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In vitro micropropagation of *Bromelia balansae* Mez.: effects of light intensity and growth regulators on plant development

Micropropagação *in vitro* de *Bromelia balansae* Mez.: efeitos da intensidade luminosa e de reguladores de crescimento no desenvolvimento vegetal

Micropropagación *in vitro* de *Bromelia balansae* Mez.: efectos de la intensidad de luz y reguladores de crecimiento en el desarrollo vegetal

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Abstract
We detail herein the *in vitro* micropropagation of *Bromelia balansae* Mez. In this regard, we investigated the germination and the action of growth regulators in inducing callogenesis. Furthermore, we thoroughly investigated the influence of light intensity on plant growth as well as the production of secondary metabolites. The germination rate of *B. balansae seeds* was 94.95% and the developed seedlings were uniform. It was possible to observe that the amount of callus formed per explant was influenced by the concentrations and combinations of distinct growth regulators. Furthermore, it was possible to observe variations in the concentration of total phenolic compounds and flavonoids in the extracts of the plants cultivated under different light intensities. Therefore, this study showcases the effect of growth conditions in the morphology and chemical constitution of *in vitro* grown *B. balansae*.
Keywords: Bromeliads; Plant tissue culture; Luminosity; Growth; Phytochemistry; Mass spectrometry.

Resumo
Detalhamos aqui a micropropagação *in vitro* de *Bromelia balansae* Mez. A este respeito, investigamos a germinação e a ação dos reguladores de crescimento na indução da calogênese. Além disso, investigamos a influência da intensidade da luz no crescimento das plantas, bem como a produção de metabolitos secundários. A taxa de germinação das sementes de *B. balansae* foi de 94,95% e as plântulas desenvolvidas eram uniformes. Foi possível observar que a quantidade de calo formado por explante foi influenciada pelas concentrações e combinações de reguladores de crescimento distintos. Além disso, foi possível observar variações na concentração de compostos fenólicos totais e flavonoides nos extratos das plantas cultivadas sob diferentes intensidades de luz. Portanto, este

estudo mostra o efeito das condições de crescimento na morfologia e constituição química da *B. balansae* cultivada *in vitro*.
Palavras-chave: Bromélias; Cultura de tecidos vegetais; Luminosidade; Crescimento; Fitoquímica; Espectrometria de massas.

Resumen
Detallamos aquí la micropropagación *in vitro* de *Bromelia balansae* Mez. A este respecto, investigamos la germinación y la acción de los reguladores del crecimiento en la inducción de la callogénesis. Además, investigamos a fondo la influencia de la intensidad luminosa en el crecimiento de la planta, así como la producción de metabolitos secundarios. La tasa de germinación de las semillas de *B. balansae* fue del 94,95% y las plántulas desarrolladas fueron uniformes. Se pudo observar que la cantidad de callo formado por explante estaba influenciada por las concentraciones y combinaciones de distintos reguladores de crecimiento. Además, fue posible observar variaciones en la concentración de compuestos fenólicos totales y flavonoides en los extractos de las plantas cultivadas bajo diferentes intensidades de luz. Por lo tanto, este estudio muestra el efecto de las condiciones de crecimiento en la morfología y constitución química de *B. balansae* cultivada *in vitro*.
Palabras clave: Bromelias; Cultivo de tejidos vegetales; Luminosidad; Crecimiento; Fitoquímica; Espectrometría de masas.

1. Introduction

In plant cells, metabolites are divided into either primary or secondary. The primary metabolites perform an essential function in the plant, such as exemplified by pathways involving synthesis and breakdown of carbohydrates, lipids, proteins and nucleic acids. On the other hand, secondary metabolites do not have a universal distribution, and represent a chemical interface between plants and the surrounding environment (Yang et al., 2018).

It is known that biotic factors such as attacks by pathogens (micro-organisms and herbivores), and abiotic factors such as circadian rhythm, temperature, water availability, sunlight, nutrients, altitude and mechanical stimuli, can interfere qualitatively and quantitatively on the production of secondary metabolites (Isah, 2019).

Concerning sunlight, both primary and secondary metabolism are reported to be influenced by it. For instance, sunlight is essential for growth and development, as it plays major roles in photosynthesis, seed germination and flower development (Silva, 2019). On the other hand, sunlight can influence the concentration and/or composition of substances through intensity, quality and photoperiod, that is, light can directly or indirectly affect the biosynthetic pathways of secondary metabolites (Gobbo-Neto & Lopes, 2007). In this sense, plants can respond in distinct ways to light stimuli, and these responses do not depend only on the presence, attenuation or absence of light, but also on the spectral quality of the radiation (Pinheiro et al., 2016; Taiz et al., 2016).

The scientific literature reports that there is influence of light on the growth and development of several plants, such as those belonging to the Bromealiceae family. Rocha (2002) reports that the species *Aechmea fasciata* (Lindl.) Baker and *Guzmania lingulata* (L.) Mez., submitted to different levels of shading, showed better development under screens with 40% shading. However, there are no studies demonstrating the influence of light on the growth and yield of secondary metabolites in the species *Bromelia balansae* Mez., a terrestrial, medicinal bromeliad found in the Brazilian Midwest (Guarim Neto; Morais, 2003; Proença; & Sajo, 2007), specifically in the Cerrado of the Brazilian Federal District, Goiás, Mato Grosso do Sul and Mato Grosso (Forzza et al., 2015).

This species is popularly known as Gravatá and Caraguatá and its fruits are used in folk medicine in the preparation of cough syrups (Coelho et al., 2010), as well as reported to have expectorant action (Guarim Neto, 2006). However, with the unsustainable extractive collection, as well as the monoculture of grains and sugar cane, there was a decline of *B. balansae* populations (Ribeiro et al., 2008).

According to Pereira (2018), due to extractivism, the destruction of the natural habitat and the lack of propagation techniques, some Brazilian species of bromeliads are threatened. According to Martinelli et al. (2013), Bromeliaceae is the

second most endangered botanical family, preceded only by Asteraceae. Therefore, according to Carneiro and Mansur (2004), the use of *in vitro* methods for the conservation of Bromeliaceae is a viable alternative to repopulate endangered species.

In vitro tissue culture of medicinal plants has helped in the clonal propagation of several genotypes, thereby allowing the conservation of the germplasm. This allowed the obtainment of new sources of variability through callus cultivation, as well as providing a way to optimize the production of secondary metabolites and enhance somaclonal variation (Rodrigues; & Almeida, 2010).

It is important to highlight that the composition and concentration of the culture medium, especially of the growth regulators, have effects on the explant response in the different stages of *in vitro* cultivation (Souza et al., 2018). Auxins and cytokinins are the two classes of growth regulators most used in tissue culture. Callus formation is regulated by the availability and interaction of these two classes of growth regulators (Caldas et al., 1990; Souza et al., 2018).

Therefore, we herein investigated the germination and the action of growth regulators in inducing callogenesis on *in vitro* germinated *B. balansae*, as well as thoroughly evaluated the influence of light intensity on plant growth as well as the production of secondary metabolites.

2. Methodology

2.1 In vitro growth

The protocol for the *in vitro* growth herein used is based on a previous report by our group (Souza et al., 2021). The seeds of *B. balansae* species were donated by the Botanical Garden of Brasília, and disinfested and inoculated at the Plant Tissue Culture Laboratory of the Instituto Federal Goiano, Campus Ceres-GO, Brazil.

Seed disinfestation consists of immersion, under agitation, in ethyl alcohol 70% (v/v) for 1 minute (min.), then 20 min. in a 5% (v/v) sodium hypochlorite solution with 2 drops of polysorbate 80 under stirring. Finally, the seeds were washed 3 times for 5 min. under agitation in sterilized deionized water.

After disinfestation, the seeds were inoculated in glass tubes containing 10 mL of MS culture medium (Murashige; Skoog, 1962), with half the salt concentration (MS ½). This medium was prepared as it follows: 15 g L^{-1} of sucrose, 7.5 g L^{-1} of Phytagel ®, 0.5 mL L^{-1} of myoinositol, 0.5 mL L^{-1} of vitamins (thiamine, nicotinic acid and pyridoxine), 0.5 mL L^{-1} of glycine and pH adjusted to 5.7 before autoclaving (121 °C, 1 atm for 20 min.), and only after autoclaving the seeds were inoculated.

The tubes containing the seeds were kept in a growth room with a temperature of 25 ± 2 °C and a photoperiod of 16 hours of daylight. The luminous intensity, which was measured by a luxmeter, was approximately 50 μ mol m-2s $^{-1}$, obtained by white LED lamps.

The seeds were evaluated monthly. However, after three months, the germination rate (%) and microbial contamination were verified by the naked eye (%). Seeds with radicle protrusion were considered germinated.

2.2 Callogenesis induction

In a laminar flow hood, after removing the residues of the MS½ medium from the *in vitro* seedlings, with an average of 2.5 cm in height, cuts were made on the surface of the leaf segments, being inoculated the root and the stem base.

To induce callogenesis, the explants were inoculated in MS½ culture medium, which was prepared as aforementioned, and then supplemented with combinations of 0 μM (0 mg L^{-1}); 1 μM (0.186 g L^{-1}); 2 μM (0.372 mg L^{-1}) and 4 μM (0.744 g L^{-1}) of naphthaleneacetic acid (NAA) and 0 μM (0 mg L^{-1}); 1 μM (0.225 mg L^{-1}); 2 μM (0.450 mg L^{-1}) and 4 μM (0.900 mg L^{-1}) of 6-benzylaminopurine (BAP).

The experiment consisted of 16 treatments. Each treatment contained one explant, totaling eight replications. All

flasks were kept in a growth room with a temperature of 25 ± 2 °C and a photoperiod of 16 hours of daylight. The luminous intensity was approximately 50 µmol m-2s $^{-1}$, obtained by white LED lamps.

At the end of 3 months, the material was evaluated regarding the percentage of microbial contamination (%), survival (number of explants developed per number of inoculated explants) (%), callogenesis (absence or presence) (%) and the number of calluses per explant.

2.3 Brmelia balansae Mez. subjected to three growth conditions

Seedlings measuring 2.5 cm in height, multiplied *in vitro*, were washed in running water until the complete removal of residues from the MS½ medium and subsequently transported to the screenhouse of the Instituto Federal Goiano, Campus de Ceres-GO (15° 21 ´ 0. 88" S; 49º 35´ 55. 96" W) and transplanted into trays of 40 cells containing 36 g of substrate Carolina Soil ® and Tropstrato Florestal® (with a ratio of 1:3, respectively), under irrigation by aspersion for 3 min, 4 times a day. It should be noted that every 15 days,10 mL of modified Hoagland and Arnon® ½ was applied to the seedlings until they were soaked.

The screen cover was constituted of transparent polyethylene with a thickness of 100 microns and the sides were shaded (black tint) with 50% brightness. The composition of Carolina Soil® was: peat, vermiculite, carbonized rice husk, organic waste and NPK (nitrogen, phosphorus and potassium). The composition of Tropstrato Florestal® was: pine bark, coconut fiber, rice husk and expanded vermiculite. And the nutrient solution by Hoagland and Arnon® contained macro and micronutrients, based on nitrogen, phosphorus, potassium, calcium, magnesium and sulfur.

After three months, the seedlings were transplanted into bags with a capacity of 6 liters with the same substrate composition. As for the irrigation process, it remained the same, as previously described.

After 5 months in the greenhouse, 15 (fifteen) *B. balansae* were transplanted into 10 kg pots. The substrate composition was: Tropstrato Florestal® substrate, sieved sand and chicken litter in the proportion 1: 0.5: 0.5, respectively. Then, 05 pots were placed under direct sunlight, 05 pots were placed under 70% luminosity and 05 pots were placed under 50% luminosity. The watering process took place in the afternoon (at 16:00) with the aid of hoses.

The image below (Figure 1) illustrates how *B. balansae* were subjected to three growth conditions.

Figure 1 - *Bromelia balansae* Mez. subjected to three growth conditions (under direct sunlight; 70% of luminosity and 50% of luminosity) at Instituto Federal Goiano, Campus de Ceres-GO.

Goiano Federal Institute, Campus Ceres-GO, (2018). Source: Authors.

The characteristics of the fabrics were: 70% (monofilament shaded fabric 70%, 100% polyethylene, black, special strong black type 70%, Solpack®) and 50% (monofilament shaded fabric 70%, 100% polyethylene, black, 50% light black type, Solpack®).

After 136 days (4 months and 12 days), the following parameters were evaluated: plant height, number of leaves, length of the largest leaf, measurement of the largest leaf width, stem diameter and rosette diameter according to Sanches (2009).

A specimen of the inflorescence of *B. balansae* was deposited in the Herbarium of the Federal University of Goiás (UFG) under the number 71921.

2.4 Plant height

The height of the plant was measured using a graduated measuring tape, which was positioned vertically at the level of the substrate, measuring from the base of the stem to the fold of the last leaf of the plant.

2.5 Number of leaves

To determine the number of leaves, a count was performed, disregarding: leaves in senescence and leaves that were not above the level of the cistern.

2.6 Length of the largest leaf

The measurement of the length of the largest leaf was verified using a graduated tape measure, which was positioned at the base of the stem until the end of the leaf.

2.7 Width of the largest leaf

The width of the largest leaf was measured using a graduated ruler. To standardize the measurements, the reading was performed in the middle position of the sheet. As the leaves of *B. balansae* curve, they were stretched to perform the reading.

2.8 Stem diameter

With the aid of a digital caliper in the closest possible position to the substrate, the diameter of the stem was determined.

2.9 Rosette diameter

The diameter of the rosette was measured with the aid of a measuring tape, being measured between two opposite leaves at the point where they fold.

2.10 Quantification assays

Firstly, the leaves of *B. balansae* cultivated under different shading conditions (under direct sunlight, 70% light and 50% light) were collected, and then dried and crushed at the Plant Tissue Culture Laboratory of the Federal Institute of Goiano, Campus Ceres- GO, Brazil.

Three leaves were randomly collected from three different individuals from each treatment. In the selection of individuals, those on the extremities were excluded. The leaves that surrounded the main axis of the plant, which visually presented the largest size, were collected, and the leaves in senescence and the leaves that were not above the level of the cistern were not collected. The collection time took place in the morning at 10 am.

Subsequently, the leaves were fragmented and dried in an oven under forced ventilation at 40°C until constant weight, totaling 7 days of drying. After the drying process, they were crushed in knives mill and the powder obtained for each sample

was packed in duly identified Kraft paper bags and stored in an airy place, protected from light (BRASIL, 2019; SONAGLIO et al., 2010).

Then, the leaves, in the form of powder, were sent to the Natural Products Research Laboratory (LPPN) of the Faculty of Pharmacy of the Federal University of Goiás (UFG) to carry out the measurements.

2.11 Total phenols (FT)

For the quantitative determination of total phenolic compounds present in the samples cultivated under direct sunlight, 70% of luminosity and 50% of luminosity of the species *B. balansae*, the method of Hagerman and Butler (1978) adapted by Mole and Waterman (1987) was used.

Sample preparation: The sample (pulverized plant material) was weighed in the amount of 2 g and then transferred to a 250 mL Erlenmeyer flask and 150 mL of distilled water was added. The mixture was heated to boiling and refluxed between 80°C and 90°C for 30 min. The resulting aqueous extract was cooled and transferred to a 250 mL volumetric flask, which was completed to volume with distilled water. Then, the resulting mixture was filtered through a qualitative filter paper, discarding the initial 50 mL.

In duly identified test tubes, 2 mL of sodium lauryl sulfate/triethanolamine solution, 1 mL of $FeCl_3$ solution and 1 mL of the respective sample were added. After 15 min at rest, the reading of the sample was performed in a spectrophotometer at 510 nm. The assay for the quantification of total phenols was performed in triplicate.

Blank assay: In a test tube, 2 mL of sodium lauryl sulfate/triethanolamine, 1 mL of iron (III) chloride solution ($FeCl_3$) and 1 mL of distilled water were added.

Preparation of the standard curve: Tannic acid was weighed in the amount of 100 mg and then transferred to a 100 mL volumetric flask, which was made up to volume with 40 mL of 50% methanol and the remainder with distilled water. Subsequently, aliquots of 10 µL, 50 µL, 100 µL, 150 µL and 200 µL of this solution were removed and transferred to test tubes containing 2 mL of sodium lauryl sulfate/triethanolamine solution, 1 mL of $FeCl_3$ solution and completed the volume to 4 mL with distilled water, the concentrations of each tube being: 0.0025 mg mL^{-1}; 0.0125 mg $mL^{-1;}$ 0.0250 mg $mL^{-1;}$ 0.0375 mg mL^{-1} and 0.0500 mg $mL^{-1,}$ respectively. After 15 min. at rest, the reading was performed in a spectrophotometer at 510 nm.

Each curve point was prepared in triplicate. From the equation of the straight line obtained from the standard curve, it was possible to calculate the concentration (mg $mL^{-1)}$ of total phenols and the percentage present in the sprayed plant material, from the following equation:

$$C = \frac{ABS - A}{B}$$

Wherein:

C= Tannic acid in mg mL^{-1}

ABS = Absorbance

A = Intercept of the curve

B= Slope of the curve

$$FT\ (\%) = \frac{C \times v \times 10^{-3} \times 100}{m\ (g)}$$

Wherein:

FT (%) = Total phenol %

C= Phenolic content in mg mL^{-1}

v=flask volume

m (g) = pulverized sample weight

Additionally, the number of milligrams of total phenols per gram of leaf was calculated.

2.12 Total Flavonoids

For the determination of total flavonoids present in samples cultivated under direct sunlight, 70% of luminosity and 50% of luminosity of the species *B. balansae*, the spectrophotometric method described by Rolim et. al. (2005) modified.

Sample preparation: The sprayed sample was weighed in the amount of 1 g. It was then transferred to a 125 mL ground-glass flask and 50 mL of methanol:0.02 M acetic acid (99:1) solution was added. Subsequently, the sample was heated in a water bath under reflux at 90 ºC for 40 min, and then filtered through analytical filter paper. In test tubes, duly identified, 1800 µL of solvent (methanol solution: 0.02 M acetic acid) plus 200 µL of samples were added, under a dilution factor of 10. Then, the absorbance was read at 361 nm. All samples were prepared in triplicate.

Blank: It was prepared with a mixture of methanol: acetic acid 0.02 mol L^{-1}.

Preparation of the Standard Curve: 10 mg of rutin were weighed and transferred to a 100 mL volumetric flask. Then, the flask was made up to volume with a solution of methanol: 0.02 M acetic acid (99:1). Subsequently, aliquots of 100 µL, 200 µL, 300 µL, 400 µL and 500 µL of this solution were removed and transferred to test tubes, which were duly identified. The test tubes were made up to 2 mL with a solution of methanol: 0.02 M acetic acid (99:1), with the concentrations of each tube being: 0.005 mg $mL^{-1;}$ 0.01 mg $mL^{-1;}$ 0.015 mg $mL^{-1;}$ 0.02 mg mL^{-1} and 0.025 mg $mL^{-1,}$ respectively. Each curve point was prepared in triplicate.

From the equation of the straight line obtained from the standard curve, it was possible to calculate the concentration (mg $mL^{-1)}$ of total flavonoids and the percentage present in the sprayed plant material, from the following formulas:

$$C = \frac{ABS - A}{B}$$

Wherein:

C= Rutin in mg mL^{-1}

ABS = Absorbance

A = Intercept of the curve

B= Slope of the curve

$$FlaT\ (\%) = \frac{C \times v \times 10^{-3} \times 100}{m\ (g)}$$

Wherein:

FlaT (%) = Flavonoid content %

C= Flavonoid concentration in mg mL^{-1}

v= Volume of the flask

m (g) = weight of pulverized sample

In addition, the number of milligrams of total flavonoids per gram of leaf was calculated.

2.13 Statistical analysis

For the number of calluses per explant, to compare plant height, number of leaves, length of the largest leaf, measurement of the largest leaf width, stem diameter and rosette diameter and the number of secondary metabolites as a function of different light intensities in that the plants, the data obtained was submitted to analysis of variance, and the averages compared by Tukey's test at 5% probability using the statistical program SISVAR.

2.14 Mass spectrometry analysis

Samples of leaves grown under direct sunlight, 70% light and 50% light for the species *B. balansae* were weighed separately in the amount of 20 mg, and extracted with 1 mL of 80% ethyl alcohol in an ultrasonic bath for 10 min. Then, the samples were centrifuged at 4000 rpm for 20 min. In the supernatants, 1 mL of hexane was added and then taken to the ultrasonic bath for 10 min. Subsequently, they were centrifuged under the same conditions described above. Finally, the hexane fractions were discarded and the polar fractions were sent to the Laboratory of Chromatography and Mass Spectrometry (LaCEM) of the Institute of Chemistry at UFG for analysis.

Mass spectrometry analysis was performed according to Silveira et al. (2019) with adaptations, using a microTOF III spectrometer (Bruker® Daltonics, Bremen, Germany) equipped with an electrospray ion source (ESI) (Bruker ® Daltonics, Bremen, Germany). Analysis was performed by direct infusion (3 μ L mim $^{-1)}$ after extraction with methanol and 1 mol L $^{-1}$ formic acid. All analysis was performed in positive mode. The parameters employed for the formation of the spray in the positive ESI (+) mode were: nitrogen gas nebulizer with a temperature of 200 ºC, pressure of 0.4 bar and drying gas flow of 4 L mim $^{-1;}$ capillary voltage of -4 kV; temperature of 200 ºC in the capillary transfer; end plate offset of -500 V; skimmer of 35 V and collision voltage of $^{-1}$ .5 V. Each spectrum was acquired using 2 microscans and processed using Data Analysis® software (Bruker® Daltonics, Bremen, Germany).

The mass spectra were processed, and the elemental compositions of the compounds were determined through the detected m/z values. The proposed structures for each formula were assigned using the database ChemSpider (2015), PubChem (2019) and Dictionary of Natural Products (2018).

3. Results and Discussion

3.1 *In vitro* growth

Tissue culture techniques have been applied in research involving medicinal plants, whose protocols allow establishing standards for mass multiplication of various species used in the production of herbal medicines. For the development of these protocols for a given species, it is first necessary to establish it *in vitro* (Morais et al., 2012).

In the present study, the *in vitro* germination rate of *B. balansae* seeds was 94.95% and the developed seedlings were uniform. Therefore, the *in vitro* germination protocol was efficient.

A study that corroborates the present work was that developed by Miranda and Silveira (2012), in which they obtained 95% of germination rate with the same species under study and the percentage of disinfestation was 100%.

Taking into account the time of exposure of the seeds to the disinfesting solution and the concentration of the disinfesting solution used, it was possible to observe that the protocol was not phytotoxic to the explants. According to Andrade (2002), the concentration of hypochlorite, the time and method of disinfestation vary with the type and age of the fabric.

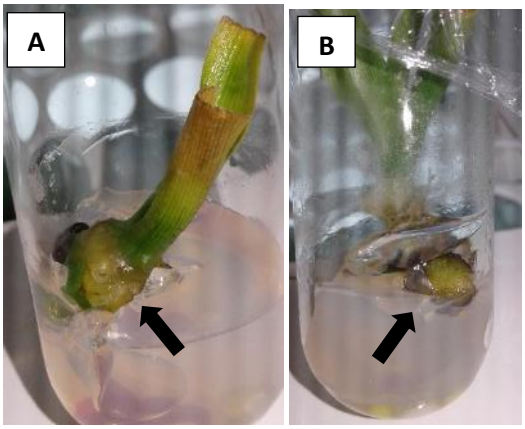
As for microbial contamination, none of the samples presented microorganisms, thus guaranteeing the establishment of the *in vitro* genotype. On the other hand, according to Sato et al. (2001), native plant species have some difficulty in *in vitro* establishment, mainly due to microbial contamination and oxidation.

3.2 Action of growth regulators on callogenesis induction

As for the test with supplementation of the culture medium with growth regulators (NAA and BAP), 3.90% (05/128) of the samples were contaminated with microorganisms and discarded. In regards to survival (number of explants developed per number of inoculated explants), all other samples (123/128), with 96.10%, lasted until the end of the analysis. Of the 123 remaining samples, 31.71% (39/123) formed calluses.

It was possible to observe 'friable' (soft) calluses, which are fragile and separate easily. The color of the calluses was variable, from yellow to green and brown (Figure 2).

Figure 2 - *Bromelia balansae* Mez. cultivated *in vitro* as a source of explant used for callus induction. A and B-. Explants with the formation of friable calluses of variable color, from yellow to green and brown, grown in MS½ medium supplemented with naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP), maintained at 25 °C ± 2 °C under a 16- hour light photoperiod a day for 3 months. Combination of 2 µM NAA and 4 µM BAP.



Source: Authors. Instituto Federal Goiano, Campus Ceres-GO, (2018), Brazil.

Thus, obtaining friable calluses enables future studies in the area of somatic embryogenesis and/or secondary metabolites.

Calluses are tissues that develop in response to physical or chemical injury. Callus culture can be started *in vitro* by placing a small part of an explant in the culture medium under aseptic conditions. Under the stimulus of endogenous growth substances or growth regulators added to the medium, cellular metabolism begins active division. During the process, cell differentiation and specialization are reversed and the explant originates a new tissue that is composed of meristematic and non-specialized cells (Santana et al., 2001).

The average amount of calluses formed per explant is shown in Table 1.

Table 1- Average number of calluses formed per explant, after 3 months, in *Bromelia balansae* Mez. as a function of different combinations of naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) .

		BAP (µM)			
		0 µM	1 µM	2 µM	4 µM
NAA (µM)	0 µM	0*A	0A	0.125 A	0.125 A
	1 µM	1A	0.375 A	0.125 A	0.125 A
	2 µM	0.625 A	1.5A	0.125 A	3.125 B
	4 µM	1.375 A	1.25 A	0A	1.875 AB

* Means followed by the same capital letter in the column and in the row do not differ statistically from each other, according to Tukey's test, at the 5% probability level.

Source: Authors.

It was possible to observe that the number of calluses formed per explant was influenced by the concentrations and combinations of NAA and BAP, and the highest average amount of calluses formed significantly was 3.125 calluses per

explant in the combination of 2 µM of NAA and 4 µM of BAP. Therefore, in the present study, it was possible to observe a greater tissue/cellular sensitivity of the *B. balansae* species when exposed to growth regulators at a concentration of 2 µM of NAA and 4 µM of BAP (Table 1).

In general, media containing auxins induce the formation of nodes, adventitious roots or calluses (Carvalho et al., 2006; Melo, 2002). According to Brum, Silva and Pasqual (2002), the most used auxin to stimulate multiplication in culture medium is NAA.

According to Taiz et al. (2016), cytokinins participate in cell division in callus in the presence of auxin, promoting the formation of buds or roots from callus in culture. It is noteworthy that the most used cytokinin is BAP, which has been shown to be essential for shoot multiplication and induction of adventitious buds (Brum et al., 2002).

According to Rodríguez (1987), a higher concentration of auxin than cytokinin results in roots or callus and, on the contrary, a higher concentration of cytokinin than auxin originates shoots.

It is noteworthy that growth regulators do not act in isolation, but with possible interaction with endogenous plant hormones, resulting in a hormonal balance. According to Caldas, Haridasan and Ferreira (1990), the development of callus in different species can be: independent of auxin and cytokinin, dependent on auxin, dependent on cytokinin or dependent on both, auxin and cytokinin.

Growth regulators are responsible for determining the development of cells, tissues or plants in a culture medium, and the concentration and combination of different types of auxins and cytokinins to supplement the culture medium, depend mainly on the plant species, the type of tissue or organ and the purpose of the research (Souza et al., 2018).

This was possible to observe in other species of the Bromeliaceae family, as in the study by Marcondes et al. (2014); rhizome buds of the species *Bromelia pinguin* L., popularly known as Gravatá, Caraguatá, caroatá and bananinha do mato were inoculated in MS½ medium containing growth regulators, being: 10 treatments consisting of five concentrations of BAP (0; 0.5; 1.0, 2.0 and 5.0 mg L^{-1}) combined with two concentrations of NAA (0; 0.5 mg L^{-1}) with five replications. After a period of 90 days, low concentrations (0.5 mg L^{-1} of NAA) in the culture medium were favorable for *in vitro* callus formation for *B. pinguin.* The addition of BAP only in MS medium proved to be a limiting factor in the development of calluses in this species. The highest callus formation was obtained in the interaction of NAA and BAP (NAA (0.5) + BAP (5.0)).

In the work by Pereira et al. (2009) observed the induction of callogenesis from the leaf base of the species *Ananas macrodontes* E. Morren (Bromeliaceae) at concentrations of 1 and 4 µM of NAA and 4 µM of BAP. The beginning of cell differentiation with the emission of buds was also verified.

In the study by Bellintani (2006), nodal segments of the species *Neoregelia mucugensis* Leme, *Orthophytum mucugense* Wand. and Conceição and *Orthophytum albopictum* Philcox (Bromeliaceae endemic to Bahia) were inoculated in test tubes containing MS½ culture medium in different combinations of BAP (0.00; 1.11; 2.22 and 4.4 µM) and NAA (0 .00; 0.33; 0.65 and 1.30 µM). After six months of inoculation, the percentage of explants that formed calluses was evaluated and it was possible to observe that the percentage of explants that originated calluses was high (60 %) for the treatment of *O. albopictum* with 1.11 µM of BAP associated with 0.65 µM NAA.

With the pineapple species cv. Pérola (Bromeliaceae), in general, the MS medium supplemented with 1.8 mg L^{-1} of NAA and 2 mg L^{-1} of BAP proved to be more efficient for inducing shoots, in number, but with the formation of a great mass of calluses. In the MS medium supplemented with 0.1 mg L^{-1} of NAA and 0.5 mg L^{-1} of BAP, shoots were smaller, without callus formation (Moreira et al., 2003). According to the authors, the concentration of auxin used was probably high, since NAA is an auxin that, if added in concentrations above a few tenths of a milligram, tends to form calluses in the explants.

Finally, much remains to be known about the mechanisms of plant hormones, growth regulators and the processes that control plant development (Andrade, 2002).

3.3 Growth of *B. balansae* as a function of light intensity

The growth analysis is used to verify the degree of tolerance of different species to changes in the amount of radiation received, resorting to several parameters to evaluate plant responses in relation to light intensity (Benicasa, 2003).

However, the cultivation of ornamental and medicinal plants often follows empirical knowledge, and these are cultivated in places where they usually present a better morphological response (Araújo et al., 2017).

Regarding the parameters: plant height, number of leaves, length of the largest leaf, measurement of the largest leaf width, stem diameter and rosette diameter of plants grown under direct sunlight, in 70% of luminosity and in 50% of luminosity, found are described in Table 2.

Table 2 - Average of the parameters: plant height, number of leaves, length of the largest leaf, measure of the largest leaf width, stem diameter and rosette diameter of *Bromelia balansae* Mez . grown under direct sunlight, at 70% and 50% light.

treatment	height (cm) ^{ns}	No of leaves	Leaf length (cm)	Leaf width (cm)	Stem diameter (cm)	Rosette diameter (cm)
direct sunlight	62.96 A	23.2 A	63.5 B	4.12 A	46.26 A	97.9 A
70% luminosity	70.2 A	24.4 A	74.4 A	3.74 A	47.34 A	110.6 A
50% luminosity	65A	23.8 A	67.5 AB	4.2 A	39.73 A	101.3 A
CV (%)	10.19	12.53	8.94	14.29	18.10	11.79

Means followed by the same capital letter in the column do not differ statistically from each other, according to Tukey's test, at the 5% probability level ^{ns}. Caption: CV (%): Variation coefficient. Source Authors.

As observed in Table 2, the means of the results obtained did not differ. With the exception of the parameter: length of the largest leaf, in which the average of the plants cultivated in 70% of luminosity displayed larger size with 74.4 cm, followed by the average of the plants cultivated in 50% of luminosity with 67.5 cm and of the average of plants grown under direct sunlight with 63.5 cm. It is noteworthy that all specimens of *B. balansae* used in the analysis survived all the conditions studied.

In the work of Ota (2018), there were also no differences between weight, length and diameter measurements between the fruits of *Ananas ananassoides* (Baker) LBSm. (Bromeliaceae) evaluated under light and shading conditions.

According to Ferreira et al. (2006) bromeliads have a great capacity for adaptation, developing in various types of habitats, from environments with total shade to even those environments where they are exposed to sunlight. Even though light is an indispensable factor for the survival of plants, each species needs a particular amount of light offered by the environment so that its morphological qualities are maintained in adequate conditions (Kerbauy, 2008).

Therefore, all light conditions were suitable for the growth and development of the species under study, and it can be inferred that the *B. balansae* plants were tolerant to the studied conditions. Oliveira and Gualtieri (2011) reported that this demonstrates that the plant species has adaptive ability.

It is interesting to observe in the work by Batagin et al. (2009) the phenotypic plasticity of *Ananas comosus* (L.) Merrill cv. IAC "Gomo-de- mel" (Bromeliaceae), because when transferred from *in vitro* to *ex vitro* conditions, the micro plants showed 100% survival, both under screen with 50% shading and under direct sunlightlight condition, noting that the change from heterotrophic to autotrophic metabolism is not a limiting factor for acclimatization of this cultivar.

Larcher (2004) states that shaded plants considerably increase leaf size as a mechanism for greater interception and absorption of light for the photosynthetic process, while plants under full sun showcase smaller leaf size, as it is sufficient for absorbing the necessary energy for photosynthesis, in addition, smaller size prevents the leaf from receiving high radiation that could lead to the photoinhibition process and consequently damage to the photosystems responsible for the absorption and

transfer of energy in the chloroplast. However, this is not very peculiar to CAM plants (crassulacean acid metabolism), such as bromeliads, which gives them the ability to develop better in high temperature environments (Pimentel, 1998).

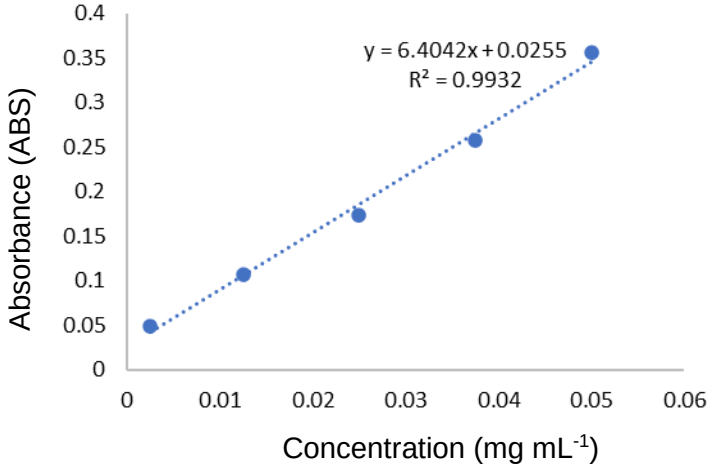
Also, perhaps this lower leaf growth of species cultivated under direct sunlightlight is related to greater exposure to UVB radiation, as according to Boeger and Poulson (2006) the increase in UVB radiation may induce structural and physiological changes in plants, influencing their growth and development, a fact observed in his study in which the leaves of the species *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) treated with UVB radiation showed lower leaf area, fresh and dry mass, trichome density on the adaxial surface and stomata density on the abaxial surface of the leaf. However, they showed the highest mean values of total blade thickness and mesophyll, higher concentrations of total chlorophyll, chlorophyll a and chlorophyll b and leaf phenolic compounds than leaves not treated with UVB radiation. As for phenolic compounds, he also observes an increase of these compounds in *B. balansae* grown under direct sunlight (Tables 2 and 3).

Finally, when plants experience changes in light conditions, they are able to a greater or lesser extent to adjust, and the adjustment made by plants to changes in the number of light leads to changes in morphology and physiology (Pacheco; & Paulilo, 2009).

3.4 Quantitative analysis

For the quantification of total phenolic compounds in *B. balansae* grown under direct sunlight, at 70% and 50% of luminosity, it was necessary to construct a standard curve of tannic acid. The curve equation was y = 6.4042x + 0.0255, with a r^2 of 0.9932, where y is equal to absorbance and x is the concentration of tannic acid, as shown in Figure 3.

Figure 3 - Standard curve for the determination of total phenols in leaf extracts of *Bromelia balansae* Mez . grown under direct sunlight, at 70% and 50% light. Tannic acid concentration in mg mL^{-1} versus Absorbance (ABS). Each point represents the mean ± standard deviation in triplicate.



Source: Authors.

The results of the quantification of total phenolic compounds in extracts of leaves of *B. balansae* grown under direct sunlight, at 70% and 50% of luminosity were represented as mean ± standard deviation according to Table 3.

Table 3 - Percentage (m/m) of total phenolic compounds (FT) present in leaf extracts of *Bromelia balansae* Mez. grown under direct sunlight, at 70% and 50% light.

Samples	FT (%)	mg total phenols in mL of extract	mg of phenols in g of leaves
Under direct sunlight	0.28% ± 0.0020 A	0.022095 A	11.04744 A
70% luminosity	0.22% ± 0.0007 B	0.017254 B	8.627151 B
50% luminosity	0.20% ± 0.0011 C	0.015797C	7.898462 C

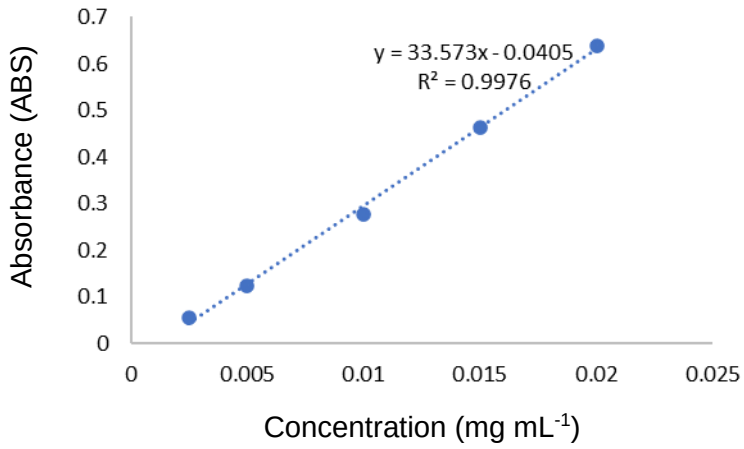
Means followed by the same capital letter in the column do not differ statistically from each other, according to Tukey's test, at the 5% probability level. Source: Authors.

Therefore, it was possible to observe that the phenolic compounds present in the extracts of the leaves of the plants cultivated in different light intensities had different significant levels. Under direct sunlight it contained the highest amount of total phenolic compounds with 0.28%, followed by plants grown with 70% luminosity, with 0.22% and those grown with 50% luminosity, with 0.20%.

For pharmaceutical industrial scale, it was possible to verify that for each gram of leaves of *B. balansae* grown under direct sunlight it contains 11.04744 mg of total phenols, followed by leaves grown in 70% luminosity with 8.627151 mg and leaves grown in 50 % luminosity with 7.898462 mg

For the quantification of total flavonoids in *B. balansae* grown under direct sunlight, at 70% and 50% luminosity, it was necessary to construct a standard rutin curve. The curve equation was y = 33.573x - 0.0405, with ar ^{2} of 0.9976, where y is equal to absorbance and x to rutin concentration, as shown in Figure 4.

Figure 4 - Standard curve for the determination of total flavonoids in leaf extracts of *Bromelia balansae* Mez . grown under direct sunlight, at 70% and 50% luminosity. Rutin concentration in mg mL^{-1} versus Absorbance (ABS). Each point represents the mean ± standard deviation in triplicate.



Source: Authors.

The results of quantification of total flavonoids in extracts of leaves of *B. balansae* grown under direct sunlight, at 70% and 50% luminosity, were represented as mean ± standard deviation according to Table 4.

Table 4 - Percentage (m/m) of total flavonoids (FlaT) present in leaf extracts of *Bromelia balansae* Mez . grown under direct sunlight, at 70% and 50% luminosity.

Samples	FlaT (%)	mg of flavonoids in mg of extract	mg of flavonoids in mg of leaves
under direct sunlight	0.65% ± 0.0065 A	0.012960 A	3.237 A
70% luminosity	0.60 % ± 0.0024 B	0.011932 B	2.980 B
50% luminosity	0.59 % ± 0.0038 B	0.011793 B	2.950 B

Means followed by the same capital letter in the column do not differ statistically from each other, according to Tukey's test, at the 5% probability level. Source: Authors.

Therefore, it was possible to observe that the total flavonoids present in the extracts of the leaves of the plants cultivated in different light intensities had different significant levels. Under direct sunlight it contained the highest number of total flavonoids with 0.65%, followed by plants grown with 70% luminosity, with 0.60% and those grown with 50% luminosity, with 0.59%. It is noteworthy that plants grown at 70% and 50% luminosity did not show significant differences.

For pharmaceutical industrial scale, it was possible to verify that for each gram of leaves of *B. balansae* grown under direct sunlight it contains 3.237 mg of total flavonoids, followed by leaves grown at 70% luminosity with 2.980 mg and leaves grown at 50% luminosity with 2.950 mg.

According to the present study, it was possible to observe that the intensity of sunlight was a significant interfering factor in the production of secondary metabolites, since plants grown under direct sunlight had a higher content. And that the presence of secondary metabolites identified and quantified in the species under study, guarantee advantages for its survival and perpetuation.

In the study by Ota (2018), the ascorbic acid content found in the fruit of *Ananas ananassoides* (Baker) LB Smith (Bromeliaceae), popularly known as pineapple-do- cerrado, under luminosity conditions was significantly higher, with 259.91 ± 27.28 mg, than that found under shading, with 37.30 ± 2.25 mg the content of phenolic compounds did not have significant differences between luminosity and shading conditions.

In the work developed by Rodrigues (2016), in shaded plants (blue shading with 75% luminosity) of *Varronia curassavica* Jacq. (Boraginaceae), popularly known as herb-whaling, the flavonoid content remained stable throughout the evaluated period. As for plants kept under direct sunlight, the flavonoid content increased with the period of exposure to natural light conditions. The author suggests that these results possibly indicate a photoprotection response of leaves to solar radiation.

Kerbauy (2008) explains that the perception of the light signal of light by the plant is given by specialized photoreceptors, which allow the light to be absorbed and photochemically activated. By selectively absorbing different wavelengths, the photoreceptor analyzes the informative content contained in the ambient light and transforms it into a primary action inside the cells. Upon becoming photochemically active, the photoreceptor triggers a cascade of biochemical events leading to metabolic responses. Photoreceptors can be: phytochromes (V and VE), cryptochromes and phototropins (blue and UV-A), UVR8 (UV-B) and Zeitlupe proteins (blue) (Mendoza-Paredes et al., 2021).

Thus, plants can respond specifically to light stimuli, as photoreceptors activate transcriptional factors that induce plant responses to light. These responses do not depend only on the presence, attenuation or absence of light, but also on the spectral quality of the radiation (Taiz et al., 2016). According to Kerbauy (2008), a higher content of secondary metabolites in plants grown under direct sunlight may be related to the presence of inactive genes that are expressed when activated and influenced by environmental signals (sunlight), as seen in the present work, and differentiated photoresponses may depend on changes in the relative quantity of photoreceptors and intrinsic biochemical properties of the plant.

In the present study, it was also observed that the content of biomolecules in the leaves of shaded plants decreased as

a result of the decrease in light intensity. According to Rodrigues (2016), this response may be related to the inhibitory effect of light on the synthesis of primary metabolites such as sugars and amino acids, since the synthesis of aromatic compounds, such as phenolic compounds and flavonoids, depends on the availability of carbon skeletons from the environment. metabolism of carbohydrates and amino acids.

Another explanation would be that shading reduced the perception and signaling of the photoreceptors responsible for the production of phenolic compounds and flavonoids, in this irradiance condition. To substantiate the data in this work, scientific studies have demonstrated the interference of sunlight and the direct relationship of photoreceptors in the production of secondary metabolites (phenolic compounds and flavonoids), such as the one developed by Li (2009), in which transgenic plants of the species Arabidopsis thaliana with overexpression of cryptochrome1 (CRY1), under high light conditions, contained higher content of phenolic compounds, flavonoids and lignin than wild-type plants. Results from liquid chromatography coupled with mass spectrometry showed that leaves from plants overexpressing CRY1 contained twice as much chlorogenic acids and ten times as much rutin than wild-type plants. Plants overexpressing CRY1 exhibited reduced ultraviolet-B injury. The author justifies that this may be due to the accumulation of secondary metabolites that have antioxidant activities in the protection of cellular structures from oxidative damage.

According to Mazza et al. (2000), the degree of damage that radiation can cause to plants depends on the efficiency of protection and repair mechanisms, such as the accumulation of substances or structures that absorb radiation, as well as the activation of antioxidant defenses. Additionally, plants exposed to solar radiation, specifically UVB, are induced to biochemical changes, such as the production of compounds that absorb and attenuate the negative effect of UVB radiation, such as: flavonoids and other phenolic compounds.

Brelsford et al. (2019) reported that cryptochromes are the main photoreceptors that regulate the accumulation of phenolic compounds in response to blue light and UV-A radiation, and the lack of functional cryptochromes impairs photosynthetic performance under bright light. They also revealed a role for UVR8 in flavonoid accumulation in response to a low dose of UV-A in Arabidopsis thaliana.

The work developed by Zhao et al. (2016), demonstrated that NtPHYB1K326, homologue of phytochrome B, was isolated from Nicotiana tabacum cv. K326 to investigate the role of light receptors in regulating polyphenol metabolism in tobacco leaves. The results indicated the involvement of NtPHYB1K326 in the regulation of polyphenol metabolism in tobacco leaves. The authors confirmed that NtPHYB1K326 can control the phenylpropanoid pathway by regulating the transcription of genes PAL4 (phenylalanine ammonia lyase 4), 4CL1 (4-coumarate: coenzyme A ligase 1) and COMT (caffeic acid 3-O-methyltransferase).

Yang et al. (2018b) also isolated CmUVR8, a homologue of UVR8, from the species Chrysanthemum morifolium Ramat, and the results indicated that CmUVR8 plays important roles in UV-B signal transduction and UV-B-induced flavonoid accumulation.

Finally, light is an environmental signal that, when perceived, triggers changes in the metabolism and development of plants, and can influence the concentration and/or composition of classes of secondary metabolites through intensity, quality and photoperiod (ISAH, 2019).

3.5 Mass spectrometry

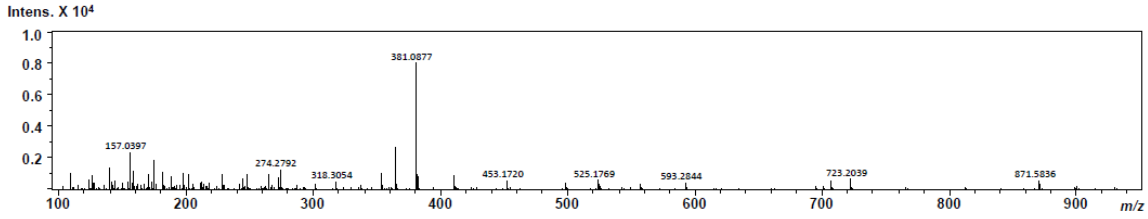
Mass spectrometry is an extremely valuable analytical technique in which molecules in a sample are converted into gas-phase ions, which are then separated in the mass spectrometer according to their mass ratio (m) over charge (z), m /z (Moraes & Lake, 2003).

Figure 5 illustrates the mass spectra of the probable compounds present in the samples cultivated under direct

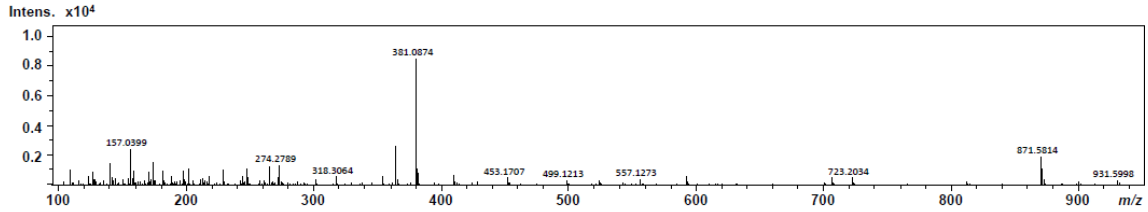
sunlight, 70% luminosity and 50% luminosity, respectively, of the species *B. balansae*.

Figure 5 - Mass spectra of the leaf extract of *Bromelia balansae* Mez . Grown in: A- full sun; B- 70% luminosity and C- 50% luminosity, respectively. Positive mode detection by mass spectrometry using a microTOF III spectrometer equipped with an electrospray ion source (ESI).

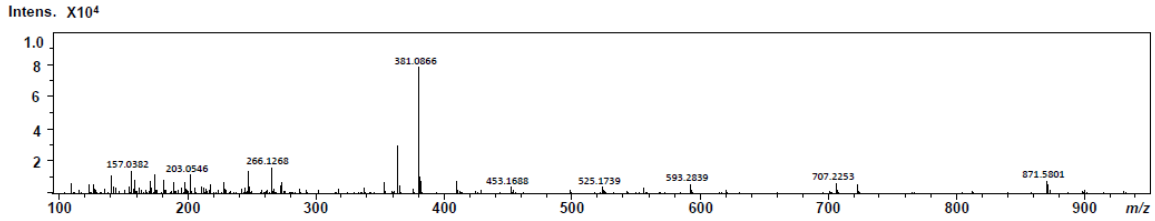
A- under direct sunlight



B- 70 % luminosity



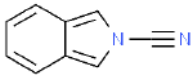
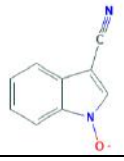
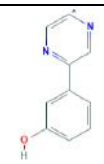
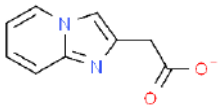
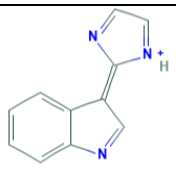
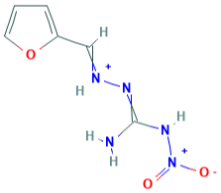
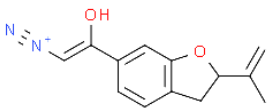
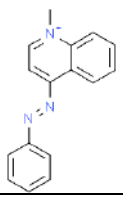
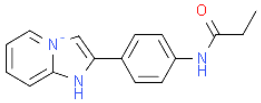
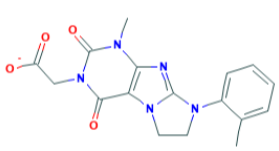
C- 50 % luminosity

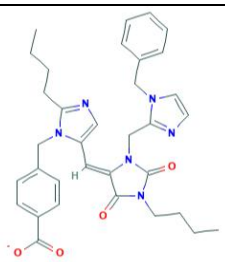


Source: Authors.

Table 5 contains the estimated molecular formulas and the probable compounds present in the samples cultivated under direct sunlight of the species *B. balansae*.

Table 5 - Estimated molecular formulas from the mass spectrum of extract from leaves grown under direct sunlight of *Bromelia balansae* Mez., positive mode, by mass spectrometry using a microTOF III spectrometer equipped with an electrospray ion source (ESI).

Experimental (*m/z*)	molecular formula	Error	structure	Possible chemicals
124.0283	$C_3H_2N_5O$	- 23.8	---	---
141.0438	$C_9H_5N_2$	6.8		Isoindole-2-carbonitrile
157.0394	$C_9H_5N_2O$	1.6		1-oxidoindole-3-carbonitrile
159.0513	$C_4H_7N_4O_3$	- 0.3	---	---
171.0548	$C_{10}H_7N_2O$	2.6		3-(pyrazin-2-yl)-phenol
175.0494	$C_9H_7N_2O_2$	4.4		Imidazo-[1,2-a]-pyridin-2-yl acetate
182.0711	$C_{11}H_8N_3$	1.2		(3*Z*)-3-imidazole - 1 -ium-2-ylideneindole
189.0633	$C_6H_5N_8$	- 0.6	---	---
198.0659	$C_6H_8N_5O_3$	- 18.7		[[amino (nitramido) methylidene] amino] - (furan-2-ylmethylidene) azioni
203.0544	$C_5H_3N_{10}$	- 3.6	---	---
229.0966	$C_{13}H_{13}N_2O_2$	2.3		(Z)-2-hydroxy-2-(2-isopropenyl-2,3-dihydro- 1 -benzofuran-6-yl)-ethenediazonium
248.1165	$C_{16}H_{14}N_3$	6.8		1-methyl-4-[(*E*)-phenyl-diazenyl]-quinoline
266.1283	$C_{16}H_{16}N_3O$	1.3		2-[4-(propionylamino)phenyl] -1H - *imidazo* [1,2-a]pyridin-4-ium
274.2793	---	---	---	---
318.3055	$C_{13}H_{36}N_9$	10.5	---	---
354.1164	$C_{17}H_{16}N_5O_4$	9.4		2-[4-Methyl-6-(2-methylphenyl) -1,3 - dioxo-7,8-dihydropurine-[7,8-a]-imidazol-2-yl] acetate

365.1114	$C_{16}H_{13}N_8O_3$	- 2.5	---	---
381,0877	$C_{10}H_{21}O_{15}$	- 0.5	---	---
411,1055	$C_{22}H_{19}O_8$	4.7	---	---
453.1729	$C_{18}H_{25}N_6O_8$	- 0.0	---	---
525.1759	$C_{26}H_{17}N_{14}$	- 0.7	---	---
593.2845	$C_{34}H_{37}N_6O_4$	4.4		4-[[5-[(*Z*)-[3-[(1-benzylimidazol-2-yl)methyl] -1 -butyl-2,5-dioxoimidazolidin-4-ylidene]methyl]-2-butylimidazole- benzoate 1 -yl]methyl]
707.2253	$C_{27}H_{39}N_4O_{18}$	0.1	---	---
723.2033	$C_{22}H_{43}O_{26}$	0.6	---	---
871.5806	$C_{49}H_{75}N_8O_6$	- 0.3	---	---

Source: ChemSpider (2015) and PubChem (2019).

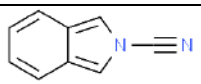
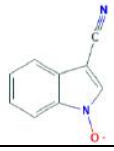
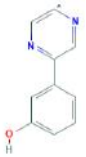
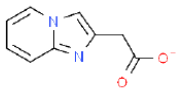
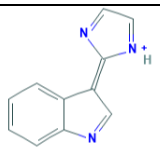
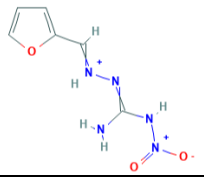
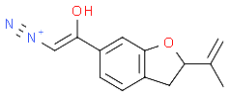
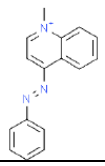
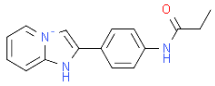
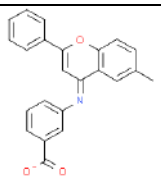
Therefore, the probable compounds found were: isoindole-2-carbonitrile; 1-oxidoindole-3-carbonitrile; 3-(pyrazin-2-yl)-phenol; imidazol-[1,2-a]-pyridin-2-yl acetate; (*3Z*)-3-imidazole - 1 -ium-2-ylideneindole; [[amino(nitramido)methylidene]amino]-(furan-2-ylmethylidene)azio; (*Z*)-2-hydroxy-2-(2-isopropenyl-2,3-dihydro-1-benzofuran-6-yl)-ethenediazonium; 1-methyl-4-[(*E*)-phenyl-diazinyl]-quinolinium; 2 [4-(Propionyl amino)phenyl]-1H-imidazo[1,2-a]pyridin-4-ium; 2-[4-Methyl-6-(2-methylphenyl)-1,3-dioxo-7,8-dihydropurino-[7,8-a]imidazol-2-yl] acetate and 4-benzoate [[5-[(*Z*) - [3-[(1-benzylimidazol-2-yl)methyl]-1-butyl-2,5-dioxoimidazolidin-4-ylidene]methyl]-2-butylimidazol-1-yl] methyl].

It is suggested that the isoindole-2-carbonitrile and 1-oxidoindole-3-carbonitrile compounds are indole alkaloids (Henriques et al., 2010). The compound 3-(pyrazin-yl)-phenol belongs to the pyrazine class (Assis et al., 2009). The compounds imidazole-[1,2-a]-pyridin-2-yl acetate and (3*Z*)-3-imidazole-1-ium-2-ylideneindole are suggestive of the class of imidazole alkaloids (Henriques et al., 2010; Shao et al., 2017). The compound [[amino (nitroamide) methylidene] amino] -(furan-2-ylmethylidene) azio is suggestive of the class of furans (SOARES, 2004) and (*Z*) -2-hydroxy-2- (2-isopropenyl-2 ,3-dihydro-1-benzofuran-6-yl)-Ethen diazonium from the benzofuran class (Kuster & Rocha, 2010).

The compound 1-methyl-4-[(*E*)-phenyl-diazinyl]-quinoline is suggestive of the class of quinoline alkaloids and the compound 2- [4-(propionyl amino) phenyl] -1H - imidazol [1,2-a] pyridin-4-ium is suggestive of the class of imidazole alkaloids (Cabral; & Pita, 2015; Henriques et al., 2010). Finally, the compounds 2-[4-Methyl-6-(2-methylphenyl) -1,3 -dioxo-7,8-dihydropurine-[7,8-a]-imidazol-2-yl] acetate and 4-[[5-[(*Z*)-[3-[(1-benzylimidazol-2-yl) methyl]-1-butyl-2,5-dioxoimidazolidin-4-ylidene] methyl]-2-butylimidazole benzoate -1 -yl] methyl] are suggestive of the class of imidazole alkaloids (Henriques et al., 2010).

Table 6 contains the estimated molecular formulas and probable compounds present in the samples cultivated at 70% luminosity of the species *B. balansae*.

Table 6 - Estimated molecular formulas from the mass spectrum of extract of leaves grown at 70% luminosity of *Bromelia balansae* Mez., positive mode, by mass spectrometry using a microTOF III spectrometer equipped with an *electrospray ion source* (ESI).

Experimental *(m/z)*	molecular formula	Error	chemical structure	Probable chemical compounds
127.0287	$C_8H_3N_2$	3.2	---	---
141.0438	$C_9H_5N_2$	13.6		Isoindole-2-carbonitrile
157,0397	$C_9H_5N_2O$	- 0.2		1-oxidoindole-3-carbonitrile
159.0508	$C_4H_7N_4O_3$	3.2	---	---
171.0542	$C_{10}H_7N_2O$	6.4		3-(pyrazin-2-yl)-phenol
175,0497	$C_9H_7N_2O_2$	2.8		Imidazo-[1,2-a]-pyridin-2-yl acetate
182.0704	$C_{11}H_8N_3$	4.9		(3*Z*)-3-imidazole - 1 -ium-2-ylideneindole
189.0636	$C_6H_5N_8$	- 2.1	---	---
198.0657	$C_6H_8N_5O_3$	- 18.0		[[amino (nitramido) methylidene] amino] - (furan-2-ylmethylidene) azioni
203.0556	$C_8H_{11}O_6$	- 3.0	---	---
219.0309	$C_{12}H_3N_4O$	- 3.3	---	---
229.0968	$C_{13}H_{13}N_2O_2$	1.7		(Z)-2-hydroxy-2-(2-isopropenyl-2,3-dihydro- 1 -benzofuran-6-yl)-ethenediazonium
248.1171	$C_{16}H_{14}N_3$	4.6		1-methyl-4-[(*E*)-phenyl-diazenyl]-quinoline
266,1287	$C_{16}H_{16}N_3O$	0.5		2-[4-(propionylamino)phenyl] -1H - *imidazo* [1,2-a]pyridin-4-ium
274.2791	---	---	---	---
318.3057	$C_{13}H_{36}N_9$	9.7	---	---
354.1155	$C_{23}H_{16}NO_3$	- 8.7		3- [(E)-(6-Methyl-2-phenyl- *4H* -chromen-4-ylidene) amino] benzoate
365.1116	$C_{16}H_{13}N_8O_3$	- 3.1	---	---
381.0874	$C_{10}H_{21}O_{15}$	0.2	---	---

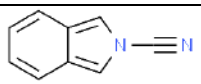
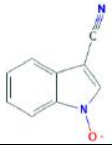
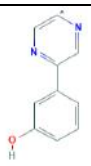
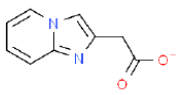
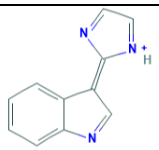
411,1043	$C_{19}H_{11}N_{10}O_2$	4.4	---	---
453.1729	$C_{18}H_{25}N_6O_8$	-0.6	---	---
499.1211	$C_{38}H_{15}N_2$	3.8	---	---
593.2842	$C_{30}H_{33}N_{12}O_2$	0.3	---	---
707.2255	$C_{27}H_{39}N_4O_{18}$	- 0.1	---	---
813.4998	$C_{56}H_{65}N_2O_3$	-1.0	---	---
871.5816	$C_{50}H_{71}N_{12}O_2$	0.2	---	---
932,605	$C_{29}H_{74}N_{25}O_{10}$	- 0.6	---	---

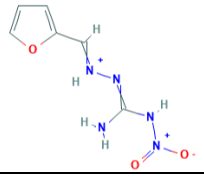
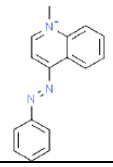
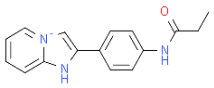
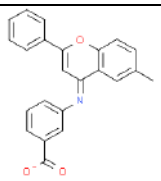
Source: ChemSpider (2015) and PubChem (2019).

Therefore, the likely compounds found in the leaves of species grown at 70% luminosity were the same compounds found in the leaves of plants grown under direct sunlight. Except for the absence of the compounds: 2-[4-methyl-6-(2-methylphenyl) -1,3 -dioxo-7,8-dihydropurine [7,8-a]imidazol-2-yl] acetate and benzoate 4-[[5-[(Z) - [3-[(1-Benzylimidazol-2-yl)methyl]-1-butyl-2,5-dioxoimidazolidin-4-ylidene]methyl]-2-butylimidazole-1- yl] methyl] and the appearance of the compound Benzoate of 3-[(*E*)-(6-methyl-2-phenyl-4H-chromen-4-ylidene) amino], which is suggestive of the group of chromones (Kuster & Rocha, 2010).

Table 7 contains the estimated molecular formulas and probable compounds present in the samples cultivated at 50% luminosity of the species *B. balansae.*

Table 7 - Estimated molecular formulas of the mass spectrum of extract from leaves grown at 50% luminosity of *Bromelia balansae* Mez., positive mode, by mass spectrometry using a microTOF III spectrometer equipped with an *electrospray ion source* (ESI).

Experimental *(m/z)*	**molecular formula**	**Error**	**chemical structure**	**Probable chemical compounds**
124.0276	$C_3H_2N_5O$	- 18.2	---	---
141.0428	$C_9H_5N_2$	6.6		Isoindole-2-carbonitrile
157.0381	$C_9H_5N_2O$	9.8		1-oxidoindole-3-carbonitrile
159.0505	$C_4H_7N_4O_3$	4.9	---	---
171.0538	$C_{10}H_7N_2O$	8.8		3-(pyrazin-2-yl)-phenol
175,049	$C_9H_7N_2O_2$	6.7		Imidazo-[1,2-a]-pyridin-2-yl acetate
182.0696	$C_{11}H_8N_3$	9.4		(3*Z*)-3-imidazole - 1 -ium-2-ylideneindole
189,063	$C_6H_5N_8$	1.1	---	---

198.0653	$C_6H_8N_5O_3$	- 16.0		[[amino (nitramido) methylidene] amino] - (furan-2-ylmethylidene) azioni
203.0546	$C_8H_{11}O_6$	2.2	---	---
219.0301	$C_{12}H_3N_4O$	0.0	---	---
229.0947	$C_9H_9N_8$	- 0.9	---	---
248.1164	$C_{16}H_{14}N_3$	7.3		1-methyl-4-[(*E*)-phenyl-diazenyl]-quinoline
266,1273	$C_{16}H_{16}N_3O$	5.7		2-[4-(propionylamino)phenyl] -1H - imidazo [1,2-a]pyridin-4-ium
274.2776	$C_{16}H_{36}NO_2$	- 13.0	---	---
354.1156	$C_{23}H_{16}NO_3$	- 9.6		3- [(*E*)-(6-Methyl-2-phenyl-4H-chromen-4-ylidene) amino] benzoate
365.1113	$C_{16}H_{13}N_8O_3$	- 2.2	---	---
381.0863	$C_7H_{13}N_{10}O_9$	- 0.3	---	---
383.0864	$C_4H_{11}N_{14}O_8$	3.8	---	---
411,1038	$C_{17}H_{19}N_2O_{10}$	- 1.0	---	---
453,168	$C_{25}H_{21}N_6O_3$	- 2.3	---	---
525,173	$C_{36}H_{21}N_4O$	- 3.8	---	---
593,283	$C_{29}H_{37}N_8O_6$	0.0	---	---
707.2236	$C_{24}H_{31}N_{14}O_{12}$	0.7	---	---
723.2031	$C_{35}H_{35}N_2O_{15}$	0.2	---	---
871.5807	C65H75O _ _ _ _	0.7	---	---

Source: ChemSpider (2015) and PubChem (2019).

Also, the likely compounds found in the leaves of species grown at 50% luminosity were the same compounds found in the leaves of plants grown under direct sunlight and at 70% luminosity.

Relating the data found with the sample of leaves grown under direct sunlight, the differences observed were: absence of compounds absence of compound (*Z*)-2-hydroxy-2-(2-isopropenyl-2,3-dihydro-1-benzofuran-6-yl)-Ethen diazonium, 2-[4-Methyl-6-(2-methylphenyl)-1,3-dioxo-7,8-dihydropurine-[7,8-a]-imidazole-2-acetateyl] and 4-[[5-[(*Z*)-[3-[(1-benzylimidazol-2-yl)methyl]-1-butyl-2,5-dioxoimidazolidin-4-ylidene]methyl]-2 Benzoate-butylimidazol-1-yl]methyl] and the presence of the compound Benzoate of 3-[(*E*)-(6-methyl-2-phenyl-4H-chromen-4-ylidene) amino], which is suggestive of the group of cromones (Kuster & Rocha, 2010) in leaves grown at 50% luminosity.

Comparing the data found with the sample of leaves grown at 70% luminosity, the differences observed were: absence of the compound (*Z*)-2-hydroxy-2-(2-isopropenyl-2,3-dihydro -1-benzofuran-6- il)-ethen diazonium in leaves grown at 50% luminosity.

In the present study, it was possible to observe that the biosynthetic routes of the secondary metabolites of the samples cultivated under direct sunlight, 70% luminosity and 50% luminosity were similar. It is noteworthy that most of the suggested compounds belong to the alkaloid class. This may be related to the positive mode used in the methodology, as substances that have basic groups, mainly amines, amides and esters, are usually analyzed in the positive mode, given the relative ease with which they are protonated (Crotti et al., 2006).

Alkaloids form a heterogeneous group of natural compounds that generally have a complex structure. They consist of

carbon, hydrogen and nitrogen, which in most cases forms part of a heterocyclic ring. Their biogenetic origin is normally from amino acids and they are provided with a great pharmacological or toxicological activity (Cabral & Pita, 2015).

Substances with a nitrogen atom not belonging to a heterocyclic system are called protoalkaloids. Nitrogenous compounds with and without heterocyclic rings that are not derived from amino acids are called pseudoalkaloids (Henriques et al., 2010).

It is noteworthy that alkaloids have already been detected in species of the Bromeliaceae family. As in the study by Vargas et al. (2016) who detected traces of alkaloids in *Bromelia pinguin* L. In the work by Cruz (2017), the results of the preliminary phytochemical characterization of extracts from the leaves of *Bromelia laciniosa* Mart. ex Schult. revealed the presence of alkaloids. As well as, Lopes Neto et al. (2015) detected in the phytochemical characterization alkaloids in the extracts of the leaves of the crown of the pineapple fruit *Ananas comosus* var. Comosus (L.) Merril.

Changes in the content and percentage of specific chemical components, depending on the radiation incident on the plants, or even the presence of some compounds found only at a certain level of radiation during plant cultivation, according to Kerbauy (2008), highlights the importance of of light as an inducer or inhibitor of specific biosynthetic routes for the synthesis of substances for pharmacological use, derived from the secondary metabolism of plants.

It is interesting to note that in the study by Ota (2018), the phenolic compounds, theobromine and rutin were not detected in *Ananas ananassoides* (Baker) LBSm fruits. (Bromeliaceae), under luminosity conditions, being identified and quantified under shading conditions. The phenolic compounds, ferulic acid and quercetin, were not found in fruits cultivated in the presence of luminosity and shading. It was noted that caffeine stood out in the fruits both under luminosity and shading conditions. Catechin and caffeic acid stood out in the fruit under shading conditions.

Finally, light is the main factor that controls the growth and development of plants, which are affected in a complex way by irradiance at all stages of growth. In addition, sunlight is a stimulus that can interfere with secondary metabolites, both qualitatively and quantitatively (Souza et al., 2011b).

4. Conclusion

The germination rate of *B. balansae seeds* was 94.95% and the developed seedlings were uniform. No sample was contaminated by microorganisms, thus ensuring the establishment of the *in vitro genotype*. As for the test with supplementation of the culture medium with growth regulators (NAA and BAP), 3.90% (05/128) of the samples were contaminated with microorganisms and discarded. With regard to survival (number of explants developed per number of inoculated explants), all other samples (123/128), with 96.10%, lasted until the end of the analysis. Of the 123 remaining samples, 31.71% (39/123) formed calluses. It was possible to observe that the amount of callus formed per explant was influenced by the concentrations and combinations of NAA and BAP, and the highest average amount of callus formed was 3.125 calluses per explant in the combination of 2 µM of NAA and 4 µM of BAP. Regarding the parameters: plant height, number of leaves, length of the largest leaf, measure of the largest leaf width, stem diameter and rosette diameter of plants grown under direct sunlight, at 70% luminosity and at 50% luminosity, the averages the results obtained did not differ significantly. With the exception of the parameter: length of the largest leaf, in which the average of plants cultivated in 70% luminosity presented the largest size with 74.4 cm, followed by the average of plants cultivated in 50% luminosity with 67.5 cm and the average of plants grown under direct sunlight with 63.5%. Therefore, all light conditions were suitable for the growth and development of the species under study, and it can be inferred that the *B. balansae plants* were tolerant to the studied conditions. It was possible to observe that the total phenolic compounds and flavonoids present in the extracts of the leaves of the plants cultivated in different light intensities varied significantly. Under direct sunlight, it contained a higher number of secondary metabolites, with 0.28% for total phenolic compounds and 0.65% for total flavonoids. The intensity of sunlight was a significant interfering factor in the

production of secondary metabolites. However, plant responses to radiation involve several physiological mechanisms that still require more detailed investigations. Through mass spectrometry, it was possible to observe that the biosynthetic routes of the secondary metabolites of the samples cultivated under direct sunlight, 70% luminosity and 50% luminosity were similar. It is noteworthy that most of the suggested compounds belong to the class of alkaloids. Little is known about the composition of secondary metabolites of *B. balansae leaves* harvested under different luminosity conditions. Therefore, both the content and the quality of these metabolites may vary according to the environmental conditions in which the plants will be submitted.

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