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Germinação *in vitro* e *ex vitro* de *Jatropha gossypiifolia* e iniciação de cultivo *in vitro*

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Abstract
Jatropha gossypiifolia L. is a widely used medicinal plant in the Amazon region. Commonly known as pião-roxo, it produces luteol, a compound with healing properties, and has therefore been included in the National List of Medicinal Plants of Interest to the Brazilian Unified Health System (SUS). Despite its potential as a herbal medicine, there are no phytotechnical studies supporting its large-scale propagation, making seed germination trials essential. For the *ex vitro* germination test, seeds were placed in different substrates over a period of 180 days to determine the most suitable soil type for germination. For the *in vitro* germination test, three concentrations of sodium hypochlorite were evaluated: 0.1%, 0.5%, and 1.0%. Nodal segments were subjected to the same aseptic treatments. As a result, clayey soil was identified as the most effective substrate, presenting the highest seed germination speed index. Since *in vitro* seed germination was not achieved, cultivation of the species was initiated using nodal segments, with the most effective treatment being 1.0 mg/L of sodium hypochlorite. Thus, it was demonstrated that biomass production of this species is feasible for pharmaceutical industry applications through both *ex vitro* and *in vitro* methods.

Keywords: Conservation; Medicinal plant; Germination test; Pião-roxo; Biomass production.

Resumen
Jatropha gossypiifolia L. es una planta medicinal común en el Amazonas. Conocida popularmente como punta morada, produce luteol que tiene actividades curativas y, por eso, fue incluida en la Lista Nacional de Plantas Medicinales de Interés para el SUS. Tiene potencial como medicina herbaria, pero no existen estudios fitotécnicos para su reproducción a gran escala y, por tanto, son necesarias pruebas de germinación de semillas. Para la prueba de germinación *ex vitro*, las semillas se colocaron en diferentes sustratos durante 180 días para determinar el mejor tipo de suelo para la germinación. Para la prueba de germinación *in vitro* se probaron tres concentraciones diferentes de hipoclorito de sodio: 0,1; 0,5 y 1,0%. Los segmentos nodales fueron sometidos a la misma asepsia. Como resultado, se indicó suelo arcilloso por presentar el mayor índice de velocidad de germinación de las semillas. Al no obtenerse germinación de semillas *in vitro*, se decidió iniciar el cultivo de esta especie utilizando segmentos nodales y el mejor tratamiento fue 1,0 mg/L de hipoclorito de sodio. Así, se encontró que es posible producir biomasa vegetal de esta especie para satisfacer las necesidades de la industria farmacéutica tanto *ex vitro* como *in vitro*.

Palabras clave: Conservación; Planta medicinal; Prueba de germinación; Peonza morada; Producción de biomosa.

Resumo
A *Jatropha gossypiifolia* L., é uma planta medicinal comum no Amazonas. Popularmente conhecida como pião-roxo, produz luteol que apresenta atividades cicatrizantes e, por isso, foi inserido na Relação Nacional de Plantas Medicinais de Interesse ao SUS. Apresenta potencial como fitoterápico, mas não possui estudos fitotécnicos para reprodução em larga escala e, por isso, testes de germinação de sementes são necessários. Para o teste de germinação *ex vitro*, sementes foram colocadas em diferentes substratos por 180 dias para averiguar o melhor tipo de solo para germinação. Para o teste de germinação *in vitro*, foram testadas três diferentes concentrações de hipoclorito de sódio: 0,1; 0,5 e 1,0%. Segmentos nodais foram submetidos à mesma assepsia. Como resultado, foi indicado o solo argiloso por apresentar o maior índice de velocidade de germinação de sementes. Como não foi obtido germinação de sementes *in vitro*, optou-se por iniciar o cultivo desta espécie utilizando segmentos nodais e obteve-se como melhor tratamento o de 1,0 mg/L de hipoclorito de sódio. Assim, verificou-se que é possível produzir biomassa vegetal desta espécie para suprir as necessidades da indústria farmacêutica tanto *ex vitro* como *in vitro*.

Palavras-chave: Conservação; Planta medicinal; Teste de germinação; Pião-roxo; Produção de biomassa.

Introduction

The Euphorbiaceae family encompasses the Crotonoideae subfamily, which includes the Jatropheae L. tribe, part of the Jatropha L. genus. This genus comprises between 165 and 175 species, distributed across Africa, India, the West Indies, Central America, the Caribbean, and South America (LEAL and AGRA, 2005) [1]. Among these species is *Jatropha gossypiifolia*, which is found throughout Brazil (BIGIO, 2023) [2].

Jatropha gossypiifolia L. is a subshrub that can reach up to 5 meters in height, with purplish branches and leaves during its juvenile stage, and secretes a milky, acrid latex. Its leaves are simple and lobed, its flowers are arranged in purple paniculate inflorescences, and its fruit is a capsule (ARAÚJO *et al.*, 2023) [3].

This species, commonly known in northern Brazil as pião-roxo, possesses medicinal properties (VARELA, 2021) [4], which, according to Bastos (2019) [5], include healing and anti-inflammatory biological activities. Lemos *et al.* (2021) [6] attribute these properties to the triterpene luteol, and as noted by Ferreira (2022) [7], the plant has been included in the National List of Medicinal Plants of Interest to the Brazilian Unified Health System.

Pião-roxo exhibits significant phytotherapeutic potential, and as such, research into its various biological and agronomic aspects has been encouraged, given the limited knowledge available regarding its life cycle. Consequently, studies on seed germination are essential.

As stipulated by the Ministry of Agriculture, Livestock and Supply through the Rules for Seed Analysis, the evaluation of light, temperature, humidity, oxygen availability, and substrate type is fundamental for conducting seed germination tests (BRASIL, 2009) [8]. According to Lima Júnior (2010) [9], the most commonly used substrates for such tests include coconut fiber, soil, and sand, which must be adequately moistened to ensure sufficient water availability for seed germination.

Mendes *et al.* (2019) [10] state that the ideal substrate must meet the specific requirements of the seed in terms of size, shape, and desiccation tolerance, while also allowing for the assessment of its availability, economic viability, standardization, and uniformity. Furthermore, it should possess suitable physical and chemical properties. According to these authors, an appropriate substrate must offer adequate structure, consistency, and porosity to facilitate water drainage, good capillarity for moisture retention, and be free of toxic substances, pathogens, invasive plants, or other pests.

As Queiroga *et al.* (2021) [11] emphasize, the substrate must not impede oxygen penetration and should not retain excessive moisture, which could result in a water film surrounding the seed. Therefore, the ideal substrate must maintain a balance between water availability and aeration.

Accordingly, the substrate selected should be the one that yields the best results in seed germination tests and seedling establishment (DE PETRI *et al.*, 2020) [12].

Modern biotechnological methodologies are increasingly employed in seed germination studies (JOÃO *et al.*, 2021) [13]. Through plant tissue culture and micropropagation techniques, it is possible to rapidly produce plant biomass on a large scale and at low cost (SANTOS, 2020) [14], for use in the pharmaceutical industry.

The initial step in obtaining inputs through this technique involves the production of axenic seedlings *in vitro* (HOFFMANN *et al.*, 2022) [15], typically derived from explants such as seeds, nodal or apical segments, leaves, roots, among others (LIMA *et al.*, 2022) [16]. These explants are placed in culture media that generally contain an auxin to promote cell elongation and a cytokinin to stimulate cytokinesis, thereby inducing rapid formation and growth of the aerial parts of the plant (PORFÍRIO *et al.*, 2019) [17].

According to Docha *et al.* (2020) [18], the first stage of micropropagation is explant asepsis, aimed at the complete elimination of microorganisms. This process typically involves the use of antibacterial agents such as sodium and calcium hypochlorite, in concentrations ranging from 0.1% to 1.0%, and various antibiotics. Additionally, 70% ethanol serves an astringent function, detaching microbial hyphae and spores from the explants. Systemic fungicides such as Derosal or Benomyl at 1% are also commonly employed.

Asepsis protocols are considered effective when they induce seed germination or promote the growth of pathogen-free nodal segments. It is customary to identify the treatment that yields the highest germination rate and the lowest contamination rate. Upon successful asepsis, the process advances to the multiplication phase, where plant biomass is produced on a large scale. Therefore, *in vitro* germination tests are as necessary and significant as *ex vitro* tests.

In light of the need to produce biomass for use in the phytotherapeutic industry, the objective of this study was to determine the most suitable substrate for seed germination and the most effective asepsis protocol for initiating *in vitro* cultivation of *Jatropha gossypiifolia* L., with a view toward large-scale biomass production for pharmaceutical applications.

Material and Methods

1. *Ex vitro* germination test:

To conduct this experiment, ripe fruits were collected in November 2012 from the parent plant located in the garden of the Institute of Health and Biotechnology at the Federal University of Amazonas, specifically in front of the Plant Tissue Culture Laboratory. The fruits were taken to the laboratory, depulped, and the seeds were sown in different types of substrates.

Four types of substrates were used in this experiment: coconut fiber, sand, clay, and a 1:1 mixture of sand and clay. These substrates were autoclaved for 40 minutes at 121 ºC and distributed in plastic trays measuring 25.0 cm × 39.0 cm × 7.5 cm, which were kept in the greenhouse of the Plant Tissue Culture Laboratory at the Institute of Health and Biotechnology of the Federal University of Amazonas.

In each substrate, 30 seeds were placed, as shown in Figure 1, totaling 120 seeds of *Jatropha gossypiifolia* L.

Figure 1 - *Ex vitro germination experiment of Jatropha gossypiifolia L., showing seed distribution in the substrate composed of equal parts sand and clay*.

The trays containing the substrates and seeds were installed in a covered outdoor area of the laboratory, where they were irrigated daily and monitored for 180 days post-sowing.

Every 15 days, data were collected on the number of germinated seeds in each substrate type to evaluate the Seed Germination Speed Index.

The Germination Speed Index (GSI) was calculated as described by Maguire (1962) [19].

The results were expressed as mean and standard deviation and analyzed using Tukey's test.

2. *In vitro* germination test:

2.1. Seed asepsis:

Fruits of *J. gossypiifolia* L. were collected from the nursery of the Plant Tissue Culture Laboratory at the Coari Institute of Health and Biotechnology, Federal University of Amazonas. In the laboratory, the fruits were depulped and the seeds washed with neutral detergent and rinsed under running water. Subsequently, they were immersed in three different concentrations of sodium hypochlorite—0.1%, 0.5%, and 1.0%—for 30 minutes under constant orbital agitation at 100 rpm. They were then immersed in 70% ethanol for 1 minute and in a 1% Benomyl solution for 1 hour, also under orbital agitation. At the end of the process, the seeds were rinsed four times with sterile distilled water and inoculated into test tubes containing MS/2 culture medium supplemented with 30.0 g/L sucrose and adjusted to pH 6.0.

The seeds were maintained in a growth chamber under a 16-hour photoperiod with cold white fluorescent lighting, at a temperature of 25 ± 2 ºC and relative humidity of 65% for 180 days to determine the germination rate. However, contamination rate was assessed only once, 30 days after *in vitro* inoculation.

Thirty seeds were used for each sodium hypochlorite treatment.

2.2. Asepsis of nodal segments:

Nodal segments of *J. gossypiifolia* L. were obtained from branches collected from mother plants in the nursery of the Plant Tissue Culture Laboratory at the Institute of Health and Biotechnology of Coari, Federal University of Amazonas. In the laboratory, the branches were cut into nodal segments shaped like small "Y" structures, washed with neutral detergent using a soft toothbrush, and thoroughly rinsed under running water.

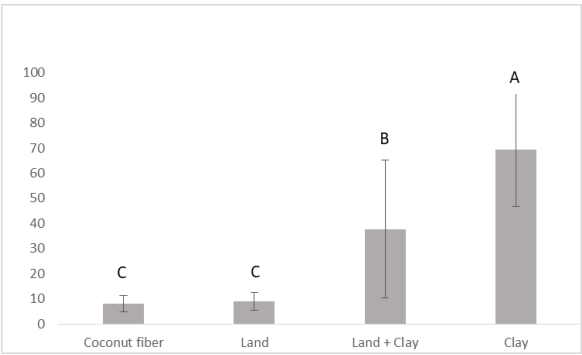
The nodal segments underwent the same aseptic treatment as the seeds, with the addition of 5.0 mg/L of ascorbic acid to the culture medium. This experiment was conducted under the same conditions as the previous one.

The results of these experiments were analyzed using simple percentage calculations followed by Tukey's test.

Results and Discussion

1. E*x vitro* germination test:

When selecting a substrate, it is essential to consider seed size, moisture requirements, light sensitivity, and the ease with which seedling development and evaluation can be conducted (SOUZA *et al.*, 2020) [20].



Graph 1: Germination rate of *Jatropha gossypiifolia* L. in different substrates.

As shown in Graph 1, after 180 days of sowing, coconut fiber and sandy soil exhibited low

germination rates, respectively (7.99 ± 3.22) and (8.99 ± 3.53), due to the limited water retention capacity of these substrates. Gonçalves *et al.* (2022) [21] reported similar findings with cedar seeds. Furthermore, as explained by Menegaes *et al.* (2017) [22], this phenomenon occurs because *Jatropha gossypiifolia* L. seeds do not encounter optimal conditions in these substrates in terms of density, total porosity, aeration space, moisture, water retention capacity, proportion of solid pores, and appropriate water pH.

Both the substrate composed of equal parts sand and clay (37.66 ± 27.44) and the clayey soil (69.33 ± 22.49) demonstrated a progressive germination rate that stabilized after 90 days (data not shown), indicating high germination performance. Silva *et al.* (2020) [23] explains that substrates composed of larger particles possess greater void space and, consequently, lower compaction and apparent density, which enhances soil aeration and facilitates seedling germination. Dutra *et al.* (2016) [24] also identified these two substrate types as ideal for the germination of Luehea divaricata Mart. et Zucc. seeds.

2. *In vitro* germination test:

Asepsis is a critical step in the *in vitro* plant cultivation process, as the absence of an effective protocol for microorganism elimination results in an insufficient number of seedlings to proceed with biomass multiplication (VIEIRA *et al.*, 2021) [25].

Although the *in vitro* germination experiment for *Jatropha gossypiifolia* L. seeds was repeated three times in triplicate, axenic seeds could not be obtained, and no germination was observed even after 180 days, indicating low viability of this species under stress conditions. Similar results were reported by Lattuada *et al.* (2019) [26] with oregano.

Due to the unsatisfactory germination results and the need to address biomass production for pharmaceutical applications, a new asepsis experiment was conducted using nodal segments. However, this resulted in 100% mortality of explants due to oxidation. This outcome can be attributed to the relationship between explant size and the action of the disinfectant agent, as small tissues are highly susceptible to degeneration caused by sodium hypochlorite, leading to phytotoxicity (SILVA *et al.*, 2021) [27].

Moreover, the progressive darkening that culminated in complete carbonization of the nodal segments may also be explained by the release of phenolic compounds, highlighting the need to retest this cost-effective asepsis method with the addition of an antioxidant agent and/or under light-exclusion conditions.

Subsequently, a second experiment identical to the first was conducted, incorporating 5.0 mg/L of ascorbic acid into the culture medium to mitigate phenolic oxidation caused by explant excision (RONDON *et al.*, 2023) [28]. The results are presented in Table 1.

Table 1: Asepsis of nodal segments of *Jatropha gossypiifolia* L.

Treatment	Live explants (%)	Bacterial contamination (%)	Fungal contamination (%)
0.1% sodium hypochlorite	100,0 a	13,33 b	50,0 c
0.5% sodium hypochlorite	100,0 a	13,33 b	30,0 b
1.0% sodium hypochlorite	100,0 a	3,33 a	10,0 a

In this second experiment, as shown in Table 1, 100% of the nodal segments survived across all treatments, indicating that ascorbic acid exerted an effective antioxidant action on this type of explant. Similar results were reported by Oliveira *et al.* (2021) [29] in studies with olive trees.

According to the table, bacterial contamination of *Jatropha gossypiifolia* L. nodal segments was 13.33% in treatments with 0.1% and 0.5% sodium hypochlorite, and 3.33% in the 1.0% treatment, demonstrating the disinfectant's efficacy against bacterial infestations in this species. As noted by Silva *et al.* (2021) [27], acceptable contamination rates are those below 10.0%.

Table 1 also shows that fungal contamination decreased proportionally with increasing sodium hypochlorite concentration—50.0%, 30.0%, and 10.0%, respectively—confirming its effectiveness against fungal contaminants. Messias *et al.* (2019) [30] reached similar conclusions in their studies with *Paulinia melifolia*.

Therefore, treatment with 1.0% sodium hypochlorite is currently recommended for initiating *in vitro* cultivation of this species using nodal segment explants.

Conclusion

Based on the findings of this study, it is concluded that high germination rates of *Jatropha gossypiifolia* L. can be achieved *ex vitro* when using clay-based substrates. However, it is recommended that further trials be conducted with nutrient-rich substrates to investigate the potential for even higher germination rates.

It is also advised that new *in vitro* seed germination tests be undertaken, as the experiments conducted in this study did not result in successful *in vitro* germination of *J. gossypiifolia* L. seeds. Nevertheless, it was possible to initiate axenic *in vitro* cultivation of this species using nodal segments, which may be employed for

large-scale biomass production to meet the demands of the pharmaceutical industry.

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