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PLANT PRODUCTION FROM *IN VITRO* CULTURE OF ISOLATED ZYGOTIC EMBRYOS OF *BUTIA YATAY* (MART.) BECC. (ARECACEAE)

Short communication

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ABSTRACT

This study aimed to develop for *in vitro* germination of zygotic embryos and to establish an acclimatization procedure for the production of *Butia yatay* palm plants under controlled conditions. *B. yatay* fruits were mechanically scarified for seeds extraction prior to isolation of zygotic embryos. Embryos were cultured on Murashige and Skoog medium with a combination of plant growth regulators. Acclimatization consisted of transferring plants to sterilized or unsterilized soil-sand substrates, with or without polyethylene cover. Embryos cultivated on Murashige and Skoog medium with 0.1 $mg \cdot L^{-1}$ 1-naphthaleneacetic acid and 0.1 $mg \cdot L^{-1}$ 6-benzylaminopurine achieved 90% germination. After fungicide treatment and transplanting into sterilized soil mix in shaded conditions, the plants were covered with polyethylene, which resulted in a 67% acclimatization success rate.

Key words: acclimatization, palm tree, sterilization

INTRODUCTION

The genus *Butia* (Mart.) Becc. (Arecaceae) is endemic to the central-eastern region of South America, consisting of 24 species distributed across Argentina, Brazil, Paraguay, and Uruguay. Certain species, such as *B. odorata* and *B. yatay*, can develop extensive populations, creating unique ecosystems known as palm groves or "butiazais" in Brazil (Batista et al. 2014). Although the global risk of extinction of *B. yatay* has not been assessed, it is classified as vulnerable in the Brazilian Red Book of Endangered Species (Martinelli & Moraes 2013). Based on current distribution data, the population of *B. yatay* in Uruguay is categorized as endangered by the International Union for Conservation of Nature (IUCN 2020). *Butia yatay* is one of the tallest species in its genus, reaching a trunk height of 12–15 meters. It is widely cultivated for ornamental purposes in urban environments, including plazas, parks, and gardens (Mandón & Campagna 2023). The fibrous and compact root system, located close to the trunk, facilitates successful transplantation of adult specimens (Márquez & Fiorentino 2007).

Recent studies have shown that *B. yatay* fruit pulp is high in carbohydrates (11.4 $mg \cdot g^{-1}$) and is an excellent source of dietary fiber, linoleic acid, and linolenic acid, which are essential precursors for the synthesis of omega-3 and omega-6, important in the diet (Mandón & Campagna 2023).

Compounds found in the fruit, including the pulp and seeds, are considered beneficial due to their antioxidant, antiproliferative, and antimicrobial properties, which offer potential applications in the food and pharmaceutical industries. Another potential product is the seed oil from *B. yatay*, which can be used in the production of soaps, detergents, cosmetics, creams, and dental adhesives (Hoffmann et al. 2014). Moreover, *Butia* oil can provide an alternative for biofuel production, as has been demonstrated for other palm species (Young & Steffen 2008; Falasca et al. 2012).

Despite their ecological and economic importance, the conservation of natural populations and the propagation of *Butia* palms pose significant challenges. The dissemination of these palms relies on seeds, known for their dormancy and inconsistent and slow germination rates (Baskin & Baskin 2014).

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Studies reveal that *B. capitata* seeds can take up to two years to germinate naturally, and germination is erratic and highly inefficient unless mechanical resistance to the endocarp is removed – a critical step for commercial seedling production (Broschat 1998). Low germination rates in *Butia* species are often associated with a combination of factors, including immature embryos, impermeability to water and gases, mechanical barriers, and chemical inhibitors (Hoffmann et al. 2014). These hurdles hinder large-scale seedling production and pose a significant obstacle to *ex situ* conservation efforts. In response, innovative approaches such as *in vitro* culture and cryopreservation are emerging as powerful tools to overcome these limitations, offering promising solutions for both rapid propagation and long-term genetic preservation. In this short communication, we present a sustainable and efficient protocol for the regeneration of *B. yatay* plants by *in vitro* culture of zygotic embryos.

MATERIALS AND METHODS

Ripe fruits of *B. yatay* (Fig. 1A) were collected from Buena Vista, Goya Department, Corrientes Province, Argentina (29º25'2" S, 59º18'19" W). Fruits from multiple plants were stored together at room temperature. Fruits were incised to remove the epicarp and mesocarp, yielding pyrenes (woody endocarp and seeds, Fig. 1B). Seeds were extracted (Fig. 1C), disinfected with 70% ethanol (3 min) and 2.5% sodium hypochlorite (20 min), and rinsed with sterile water. Zygotic embryos were isolated by sectioning the seeds at the micropylar region with sterile forceps (Fig. 1D). Disinfected seeds and isolated zygotic embryos (Figs. 1C–E) were cultured in Murashige and Skoog (MS) (1962) medium (3% sucrose) as a control or supplemented with 0.1 $mg \cdot L^{-1}$ indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), or 1-naphthaleneacetic acid (NAA), alone or in combination with 0.1 $mg \cdot L^{-1}$ or 1 $mg \cdot L^{-1}$ 6-benzylaminopurine (BAP). The pH of the media was adjusted to 5.8, gelled with 0.6% agar, and sterilized. Cultures were maintained at 27±2 °C with a 14-h photoperiod (116 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation). Germinating embryos were transferred to fresh medium after 60 days. Contamination, germination, and plant conversion were recorded after 60 and 120 days. A total of 30 zygotic embryos were used for each medium.

Plants with well-developed roots and leaves (~5 cm) were rinsed and treated with 2 $g \cdot L^{-1}$ captan fungicide. They were potted in a 1:1 soil-sand mix, with two types of substrate (sterilized or unsterilized) and two acclimatization methods (1 – ventilated chamber, 80% humidity, 80% shade, 30 days, 2 – sealed polyethene bags, 80% shade, gradually ventilated, 4 weeks). For each acclimatization method, 16 randomly selected plants were used. Data were analyzed using ANOVA and Duncan's test ($P \leq 0.05$) in InfoStat 2017 (Di Rienzo et al. 2017). Results are presented as means ± SD.

RESULTS AND DISCUSSION

This study showed that *in vitro* cultivation of disinfected *B. yatay* seeds resulted in 100% contamination, making them unsuitable for plant production. Similarly, *B. eriospatha* seeds showed a 62.6% contamination rate *in vitro* (Waldow et al. 2013). These challenges highlight the need for alternative methods, such as the use of isolated zygotic embryos, which have successfully established aseptic cultures without contamination, as opposed to whole seeds.

On the basal MS medium, 50% of the zygotic embryos germinated (Fig. 1F & 2A). After 40 days, the embryos (initially 1 mm) had elongated to 5 mm and turned white (Fig. 1F), while the remaining 50% showed no physiological activity (Fig. 1E & 2A). On containing 0.1 $mg \cdot L^{-1}$ BAP alone or with NAA, 0.1 $mg \cdot L^{-1}$ IAA, 1 $mg \cdot L^{-1}$ BAP with 0.1 $mg \cdot L^{-1}$ NAA, germination increased to about 60–70% compared to the control. The most stimulating medium was the one containing 0.1 $mg \cdot L^{-1}$ BAP and 0.1 $mg \cdot L^{-1}$ NAA, where germination reached more than 90% (Fig. 2A). Eslabão et al. (2018) noted that *B. yatay* has the lowest germination rate (20%) among *Butia* species. According to the definitions of seed dormancy proposed by Baskin and Baskin (2004), *B. yatay* is considered to exhibit a type of dormancy caused by a combination of physical and physiological dormancy due to the water-impermeable woody endocarp and hormonal requirements for normal seedling growth.

The absence of growth regulators in the medium prevented the development of embryos into plants within 120 days (Fig. 1F & 2B). The presence of BAP or auxins (NAA, IAA, IBA), alone or in combination with BAP, significantly boosted the development of plants (Fig. 2B). After 90 days, the embryos turned green and produced the first leaf (Fig. 3A, B). The combination of 0.1 $mg \cdot L^{-1}$ BAP and

NAA resulted in more than 90% plant formation (Fig. 2B), outperforming the combinations of IAA and IBA. Conversion rates in different combinations of BAP and auxin conversion ranged from 23% to 60%. Higher concentrations of BAP, i.e. 1 mg·L^{-1}, reduced conversion compared to 0.1 mg·L^{-1} BAP, regardless of auxin. Other studies confirm that auxins or cytokinins enhance plant conversion in *Butia* species (Eslabão et al. 2018; Fior et al. 2018).

After 120 days, plants with robust root and leaf growth were acclimatized. Acclimatization is challenging for Arecaceae, requiring careful adjustments (Plata et al. 2006). In this study, 68.7% of plants on sterile substrate covered with polyethylene adapted successfully, whereas only 25% survived in the acclimatization chamber without any cover. No seedling survived on unsterilized substrate (data not presented). Mortality during acclimatization is often due to microbial exposure, highlighting the need for sterilization or agrochemicals (Kumar & Rao 2012). *In vitro* regenerated plants often wilt or die when transferred to the greenhouse due to environmental changes. The most effective method involved transplanting captan-treated (2 g·L^{-1}) plants into a sterilized soil-sand mix covered with polyethene bags under 80% shade cloth, with periodic ventilation. Figure 3E shows *B. yatay* plants acclimatizing, while Figure 3F shows a 1.7 m tall plant thriving outdoors 12 years after transplanting.

This experiment demonstrated high germination capacity and successful plant conversion, and the possibility of commercial micropropagation could be helpful in the ecological restoration and commercial propagation of *Butia yatay*.

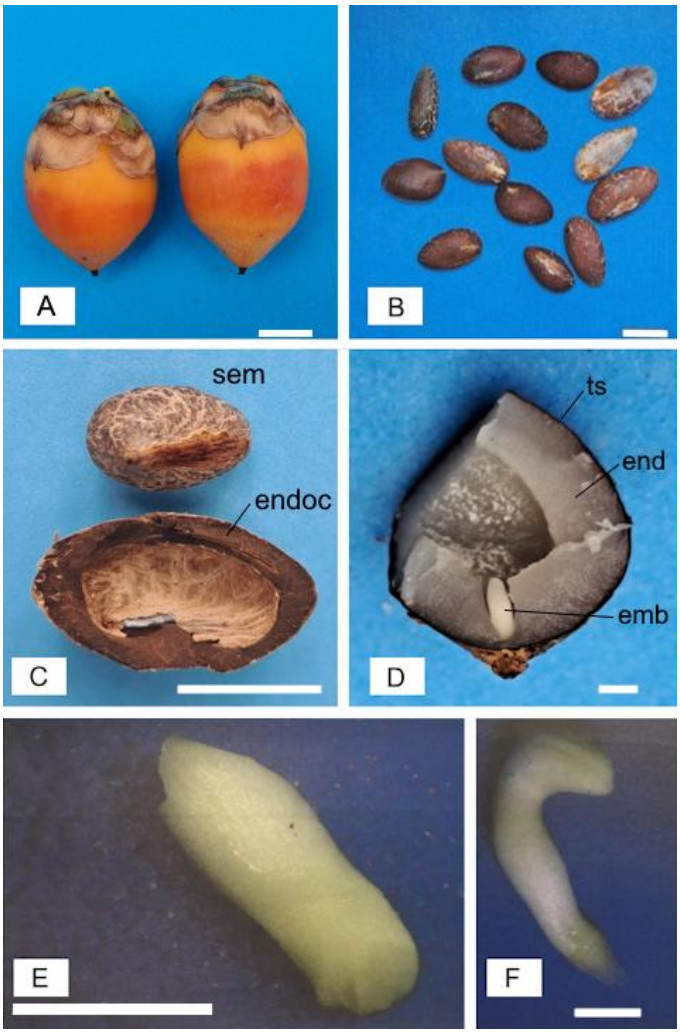

Figure 1. Extraction of seeds and isolation of zygotic embryos of *Butia yatay* for disinfection and *in vitro* culture: pyrene set (A, B), extraction of seeds from pyrene (C), dissection of seeds in the micropylar part to isolate the zygotic embryo (D), isolated zygotic embryo (E), isolated zygotic embryo that has started the germination process (F)
Bars: 1 cm (A–C), 5 mm (D–F); sem – seed, endoc – woody endocarp, ts – seminal integument, end – endosperm, emb – zygotic embryo

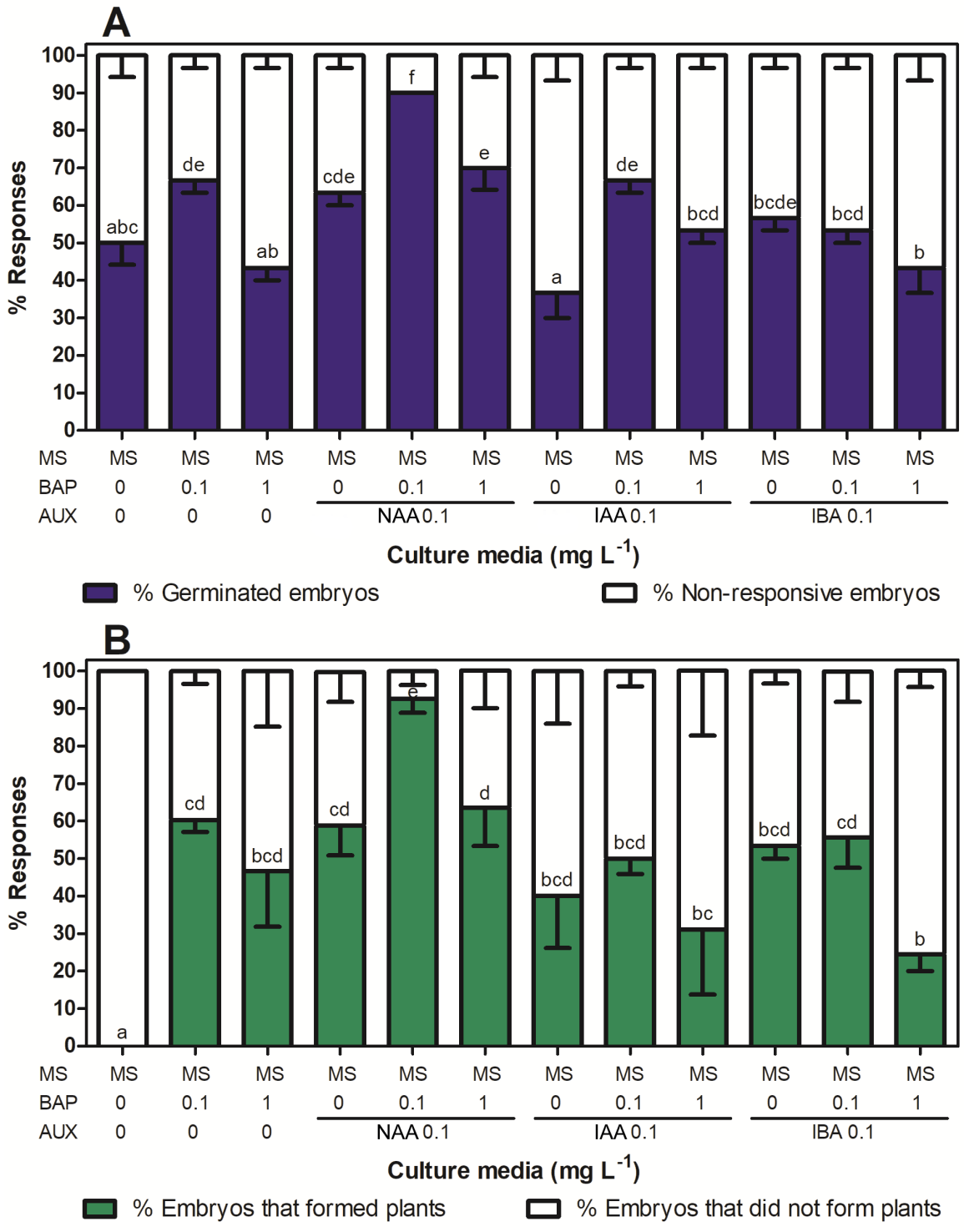

Figure 2. Effect of culture medium on germination and conversion to plants of zygotic embryos isolated from *Butia yatay*: percentage of embryos that germinated or did not respond after 60 days of culture (A), percentage of embryos that formed or did not form plants after 120 days of culture (B)

Different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$); MS – Murashige and Skoog basal medium, AUX – auxin, NAA – 1-naphthaleneacetic acid, IAA – indole-3-acetic acid, IBA – indole-3-butyric acid, BAP – 6-benzylaminopurine

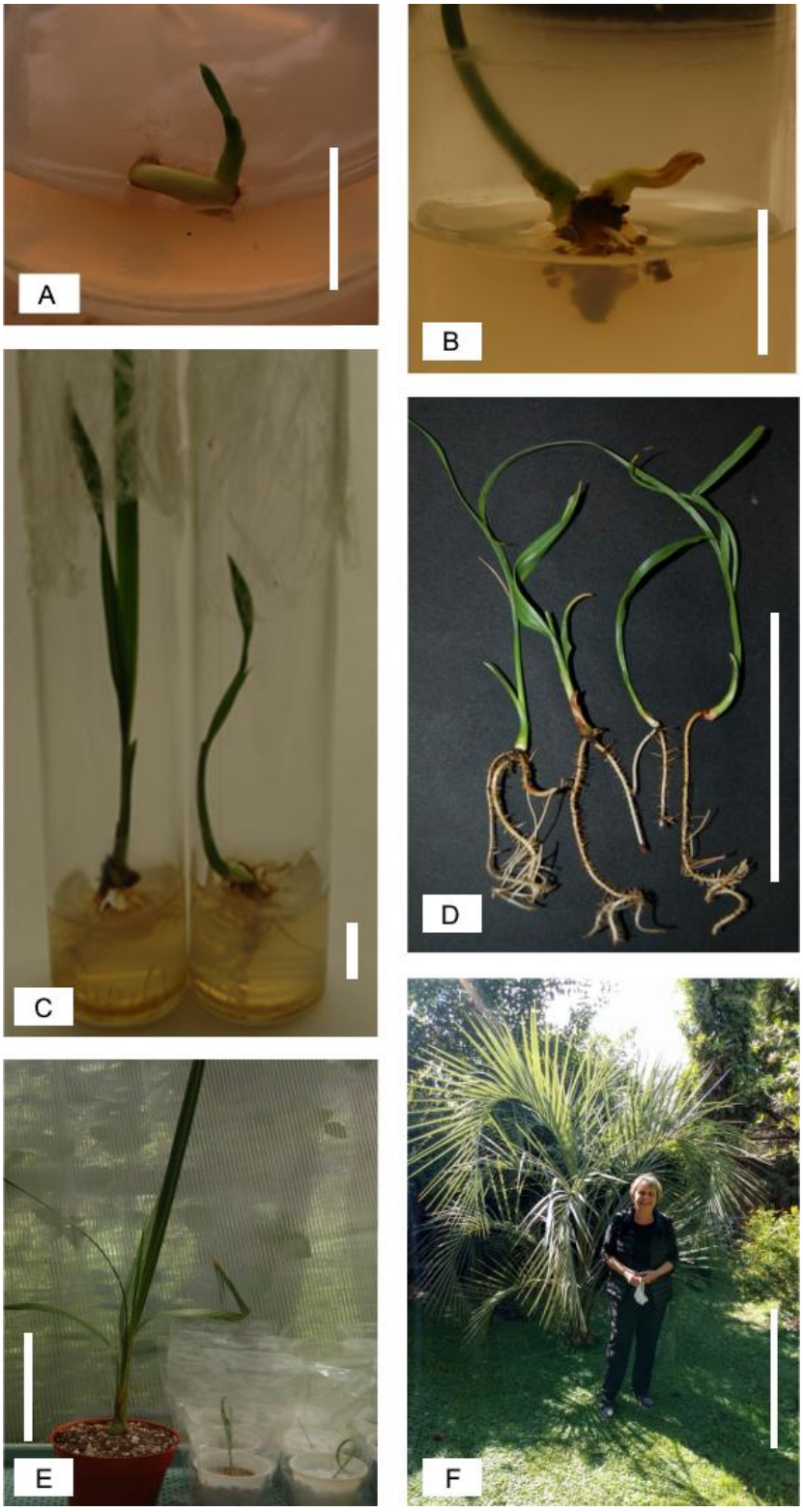

Figure 3. *In vitro* morphogenic responses and acclimatization of plants obtained from zygotic embryo cultures isolated from *Butia yatay*: zygotic embryos that became green and produced the first leaf after 90 days of *in vitro* culture (A, B), plants obtained from *in vitro* with marked root and leaf growth, selected and conditioned for acclimatization, after 120 days of culture (C, D), plants obtained from *in vitro* in the acclimatization phase with transparent polyethylene bags as a humid chamber and an acclimatized plant ready for transplanting to the field (E), plant transplanted to outdoor soil after 12 years of *in vitro* culture (F)
Bars: 1 cm (A–C), 10 cm (D, E), 1 m (F)

CONCLUSIONS

A meticulous method of germination and seedlings initiation was developed that enables commercial propagation of *Butia yatay* from zygotic embryos. The protocol consisted of extraction of embryos from disinfected seeds and their culture on MS medium with 0.1 $mg \cdot L^{-1}$ 1-naphthaleneacetic acid and 0.1 $mg \cdot L^{-1}$ 6-benzylaminopurine for 60 days, followed by growth of seedlings on fresh medium for another 60 days and acclimatization of young plants in sterile soil – sand substrate under foil and after captan application for 120 days.

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REFERENCES

Baskin J.M., Baskin C.C. 2004. A classification system for seed dormancy. Seed Science Research 14(1): 1–16. DOI: 10.1079/ssr2003150.

Baskin J.M., Baskin C.C. 2014. What kind of seed dormancy might palms have? Seed Science Research 24(1): 17–22. DOI: 10.1017/s0960258513000342.

Batista W.B., Rolhauser A.G., Biganzoli F., Burkart S.E., Goveto L., Maranta A. et al. 2014. Savanna plant community types at El Palmar National Park (Argentina). Darwiniana 2(1): 5–38. DOI: 10.14522/darwiniana.2014.21.569. [in Spanish with English abstract]

Broschat T.K. 1998. Endocarp removal enhances *Butia capitata* (Mart.) Becc. (pindo palm) seed germination. HortTechnology 8(4): 586–587. DOI: 10.21273/horttech.8.4.586.

Di Rienzo J.A., Casanoves F., Balzarini M.G., González L., Tablada M., Robledo C.W. 2017. InfoStat versión 2017. InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. [in Spanish]

Eslabão M.P., Sarto M.T., Dutra L.F., Heiden G. 2018. Germinação in vitro de *Butia odorata* (Arecaceae) sob influência de 2,4-D. 20th Postgraduate Meeting, 4th Integrated Week, Federal University of Pelotas, 4 p. [in Portuguese]

Falasca S.L., Miranda del Fresno C., Ulberich A. 2012. Possibilities for growing queen palm (*Syagrus romanzoffiana*) in Argentina as a biodiesel producer under semi-arid climate conditions. International Journal of Hydrogen Energy 37(19): 14843–14848. DOI: 10.1016/j.ijhydene.2011.12.092.

Fior C.S., Costa Calil A., Dutra de Souza P.V., Schwarz S.F. 2018. Impact of ANA and sucrose concentrations on the *in vitro* rooting of *Butia odorata* (Barb.Rodr.) Noblick. Iheringia, Série Botânica 73(1): 13–21. DOI: 10.21826/2446-8231201873102. [in Portuguese with English abstract]

Hoffmann J.F., Barbieri R.L., Rombaldi C.V., Chaves F.C. 2014. *Butia* spp. (Arecaceae): An overview. Scientia Horticulturae 179: 122–131. DOI: 10.1016/j.scienta.2014.08.011.

IUCN 2020. The IUCN Red List of Threatened Species Conservation. Version 2020-2. International Union for Conservation of Nature. https://www.iucnredlist.org

Kumar K., Rao I.U. 2012. Morphophysiologicals problems in acclimatization of micropropagated plants in -*ex vitro* conditions- A reviews. Journal of Ornamental and Horticultural Plants 2(4): 271–283.

Mandón E., Campagna M.N. 2023. Fruits from ornamental palms trees *Butia yatay* and *Syagrus romanzoffiana* (Arecaceae) as a source of nutrients and bioactive compounds. Boletín de la Sociedad Argentina de Botánica 58(4): 533–546. DOI: 10.31055/1851.2372.v58.n4.41049. [in Spanish with English abstract]

Márquez F., Fiorentino J. 2007. Palmeras porteñas. Colección Azulejo 3. Biodiversidad Urbana, Buenos Aires, Argentina, 52 p. [in Spanish]

Martinelli G., Moraes M.A. 2013. Red Book of Brazilian Flora. Rio de Janeiro Botanic Garden Research Institute, CNCFlora, Brazil, 1100 p. [in Portuguese]

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15(3): 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

Plata A., Rey L., Ayala Díaz I.M. 2006. Avances en el rescate de embriones en palma de aceite: una herramienta eficiente en material genético de difícil germinación. Cenipalma 139: 1–4. [in Spanish]

Waldow D.A.G., Reiniger L.R.S., Golle D.P., Curti A.R. 2013. *In vitro* culture of zygotic embryos of *Butia eriospatha*. Semina: Ciências Agrárias 34(5): 2179–2188. DOI: 10.5433/1679-0359.2013v34n5p2179.

Young C.E.F., Steffen P.G. 2008. Biocombustibles as strategy of development: A course towards sustainability or to a new periphery? Polis, Revista Latinoamericana 21; 10 p. http://journals.openedition.org/polis/2897 [in Spanish with English abstract]