IN VITRO AND *EX VITRO* GERMINATION OF *JATROPHA GOSSYPIIFOLIA* AND INITIATION OF *IN VITRO* CULTIVATION

ORIGINAL ARTICLE

MALOSSO, Milena Gaion[1], SILVA Maria da Paz Felix da[2], BARBOSA, Edilson Pinto[3], FURTADO, Maria Aparecida Silva[4], ROJAS, Jack Berllen Santos[5]

MALOSSO, Milena Gaion *et al*. ***In vitro* and *ex vitro germination* of *Jatropha gossypiifolia* and initiation of *in vitro* cultivation**. Revista Científica Multidisciplinar Núcleo do Conhecimento. Year 09, Ed. 08, Vol. 01, pp. 67-82. August 2024. ISSN: 2448-0959, Acess link: https://www.nucleodoconhecimento.com.br/biology/in-vitro-and-ex-vitro-germination, DOI: 10.32749/nucleodoconhecimento.com.br/biology/in-vitro-and-ex-vitro-germination

ABSTRACT

Jatropha gossypiifolia L. is a common medicinal plant in the Amazon. Popularly known as pião-roxo, it produces luteol that has healing activities and, therefore, was included in the National List of Medicinal Plants of Interest to the SUS. It has potential as an herbal medicine, but there are no phytotechnical studies for large-scale reproduction and, therefore, seed germination tests are necessary. For the *ex vitro* germination test, seeds were placed in different substrates for 180 days to determine the best type of soil for germination. For the *in vitro* germination test, three different concentrations of sodium hypochlorite were tested: 0.1; 0.5 and 1.0%. Nodal segments were subjected to the same asepsis. As a result, clayey soil was indicated as it presented the highest seed germination speed index. As *in vitro* seed germination was not obtained, it was decided to start the cultivation of this species using nodal segments and the best treatment was 1.0 mg/L of sodium hypochlorite. Thus, it was found that it is possible to produce plant biomass of this species to meet the needs of the pharmaceutical industry both *ex vitro* and *in vitro*.

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

1. INTRODUCTION

Euphorbiacea family includes the Crotonoidae subfamily, which includes the Jatropheae L. tribe, which is part of the *Jatropha* L. genus. The latter is home to between 165 and 175 species, distributed across Africa, India, the West Indies, Central America, the Caribbean and South America (Leal and Agra, 2005). Within this genus is *Jatropha gossypiifolia*, which is distributed throughout Brazil (Bigio, Secco, Moreira, 2023).

Jatropha gossypiifolia L. is a subshrub up to 5 m tall, with purplish branches and leaves when young, provided with milky, acrid sap. Its leaves are simple and lobed, the flowers are arranged in purple-colored paniculate summits and the fruit is of the capsule type (Araújo *et al*., 2023).

This species, popularly known in the northern region of Brazil as the pião-roxo, has medicinal properties (Varela, 2021) which, according to Bastos (2019), has healing and anti-inflammatory biological activities, of which Lemos *et al*. (2021) explain that they come from the triterpene luteol and, therefore, as stated by Ferreira (2022). For this reason, this plant is listed in the National List of Medicinal Plants of Interest to the Brazilian Unified Health System (Brasil, 2022).

Pião-roxo has great phytotherapeutic potential and, therefore, studies on its various aspects have been encouraged, since very little is known about the life cycle of this species. Therefore, it is necessary, among others, to study seed germination.

As stipulated by the Ministry of Agriculture, Livestock and Supply through the Rules for Seed Analysis, the analysis of light, temperature, humidity, oxygen availability and the type of substrate are fundamental for seed germination tests (Brasil, 2009). According to Lima Júnior (2010), the substrates most commonly used in seed germination tests are, among others, coconut fiber, earth and sand, which must be adequately moist to provide sufficient water for the seeds to germinate.

According to Mendes *et al*. (2019), the ideal substrate is one that meets the needs regarding the size, shape and tolerance to desiccation of the seed, in addition to allowing the evaluation of its availability, economic viability, standardization and homogenization, and that has physical and chemical characteristics that are not very viable. According to these authors, the appropriate substrate has sufficient structure, consistency, porosity to drain excessive water, good capillarity for water retention and does not contain toxic substances or be harmless to diseases, invasive plants or other pests.

Therefore, as stated by Queiroga *et al*. (2021), the substrate must not restrict the penetration of oxygen and therefore cannot contain excess moisture in order to prevent the water film from surrounding the seed, so the appropriate substrate must maintain the proportion between water availability and aeration.

Therefore, the substrate that should be used is the one that provides the best results in a seed germination test and obtaining seedlings (De Petri *et al*., 2020).

More modern Biotechnology methodologies are currently also widely used for seed germination (João *et al*., 2021). Through plant tissue culture, using the micropropagation technique, it is possible to quickly produce plant biomass on a large scale and at low costs (Santos, 2020) for use in the pharmaceutical industry.

The first step in obtaining inputs from this technique is to obtain axenic seedlings in vitro (Hoffmann *et al.*, 2022), which are commonly derived from explants such as seeds, nodal or apical segments, leaves, roots, among others (Lima, Da Rosa, Bonome, 2022), which are placed in culture media that generally contain an auxin that promotes cell elongation and a cytokinin, which stimulates cellular cytokinesis, inducing the rapid formation and growth of the aerial part of the plant (Porfírio *et al*., 2019).

According to Docha *et al*. (2020), the first stage of micropropagation is explant asepsis. In this stage, whose objective is the total elimination of microorganisms, antibacterial substances such as sodium and calcium hypochlorite are used, with concentrations that generally vary between 0.1 to 1.0%, and even various antibiotics. Here, 70% alcohol has an astringent function, that is, to detach hyphae and spores of microorganisms from the explants. And finally, systemic fungicides such as Derosal or Benomyl 1% are also commonly used.

Asepsis protocols are considered valid when they induce seed germination or the growth of pathogen-free nodal segments and it is customary to indicate the treatment that induces the highest germination rate and lowest contamination rate. Having achieved success in this stage, we then move on to the multiplication phase, where plant biomass is produced on a large scale. Therefore, *in vitro* germination tests are equally necessary and as important as *ex vitro* germination tests.

Thus, aiming to produce biomass to serve as an input for the phytotherapy industry, the objective of this work was to determine the best substrate for seed germination as well as the best type of asepsis for starting the *in vitro* cultivation of *Jatropha gossypiifolia* L., aiming at the subsequent large-scale production of plant biomass to be used as input by the pharmaceutical industry.

2. MATERIAL AND METHODS

2.1 *EX VITRO* GERMINATION TEST

To carry out this experiment, in November 2012, ripe fruits were collected in the matrix access located in the garden of the Institute of Health and Biotechnology of the Federal University of Amazonas, more precisely, in front of the Plant Tissue Culture Laboratory, where were taken, pulped and the seeds were planted on different types of substrates.

In this experiment, four types of substrates were used: coconut fiber, sand and clay, sand + clay (1:1). These substrates were autoclaved for 40 minutes at 121 ºC and distributed in plastic trays measuring 25.0 cm x 39.0 cm x 7.5 cm, which were kept in the greenhouse of the Plant Tissue Culture Laboratory of the Biotechnological and Health Institute at Federal University of Amazonas.

In each substrate, 30 seeds were deposited, as shown in Figure 1, in order to use a total of 120 seeds of *Jatropha gosypiidolia* L.

Figure 1 - *Ex vitro* germination experiment of *Jatropha gossypiifolia* L. showing the distribution of seeds in the substrate with equal proportions of sand and clay

Source: Authors, 2024.

The trays with the substrates and seeds were installed in an external covered area of the laboratory, where they were daily irrigated and monitored for 180 days after planting.

Every 15 days, data was collected regarding the number of seeds germinated in each type of substrate to evaluate the Germination Speed Index of the seedlings.

The Germination Speed Index (IVE) was calculated, as described by Maguire (1962).

The results were given from the mean and standard deviation test followed Tukey Test.

3. *IN VITRO* GERMINATION TEST

3.1 SEED ASEPSIS

Fruits of J*. gossypiifolia* L. were collected in the nursery of the Plant Tissue Culture Laboratory of the Coari Institute of Health and Biotechnology, Federal University of Amazonas. In this laboratory, the fruits were pulped and the seeds washed with neutral detergent and rinsed under running water. Then, they were immersed respectively in three different concentrations of sodium hypochlorite: 0.1, 0.5 and 1.0% for 30 minutes and kept under constant orbital agitation at 100 rpm, then immersed in 70% alcohol for 1 minute and immersed in 1% Benomyl solution for 1 hour and kept under the same orbital agitation. At the end of this process, they were washed 4 times with sterile distilled water and then inoculated into test tubes containing MS/2 culture medium, added with 30.0 g/L of sucrose and pH adjusted to 6.0. The seeds were kept in growth salt, with a photoperiod of 16 hours/light from cold white lamps, temperature of 25 ± 2ºC and relative humidity of 65% for 180 days to determine the germination rate. However, the contamination rate was counted only once, 30 days after *in vitro* inoculation. 30 seeds were used for each sodium hypochlorite treatment.

3.2 ASEPSIS OF NODAL SEGMENTS

Nodal segments of *J. gossypiifolia* L. from twigs collected from mother plants in the nursery of the Plant Tissue Culture Laboratory of the Institute of Health and Biotechnology of Coari, Federal University of Amazonas were the source of explants for this experiment. In this laboratory, the branches excised into nodal segments shaped like small "Y" were washed with neutral detergent using a soft toothbrush and rinsed thoroughly in running water. Then, the nodal segments received the same asepsis treatment described for the seeds, but 5.0 mg/L of ascorbic acid was added to the culture medium. This experiment was carried out in the same way as the previous one.

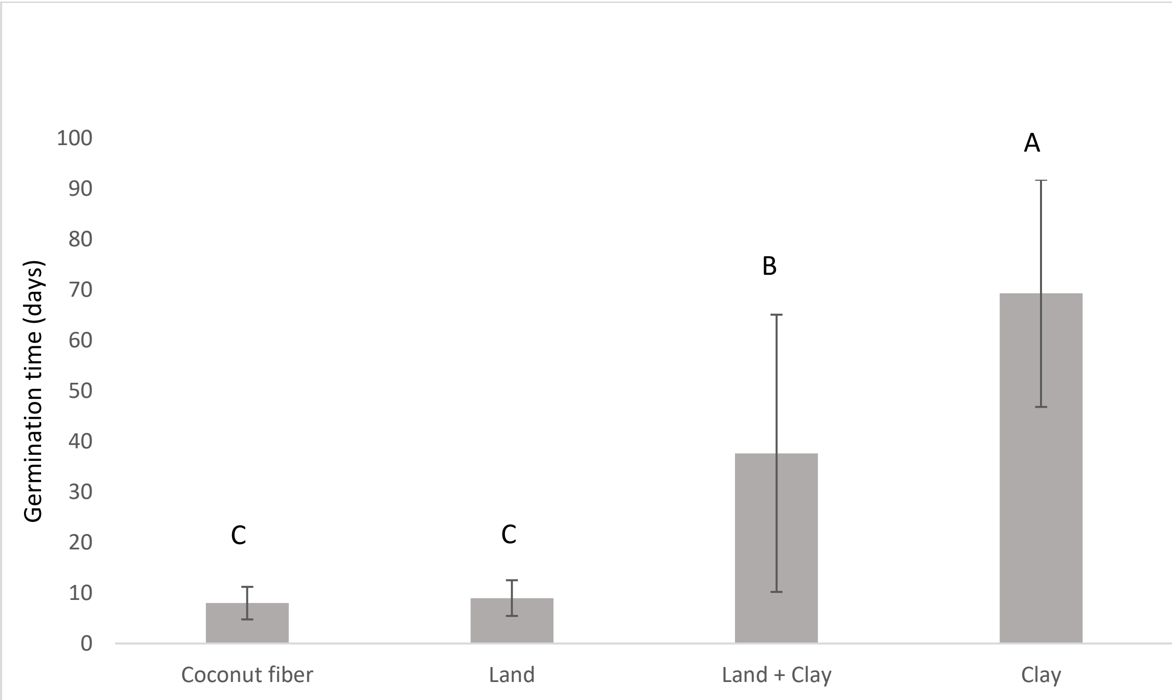
The results of these experiments were analyzed using simple percentages followed by Tukey Test.

4. RESULTS AND DISCUSSION

4.1 EX *VITRO* GERMINATION TEST

The size of the seed, its requirement regarding humidity, sensitivity to light and the ease offered for the development and evaluation of the seedling must be taken into consideration when choosing the substrate (Souza *et al*., 2020).

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



* Substract type. Source: Authors, 2024.

In statistical theory, the letter A represents a result that is better than b, which in turn is better than c and so on. Therefore, those results of statistical analyzes represented by the letter A are the most suitable as an answer to the experimental question.

As can be seen in Graph 1, after 180 days of planting, coconut fiber and sandy soil showed low twinning rates, respectively (7.99 ± 3.22 and (8.99 ± 3.53) at the end of this experiment due to the difficulty of these substrates retaining water. Gonçalves *et al*. (2020) obtained the same results with cedar. Furthermore, as explained by Menegaes *et al*. (2017) this also occurs because *Jatropha gossypiifolia* L. seeds do not find ideal conditions of density, total porosity, aeration space, humidity, water retention capacity, proportionality of solid pores and adequate water pH in these types of substrate.

Both substrate consisting of identical proportions of sand + clay (37.66 ± 27.44) and clayey soil (69.33 ± 22.49) showed a progressive germination rate that stabilized after 90 days (data not shown) in both substrates, which showed a high germination rate. Silva *et al*. (2023) explain that substrates made up of larger particles have more empty space and, therefore, a lower degree of compaction and apparent density, leading to greater soil aeration, which makes seedling germination easier. Dutra *et al*. (2016), also found that these two types of substrate are ideal for the germination of *Luehea divaricata* Mart. *et*. Zucc. seeds.

4.2 *IN VITRO* GERMINATION TEST

Asepsis is a critical step for the in vitro plant cultivation process, as the lack of an adequate protocol for eliminating microorganisms leads to an insufficient quantity of seedlings to continue the multiplication process (Vieira *et al*., 2021) of biomass.

Although the *in vitro* germination experiment of *Jatropha gossypiifolia* L. seeds was reassembled three times and in triplicate, it was not possible to obtain axenic seeds of this species *in vitro* and, even after 180 days of conducting this experiment, there was no germination, which demonstrates low viability of this species under stress conditions, as explained by Lattuada *et al.* (2019), who obtained the same results with oregano.

As the germination results were not satisfactory and aiming to solve the problem of producing plant biomass for input in the pharmaceutical industry, a new asepsis experiment was set up, however, now using the nodal segments, which resulted in 100% of segment-type explants killed by oxidation. This fact can be explained by the relationship between the size of the explant and the action of the disinfectant agent, since small tissues are very susceptible to degeneration caused by sodium hypochlorite, which leads to phytotoxicity (Silva *et al*. 2021).

Furthermore, the successive darkening that culminated in the total carbonization of the nodal segments can also be attributed to the release of phenolic compounds, which leads to the need to test this cheaper asepsis again, but now with the addition of some antioxidant agent and/or in the absence of light.

Then, a second experiment identical to the first was carried out, including only 5.0 mg/L of ascorbic acid in the culture medium, aiming to solve the problem of phenolic oxidation caused by excision of the explant (Rondon *et al*., 2019). And, as a result, the data in Table 1 were obtained.

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

Treatment	**Leve explantes (%)**	**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

Source: Authors, 2024.

In this second experiment, as seen in Table 1, it was verified that 100% of the nodal segments survived in all treatments, indicating that ascorbic acid presented an efficient antioxidant action in this type of explant. The same results were found by Oliveira Ribeiro, De Souza, (2021) in their studies with olive trees.

According to this Table, the contamination rate of the nodal segments of *Jatropha gossypiifolia* L., obtained through visual observation by bacteria was 13.33% in treatments with 0.1 and 0.5% sodium hypochlorite and 3.33% in the treatment with 1.0% of this same reagent, indicating that the disinfectant agent is efficient against bacteria that infest this plant species, since, according to Silva *et al*. (2021) acceptable contamination rates are those less than 10.0%.

It can also be seen in Table 1 that the fungal contamination rate was inversely proportional to the increase in sodium hypochlorite concentration, that is, it was 50.0, 30.0 and 10.0%, respectively, at concentrations of 0.1, 0.5 and 1.0% sodium hypochlorite, proving its efficiency also against this type of contaminant. Messias *et al*. (2019) reached the same conclusion in their studies with *Paulinia melifolia*.

Therefore, treatment with 1.0% sodium hypochlorite is currently indicated for initiating *in vitro* cultivation of this species with this type of explant.

5. CONCLUSION

With this work, it is concluded that it is possible to obtain high germination rates of *Jatropha gossypiifolia* L. *ex vitro* in clay substrate. However, we suggest that new tests be carried out on substrates with a high nutrient content to investigate the possible occurrence of a higher germination rate.

It is recommended that new in vitro seed germination tests be carried out, since the experiments carried out during this study did not induce in vitro germination of J. gossypiifolia L seeds. However, it was possible to initiate in vitro axenic cultivation of this species using nodal segments, which can be used to produce plant biomass on a large scale to meet the needs of the pharmaceutical industry.

ACKNOWLEDGMENTS

We would like to thank the Fundação de Amparo a Pesquisa do Amazonas (FAPEAM) for funding through the Scientific Initiation Scholarship Program. We also thank to Dr. Thierry Ray Jehlen Gasnier for guidance in carrying out the statistical analyses.

REFERENCES

ARAÚJO, Paloma Andrade Santos; DE OLIVEIRA, Vinícius Araújo; PONTES, Márcio Michel; DE GÓIS, Alexsandro Melquiades; DA SILVA, Gisele Nayara Bezerra.

FALCÃO, Rosângela Estevão Alves. **Pinhão-roxo (Jatropha gossypiifolia L.):** uma revisão de literatura dos usos tradicionais, atividade biológica e caracterização fitoquímica. In: Pesquisas e avanços e química de produtos naturais. 1º Edição. Jardim do Serindó: Agro Science. 463 p., 2023. Available in: https://agronscience.com/livro-cbqnat/. DOI: doi.org/10.53934/9786599965814. Accessed at: 24 ago. 2023.

BASTOS, Maria Lysete de Assis. Evidências científicas acerca das atividades biológicas de uma planta nativa do Nordeste Brasileiro – o pião roxo. **Revista Enfermagem Atual in Derm. Suplemento**, 2019;87, 9 p., 2019.

BIGIO, N. C.; SECCO, R. S.; MOREIRA, A. S. *Jatropha in* **Flora e Funga do Brasil**. Jardim Botânico do Rio de Janeiro, 2023. Available in: <https://floradobrasil.jbrj.gov.br/FB17581>. Accessed at: 24 ago. 2023.

BRASIL. Ministério da Saúde. **Plantas Medicinais de Interesse do SUS – RENISUS**. Publicado em 09/08/2021 e atualizado em 06/12/2022. Available in: https://www.gov.br/saude/pt-br/composicao/sectics/pnpmf/ppnpmf/renisus. Accessed at: 11 jul. 2024.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. 1º Edição. Brasília: MAPA, 398 p., 2009. Available in: regras-para-analise-de-sementes.pdf. Acesso em 25 ago. 2023.

DE PETRI, Eliane Cristina Moreno; CARDOSO, Elisa dos Santos; TIAGO, Auana Vicente; DA ROCHA, Vinícius Delgado; ROSSI, Ana Aparecida Bandini. Influência do armazenamento e substrato na emergência de plântulas de maracujá-amarelo. **South American Journal of Basic Education, Technical and Technological**, v. 7, n. 2, p. 458–468, 2020. Available in: https://d1wqtxts1xzle7.cloudfront.net/88938064/2528-libre.pdf?1658703305=&response-content-disposition=inline%3B+filename%3DInfluencia_Do_Armazenamento_e_Do_Substra.pdf&Expires=1694183623&Signature=Gf1OY1iGPbEyynaJQVeblfZ88DmDuG98lPWTSizWXiuJI3TpCaqryVRkzDm52zOf4~haJLv42u2RWmB4fDpPzgjBWNlfIzykYxOTbNb8KEtL4rfJ~YZGacHs4eRm4taOZx12bYjKTG0yeBwtyMq64q~zTU78f7YLhO9YNDQ4fD5ZoH59c4vZpDGgq50TeQUtPKVSvQTohzacusBGaTYW9Cx0WGKR3nk7v09CplaVuUVEikJlk2YYjFT6wLAQGexo~7OjlF5qtfS7Kb-lfVZ6TPR0gBpjyn5cHcwbPKIjRBB~hfCZRJKzUSohaIgAWm0JUCsSJp7w2c2oQbUyRPPPeg__&Key-Pair-Id=APKAJLOHF5GGSLRBV4ZA. Accessed at: 08 set. 2023.

DOCHA, Anna Luiza Mota; DE OLIVEIRA, Leandro Silva; DE SOUZA, Naiara dos Santos; BRONDANI, Gilvano Ebling. Estabelecimento in vitro de Ceiba riboflora Carb.-Sobr. e L. P. Queiroz: uma espécie endêmica do vale do rio São Francisco. **Caderno de Ciências Agrárias**, v. 12, p. 1–5, 2020. Available in: https://pdfs.semanticscholar.org/f631/988d28083d46dcceede70253104fe4d55cee.pdf. Accessed at: 08 set. 2023.

DUTRA, Adriana Falcão; ARAÚJO, Maristela Machado; RORATO, Daniele Guariente, MIETH, Patrícia. Germinação de sementes e emergência de plântulas de Luehea divaricata Mart. et. Zuc. em diferentes substratos. **Ciência Florestal**, v. 26, n. 2, p.

411–418, 2016. Available in: https://www.scielo.br/j/cflo/a/bcMxMcp9kyfN3bWqKJ4HRgg. Accessed at: 28 ago. 2023.

FERREIRA, Ilke Mara Rodrigues. Estudo farmacológico e toxicológico da Jatropha gossypiifolia: uma revisão integrativa. **Revista Ciência (In) Cena**, v. 1, n. 16, p. 71 – 87, 2022. Available in: https://estacio.periodicoscientificos.com.br/index.php/cienciaincenabahia/article/view/1311/1110. Accessed at: 24 ago. 2023.

GONÇALVES, Viviane Evellyn Costa; DE CARVALHO, Cleverson Agueiro; DE BRITO, Rychaellen Silva; DA SILVA, Márcio Chaves; ANDRADE, Reginaldo Almeida. **Influência de diferentes níveis de umidade para a germinação de cedro (Cedrela fissilis)**. In: Anais do 9º Congresso Florestal Brasileiro. p. 582 – 585, 2020. Available in: file:///C:/Users/User%20-%20005/Downloads/5596f81d-cd02-45ee-b535-fb5c0ac4a0fb.pdf. Accessed at: 28 ago. 2023.

HOFFMANN, Luana Tiara; BITTENCOURT, Ricardo; GROTTI, Yasmin Tassi; SPELICH, Carmen Lúcia. Germinação in vitro de Raulinoa echinata R. S. Cowan (Rutaceae): sementes e embriões zigóticos. **Ciência Florestal**, v. 32, n. 3, p. 1187 – 1204, 2022. Available in: https://web.p.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=01039954&AN=160108573&h=sAJC95HIgwP4rSmQdGg3G8PX7xDwGcaBoDMtmRWeB4MHoe0dlp67s8wT1yeBb%2frRfI4RkA833KgEFT82OKHHcA%3d%3d&crl=c&resultNs=AdminWebAuth&resultLocal=ErrCrlNotAuth&crlhashurl=login.aspx%3fdirect%3dtrue%26profile%3dehost%26scope%3dsite%26authtype%3dcrawler%26jrnl%3d01039954%26AN%3d160108573. Accessed at: 08 set. 2023.

JOÃO, Amanda Aparecida; FERRERA, Lucas Fantin Machado; CORRÊA, Pedro Henrique Florêncio; LUIZ, Rita de Cássia Portes; DE OLIVEIRA NETO, Sebastião Soares. Biotecnologia Vegetal 4.0: uma abordagem sobre "speed breeding". **Research, Society and Developmente**, v. 10, n. 12, 2021. Available in: https://rsdjournal.org/index.php/rsd/article/view/20120/18009. Accessed at: 08 set. 2023.

LATTUADA, Daiane Silva; GUASSO, Leonardo Zucuni; DE OLIVEIRA, Kédima Melo; DA SILVA, Valmira Machado; DE SOUZA, Paulo Vitor Dutra. Tipos de explantes para estabelecimento in vitro para orégano e hortelã. **Pesquisa Agropecuária Gaúcha**, v. 25, n. 3, p. 91–103, 2019. Available in: https://revistapag.agricultura.rs.gov.br/ojs/index.php/revistapag/article/view/88/68. Accessed at: 08 set. 2023.

LEAL, Crislaine Kieva Abreu; AGRA, Maria de Fátima. Estudo farmacobotânico comparativo das folhas de Jatropha molissima (Pohl.) Baill. e Jatroplha ribifolia (Pohl.) Baill. (Euphorbiaceae). **Acta Farm. Banaerense**, v. 24, n. 1, p. 5 –13, 2005. Available in: http://www.latamjpharm.org/trabajos/24/1/LAJOP_24_1_1_1_6WQ842B4X2.pdf. Accessed at: 24 ago. 2023.

LEMOS, Ari Sérgio de Oliveira; DE OLIVEIRA, Naiara Norberto Tavares; NETTO, Lívia Lacerda; REIS, Samara Evangelista; DE MEDEIROS, Valquíria Pereira; FABRI, Rodrigo Luiz; CHEDIER, Luciana Moreira. Avaliação do perfil químico e citotóxico de Jatropha multifida L. (Euphorbiaceae). **Brasilian Journal of Development**, v. 7, n. 10, p. 94971–94984, 2021. Available in: https://d1wqtxts1xzle7.cloudfront.net/79162067/pdf-libre.pdf?1642689252=&response-content-disposition=inline%3B+filename%3DAvaliacao_do_perfil_quimico_e_citotoxico.pdf&Expires=1692934763&Signature=F9301XHPCRTOgl880kZIABgSCFoHoslJT-egiBxDM2vEdt82753zv4LNN2BgChM87sjk5tFB7-bTpy-SAF-O0kOaYEIKtZlR~rFOwRiIbCf58qjpVUB7R6Sod2bYm~U8bfw42MfkzCAGzx6I5~eUAOvt0nVoMiJBF2pXwy-6hNsnQ5G9RVMmC7WNbDoMr3Pbxhl3j1ze~UhclIA37phqEa1edK4MEbN3E7w8lzXBSKbQNry0OIwSJ-QRx-eftwQ~sB0tvEksIEzbgg9zV9VxbXprJNEF21XwSO-jZGWD0IJ1AdJ7W5djlG9KN3Lb5ORwDZJhd9mQ1EKMIAvizo1vXw__&Key-Pair-Id=APKAJLOHF5GGSLRBV4ZA . DOI:10.34117/bjdv7n10-010. Accessed at: 24 ago. 2023.

LIMA, Cláudia Simone Madruga; DA ROSA, Gabriela Gerhardt; BONOME, Lisandor Tomas da Silva. **Aspectos técnicos da cultura de Romanzeira**. 1ª Edição. Chapecó: Editora UFFS. 138 p. 2022.

LIMA JUNIOR, Manoel de Jesus. **Manual de procedimentos para a análise de sementes florestais**. 1ª Edição. Manaus: EDUFAM, 146 p., 2010. Available in: https://www.researchgate.net/profile/Fatima-Pina-Rodrigues/publication/232768692_MANUAL_DE_PROCEDIMENTOS_PARA_ANALISE_DE_SEMENTES_FLORESTAIS/links/0912f50953d0105ee3000000/MANUAL-DE-PROCEDIMENTOS-PARA-ANALISE-DE-SEMENTES-FLORESTAIS.pdf. Accessed at: 25 ago. 2023.

MAGUIRE, James D. Speed of germination: aid in selection and evaluation for seedling emergence and vigour. **Crop Science**, v. 2, n. 2, p. 176-177, 1962. Available in: https://doi.org/10.2135/cropsci1962.0011183X000200020033x. Accessed at: 28 ago. 2023.

MENDES, Rafaella Gouvea; BONETTI, Leila Leal d Silva; GASTL-FILHO, Josef; DE MENEZES, Danylla Paula; de SANTI, Sávio Luiz; REZENDE, Arthur Silva; MENEZES, Leonardo Henrique Queiroz; SILVA, Aurélio Freitas Pereira. Germinação e vigor de sementes de Araticum-cagão influenciadas por GA3 em diferentes substratos. Bazilian Journal of Animal and Enviromental Research. v. 2, n. 1, p. 632 – 645, 2019. Available in: https://ojs.brazilianjournals.com.br/ojs/index.php/BJAER/article/view/1449/1336. Acesso em 25 ago. 2023.

MENEGAES, Janine Farias; NUNES, Ubirajara Russi; BALLÉ, Rogério Antônio, LUDWIG, Eduardo José; SANGOIO, Pablo Reno; SPEROTTO, Lucas. Germinação de Sementes de Cartamus tinctorius em diferentes substratos. **Acta Iguazu.**, v. 6, n. 3, p. 22–30, 2017. Available in:

https://saber.unioeste.br/index.php/actaiguazu/article/view/17705/11728. Accessed at: 28 ago. 2023.

MESSIAS, Thiago da Silva; REZENDE, Rodrigo Kelson Silva; DA SILVA, Luciely Faustino; NUNES, Geissiany Pereira; JESUS, Mailson Vieira. Estabelecimento de protocolo para descontaminação de explantes foliares de Paullinia meliifolia. Saber Científico. v. 8, n. 1, p. 9–14, 2019. Available in: https://periodicos.saolucas.edu.br/index.php/resc/article/view/1255/1097. Accessed at: 08 set. 2023.

OLIVEIRA, Nilma Portela; RIBEIRO, Santuza Aparecida Furtado; DE SOUZA, Marília Maia. Controle de contaminação e oxidação no cultivo in vitro de oliveira (Olea europaea L.) cv. "Koroneiki". **Research, Society and Development**, v. 10, n. 5, p. 1 – 10, 2021. Available in: https://rsdjournal.org/index.php/rsd/article/view/14929/13423. Accessed at: 08 set. 2023.

PORFÍRIO, Kennedy de Paiva; TITON, Miranda; DE CASTRO, Ana Carolina Macedo; PEREIRA, Israel Marinho; PFEILSTICKER DE KNEGT, Rafael Antoniu. Multiplicação in vitro de Xyolopedia aromatica em diferentes meios de cultura e concentrações de BAP. **Pesq. flor. bras.**, Colombo, v. 39, e201901895, p. 1-7, 2019. Available in: https://pfb.cnpf.embrapa.br/pfb/index.php/pfb/article/view/1895/898. Accessed at: 08 set. 2023.

QUEIROGA, Vicente de Paula; GOMES, Josivalda Palmeiras; FIGUEIREDO NETO, Acácio; QUEIROZ, Alexandre José de Melo; MENDES, Nouglas Veloso Barbosa; de ALBUQUERQUE, Ester Maria Barros. Mirtilo (Vaccinum spp.) tecnologias de plantio em típicas regiões serranas. **Revista Eletrônica A Barriguda**, 237 p., 2021. Available in: https://www.researchgate.net/profile/Nouglas-Mendes-2/publication/354697031_MIRTILO_Vaccinium_spp_TECNOLOGIAS_DE_PLANTIO_EM_TIPICAS_REGIOES_SERRANAS_Editores_Tecnicos/links/61487562a3df59440b9bef50/MIRTILO-Vaccinium-spp-TECNOLOGIAS-DE-PLANTIO-EM-TIPICAS-REGIOES-SERRANAS-Editores-Tecnicos.pdf. Accessed at: 25 ago. 2023.

RONDON, Melaca Juliana Peixoto; DE SOUZA, Tacia Ivila; ARAÚJO, Danielly Aparecida Amorim; ARAÚJO, Ingrid Slusarski; FERNANDES, Daiane Ávila. Benefícios do carvão ativado no meio de cultura para os explantes de banana prata, nanica e terra. **Connectionline**, n. 21, p. 71–81, p. 2019. Available in: https://www.periodicos.univag.com.br/index.php/CONNECTIONLINE/article/view/1391/1500. Accessed at: 08 set. 2023.

SANTOS, Cleberson Corrêa (Org.). **Agrobiodiversidade:** manejo e produção sustentável. Volume 1. 1º Edição. Mato Grosso: Editora Pantanal. 146 p. 2020. Available in: https://editorapantanal.com.br/ebooks/2020/agrobiodiversidade-manejo-e-producao-sustentavel/ebook.pdf. Accessed at: 08 set. 2023.

SILVA, Jailton Jaime das Neves; NAVROSKI, Maricio Carlos; DE AQUINO, Marina Gabriela Cardoso; DENEGA, Lucas; DA FONSECA, Pedro Herique Tavares; DE OLIVEIRA, Luciana Magda; PEREIRA, Mariane de Oliveira. Resgate vegetativo, estabelecimento in vitro e estaquia de Drimys brasiliensis Miers. **Ciência Florestal**, v. 33, n. 1, p. 21–25, 2023. Available in: scielo.br/j/cflo/a/CXZ46ZrRhYqkPwK9nDXb5YN/?format=pdf&lang=pt. Accessed at: 08 set. 2023.

SILVA, Ana Cláudia Lopes; MANFIO, Cândida Elisa; LEÃO, José Ricardo Avelino; DE CARVALHO, Josiane Celerino; GONÇALVES, José Francisco de Carvalho; RAPOSO, Andréa. Indução de calogênese em segmentos foliares de seringueira (Hevea spp.) na Amazônia Sul Ocidental. **Research, Society and Development**, v. 10, n. 9, p. 1 – 10, 2021.

SOUZA, Gilda Gonçalves; REDIG, Meirevalda do Socorro Ferreira; BRITO, Sinara de Nazaré Santana; MONTEIRO, Arlesson Sidney Almeida; BRONZE, Antônia Benedita da Silva; LOPES, Elessandra Laura Nogueira; VASCONCELOS, Omar Machado. Determinação do índice de velocidade de germinação e dos parâmetros genéticos de sementes de Bacaba em diferentes substratos na Amazônia Oriental. **Brazilian Journal of Development**, v. 7, n. 3, p. 25887 – 25898, 2020.

VARELA, Alex Gonçalves. A trajetória de Joaquim Monteiro Caminhoá: um botânico no império do Brasil. **Brasilian Journal of Development**, v. 7, n. 1, p. 9905-9924, 2021. Available in: https://ojs.brazilianjournals.com.br/ojs/index.php/BRJD/article/view/23794. Accessed at: 24 ago. 2023.

VIEIRA, Michel Rafael Soares; SILVESTRIM, Eneida Guerra; DE LIMA FILHO, Arlindo Almeida; LOPES, Aixa Braga; SILVESTRIM, Fernanda Guerra. Métodos de assepsia na multiplicação in vitro da bananeira ´Pacovan´ (Musa spp.). **Research, Society and Development**, v. 10, n. 16, 9 p., 2021. Available in: https://rsdjournal.org/index.php/rsd/article/view/23765/20947. Accessed at: 08 set. 2023.

Material received: April 11, 2024.

Peer-Approved Material: July 15, 2024.

Edited material approved by the authors: July 29, 2024.

---

[1] Advisor. PhD in Biotechnology from the Federal University of Amazonas; Master in Biotechnology from the University of Ribeirão Preto; Specialist in Phytotherapy and Medicinal Plants from the Faculty of Minas Gerais (Lato sensu); Bachelor in Biology and Degree in Biological Sciences from the University of Araraquara. ORCID: https://orcid.org/0000-0003-1613-1331. Currículo Lattes: https://lattes.cnpq.br/1873078781409836.
[2] Bachelor of Biotechnology. ORCID: https://orcid.org/0000-0002-0428-9118. Currículo Lattes: http://lattes.cnpq.br/8334191085103677.

[3] PhD in Economic Development; Master in Economics; Bachelor in Economics. ORCID: https://orcid.org/0009-0003-1056-2840. Currículo Lattes: http://lattes.cnpq.br/2821682713242701.
[4] PhD in Linguistics; Bachelor of Arts. ORCID: https://orcid.org/ 0000-0002-4725-5321. Currículo Lattes: http://lattes.cnpq.br/7809909271012069.
[5] Master in Biotechnology; Bachelor in Biotechnology. ORCID: https://orcid.org/0000-0002-5295-4792. Currículo Lattes: http://lattes.cnpq.br/2408139391652337.