calos provenientes de luz branca a diferentes qualidades de luz e tempo de cultivo não proporcionou variações significativas de concentração e de rendimento de ácido ferúlico.

Palavras-chave*:* Cultivo *in vitro*; Elicitação; Fenólicos totais.

Abstract

Interest in harnessing biological processes for the production of bioactive compounds from natural sources has increased considerably. The manipulation of light quality in callus culture

is considered a promising strategy for *in vitro* metabolite production. The objective of this study was to investigate the influence of light quality on the growth, histochemistry, and ferulic acid production of callus cultures of *P. caimito*. For *in vitro* callus induction, 1-cm^2 leaf fragments were cultured in 50% MS medium supplemented with 2,4-dichlorophenoxyacetic acid and benzylaminopurine in the absence or presence of light (white, blue, green, yellow, or red). Methanol extraction was performed with partitioning of the extract and subsequent quantification of ferulic acid using a liquid chromatograph coupled to a mass spectrometer. The presence of light promoted greater growth than the absence of light. In the interaction between light quality and culture time, linear biomass growth until 28 days was observed under yellow, red, and blue lights and in the dark. The highest callus biomass values were observed under yellow and red lights. The histochemical tests showed the presence of phenolic compounds, alkaloids, flavonoids, and terpenes. The exposure of calli cultured under white light to different light qualities and culture times did not result in significant differences in the concentration or yield of ferulic acid.

Keywords: *In vitro* culture; Elicitation; Total phenolics.

Resumen

Él interés en el desarrollo de los bioprocesos para la producción de compuestos bioactivos utilizando fuentes naturales, ha aumentado considerablemente. La utilización de la calidad de la luz, en la cultura de callos, es considerada una de las estrategias promisorias para la producción de metabolitos *in vitro*. En este contexto, este estudio tiene por objetivo investigar la influencia de la cualidad de la luz en el crecimiento, histoquímica y el contenido de ácido ferúlico en la cultura de callos de *P. caimito*. Para la inducción de los callos *in vitro*, se utilizó fragmentos foliares (1 cm^2) en medio MS 50% suplementado con ácido 2,4-diclorofenoxiacético y benzilaminopurina, en la presencia de las luces: blanca, azul, verde, amarilla y rojo, incluso en la oscuridad. Se realizó la extracción metanólica con la división del extracto y posterior cuantificación del ácido ferúlico, ejecutada en cromatógrafo de líquidos acoplado al espectrómetro de masas. La presencia de la luz proporcionó un crecimiento más expresivo con relación a los callos inducidos en ausencia de la luz, y, en la interacción entre las cualidades de luz y tiempo de cultivo, se observó que hubo crecimiento lineal de biomasa hasta los 28 días en las luces amarilla, roja y azul, y en la oscuridad. Los valores más expresivos de biomasa de callos fueron observados bajo las luces amarilla y roja. En los testes histoquímicos se verificó la presencia de compuestos fenólicos, alcaloides, flavonoides y terpenos. La exposición de los callos provenientes de la luz blanca a distintas cualidades de

luz y tiempo de cultivo, no proporcionó variaciones significativas de concentración y de rendimiento de ácido ferúlico.

Palabras clave: Cultura *in vitro*; Elicitación; Fenólicos totales.

1. Introduction

The use and study of medicinal plants in Brazil are mainly driven by the high plant diversity and low cost associated with treatments based on them (Santos et al., 2011). The search for phytotherapeutic agents through the screening of natural sources may result in the discovery of clinically useful drugs for the treatment of several diseases, such as cancer, and in the discovery of new antimicrobial agents (Harvey et al., 2015).

Studies on different species of the genus *Pouteria*, such as *P. campechiana* (Kunth) Baehni, *P. sapota* (Jacq.) HE Moore & Stearn, and *P. viridis* (Pittier) Cronquist identified the presence of phenolic antioxidant compounds such as gallic acid, gallocatechin, catechin, epicatechin, dihydromyricetin, catechin-3-O-gallate, and myricitrin (Ma et al., 2004;). In fresh fruits of the species *P. gardneriana* (A. DC.) Radlk., a phenolic compound concentration of 284 mg 100 g^{-1} was found (Rocha et al., 2011).

Pouteria caimito (Ruiz & Pav.) Radlk, a species with commercial potential, is present in almost all Brazilian states and can also be found in Central America, northern Australia, and Malaysia (Almeida et al., 2008). *P. caimito* is used in folk medicine to relieve cough, fever, and bronchitis and as a vermifuge (Nascimento et al., 2011). The benzene extract of the fruit has α-amyrin, lupeol, erythrodiol, and dammarenediol, and taraxerol, taraxenone, and β-sitosterol are considered important phytochemicals in its bark extracts (Silva et al., 2009).

Light quality and intensity, as well as photoperiod, are key environmental factors to plant growth and development, as they directly influence the phytochemical concentrations, morphogenesis, growth, and cell and tissue differentiation (Wojciechowska et al., 2015). The synthesis of secondary metabolites in plants often originates as a defense mechanism against different types of stress, including light stress (Verma & Shukla, 2015).

Under *in vitro* culture conditions, the stimulating effect of light on compound formation has been evidenced in calli of *Stevia rebaudiana* Bertoni, where the total phenolic content increases under blue light (Ahmad et al., 2016). Blue and red light-emitting diodes increase the total phenolic content and chlorogenic acid content in pea sprouts (Liu et al., 2016). Although radiation, such as violet, green, yellow, and orange light, is only related to secondary beneficial effects on plant growth and development (Gazolla et al., 2017), it was

found, for example, that in callus culture of *Artemisia absinthium* L., the green spectrum favors the production of total phenolics, flavonoids, chlorophylls, and antioxidant activity (Tariq et al., 2014).

Ferulic acid [3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid] is a phenolic compound derived from caffeic acid that is often found in plants (Mancuso & Santangelo, 2014). It may be found as a monomer, dimer, free oligomer, or constituting polymers; covalently linked by ester bonds to polysaccharides, polyamines, or glycoproteins, or ether-linked to lignin (Paiva et al., 2013). Ferulic acid has reported medicinal properties, such as antidiabetic, hepatoprotective, anticarcinogenic, antiapoptotic, antiaging (Umre et al., 2018), antioxidant (Goujot et al., 2019) suggesting the high potential of this compound.

There are no reports on the influence of light quality on the growth or secondary metabolism of *P. caimito*, especially under *in vitro* conditions. Studies involving spectral adequacy can support relevant strategies to optimize plant growth and the production of secondary metabolites (Gazolla et al., 2017). Given the above, the objective of this study was to investigate the influence of light quality on the growth, histochemistry, and ferulic acid content in callus culture of *P. caimito.*

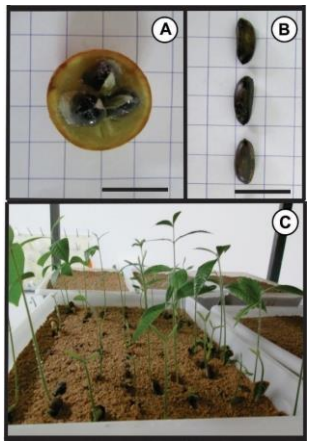
2. Material and Methods

Plant material

The experiment was conducted at the Plant Tissue Culture Laboratory of Federal Institute Goiano, Rio Verde Campus, Goiás state, Brazil. The scientific method used in this work was a quantitative and qualitative laboratory research, with the application of statistic methods to analyze the collected data (Pereira et al., 2018). The seeds were obtained from fruits of *P. caimito* collected at Fortaleza farm, geographic coordinates 18°11′59.9″S - 050°35′29.1″W, 563 m altitude. The voucher specimen was deposited at the Herbarium of Federal Institute Goiano, Rio Verde Campus, under registration number 546.

After collection, the fruits were sectioned (Figure 1-A) and the seeds were depulped (Figure 1-B) under running water with the aid of a sieve and sown in plastic trays (53x37x8 cm) with sieved coarse sand as substrate (Figure 1-C).

Figure 1. *Pouteria caimito* (Ruiz & Pav.) Radlk. **A**: Sectioned fruit. **B**: Seeds. **C**: Seedlings germinated in trays and grown in a controlled environment. Bar = 4 cm.



Source: Authors (2020).

After the emergence, a total of 250 mL of MS nutrient medium was added per tray every two weeks, until the seedlings complete 60 days and being used as a source of explants. The trays were kept at 25º C ± 3 under natural light during this period.

Callus induction

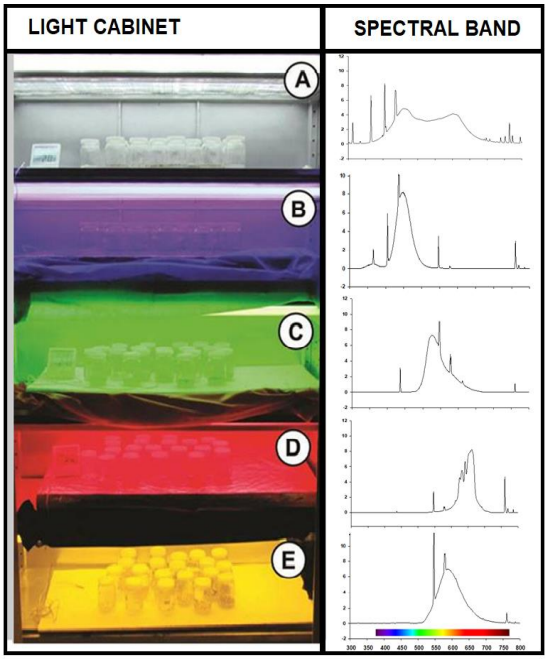
The induction callus according to Sanonne et al. (2012). For asepsis, leaves collected from seedlings grown in a climatized room were washed in running water and immersed in a 3% (v/v) commercial detergent solution for 20 minutes, then in 70% (v/v) alcohol for 1 minute. The leaves were then immersed in 0.5% sodium hypochlorite solution with active chlorine for 15 minutes, followed by three rinses with sterile distilled water in a laminar flow chamber. After leaf asepsis, leaf fragments (1 cm^2) were inoculated into glass flasks containing 40 mL of 50% MS medium, 30 g L^{-1} sucrose and 3.5 g L^{-1} agar and adjusted to pH 5.7 ± 0.03. The culture medium was supplemented with 0.5 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg L^{-1} of benzylaminopurine (BAP). The calli produced were transferred every 20 days to a new medium with the same concentrations.

Callus culture under different light qualities

Calluses cultured in the presence and absence of white light for 14 successive platings were transferred to a dark environment or to an environment illuminated by white (300-800

nm), blue (400-490 nm), green (490-560 nm), yellow (560-590 nm), or red (600-750 nm) Taschibra® tubular fluorescent lamps (Indaial, Santa Catarina, Brazil) under 45 ± 5 μmol m^{-2} s^{-1} irradiance and a 16-h photoperiod (Figure 2).

Figure 2. Culture environment of *P. caimito* calli (Ruiz & Pav.) Radlk cultured under different light qualities. **A**: White. **B**: Blue. **C**: Green. **D**: Red. **E**: Yellow.



Source: Authors (2020).

The spectral composition of each quality of light supplied to the callus will act as a modulator of the metabolic responses, stimulating different biosynthetic routes. For this reason, the spectral quality was determined using an spectroradiometer (USB2000, Ocean Optics, Dunedin, Florida, USA) and the light intensity was adjusted using a QSO-S PAR sensor (Decagon Devices, Pullman, Washington, USA). At 28 days of culture, material was collected for histochemical tests and determination of the concentration and yield of ferulic acid.

Histochemistry

For the histochemical tests, fresh calli were manually excised using a disposable razor blade. The reagents potassium dichromate ($K_2Cr_2O_7$) and ferric chloride ($FeCl_3$) were used for phenolic compounds (Gabe, 1968), aluminum chloride ($AlCl_3$) for flavonoids, Nadi reagent

for the evaluation of terpenes and resin acids (David & Carde, 1964), and Wagner's reagent for nitrogen compounds/alkaloids (Furr & Mahlberg, 1981).

Extraction of ferulic acid from **P. caimito** *calli*

All plant material was extracted according to Giri et al. (2012). The calli were dried in an oven at 40 °C for 48 h, macerated, weighed (20 mg), and transferred to a Falcon tube with 2 mL of 80% methanol (v/v).

The mixtures were sonicated for 30 minutes and then centrifuged (4000 rpm, 20 minutes), and 1 mL of the supernatant was collected. Partition extraction was performed using 200 µL of methanol extract, 800 µL of distilled water, and 400 µL of ethyl acetate and then centrifuged at 10,000 rpm for 15 minutes at 4 °C. The extract was stored (4-8 °C) until analysis.

Quantification of ferulic acid by high-performance liquid chromatography (liquid chromatography–electrospray ionization–mass spectrometry)

The analysis was performed in a liquid chromatograph coupled to a mass spectrometer (Flexar SQ 300, Perkin Elmer®) in negative ionization mode. Separation was performed with injections of 40 µL of the extract in a Brownlee SSP C18, 2.7 µm, 3.0 × 50 mm column, with gradient elution with 0.1% acetic acid in ultrapure water (solvent A) and methanol (solvent B) at 0.4 mL min^{-1}. The composition of the initial mobile phase was 10% solvent B, and this condition was held for 2 minutes for column balance before each injection. A linear gradient of 10 to 100% of solvent B was achieved over 3 minutes. Solvent B was kept at 100% for 3 minutes. The yield per flask was calculated by multiplying the mass of the calli per flask by the concentration of ferulic acid.

Data analysis

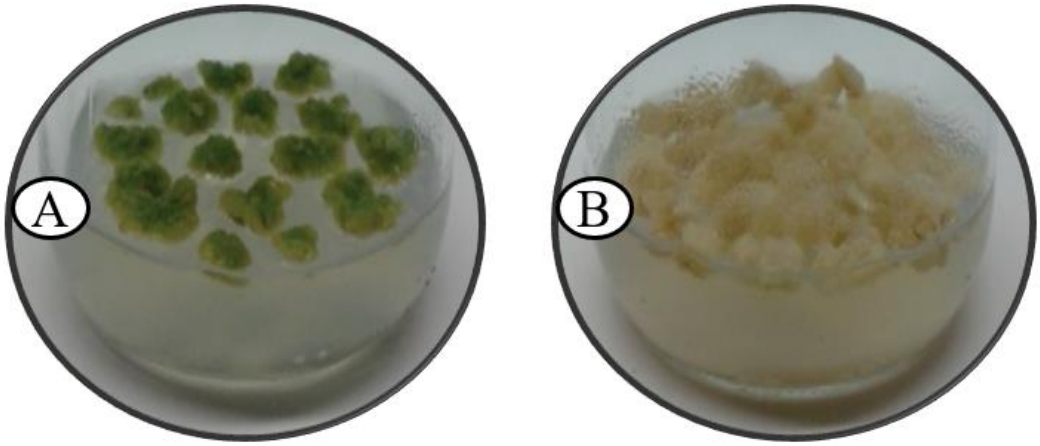
The experimental design was completely randomized, in a 6 × 5 factorial arrangement, with six spectral bands (white, blue, green, red, yellow, and dark light) and five culture times (0, 7, 14, 21, and 28 days). Significant effects were detected by analysis of variance. Means were compared between light-quality groups using the Scott-Knott test at 5% probability and

subjected to regression model fitting for the growth times. Correlation network analysis was performed using the software Genes (Genetics and Statistics) (Cruz, 2013).

3. Results and Discussion

The light condition promoted different responses in the morphology of the calli in this study. The calli that were originated and kept under white light were green (Figure 3A), while those derived from dark induction were white (Figure 3B).

Figure 3. Calluses of *Pouteria caimito* after 20 days of subculture. **A**: Calli originating from light culture (white). **B**: Calli originating from dark culture.



Source: Authors (2020).

This result is in agreement that calluses may present varied morphology, size and colors, depending on the species, period of cultivation, composition of the culture medium and environmental conditions of growth.

The white coloring of the calluses might have occurred due to the inhibition of photosynthesis and degradation of chlorophyll content in dark cultures (Khan et al., 2019). Light is a source of energy for photosynthetic organisms, and the quality of irradiated light directly or indirectly affects the functioning of the photosynthetic apparatus and other biological structures, making it necessary for the plant to adapt to the predominant light of its environment (Weston et al., 2000).

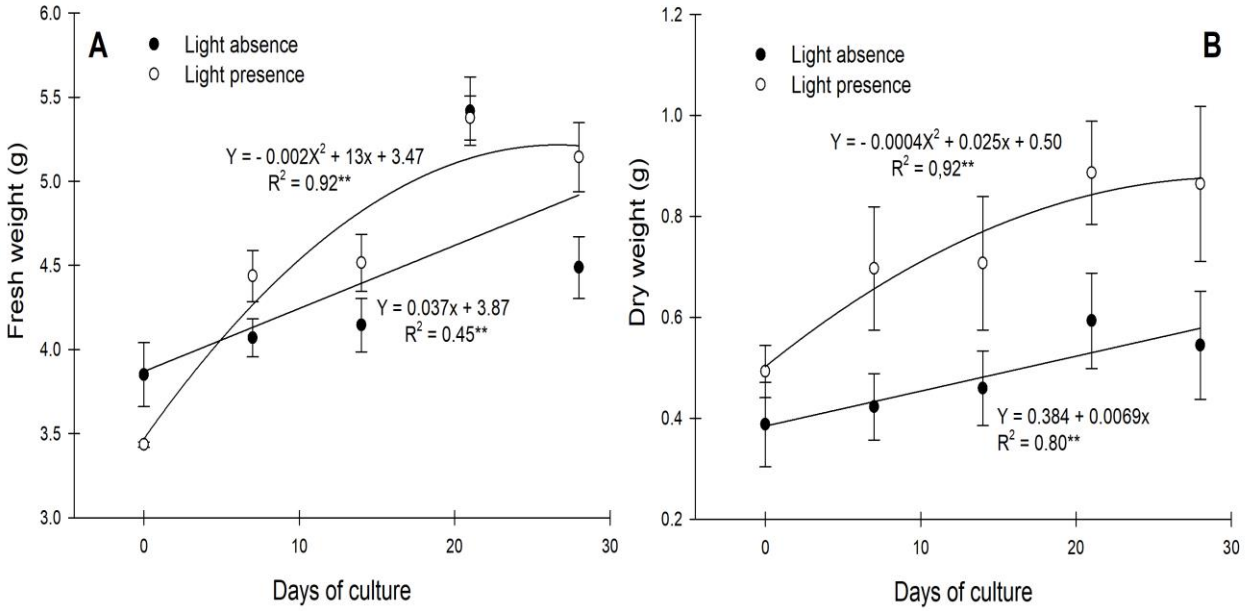
Our study is connected to the one described by these authors and, we append that, although the light isn't a prerequisite to the cellular division and the callogenesis, its presence

stimulates the biosynthesis of the chlorophyll in the vegetal cells. The absence of chlorophylls is frequently observed in seedlings cultivated in the absence of light, which develops the etiolated aspect. The presence of chlorophylls may have contributed to the photoautotrophism of callus, since we will see below that under the presence of light there was a greater accumulation of biomass.

Growth under different light conditions

Callus growth showed a quadratic response to the presence of light and a linear response to the absence of light throughout the study time. Calli induced in the presence of light had greater fresh and dry biomass accumulation (Figs. 4A and B, respectively).

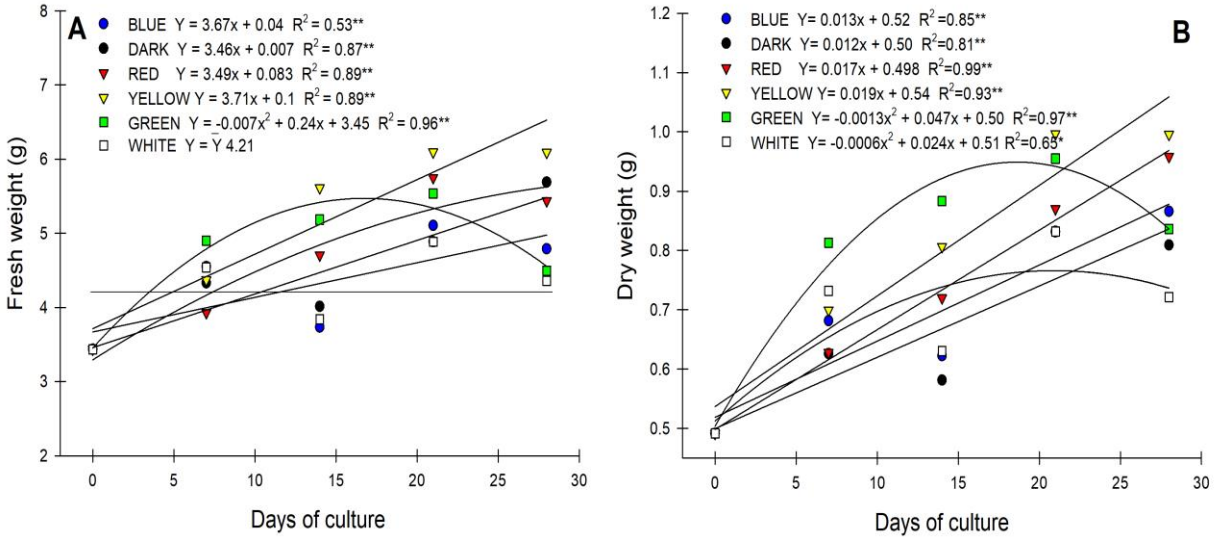
Figure 4. Fresh (A) and dry weight (B) of *P. caimito* calli grown in the presence or absence of light, evaluated every 7 days over the 28-day culture period. Significance: **p < 0.01.



Source: Authors (2020).

To measure the elicitation induced by the different light qualities, only the *P. caimito* calli previously grown under light are represented due to their greater biomass accumulation (Figure 5).

Figure 5. Fresh (A) and dry weight (B) of *Pouteria caimito* calli grown for 28 days under different light qualities. Significance: *p < 0.05; **p < 0.01.

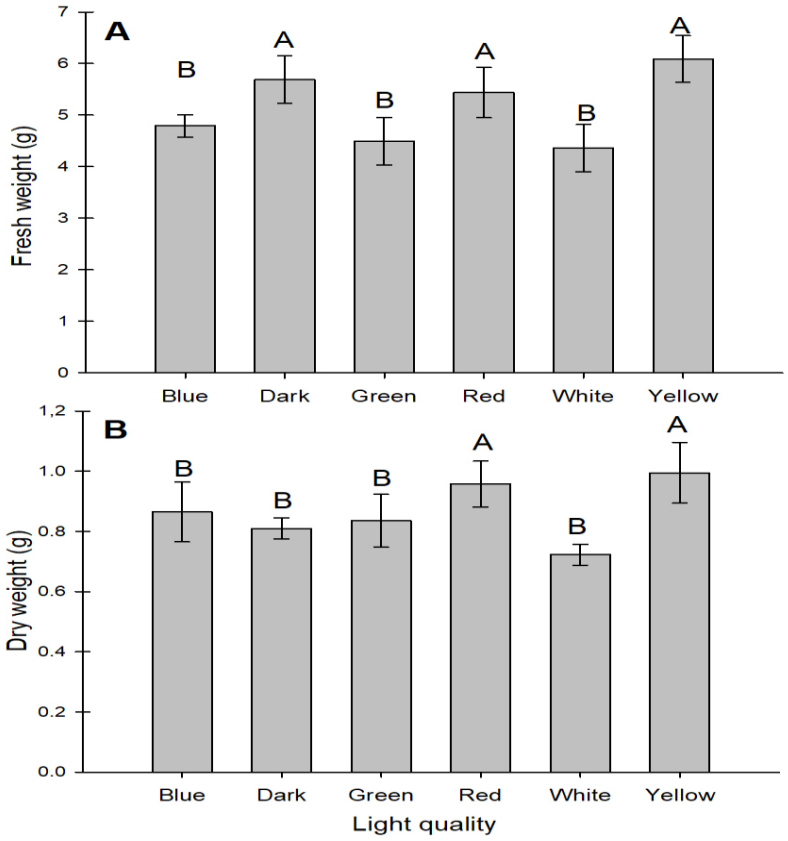


Source: Authors (2020).

There was an interaction between light quality and culture time ($p < 0.05$). Under green light, fresh and dry biomass accumulation showed a quadratic response to culture time, with maximum accumulation at 17 and 18 days, respectively (Figure 5). The fresh weight under white light did not vary with time ($p > 0.05$), but the dry weight showed a quadratic response, with maximum accumulation at 20 days (Figure 5B). The results under these conditions were similar to those observed in calli of *Stevia rebaudiana* Bertoni exposed to different light qualities, which showed a declining phase after 21 days (Ahmad et al., 2016). However, under the yellow, red, and blue lights and in the dark, the biomass of calli of *P. caimito* increased linearly up to day 28 of culture ($p < 0.01$).

The highest fresh weight values were observed in calli cultured in the dark and under the yellow and red lights (Figure 6A). Greater dry weight accumulation was observed under yellow and red light (Figure 6B), where calli doubled their weight from time 0 until day 28, increasing from an initial mean weight of 0.49 g to a final mean weight of 0.98 g.

Figure 6. Fresh (A) and dry weight (B) of calli of *P. caimito* at 28 days of culture under different light qualities. Groups marked with the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). Error bars represent the standard error of the mean.



Source: Authors, 2020.

The lack of correlation between fresh and dry weight in the dark, may be related to the accumulation of water inside cells, resulting from the deceleration of cell proliferation as a result of nutrient depletion, associated with the production of toxic products and/or reduction of O_2 inside the callus cells (Smith, 1992). Under the other light conditions, there was no change in callus biomass, though mean dry weight ended at 0.81 g, a 65% increase over that at time 0.

Fazal et al. (2016) also observed a positive effect of yellow light on biomass during growth kinetics in callus culture of *Prunella vulgaris* L. and highlighted that the effect of light quality may vary according to the investigated species. In callus cultures of *Rhodiola imbricata* Edgew, red light stimulated the biomass accumulation, and this response was attributed to the increased amount of the far-red-absorbing form of phytochrome, which participates in the synthesis and activities of growth-related enzymes (Kapoor et al., 2018). The considerable proportion of red bands in the yellow light source may be related to the

favorable growth under these conditions, which explains the similar biomass accumulation responses.

Histochemistry

In the histochemical evaluation of *P. caimito* calli cultured in the presence of light with subsequent exposure to different light qualities, the presence of the main compounds related to secondary metabolism (phenolics, nitrogenous compounds, and terpenes) was observed. The concentration of starch was similar between light-quality groups at days 0, 14, and 28. The presence of starch was also observed in *Elaeis guineensis* Jacq., acting as a source of carbon and energy supply for cell division due to its high metabolic activity (Padua et al., 2013).

This may explain the presence of starch in calli of *P. caimito* throughout the evaluated period. Because photosynthesis is inactive in the dark, the starch accumulation was attributed to addition of sugar to the culture medium, as exogenous sucrose increases the production of starch reserves in plants propagated *in vitro* (Yoon et al., 2009).

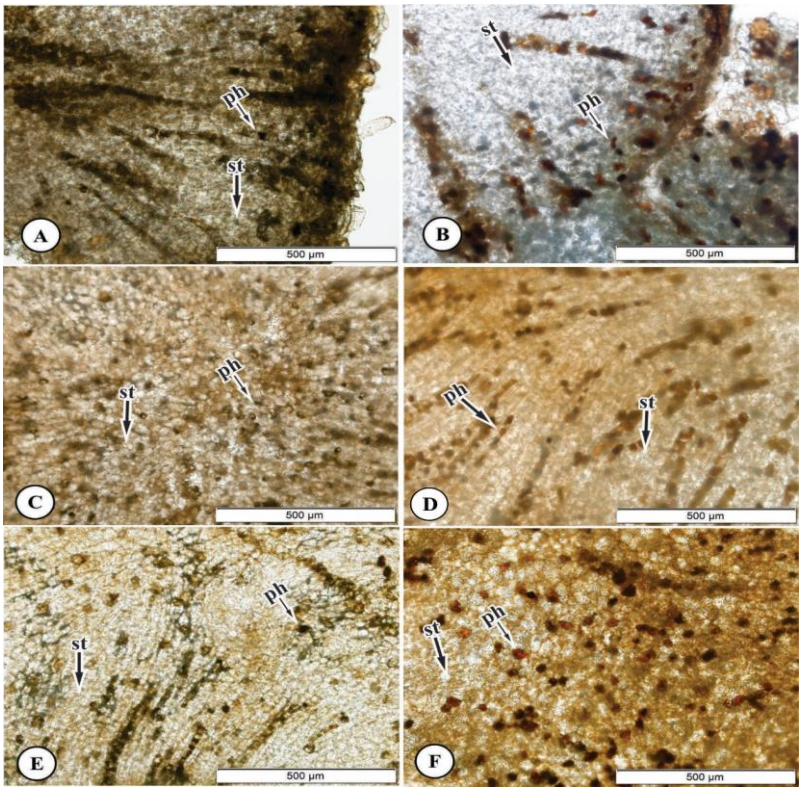
Phenolic compounds were found by potassium dichromate staining in the calli grown under red light and in the dark at 14 days, under white light at 0 days, and under all light qualities at 28 days. By iron chloride III staining, phenolic compounds were detected under all light qualities evaluated (Table 1) (Figure 7).

Table 1. Histochemical tests in calli grown under different light qualities (white, red, blue, green, yellow, and dark) for the detection of the major classes of secondary metabolites.

Light	Phenolics ($K_2Cr_2O_7$)			Phenolics ($FeCl_3$)			Alkaloid (Wagner)			Flavonoid ($MgCl_2$)			Flavonoid ($AlCl_3$)			Terpenes (Nadi)		
	0	14	28	0	14	28	0	14	28	0	14	28	0	14	28	0	14	28
White	+	-	+	++	+	++	+	-	+	-	-	-	-	++	-	+	+	+
Red		+	++		++	+		-	+		-	+		+	-		+	++
Blue		-	++		+	++		-	+		+	-		+	+		+	++
Green		-	++		+	++		-	+		+	-		+	+		++	+
Yellow		-	+		++	+		-	+		+	-		+	+		+	++
Dark		+	++		+	+		-	+		+	+		-	+		++	+

Legend: + (presence); - (absence). Source: Authors (2020).

Figure 7. Sections of calli of *P. caimito* at 0 (A and B), 14 (C and D), and 28 (E and F) days stained with iron chloride III (A, C and E) and potassium dichromate (B, D and F) for the detection of phenolic compounds. **ph**: phenolics; **st**: starch.



Source: Authors (2020).

Alkaloids were detected under all light qualities evaluated at 0 and 28 days of culture, and terpenes were present in all treatments throughout the culture period.

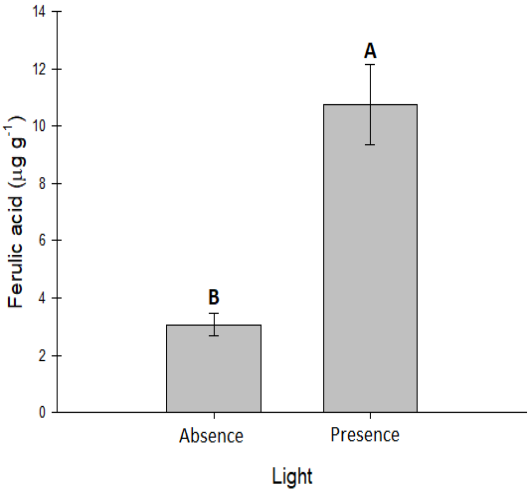
In calli stained with aluminum chloride, flavonoids were identified under white, yellow, blue, green, and red light at 14 days of culture and under blue, green, and yellow light and in the dark at 28 days. Staining with magnesium chloride identified flavonoids in calli cultured under blue, green, and yellow light and in the dark at 14 days of culture and under red light and in the dark at 28 days.

Light quality is one of the factors that affects primary and secondary metabolism in plant cell cultures. The results of this study are in agreement with those found in leaves of *Withania somnifera* (L.) Dunal, where alkaloids and phenolic compounds have been identified (Munien et al., 2015). Higher production and accumulation of secondary metabolites have been observed in *in vitro* callus culture of *Solanum aculeatissimum* Jacq than in leaves cultured *in vitro* and *in situ* (Dantas et al., 2017).

Ferulic acid quantification

Observing only isolated effects in relation to the induction environment in the presence and absence of white light after elicitation with different light qualities, it was found that calli exposed to light produced a more ferulic acid (9.49 μg g^{-1}) than calli in the absence of light (2.60 μg g^{-1}) (Figure 8).

Figure 8. Concentration of ferulic acid (μg g^{-1}) detected in calli of *P. caimito* induced in the absence and presence of light after 28 days of culture under different light qualities.

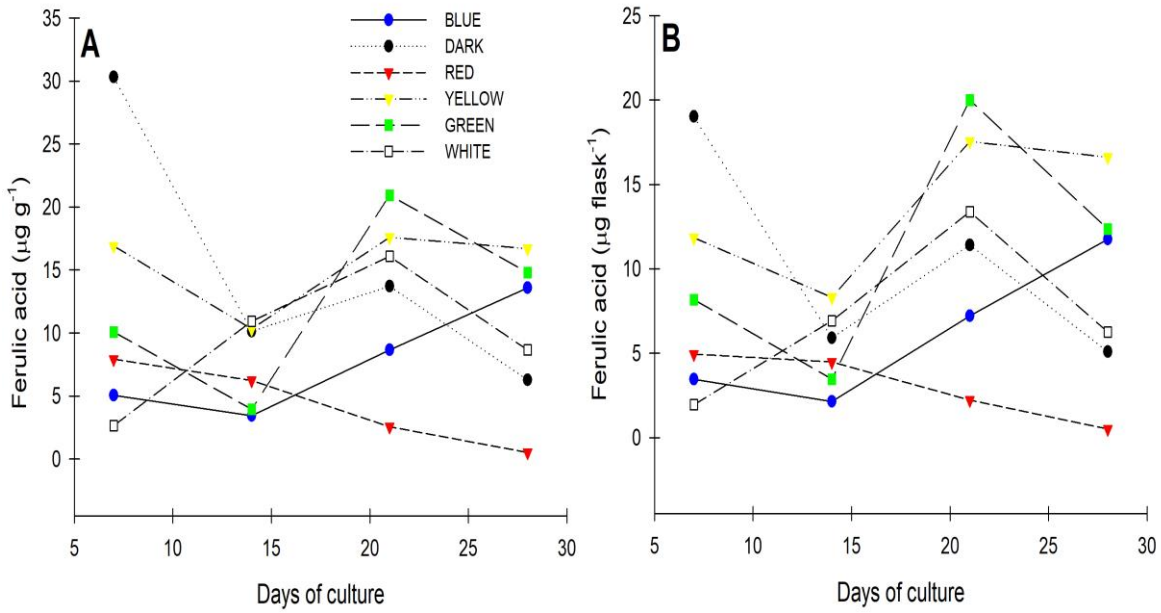


Source: Authors (2020).

In addition to acting on growth and development, light quality is involved in the modulation of biosynthesis of secondary metabolites. Greater responses have been observed in the production of total secondary metabolites in calli of *Solanum xanthocarpum* Schrad. & H. Wendl cultured in the presence of light than in the dark (Usman et al., 2020).

The exposure of calli obtained under white light to different light qualities and culture times did not result in variations in the concentration (Figure 9A) or yield of ferulic acid (Figure 9B).

Figure 9. Concentration (μg g^{-1}) [A] and yield of ferulic acid (μg $flask^{-1}$) [B] in calli of *P. caimito* as a function of light quality and culture time.



Source: Authors (2020).

Kapoor et al. (2018) found that blue light stimulated the production of phenolics in callus cultures of *Rhodiola imbricata* Edgew. Ahmad et al. (2016) reported that blue light stimulates the production of phenolics and flavonoids in calli of *Stevia rebaudiana* (Bert). Some studies have related blue light to the expression phenylalanine ammonia-lyase, a key enzyme in the biosynthesis of phenylpropanoids (Nadeem et al., 2019). In contrast, continuous white light has increased the production of phenolics and flavonoids in callus cultures of *Fagonia indica* Burm f. (Khan et al., 2019).

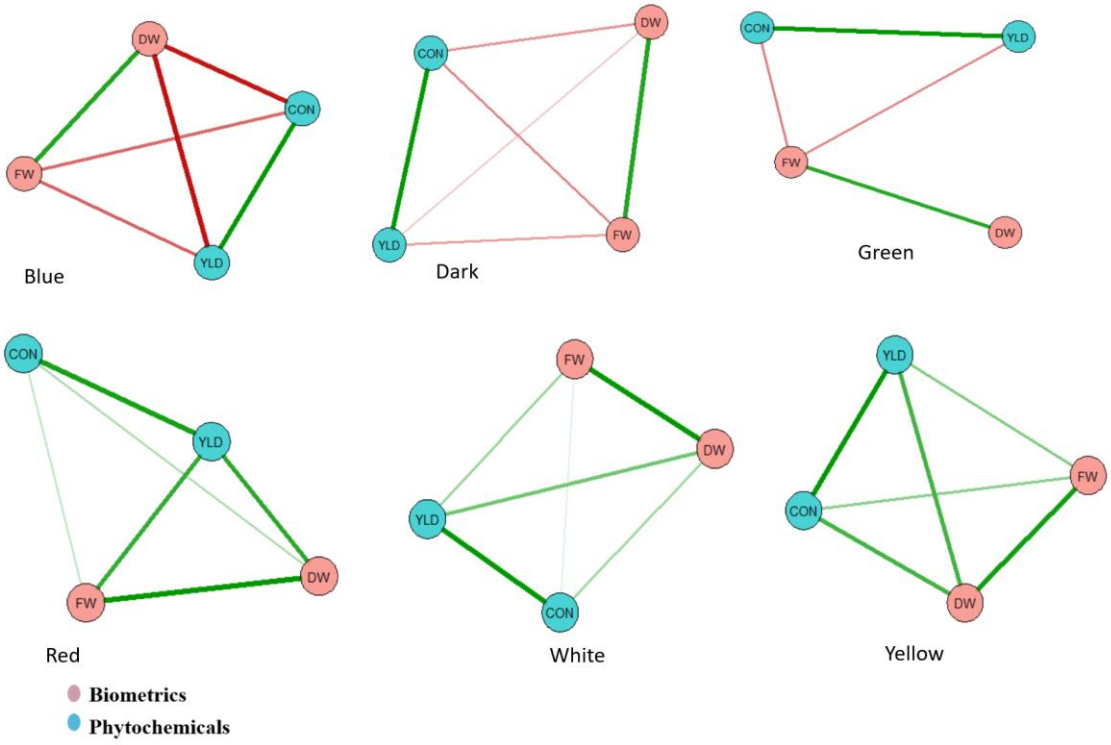
The absence of differences in the concentration of ferulic acid in the present study can be attributed to its conversion into other compounds under certain light qualities or to insufficient light intensity to promote its biosynthesis. The elicitation of ferulic acid in *P. caimito* could depend on electromagnetic radiation of a higher energy level; for example, ferulic acid has increased in callus cultures of *Vitis vinifera* L. when exposed to UV-C radiation (Cetin, 2014). Gamma-irradiation of callus cultures of *Hypericum triquetrifolium* Turra promotes an increase in the accumulation of bioactive compounds (Azeez et al., 2017).

The mean concentration of ferulic acid (9.5 μg g^{-1}) observed in *P. caimito* calli was similar to that observed in *Vitis vinifera* L. (Cetin, 2014), reinforcing that the present study is

relevant because it is the first to address the accumulation of ferulic acid in calli of this species.

Through the correlation network graph, it is possible to identify the variables and the ways in which they are connected (Figure 10).

Figure 10. Correlation networks constructed using biometric variables and the biosynthesis of ferulic acid in callus culture of *P. caimito*. The red and green lines represent negative and positive correlations, respectively. The line thickness is proportional to the correlation strength. Biometric characteristics: FW, fresh weight; DW, dry weight. Phytochemical characteristics: CON, concentration; YLD, yield of ferulic acid.



Source: Authors (2020).

The correlation network analysis showed a significant positive correlation for fresh and dry weight ($0.98 \leq r < 1$) only under white light. There were strong positive and significant correlations between the concentration and yield of ferulic acid under all tested light conditions ($0.9 \leq r < 1$), except under red light ($p > 0.05$).

The lack of correlation between the biometric characteristics and the biosynthesis of ferulic acid under the tested light conditions indicates that this metabolite is not involved in the primary metabolism of calli. It also indicates the possibility of reducing the culture time

for ferulic acid production, because the concentration and yield did not vary significantly after 7 days of culture. Additional studies should be performed for further exploration and adjustment of the culture time for other metabolites of interest.

4. Conclusion and Sugestions

Histochemical tests identified the presence of secondary metabolites in *Pouteria caimito* calli grown under different light conditions, including phenolic compounds, alkaloids, flavonoids, and terpenes. The most pronounced biomass accumulation occurred between 18 and 28 days.

The induction of ferulic acid biosynthesis was favored in the presence of light, and its concentration was maintained, even in the dark, when the calli were cultured for only one week. Studies involving higher light intensity or radiation with shorter wavelengths in the culture environment may complement studies with abiotic elicitation in *P. caimito* calli.

Therefore, further works with higher irradiance or with the usage of ultraviolet radiation must be performed in order to verify alterations in the accumulation of ferulic acid and other metabolites, as alkaloids, flavonoids and terpenes, evidenced in our histochemical tests.

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