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Germination and emergence of *Mouriri elliptica* mart., a rare medicinal fruit tree native to the Brazilian Cerrado biome

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This study is aimed to describe the germination process of *Mouriri elliptica* Martius, a species in the Melastomataceae family that is native to the *Cerrado* (tropical savannah) and popularly known in Portuguese as *croada*, *coroa de frade* [friar's crown], *croadinha* [little croada], *puçá-preto* [black net], or *jabuticaba-do-Cerrado*. The species has significant potential for use as food and medicine; however, there is a dearth of scientific studies on this plant. Two experimental tests were performed to clarify the germination process of this species: Germination was evaluated *in vitro* by integrating fruit ripening and culture medium, and the emergence and initial growth of *M. elliptica* Mart. seedlings obtained from seeds soaked for different lengths of time were observed in a greenhouse. It was verified that the *in vitro* environment was not effective for germination independent of the culture medium, even using known and effective methodologies for breaking dormancy in seeds of this species. For the emergence of seedlings, it was found that soaking seeds in distilled water for two days was the optimal length of time for seedling production.

Key words: Breaking dormancy, Melastomataceae, propagation, tetrazolium test.

INTRODUCTION

Mouriri elliptica Mart. is popularly known in Portuguese as *coroa de frade* [friar's crown] or *croadinha*, and in a study by Rufino et al. (2011), the species *M. elliptica* Mart. is referred to as *puça* [net]. This plant belongs to the family Melastomataceae; it can reach 4 to 6 m in length and is found in the Brazilian states of Goiás, Mato Grosso and Mato Grosso do Sul (Silva et al., 2001). According Silva et al. (2001), this plant has occurence in Cerrado and

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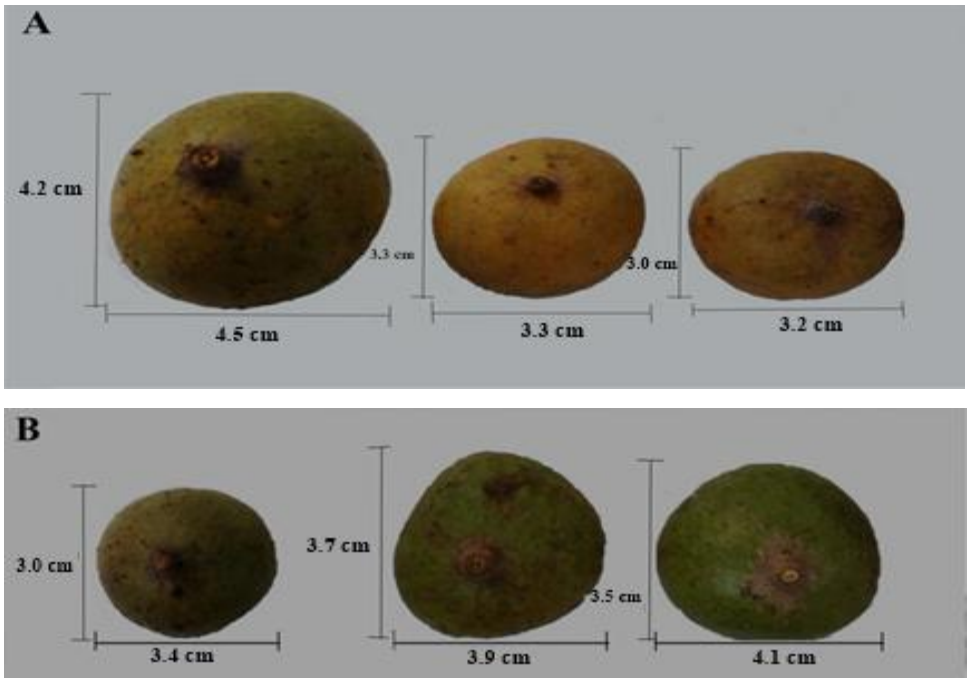

Figure 1. *Mouriri elliptica* Mart. fruits. (A) Ripe Fruits with partially orange epicarp coloration; and (B) unripe fruits with partially green epicarp coloration.

cerradão fitofisiony, yout frutification occur at September and December. According to the International Union for Conservation of Nature (IUCN, 2014), certain species of the genus are considered threatened with extinction, including *Mouriri completens* (Pitt.), *M. laxiflora* (Morley), *M. gleasoniana* (Standl.) and *M. panamensis* (Morley); however, *M. elliptica* Mart. does not appear to be threatened at this time.

The food and medicinal potential of *M. elliptica* Mart. Has been empirically established and was first described by Almeida et al. (1998) and Silva et al. (2001), who highlighted the lack of research. Currently, the shortage of publications on this plant could reflect the difficulty of finding it in its natural habitat. Articles in relevant journals are restricted to phytosociological surveys of the Pantanal biome and characterizations of its potential for vitamin C production and as an ulcer therapy (Moleiro et al., 2009; Rufino et al., 2011).

Interest in the conservation of native Cerrado species prompted the study by Vasconcelos et al. (2010) on the dormancy and breaking of dormancy in *M. elliptica* Mart. seeds, which was the first such report and verified that soaking seeds in distilled water for up to 48 h and mechanical scarification increased germination rates compared with seeds that did not receive such treatment. However, the combined effect of soaking and scarification was not evaluated.

For the genus *Mouriri*, only any studies have been conducted on its germination and chemical composition (Salis and Mattos, 2010; Vasconcelos et al., 2010; Rufino et al., 2011; Silveira et al., 2013), and there is a lack of research related to *in vitro* propagation in this genus (comment author). Therefore, the aim of this study was to evaluate the influence of the fruit ripening stage as well as the influence of seed scarification and soaking on the germination of seeds *in vitro* and in a greenhouse.

MATERIALS AND METHODS

Tests were performed in the Plant Tissue Culture Laboratory of the Federal Institute of Education, Science and Technology of Goiás, Rio Verde Campus, Goiás (GO), Brazil (*Instituto Federal de Educação, Ciência e Tecnologia Goiano, Câmpus Rio Verde*, GO). The *M. elliptica* Mart. fruits for Test 1 were collected in October 2013, and fruits for Test 2 were collected in November of the same year in the district of Planalto Verde, municipality of Montividiu, GO, Brazil at 17º 19.201' S, 51 33.500' W and 982 m.a.s.l.

A digital caliper was used to obtain biometric parameters, which included the mass (g) and longitudinal and equatorial diameter of the fruits and seeds. The ratio between the equatorial diameter and longitudinal diameter was obtained to determine the fruit shape.

Test 1: *In vitro* germination of *Mouriri elliptica* Mart. seeds in different concentrations of sucrose and fruit ripening

M. elliptica Mart. seeds were obtained from ripe fruits (epicarp partially orange) (Figure 1A) and unripe fruits (epicarp partially green) (Figure 1B). After biometric analyses of the fruits, they were manually de-pulped using a sieve and running water.

Once de-pulped, four hundred seeds were used, being two hundred from mature fruits and two hundred green fruits, were washed in running water and subsequently subjected to a

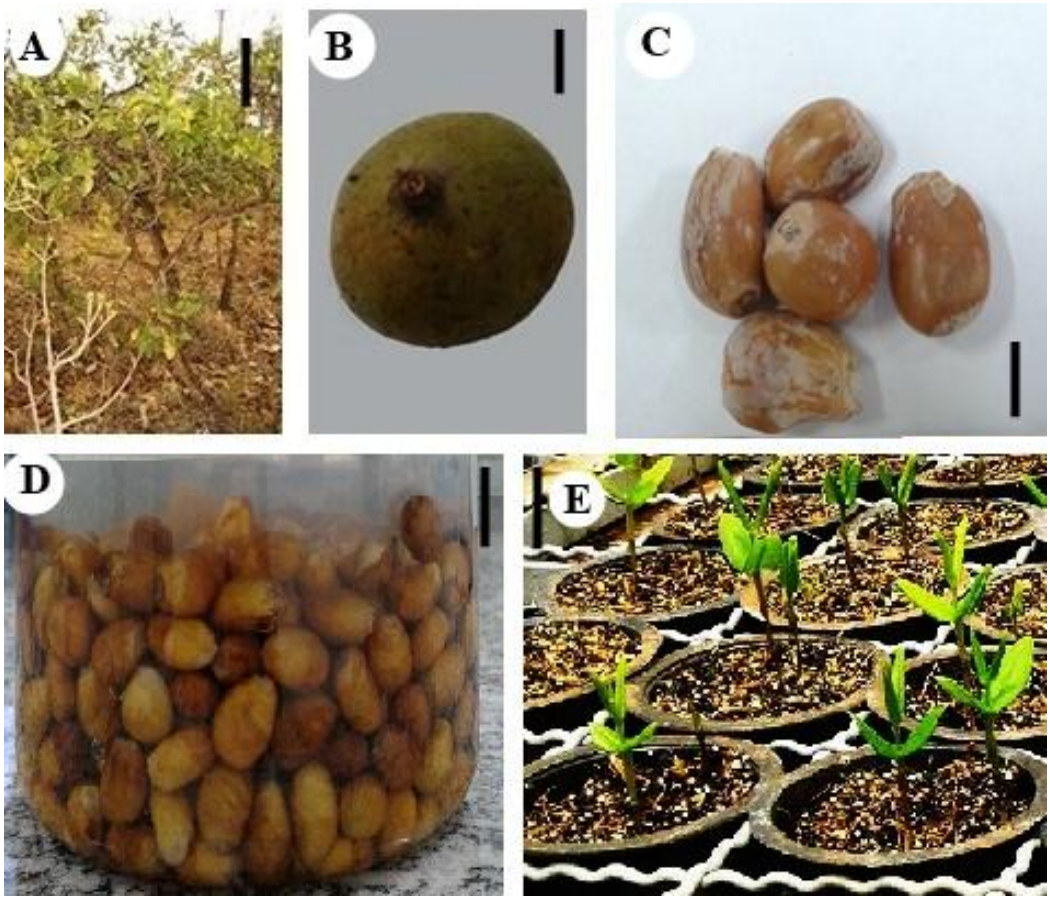

Figure 2. (A) Collection the mother plant; bar = 0.5 m. (B) Ripe fruit; bar = 11.2 mm. (C) Scarification of seeds with #60 sandpaper; bar = 10.0 mm. (D) Soaking of seeds for up to six days in distilled water at 30°C; bar = 25.0 mm. (E) Emergence of *Mouriri elliptica* Mart. seedlings in the greenhouse; bar = 2.8 cm.

dormancy breaking test following the methodology of Vasconcelos et al. (2010). Briefly, the seeds were soaked in distilled water for 48 h at room temperature (25±3°C); disinfected in a laminar flow cabinet, with the seeds remaining for one minute in 70% alcohol and four minutes agitated in commercial 100% sodium hypochlorite (Qboa, Industries Anhembi, Curitiba-PR, Brasil); and then rinsed three times with distilled and autoclaved water. For *in vitro* germination, the seeds were inoculated in test tubes containing 10 mL distilled and autoclaved water in 0, 30, 60 and 90 gL^{-1} sucrose concentrations. To avoid submersion of the seeds, "germitest" paper was used as a support. The seeds remained in these conditions for 60 days in a grow room at a temperature of 25 ±3ºC and photoperiod of 16 h.

The percent germination and seed contamination were evaluated at 60 days after inoculation (DAI). The experiment was conducted in an completely randomized design with a 2 × 4 factorial design of maturation [2] and sucrose concentrations [4], with four repetitions. To investigate seed viability at the end of the test, a tetrazolium test with four repetitions of ten seeds was performed. A tetrazolium solution of 0.075% was used at 30ºC for 24 h.

Test (2): Scarification and soaking of *M. elliptica* Mart. seeds

Seeds from ripe fruits (Figure 2B) and de-pulped fruit (with the aid of a sieve) were used. To evaluate the effect of scarification (Figure 2C), one group of seeds was sanded opposite the hilum using 60 grit sandpaper based on the techniques of Santos et al. (2004). To evaluate the effect of soaking, the seeds were maintained in a beaker containing distilled and autoclaved water and placed in a Mangelsdorf germinator at a temperature of 30ºC forzero, two, four and six days, with water changed every two days (Figure 2D).

To determine the soaking curve, four repetitions of ten seeds were used. The seeds were weighed hourly for the first six hours, every two hours for the next six hours and every 12 h until reaching 72 h from the beginning of the soaking process.Subsequently, the seeds were weighed daily. To determine the water content, the greenhouse method 105±2°C was used, and the wet weight was used as a base until a constant mass was achieved.

After the established soaking times had elapsed, four samples from 15 seeds were placed in plastic tubes containing the commercial substrate Trimix® andmaintained in a greenhouse with three daily irrigations. Before seeding, the seeds were treated with fungicide [active ingredients(carboxin + thiram): 200+200 gL^{-1}] at doses of 500 mL to 100 kg seeds and 500 mL distilled water to 100 kg seeds (Fungicide, Chemtura Indústria Química, São Paulo – SP, Brasil).

An evaluation was performed 60 days after seeding (DAS), and the percent emergence, length and numbers of leaves were analyzed (Figure 2E). The experimental design was entirely

Table 1. Mass of unripe and ripe fruits from Test 1 and ripe fruits and seeds of Test 2 of *Mouriri elliptica* Mart. Equatorial diameter andlongitudinal diameter. Rio Verde, Goiás, 2014.

	Test 1 - October collection			
	Equatorial diameter (mm)	**Longitudinal diameter (mm)**	**Mass (g)**	**Ratio (e.d./l.d.)**
Ripe fruits	35.5 ± 0.67 ^{ns}[1]	28.27 ± 0.50^{ns}	21.96±1.19^{ns}	1.25
Unripe fruits	34.94 ± 0.60	29.1 ± 0.42	21.43±0.95	1.20
Test 2 - November collection				
Ripe fruits	34.89 ± 0.63	28.67 ± 0.41	20.5±1.01	1.22
Seeds	11.9 ± 0.16	15.77 ± 0.21	1.14±0.04	0.75

[1]±Standard error of the mean. ^{ns}Not significant by t test with 5% de probability.

Table 2. Percent viability of *Mouriri elliptica* Mart. seeds from Test 1 after the tetrazolium test.

	Vigor	**Viable (%)**	**Unviable (%)**	**Dead (%)**
Unripe	0	5	0	95 ± 2.89[1]
Ripe	0	10	0	90 ± 4.08

[1]±Standard error of the mean.

randomized in a factorial arrangement of two(scarification) by four (soaking times of zero, two, four and six days), with four repetitions of 15 seeds.

RESULTS AND DISCUSSION

Biometrics

The biometric analyses showed diameters with homogenous values. In Test 1 (Table 1), the fruits had an average equatorial diameter of 35.22 mm, longitudinal diameter of 28.68 mm and mass of 21.69 g. In Test 2, homogeneity of the fruit diameters was also verified. According to the biometric parameters, *M. elliptica* Mart. fruits have an oval shape and are flattened, which was confirmed by the ratio of diameters that reached values above 1.20.

Test 1: *In vitro* germination of *Mouriri elliptica* Mart. in different concentrations of sucrose and fruit maturation

After 60 days of cultivation, *in vitro* germination of the seeds was not observed; therefore, it was not possible to fit a mathematical model to explain the data as a function of increases in sucrose concentration, due to low germination percentages. However, a high percentage of contamination (30.62%) was observed.

The high rate of contamination observed in this study could be the result of the disinfection method and high resistance and impermeability of the tegument. To guarantee more effective disinfection, Couto et al. (2004) removed the tegument of seeds, which resulted in minimal contamination of 9.52% during the *in vitro* germination of seeds of mahogany (*Swietenia macrophylla* King).

The resistance of the tegument implies a lack of water absorption, which is necessary in the germination phase. Because no studies have reported on the *in vitro* micropropagation of this species, additional tests should be developed to investigate the efficacy of breaking tegumentary dormancy and achieving *in vitro* germination.

Because *in vitro* germination was not demonstrated, the tetrazolium test was performed (Table 2), which identified seed viability at 10% from ripe fruits and only 5% for seeds from unripe fruits. Thus, performing the tetrazolium test according to the adapted methodology permitted sufficient coloration to distinguish vigor; however, the seeds suffered from high mortality in this cultivation environment (Figure 3).

Test 2: Scarification and soaking of *M. elliptica* Mart. seeds

According to an analysis of variance, differences did not occur in mass and water content between scarified and intact seeds. An increase in these variables was observed in the first hour, with mass values of 11.04 to 13.07 g for scarified seeds and 8.28 to 9.7 g for non-scarified seeds (Figure 4A) and water content values (Figure 4B) from 19.01 to 31.61% in scarified seeds and 17.52 to 29.54% in non-scarified seeds. After the first hour of soaking, the mass values and water content remained stable.

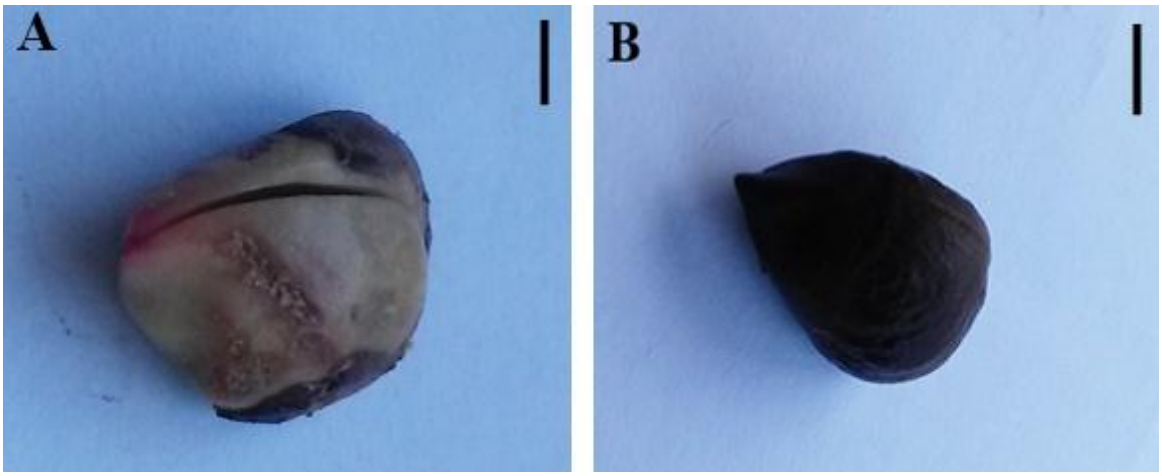

Figure 3. Seeds of *Mouriri elliptica* Mart.after the tetrazolium test. (A)Viable seeds; bar = 4.5 mm. (B) Dead seeds; bar = 4.1 mm. Rio Verde, Goiás, 2013.

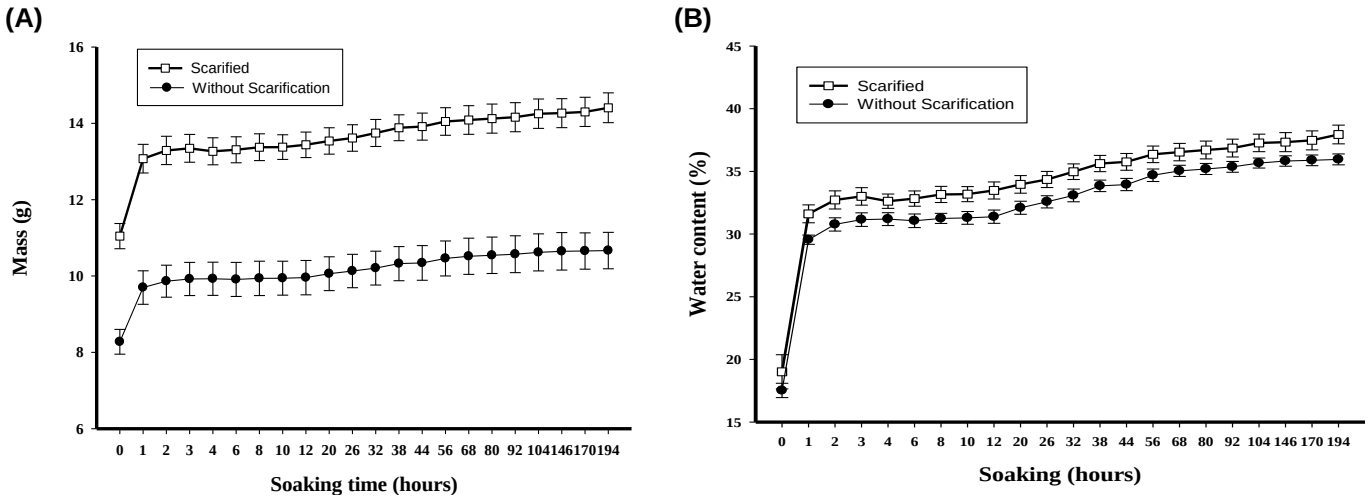

Figure 4. Soaking of scarified and intact *M. elliptica* Mart. seeds. A) Mass gain of seeds (g). B) Water content of seeds (%).

There was an interaction between soaking time and scarification that influences the emergence and length of seedlings. However, only soaking time affected the number of seeds.

The scarified seeds could not be fit to a mathematical model to explain the behavior of the data on seedling emergence and length, which reached an average of 50.83% and 4.12 cm, respectively (Figure 5A to B). For non-scarified seeds, an increase in percent emergencewas observed after soaking the seeds for two to six days, whereas a greater length (4.6 cm) was observed in the seedlings obtained from seeds soaked for two days. A decrease in the number of leaves was observed with an increase in soaking time, with values of 2.90 cm at time zero and 1.76 cm at six days (Figure 5C).

For *M. elliptica* Mart., gibberellic acid, sulfuric acid and water immersion, cooling and heating and mechanical scarification were studied by Vasconcelos et al. (2010), who achieved success with pre-soaking in gibberellic acid, soaking in water for 48 h and mechanical scarification of seeds. However, increased soaking times, which is a simple and inexpensive method, were not evaluated; thus, the study presented here is essential, and the results verified that an increase in soaking time of non-scarified seeds for up to six days has an impact on seedling emergence in this species, which exhibits tegumentary dormancy.

The efficacy of breaking tegumentary dormancy by mechanical scarification was effective for the species *Stryphnodendron adstringens* Mart.,*Dimorphandra mollis* Benth,*Sterculia foetida* L. and *Bowdichia virgilioides* Kunth., showing that this type of dormancy is common in

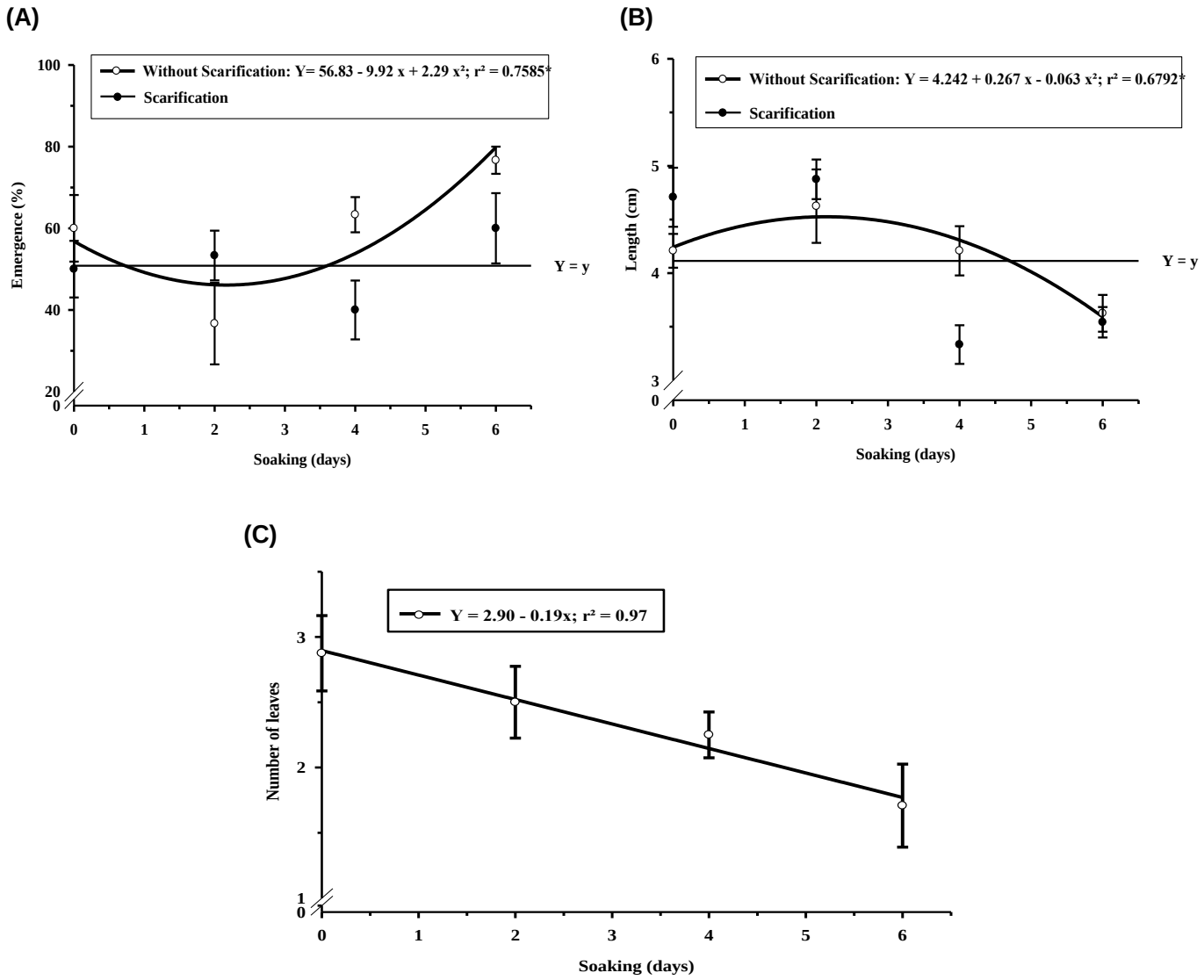

Figure 5. Effect of soaking scarified and intact seeds on the (A) emergence, (B) length of seedlings and(C) number of leaves per seedling of *M. elliptica* Mart.

native species of the Cerrado (Santos et al., 2004; Albuquerque et al., 2007; Martins and Nakagawak, 2008; Oliveira et al., 2008).

Conclusion

In vitro germination of *M. elliptica* Mart. seeds did not occur, and this behavior was independent of fruit ripeness and sucrose concentration in the culture medium. In non-scarified *M. elliptica* Mart. seeds, increased values for percent emergence occurred with increases in seed soaking time for up to six days. Seedlings with a greater leaf number and length were obtained from scarified seeds and seeds soaked for zero and two days, respectively.

Conflict of Interests

The authors have not declared any conflict of interests.

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