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Total phenolic content in fruits and in *in vitro* cultures of *Bromelia karatas* L.

Contenido total de fenoles en frutos y cultivos *in vitro* de *Bromelia karatas* L.

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Abstract

Bromelia karatas L. is a species native from America, used in traditional medicine as an alternative in the treatment of diabetes mellitus. Overexploitation and change in land use have led to a drastic decline in species. In the present work, *in vitro* cultures were established from *B. karatas* seeds and their total phenolic content was determined. To promote the germination of the seeds several scarification treatments were tested. Furthermore, the seeds were cultured on Murashige & Skoog (MS) media containing different concentration of N-6 benzyladenine (BA) with 2,4-dichlorophenoxyacetic acid (2,4-D). Obtaining a higher percentage of germination (100%) when exposing the seeds to H_2SO_4 (98%, 5 min) was obtaining. Additionally, in *in vitro* cultures, the germination time was considerably reduced (36 times), compared to the data reported in the field. Highest percentage of callus induction of leaf sections (1.5 cm^2) of adult specimens and plants germinated *in vitro* in MS media containing BA (1.0 mg $\cdot$ L^{-1}) + 2,4-D (0.0, 0.5, 1.0, and 2.0 mg $\cdot$ L^{-1}) was established. Mature fruits of *B. karatas* resulted in the greatest phenolic content (1,110 mg GAE $\cdot$ 100 g^{-1}, biomass, dry weight (DW)) measured by the Folin-Ciocalteu reagent method, while the smallest content was recorded for callus and the leaves of *in vitro* seedling germinated recorded 205 and 741 mg GAE $\cdot$ 100 g^{-1} DW, respectively. These results provide novel data, which can be part of programs for sustainable use and conservation of this species of ethnobotanical importance.

Keywords: *Bromelia karatas*, *in vitro* culture, seed scarification, total phenolic content.

Resumen

Bromelia karatas L. es una especie nativa de América, la cual es empleada en la medicina tradicional como una alternativa en el tratamiento de la diabetes mellitus. La sobreexplotación de la especie y el cambio en el uso del suelo de los sitios donde crece, han conducido a una declinación drástica de su distribución. En el presente trabajo, se establecieron cultivos *in vitro* de semillas de *B. karatas*, y se determinó el contenido total de fenoles en diferentes tejidos de la planta y de los cultivos *in vitro*. Para ello, se evaluaron diferentes tratamientos de escarificación para promover la germinación de las semillas. Posteriormente, las semillas fueron cultivadas en medio Murashige & Skoog (MS) conteniendo diferentes concentraciones de N-6 benciladenina (BA) con ácido 2,4-diclorofenoxiacético (2,4-D). Se obtuvieron altos porcentajes de germinación (100%) al escarificar las semillas con H_2SO_4 (98%, 5 min). Adicionalmente, en los cultivos *in vitro* el tiempo de germinación se redujo considerablemente (36 veces), al comparar con los datos reportados en campo. Por otro lado, el significativamente mayor porcentaje de inducción de callo se observó en los cultivos conteniendo medio MS con BA (1.0 mg $\cdot$ L^{-1}) + 2,4-D (0.0, 0.5, 1.0 y 2.0 mg $\cdot$ L^{-1}). Mientras que el mayor contenido de compuestos fenólicos se determinó en los frutos maduros (1,110 mg GAE $\cdot$ 100 g^{-1} biomasa, peso seco (DW)), en tanto que los callos y las hojas de plántulas germinadas *in vitro* registraron 205 y 741 mg GAE $\cdot$ 100 g^{-1} DW, respectivamente. Estos resultados proporcionan información novedosa que puede ser parte de programas para la conservación y uso sustentable de esta especie de importancia etnobotánica.

Palabras clave: *Bromelia karatas*, cultivo *in vitro*, escarificación, contenido de fenoles.

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1 Introduction

Bromelia karatas L. (Synonym: Bromelia plumieri (E. Morren) L.B. Smith) (Espejo-Serna *et al*., 2005) belongs to the family Bromeliaceae. The genus Bromelia comprises 50 species that are distributed from Mexico to Argentina and in the Antilles (Davidse *et al*., 1994; Montes *et al*., 2014). The bromeliads have ethnobotanical importance, since the family has different species of ornamental value, antibacterial activity, medicinal, food and agricultural; and even some species are often used as live fences (Anastácio & García de Santana, 2010; Avalos-Flores *et al*., 2022; Hornung-Leoni, 2011). *B. karatas* is commonly known as timbiriche, this is used in traditional medicine, and to the fruit have been attributed antidiabetic and antioxidant activities, among others (Escandón-Rivera *et al*., 2019; Moyano *et al*., 2012). Also, the presence of a protease called karatasin has been detected in the fruit (Meza-Espinoza *et al*., 2018). On the other hand, the species has agro-socioeconomic importance, since fiber is obtained from the plant and the edible fruit is commercialized (Montes *et al*., 1990). It also has ecological importance, by avoiding soil erosion and being a unique habitat for several animal species (Benzing, 2000). Unfortunately, even though *B. karatas* has different attributes, among which the use of the fruit as a coadjuvant in the treatment against diabetes and other conditions stands out, the populations of the species have decreased drastically, mainly in Central and South America, where there is a continuous reduction, either by adoption of new technologies for the delimitation of land, introduction of new species or the qualification of land for agricultural and livestock work, as well as housing use in areas where it grows wild. As a result, there is significant erosion of the native germplasm of the species and a loss of traditional knowledge (Montes *et al*., 2014). Due to its problems, it is important to make efforts for its conservation and sustainable use, as well as to develop studies that allow the validation of its use in traditional medicine.

Plant tissue culture has proven to be very useful in the conservation of plant species, whose populations are at risk, giving alternatives for conservation and sustainable use, as well as providing plant material to phytochemically validate medicinal use without undermining natural populations. During the last years, several works have been carried out on the germination of Bromeliaceae species. In general, the studies show high variability in terms of light requirements, temperature, use of plant growth regulators, scarification processes, and the germination percentages and times of each species (Coelho *et al*., 2011; Klekailo *et al*., 2012; Pompelli, 2006). This is due to the fact that within the family there are species that have seeds with physical and chemical characteristics of the tegument, which gives it to a structure or compact consistency that is impervious to water and gases, which inhibits mechanical and chemical germination (Coehlo *et al*., 2011). There are a few studies regarding *in vitro* culture in bromeliads. In *Vriesea reitzii*, *Puya berteroniana*, *Acanthostachys strobilacea*, *V. incurvata*, *Aechmea veitchii*, *Racinaea crispa*, *Tillandsia eizii* and *Ananas comosus*, shoot formation has been reported (Calderón-Arias *et al*., 2011; Cueva *et al*., 2006; Da Silva *et al*., 2009; Dal Vesco *et al*., 2015; dos Santos *et al*., 2017; Pickens *et al*., 2006; Roostika & Mariska, 2003; Sasamori *et al*., 2018; Viehmannova *et al*., 2016); while the callus production was generated in *Pseudoanana sagenarius*, *T. eizii* and *T. cyanea* (Avico *et al*., 2006; Cueva *et al*., 2006; Pickens *et al*., 2006); and the induction of somatic embryos in *A. comosus* (Roostika & Mariska, 2003). On the other hand, the synthesis of phenolic compounds has been detected in different species of bromeliads., e.g. *Aechmea gamosepala* and *V. platynema* (Giongi *et al*., 2019), *T. recurvate*, *T. schedeana*, *T. fasciculate* (Pérez-López *et al*., 2020), *Aechmea blanchetiana* (Magalhaes *et al*., 2012), and *Ananas porteanus* (Santana *et al*., 2011). However, from our knowledge there are no reports of *in vitro* culture in *B. karatas*. Therefore, the objective of the present work was to evaluate different types of scarification in the seed germination of *B. karatas*, and different plant growth regulators (PGR) to establish *in vitro* cultures to assess the total phenolic content (TPC).

2 Materials and methods

2.1 Plant material and seed viability

Mature infrutescences of reproductive specimens of *Bromelia karatas* L. were collected in the community of El Platanar, Malinalco, State of Mexico (18°49´45”N 99°27´31”W at 1292 msnm). A specimen was deposited in the herbarium of Facultad de Ciencias, Universidad Autónoma del Estado de

México, which was identified by Dra. Laura White Olascoaga. The fruits were washed with running water and commercial detergent. The seeds were extracted and washed with running water, to remove excess pulp, and dried at room temperature. The viability of the seeds obtained was determined by the Tetrazolium test. A batch of 10 seeds of each purchased batch was immersed in aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (1%, w/v) at pH 6.5, for one hour at 40 °C and in continuous darkness. Subsequently, the seeds were washed with distilled water to remove excess salt (Victoria *et al*., 2006), then they were sectioned longitudinally and later observed by optical microscopy. The percentage of viability was calculated as the number of seeds presented embryo coloration with respect to the total of seeds treated. The determination was made in triplicate.

2.2 *Scarification of the seeds*

The seeds were subjected to different types of scarification: (a) mechanical: removal of a lateral fragment of the seed coat; (b) thermal: immersion in water at 30 or 100 °C, for 1 or 5 min; (c) aqueous: immersion in water at room temperature for 24 h; and (d) chemistry: immersion in 30% or 98% H_2SO_4 solution (v/v), for 1 or 5 min. Furthermore, the seeds were superficially disinfected when immersed in a 1% (w/v) commercial detergent solution for 15 min, followed by a 70% (v/v) ethyl alcohol solution for 30 s, subsequently in immersion with a solution of sodium hypochlorite (0.6 or 1.2% v/v, during 10 or 20 min), and finally under aseptic conditions, under a laminar flow hood, the seeds were carefully rinsed with sterile distilled water five times and germinated aseptically in culture tubes (25 x 150 mm), containing Murashige & Skoog (MS; Murashigue & Skoog, 1962) culture medium at half its concentration, enriched with 10 g $\cdot$ L^{-1} of sucrose and 2 g $\cdot$ L^{-1} of phytagel. All media were adjusted to pH 5.8 with a 0.1 N NaOH solution, before being sterilized in an autoclave at 121 °C for 20 min. The cultures were incubated in two different light conditions, under a photoperiod of 16 h light at a luminous irradiance of 50 μmol m^{-2} s^{-1}, or in total darkness, at 25 ± 2 °C. At 48 h of incubation, all the seeds were bathed with 200 μL of a solution of gibberellic acid (0.2 mg $\cdot$ mL^{-1}; GA_3). Germination was recorded by observing the protrusion of the radicle and the germination percentage was estimated (Klekailo *et al*., 2012): $G(\%) = N_{ger}/N_{sem}$. Each treatment was carried out in batches of five tubes and each tube was inoculated with 5 seeds, the experiment was carried out in triplicate.

2.3 *Induction of callus*

Seed scarified and disinfected superficially, as well as leaf sections (1.5 cm^2) of adult specimens and plants germinated *in vitro* were inoculated in culture tubes (25 x 150 mm) containing MS culture medium supplemented with plant growth regulators (PGR): 6-benzyladenine (BA, 0.0, 0.5, 1.0 mg $\cdot$ L^{-1}) with 2,4-dichlorophenoxyacetic acid (2,4-D, 0.0, 0.5, 1.0, 1.5, and 2.0 mg $\cdot$ L^{-1}) and containing 30 g $\cdot$ L^{-1} of sucrose, 2 g $\cdot$ L^{-1} of phytagel, 500 mg $\cdot$ L^{-1} of polyvinylpyrrolidone (PVP), and 250 mg $\cdot$ L^{-1} of cysteine. The cultures were incubated at 25±2 °C and under a photoperiod of 16 h light. Each treatment derived from the combination of the PGR, was elaborated in batches of 5 tubes and the experiment was carried out in triplicate. The callus cultures were subcultured at 30 d in the baby food jars containing MS culture medium with PGR for biomass proliferation.

2.4 *Acclimation of in vitro germinated seedlings*

Seedlings germinated under aseptic conditions in a culture medium free of PGR, and furthermore, every 30 d were transferred to baby food jars containing 25 mL of MS medium culture with activated charcoal. Seedlings of 90 days old and with a length of 6-12 cm, were extracted from the culture flasks, washed with running water to eliminate the excess of culture medium and subsequently sown in pots containing peat-moss previously sterilized in autoclave. The pots were covered with polyethylene bags for 4 weeks, and they were moistened every 4 d. At the end of the period, the polyethylene bag was removed and the percentage of survival was recorded. The experiment was carried out in triplicate.

2.5 *Total phenolic content in different tissue extracts*

Biomass samples (500 mg, dry weight: immature fruit, mature fruit, leaves of *in vitro* germinated seedlings and callus) were dried by lyophilization (Labconco, USA) or by convection oven at 60 °C (Lumistell, Mexico) for 72 h. The dried samples were pulverized with the help of a mortar and poured into amber glass jars, adding methanol (1:30). The sample was kept in dark and constant agitation at 100 rpm for

24 h; subsequently it was subjected to ultrasonic bath (SKZ210HP Shanghai Kudos Ultrasonic Instrument, China) at 40 °C and 53 kHz, for 40 min. At the end of the period, the sample was filtered under vacuum. In the obtained extracts, the content of total phenols was determined by the Folin-Ciocalteu technique (Ainsworth and Gillespie, 2007). The reaction mixture consisted of 100 μL of crude extract, 250 μL of Folin reagent, 750 μL of 7 % Na_2CO_3 solution (w/v), adjusting to a final reaction volume of 2 mL, with distilled water. The mixture was vigorously stirred (Labnet International, USA), then it was incubated in the dark at room temperature for 45 min. Finally, the absorbance of the reaction mixtures was measured in a UV-Vis spectrophotometer (Cintra 1010, GBC Australia) at 760 nm. All reactions and determinations were carried out in duplicate. The content of phenols was expressed in terms of milligrams of gallic acid (20-100 mg GA; $R^2 > 0.99$) equivalents per 100 grams of biomass, dry weight (mg GAE · 100 g^{-1}, DW).

2.6 Statistical analysis

Data on percentage germination, callus induction, and phenols content were subjected to analysis of variance using the Statgraphics statistical software (Centurion XVI.II). The comparison of means was made using Fisher's Least Significant Difference test (LSD; $p \leq 0.05$).

3 Results and discussion

3.1 Seed scarification and in vitro germination

The *B. karatas* seeds showed integument dormancy or physical dormancy (coat impermeable to water). Also, the seeds were subjected at tetrazolium viability test (Fig. 1), showed at 7 d of collected an 100% of viability. Then, the seed were treated with different seed scarification process. From the all evaluated treatments in the scarification and surface disinfection of the seeds of *B. karatas*, the treatment that registered the significantly higher percentage of germination (100%), and the significantly lower percentage of contamination (0%), was the chemical scarification (98% H_2SO_4 for 5 min), followed by disinfection in 0.6% sodium hypochlorite solution for 10 min (Table 1). However, the seed germination was asynchronous.

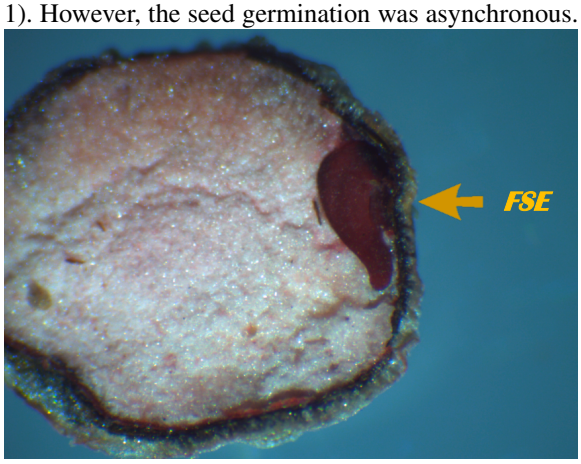

Figure 1. *Bromelia karatas* seed at 7 d of collected and treated with tetrazolium viability test (20x). FSE: Full stained embryo (arrow).

The germination was observed from the 5 d until 10 d of culture, which represents approximately 36-18 times less time, in relation to the field conditions (~ 180 d, data provided by farmers). Regarding the light condition of incubation, this showed no effect on the germination of the seeds, the observed response was the same in both conditions. While the remaining treatments of scarification, showed a range in the germination time of 5-80 d and the germination percentage ranged between 10-80%.

Nevertheless, after 2 months of storage, the seeds, when exposed to H_2SO_4 during the scarification process, show significant damage to the seed cover. By increasing the storage time of *B. karatas* seeds (6 months), the percentage of germination decreased to 45%. This coincides with the tetrazolium viability test, in data determined at 180 d (55%). According to Victoria *et al.* (2006), the test is adequate to assess the quality of embryos in *Hechtia perotensis* seeds. The germination is related to the progressive loss of the ability to germinate (viability) and the time it takes to lose its viability (longevity), these characteristics are specific for each species, together with the morphological and physiological conditions of the seeds at the moment of sowing, as well as the environmental conditions of the collection site (Sosa-Luría *et al.*, 2012). The viability found in *B. karatas* is similar to the reported data for six species of the genus *Tillandsia* (61-89 %), which was assessed by the X-ray technique (Sosa-Luría *et al.*, 2012); as well as for *H. perotensis* (36-87 %; Elizalde *et al.*, 2016).

Table 1. Effect of different types of scarification on *in vitro* germination of *B. karatas* seeds, after 30 days of culture.

Scarification type	Experimental conditions	Time	Germination (%)*
None (Control)	-	-	0.0^{a}
Mechanical	Removal of a lateral fragment of the seed coat	-	0.0^{a}
	Water temperature (°C)		
Aqueous	20°C	24 h	0.0^{a}
		1 min	0.0^{a}
	30°C	5 min	0.0^{a}
Thermal		1 min	25 ± 10^{b}
	100°C	5 min	50 ± 0^{c}
	H_2SO_4 concentration (%, v/v)		
Chemical	30%	1 min	10 ± 8.0^{ab}
		5 min	80 ± 20^{d}
	98%	1 min	70 ± 10^{d}
		5 min	100 ± 0^{e}

*The data represent the average of three ± DS replicates. Averages in columns, with different letter are significantly different ($p \leq 0.5$).

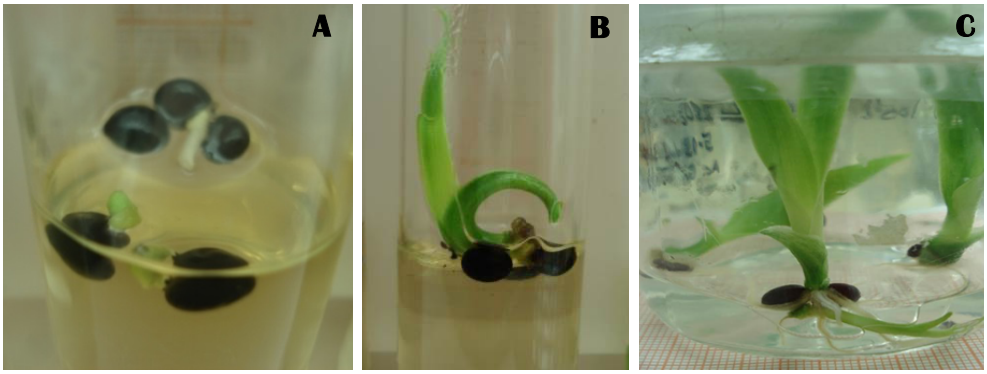

Figure 2. *In vitro* cultures of *Bromelia karatas*, (A) Seeds germinating; (B) seedling at 15 d of culture; (C) 30 d age seedlings, growing in MS medium, incubated at 25 °C and under a 16 h light photoperiod.

In general, *in vitro* germinated seedlings showed an adequate development, with the generation of long leaves of spiny margins and distributed in rosette (Fig. 2). The obtained results in the present work are within the reported ranges for species of the Bromeliaceae family, in works where a thermal scarification has been used (25-35 °C) the germination time of the seeds ranged between 3-110 d, and the percentages of germination registered between 100-15%. 100% for *B. pinguin* seeds cultured in Petri dishes covered with filter paper to maintain humidity (García-Franco *et al*., 1991); for *B. antiacantaha* seeds (80%) exposed to different temperature (10-35°C) and 8h photoperiod (Da Rosa & Ferreira, 1998); *T. eizii* seeds with an increase of NaOCl concentration in the sterilization method and culture under *in vitro* conditions (80-94%) (Pickens *et al*., 2003); the germination of *V. scalaris* seeds started seven days after *in vitro* inoculation on a germination medium (60%) (Da Silva *et al*., 2009); while in newly collected seed of *Ananas ananassoides* that were immersed in water at 90°C for 2 minutes was obtained a 92% of seed germination (Anastácio & García de Santana, 2010); in *B. laciniosa* seeds treated with immersion in acetone during 60 minutes was the treatment more efficient to promote the germination (83%) (Dutra *et al*., 2010), in *B. balansae* seeds treated with sulfuric acid for 1 min (70%) (Coelho *et al*., 2011); *T. bourgaei* (83.3%), *T. makoyana* (79.8%), *T. carlos-hankii* (79.5%), *T. prodigiosa* (75.8%), *T. fasciculata* (45%), *T. violacea* (15.2%), when the seeds were germinated at constant temperature of 25 °C and neutral photoperiod (Sosa-Luría *et al*., 2012).

Regarding the seed germination response with the scarification treatment that recorded the highest

percentages (100% germination, 98% H_2SO_4 for 5 min), it is similar to that reported for *B. balansae* (Coelho *et al*., 2011), in contrast, in *B. serra* did not promoted the germination (Klekailo *et al*., 2012), while in *Ananas ananassoides*, under the same treatment, germination was lower (76%). This may be due to the fact that the seeds of *B. karatas*, *B. balansae* and *B. serra* present tegumentary dormancy, whereas in *A. ananassoides* this characteristic is not reported (Anastácio & García de Santana, 2010). Dormancy is a strategy that can increase the possibilities in the establishment of the plant species.

3.2 Acclimation of germinated plants in vitro

On the other hand, the 90-day-old seedlings of *B. karatas in vitro* germinated were developed in a normal way, reaching a size of 6-12 cm. Furthermore, they were harvested and planted in pots for their *ex vitro* acclimatization, achieving 100% survival at 30 days of culture, and an average growth of 1 cm. However, some they showed symptoms of stress such as loss of leaves or oxidation of the leaf apex (Fig. 3). The process of successful acclimatization, together with the unbeatable percentages of germination *in vitro*, can be used in processes of massive propagation of *B. karatas*. Similar to study with *T. eizii* were obtained shoot elongates and plants, by *in vitro* cultures, and were successfully acclimatized and planted into the greenhouse (Pickens *et al*., 2006). While, in *V. incurvata* plantlets showed positively influenced for higher sucrose concentration (60 g L^{-1}) in the *ex vitro* development (Sasamori *et al*., 2018). The *Puya berteronina* regenerated plantlets enabling *ex vitro* transfer, showed after 8 weeks of culture in a glass house survival rate reached 98.3% (Viehmannova *et al*., 2016).

3.3 Induction of callus in explants

All the combinations of the concentrations of PGR tested, induced callus formation in *B. karatas* seeds, showing significant differences with respect to the control and between treatments. The generated callus was characterized by its yellow-green coloration, compact texture and because it was organogenic with generation of little multi shoots (Fig. 4). The PGR combinations whit significantly highest percentages of callus induction (78-65%), were the media supplemented with 1.0 mg · L^{-1} of BA with 2.0 mg · $L^{-1}$of 2,4-D (B1D2), 1.0 mg · L^{-1} of BA with 1.0 mg · L^{-1} of 2,4-D (B1D1), 1.0 mg · L^{-1} of BA with 0.5 mg · L^{-1} of 2,4-D (B1D0.5),

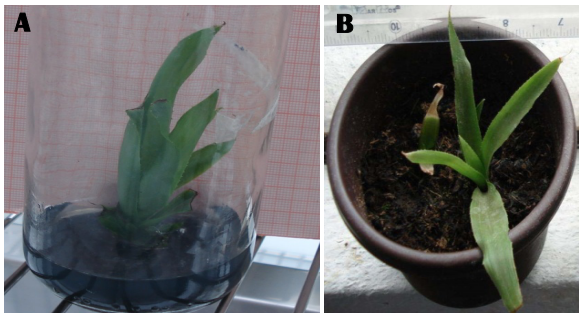

Figure 3. *Bromelia karatas* plants, (A) seedling developed *in vitro*, at 90 days of age, (B) seedlings acclimated *ex vitro* in peat moss, after 30 days of extraction of the *in vitro* conditions.

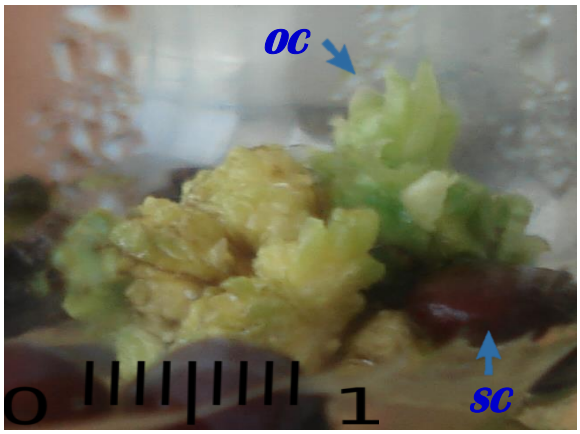

Figure 4. Formation of organogenic callus in *Bromelia karatas* seeds, at 30 days of culture in MS medium supplemented with 1.0 mg · L^{-1} of BA and 2.0 mg · L^{-1} of 2,4-D. OC= organogenic callus and SC= seed coat (bar: 1 cm).

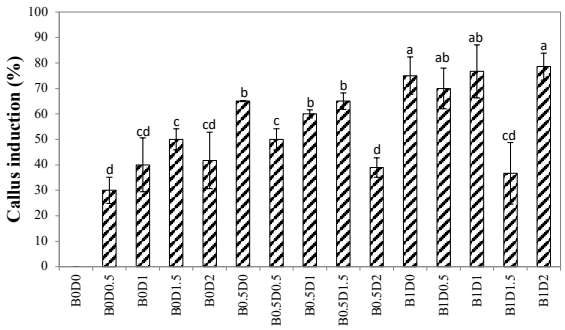

Figure 5. Callus induction in *Bromelia karatas* seeds, at 30 days of culture in MS medium supplemented with BA and 2,4-D (BxDx).

Table 2. Total phenolic content in wild plant tissues and *in vitro* cultures of *B. karatas*.

Plant material	Drying method	mg EAG/100 g biomass (DW)
Immature fruit	lyophilization	472 ± 02.60^{c}
	convection oven	94 ± 01.20^{f}
Mature fruit	lyophilization	1110 ± 29.32^{a}
	convection oven	219 ± 26.31^{d}
Callus cultures	lyophilization	205 ± 00.20^{d}
	convection oven	184 ± 09.74^{e}
Leaves of *in vitro*	lyophilization	741 ± 10.00^{b}
germinated seedlings	convection oven	204 ± 13.31^{d}

The data represents the average of three ± DS replicates. Average in columns, with different letter are significantly different (p≤0.5), using the LSD test.

and 1.0 mg · L^{-1} of BA with 0.0 mg · L^{-1} of 2,4-D (B1D0) (Fig. 5). In general, BA and 2,4-D exerted a positive effect on the generation of callus in *B. karatas* seeds. However, the role in the induction of callus of the cytokinin BA was more evident compared to the effect of auxin 2,4-D. By increasing the concentration of BA, a directly proportional relationship is observed in callus induction, it means that there is an increase in the percentage of induction. In other species of Bromeliads the effect of BA and 2,4-D has been assessed in conjunction with other regulators, in leaf explants and apical buds of *Aechmea veitchii* and *Racinaea crispa*, the formation of shoots and callus is reported (Calderón-Arias *et al*., 2011); while using explants of the crown (longitudinal section of leaves from base with nucleus) of *A. comosus*, the proliferation of somatic embryos and shoots is reported (Roostika and Mariska, 2003). Also, in *Yucca carnerosana* the combination of BA y 2,4-D showed the highest induction frequency (66%) in stems explants (López-Ramírez *et al*., 2021). Among the cytokinins, BA has been the most frequently used and successful in micropropagation studies.

On the other hand, in the present study we also assessed leaf sections of adult specimens and leaf sections of *in vitro* germinated seedlings of 60-day-old; however, no morphogenetic response was observed under the assessed PGR design. The foliar explants of adult specimens showed a high rate of oxidation and necrosis at 30 days of culture. While leaf explants derived from *in vitro* germinated plants showed signs of etiolation and later oxidation.

3.4 Total phenolic content in crude extracts of tissues and in vitro cultures

Because of it is reported that the periodic consumption of the fruit of *B. karatas*, often helps patients with diabetes mellitus, total phenolic content (TPC) in mature and immature fruit was determined. On the other hand, in order to know the synthesis capacity of phenolic compounds in *in vitro* cultures, the production of these compounds was determined in biomass derived from callus cultures and leaves of *in vitro* germinated seedlings. The order of TPC in the different tissues was as follows: mature fruit > leaves of *in vitro* germinated seedlings » immature fruit > callus. In general terms, the mature fruit contains 1.3, 2.16 and 3.16 fold more phenolic compounds than the leaves of *in vitro* germinated seedling, immature fruit and callus, respectively. Nevertheless, this finding suggests the callus cultures of *B. karatas* retain the ability to produce secondary metabolites as reported in *in vitro* cultures of *Coryphanta macromeris*, *Calophyllum brasiliense* and *Catharanthus roseus* (Cabañas-García *et al*., 2020; Cisneros-Torres *et al*., 2020; Zavala-Ortiz *et al*., 2021). Also, the order showed in TPC content by different tissues in *B. karatas*, suggests that this type of secondary metabolites is associated at cellular differentiation.

From the four assessed samples, in the mature fruit dried by lyophilization, the significantly higher content of total phenols was obtained (1,110 mg GAE · 100 g^{-1} biomass, dry weight (DW)). During the preparation of the sample two drying processes were used, in general, the results show that lyophilization is a treatment that protects or diminishes the risk of loss of phenolic compounds compared to drying in convection oven at 60 °C during 72 h. The samples that were dried by lyophilization showed, on average, 4 fold more phenolic compounds than the samples dried in a convection oven (Table 2). Other species of the family Bromeliaceae have been analyzed to know the content of phenols. In ethanolic extracts of *B. laciniosa* flowers, 53.75 mg GAE · g^{-1} extract, was determined (De Oliveira-Júnior *et al*., 2017a), while

in hydroalcoholic extracts of leaves of *Neoglaziovia variegata* 61.66 mg GAE · g^{-1} extract were found (De Oliveira-Júnior *et al*., 2017b) and in extracts with ethylacetate 608.50 mg GAE · g^{-1} extract (De Oliveira-Júnior *et al*., 2013a), extracts of leaf with dichloromethane from *Encholirium spectabile* were found 188.50 mg GAE · g^{-1} extract (De Oliveira-Júnior *et al*., 2013b), and 12 mg · g^{-1} DW, were determined in methanolic extracts of *Tilandsia albida* leaves (Kováčik *et al*., 2011). The content of phenols in ethanol extracts of immature and mature fruits of *B. karatas* has also been determined, reporting 290.3 and 407.9 mg GAE · 100 g^{-1} extract DW, respectively (Moyano *et al*., 2012).

Conclusions

The data obtained in this study indicated that the seed of *Bromelia karatas* present seed coat dormancy that is overcome with sulfuric acid immersion 98% for 5 minutes under *in vitro* culture. This is the first report in the calli and seedling culture until the *ex vitro* acclimation with 100% survival of *B. karatas*. Also, the *in vitro* cultures demonstrated that retain the ability to synthesize phenolic compounds. The protocol described here could be employed for effective mass propagation of *B. karatas* for commercial and conservation purposes.

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