



In vitro propagation from nodal segments of *Lippia organoides* (chemotype A)

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ABSTRACT: This research described an efficient micropropagation protocol for *Lippia organoides* (Verbenaceae). Sterile seeds were used to obtain germinated seedlings in Murashige and Skoog medium (MS) supplemented with sucrose and agar. The nodal segments obtained from seedlings were grown on MS medium supplemented with different concentrations of gibberellic acid (GA), benzylaminopurine (BAP) and 1-naphthalenetic acid (NAA) with BAP. The callus induction, shoots length, shoots number and root length, were analyzed. The treatments showed high percentage of callus formation at 0.5 to 1.5 mg L⁻¹ of BAP alone or in combination with NAA (0.1 mg L⁻¹). The highest value of shoot number per nodal segments was obtained at 1.5 mg L⁻¹ of BAP (4.3 ± 0.8). The obtained plantlets were better rooted in vitro in the absence of plant growth regulators (PGRs) and they showed acclimatization rate of 90%. We reported a protocol for in vitro propagation and acclimatization of *L. organoides* for A chemotypes from Colombia.

Key words: micropropagation, shoot multiplication, nodal culture.

Propagação *in vitro* de segmentos nodais de *Lippia organoides* (quimiotipo A)

RESUMO: Esta pesquisa descreve um protocolo de micropropagação eficiente para *Lippia organoides* (Verbenaceae). Sementes estéreis foram utilizadas para a obtenção de mudas germinadas em meio Murashige e Skoog (MS) suplementado com sacarose e ágar. Os segmentos nodais obtidos das mudas foram cultivados em meio MS, suplementado com diferentes concentrações de ácido giberélico (GA), benzilaminopurina (BAP) e ácido 1-naftalenacético (ANA) com BAP. Foram analisados a indução de calo, comprimento de brotação, número de brotação e comprimento de raiz. Os tratamentos mostraram alta porcentagem de formação de calo com 0,5 a 1,5 mg L⁻¹ de BAP sozinho ou em combinação com ANA (0,1 mg L⁻¹). O maior valor de número de brotações por segmento nodal foi obtido com 1,5 mg L⁻¹ de BAP (4,3 ± 0,8). As mudas obtidas apresentaram melhor enraizamento *in vitro* na ausência de reguladores de crescimento vegetal (PGRs) e demonstraram taxa de aclimação de 90%. Contudo, relata-se um protocolo para propagação *in vitro* e aclimação de *L. organoides* para quimiotipos A da Colômbia.

Palavras-chave: micropropagação, multiplicação de ramos, cultura nodal.

The genus *Lippia* belongs to the Verbenaceae family and comprises about 200 species, including grasses, shrubs, and small trees. It mainly occurs in South and Central America and tropical Africa (ATKINS, 2004). Several studies have revealed that *Lippia* spp. contain bioactive compounds with a wide range of biological activities and potential sources for treating different diseases (MASUNDA et al. 2020; LEYVA-JIMÉNEZ et al. 2019; PASCUAL et al. 2001).

Lippia organoides is an aromatic shrub native to Central America and northern region of South America (VICUÑA et al. 2010). It is characterized by its antibacterial, antifungal, antiviral, antiparasitic,

antioxidant, antigenotoxic, and anticancer biological activities (CELIS et al. 2007; MENESES et al. 2009; OLIVEIRA et al. 2007; QUINTERO-RUIZ et al. 2017; RAMAN et al. 2017; SANTOS et al. 2004; STASHENKO et al. 2014), attributed to components of its essential oil and extracts. It has also been shown that *L. organoides* has at least five chemotypes according to its essential oil major component (A, B, C, D and E) used in the cosmetics and pharmaceutical industries (RIBEIRO et al. 2014; SILVA et al. 2009; STASHENKO et al. 2010).

This plant also contains flavonoids such as pinocembrin (LEITÃO et al. 2017;

STASHENKO et al. 2013), a. A natural compound with pharmacological properties, including ischemic stroke, neurodegenerative diseases, and atherosclerosis (LAN et al. 2017). It is present only in the extract of *Lippia origanoides* chemotype A, which is exclusive of Colombia (STASHENKO et al. 2013). In addition, chemotype A also has the highest profitability in the extraction process of its products (ARIAS et al. 2020).

Attempts to propagate *L. origanoides* by conventional methods as “sticks and cuttings” and seeds have shown a lower rooting capacity and a delayed development (HERRERA-MORENO et al. 2013; SILVA et al. 2015). Therefore, it is essential to use alternative techniques to produce large amounts of biomass in a short time that allows supplying the industry demand. An option is plant propagation by tissue culture, a useful tool to optimize plant production of superior qualities, free from microorganisms and with high multiplication rates (LONE et al. 2020). However, a major limitation in large scale application of this technology is high mortality experienced by micropropagated plants during or following laboratory to land transfer (CHANDRA et al. 2010).

Although, the *in vitro* micropropagation technique has been efficient and described for some species of the genus *Lippia* (CASTELLANOS-HERNÁNDEZ et al., 2013; GUPTA et al. 2001; JOSÉ et al. 2019; JULIANI et al. 1999; PEIXOTO et al. 2006; RESENDE et al. 2015), currently, there is only a single report on the effects of different PGRs (benzylaminopurine, indole acetic acid and kinetin) on *in vitro* development of *L. origanoides* (CASTILHO et al. 2019). It is important to point out that this approach does not describe a useful methodology for *ex vitro* acclimatization. Considering that plant's response to *in vitro* culture varies between samples collected from different geographic locations (HAQUE & GOSH 2018; MANVI & PARASHARAMI 2019), this work established a protocol for *in vitro* propagation and *ex vitro* acclimatization of *L. origanoides* for A chemotype exclusive from Colombia.

The inflorescences of *L. origanoides* plants (A chemotype), were collected from experimental gardens at “Centro Nacional de Investigaciones para la Agro industrialización de Especies Vegetales Aromáticas Medicinales Tropicales (CENIVAM)”, located at Universidad Industrial de Santander, (Bucaramanga, Colombia). These inflorescences were dried in the oven for 24 h at 34 °C until their use.

For seed germination, we used the MS medium (Murashige and Skoog, 1962) prepared at ½ strength and supplemented with 1.5% w/v sucrose and 0.7 % w/v agar (Caisson) at pH 5.8, as it was

indicated by CASTELLANOS-HERNÁNDEZ et al., (2013). The seeds were immersed in 70% ethanol (1 min), followed by immersion in 4% sodium hypochlorite (12 min) and then were washed three times with sterile and distilled water. The seeds were germinated in 60 × 70 mm flask containing 20 mL of MS medium. The containers were maintained at 25 ± 2 °C with a 16 h photoperiod, provided by white and cold fluorescent tubes with an intensity of 50 μmol m⁻¹ s⁻¹ (Sylvania Toledo Glass Tube 9w TB G13). For determination of germination and contamination rates, five replications of 10 seeds were used and germination was scored by visual observation of radicle emergence posterior to 20 days. After six weeks, the nodal segments of the seedlings (with average length of 4.1 cm) were used as a source of explants for the multiplication stage.

The culture media (Table 1) was the MS medium with 3% w/v sucrose supplemented with NAA at 0.1 mg L⁻¹ and different concentrations of GA and BAP, as follows: GA (0.5, 1, 1.5 mg L⁻¹), BAP (0.5, 1, 1.5 mg L⁻¹). Nodal segments (approximately 1 cm) were obtained from 6 weeks old *in vitro* seedlings and were inoculated into different media. Each treatment was composed by one explant per flask with four replicates and three repetitions. After the sixth week, we estimated the callus percentage, the number of shoots, and the shoot's length and roots per explants.

Plantlets with well-developed roots (root length greater than 3 cm) were removed from the culture medium and washed with distilled and sterilized water to remove the adhering medium. They were transplanted in sealable plastic bags with different substrates such as river sand, black peat and vermicompost, and all were previously sterilized (Figure 1). Fifteen plantlets were transplanted for each substrate. The bags were transferred to a greenhouse and were opened gradually until ten days and were irrigated with tap water every 3 days. After 6 weeks, plantlets were transferred to field conditions.

All experiments were performed under a completely randomized design. The collected data were normalized (Log₁₀), and a factor variance analysis was performed at a significance level of P < 0.05, followed by a Tukey multiple range test. The RStudio 1.0.143 program for Windows was used.

In vitro germination of *L. origanoides* seeds collected from the field was performed to obtain axenic seedlings as sources of explants for plant propagation. After ten days the average germination rate of seedlings explants was 60%. Contamination was observed in less than 20%

Table 1 - Treatments used for germination, multiplication and rooting of nodal segments of chemotype A *L. origanoides* explants.

-----Concentration (mg/L)-----			Callus (%)	No of explant shoots	Shoot length (cm)	Root length (cm)
GA	BAP	NAA				
0.5	0	0	0	1.7 ± 0.2 bc	5.8 ± 1.3 a	8.6 ± 3.1 a
1	0	0	0	1.2 ± 0.3 c	3.2 ± 1.0 bc	2.0 ± 0.3 bc
1.5	0	0	0	1.4 ± 0.4 bc	4.4 ± 0.7 ab	3.5 ± 1.7 ab
0	0.5	0	89%	2.3 ± 1.3 abc	2.9 ± 0.1 bcd	1.9 ± 0.7 bc
0	1	0	92%	3.4 ± 1.3 ab	2.3 ± 0.4 cde	2.3 ± 1.0 bc
0	1.5	0	100%	4.3 ± 0.8 a	3.1 ± 1.1 bc	4.1 ± 1.4 ab
0	0.5	0.1	100%	1.5 ± 0.4 bc	1.8 ± 0.4 de	0.9 ± 0.4 cd
0	1	0.1	100%	1.9 ± 1.0 abc	1.6 ± 0.2 e	1.8 ± 0.7 bc
0	1.5	0.1	100%	2.2 ± 0.9 abc	1.4 ± 0.3 e	0.4 ± 0.1 d
0	0	0	0	1.7 ± 0.5 bc	4.7 ± 0.7 ab	6.5 ± 4.6 ab

The letters represent homogeneous groups; within each column, different letters indicate a significant difference at $P < 0.05$ using ANOVA and the Tukey HSD test.

Values are expressed as the mean ± standard deviation of the mean.

of seeds. Similar results were obtained with *L. graveolens* (CASTELLANOS-HERNÁNDEZ et al., 2013) since we used the same medium.

Nodal explants showed a high percentage of callus formation in treatments with BAP alone or in combination with NAA (Table 1). Callus initiation was observed from the explants within 20-30 days of inoculation. Maximum callus induction (100%) was

detected from nodal explants cultured on MS medium with 0.1 mg L^{-1} NAA and any concentration of BAP. In other *Lippia* species the addition of BAP also favored callusing at the lower end of the nodal stem explants (CASTELLANOS-HERNÁNDEZ et al. 2013, GUPTA et al. 2001; JOSÉ et al. 2019; MARINHO et al. 2011; RESENDE et al. 2015), including the study of *L. origanoides* from Brazil (CASTILHO et al. 2019).

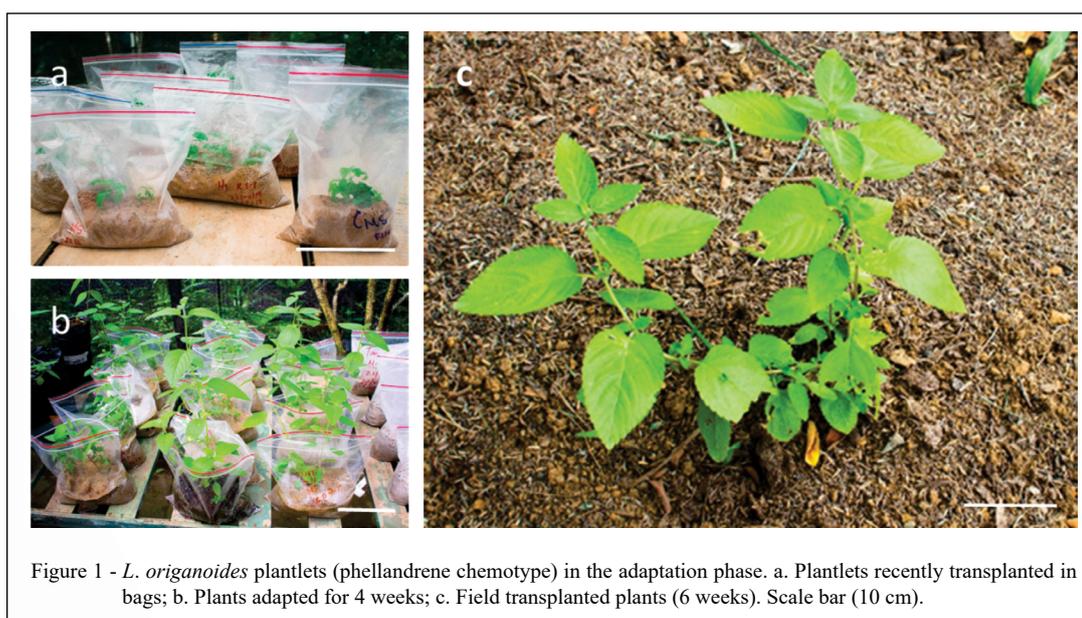


Figure 1 - *L. origanoides* plantlets (phellandrene chemotype) in the adaptation phase. a. Plantlets recently transplanted in bags; b. Plants adapted for 4 weeks; c. Field transplanted plants (6 weeks). Scale bar (10 cm).

Culture of nodal explants on media supplemented with 1.5 mg L^{-1} of BAP resulted in significant differences in the number of shoots compared to control medium (Table 1). The highest values for shoot number per explants was obtained in the medium supplemented with BAP despite the fact that they did not have significant differences with other treatments. The addition of 0.1 mg L^{-1} NAA in combination with BAP showed no statistically significant difference from treatments with BAP alone in terms of number of shoots (Figure 2; Table 1).

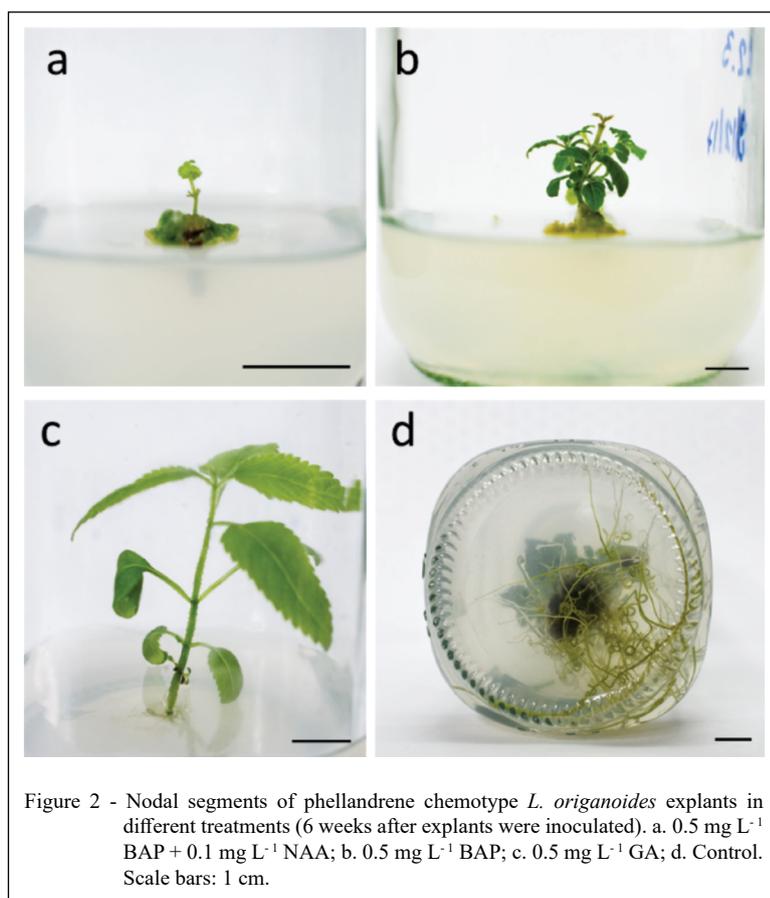
The beneficial effects of cytokinin during *in vitro* plant propagation, as report here, are in agreement with the results obtained in other species of *Lippia*, such as *L. junneliana* (JULIANI et al. 1999), *L. alba* (GUPTA et al. 2001), *L. javanica* (ARA et al. 2010), *L. nodiflora* (PRIYA & RAVINDHRAN, 2011) and *L. filifolia* (PEIXOTO et al. 2006), but in this case, the combination with NAA results in the highest multiple shoot response.

Nevertheless, *in vitro* propagation of *L. organoides* from Brazil, the best result showed shoot

regeneration was with a medium free of PGRs (CASTILHO et al. 2019), demonstrating that plant's response to *in vitro* culture varies between different geographic locations, regardless of being the same species.

Healthy elongated shoots were obtained on MS medium with 0.5 and 1.5 mg L^{-1} of GA and medium free of PGRs (4.7 ± 0.7). The effect of GA has not been reported in other *Lippia* species. This result is consistent with similar findings in *L. dulcis* (URREA et al. 2010) and *L. junneliana* (JULIANI et al. 1999), where the mean shoot length was higher in a medium free of PGRs. However, it is the opposite of CASTILHO et al. (2019) reported since the medium without PGRs did not affect the shoot length. Once more, indicating the need to establish propagation protocols for plants of the same species with differences in their localities.

Conversely using 1 mg L^{-1} of BAP or BAP in combination with NAA showed a lower capacity of the development of the length of the shoot. In contrast, the use of NAA and BAP + NAA in *in vitro* cultures explants from *L. filifolia* (PEIXOTO et al. 2006) and



L. rotundifolia (RESENDE et al. 2015); respectively, improved shoot length. Since the incorporation of BAP into the culture medium, it is used primarily to induce shoot proliferation (BHOJWANI et al. 2013); therefore, it is expected that it will not have a positive effect on stem elongation by itself.

The highest value for shoot rooting was observed at 0.5 mg L⁻¹ of GA (8.6 ± 3.1) and control treatment (6.5 ± 4.6). Increasing the GA concentration reduced the length of roots. Although, nodal explants have a positive response on MS medium with 0.5 mg L⁻¹ of GA, our results suggested that it is unnecessary PGRs to obtain elongated and rooting shoots of *L. origanoides* since there was not significant differences with control. This was also reported for *L. dulcis* (URREA et al. 2010), where MS medium was the best treatment.

After hardening in sand and peat substrates for four weeks, regenerated *L. origanoides* plantlet survival was over 90% but only 60% in vermicompost substrate (Figure 1). Despite the high mortality often observed upon transfer plants to *ex vitro* conditions (CHANDRA et al. 2010), plants obtained *in vitro* from *L. origanoides* do not present this difficulty, since it was only necessary to gradually decrease the humidity to get healthy plants. All the plants survival the transfer to the field.

The method described in the present study provided an efficient reproducible protocol for *in vitro* micropropagation of *L. origanoides* (A). This technique is an alternative to traditional propagation methods for this promising aromatic species. The application of the protocol will facilitate research into the any major volatile component that proved to have industrial value and it could stimulate their development on a commercial scale.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The projects were also approved by the Ethical Committee (Record N°. 22) from UIS.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the

collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

- ARA, N. et al. A study of plant growth hormones on *in vitro* clonal propagation of fever tea (*Lippia javanica*): A medicinal shrub. **American-Eurasian Journal of Sustainable Agriculture**, v.4, p.274–279, 2010. Available from: <https://www.researchgate.net/publication/230601374_A_Study_of_Plant_Growth_Hormones_on_in_Vitro_Clonal_Propagation_of_Fever_Tea_Lippia_Javanica_a_Medicinal_Shrub>. Accessed: Mar. 06, 2021.
- ATKINS, S. Verbenaceae. In: KUBITZKI, K; KADEREIT, J.W. **The families and genera of vascular plants**. Germany: Springer-Verlag, 2004. p.449–468.
- BHOJWANI, S.S. et al. **Plant tissue culture: An introductory text**. Agra: Springer, 2013. 309p.
- CASTELLANOS-HERNÁNDEZ, O. A. et al. *In vitro* clonal propagation and regeneration of the commercially important plant Mexican oregano (*Lippia graveolens*). **In Vitro Cellular and Developmental Biology-Plant**, v.49, p.620–625, 2010. Available from: <<https://link.springer.com/article/10.1007/s11627-013-9538-4>>. Accessed: Mar. 06, 2021. doi: 10.1016/j.indcrop.2019.01.006.
- CASTILHO, C. V. V. et al. *In vitro* propagation of a carvacrol-producing type of *Lippia origanoides* Kunth: A promising oregano-like herb. **Industrial Crops and Products**, v.130, p.491–498, 2019. Available from: <<https://doi.org/10.1016/j.indcrop.2019.01.006>>. Accessed: Mar. 06, 2021. doi: 10.1016/j.indcrop.2019.01.006.
- CHANDRA, S. et al. Acclimatization of tissue cultured plantlets: From laboratory to land. **Biotechnology Letters**, v. 32, p. 1199–1205. 2010. Available from: <<http://link.springer.com/10.1007/s10529-010-0290-0>>. Accessed: Mar. 06, 2021. doi: 10.1007/s10529-010-0290-0.
- GUPTA, S. K. et al. *In vitro* micropropagation of *Lippia alba*. **Current Science**, v.81, p.206–210. 2001. Available from: <<https://link.springer.com/article/10.1079/IVP2006814>>. Accessed: Mar. 06, 2021. doi: 10.1079/IVP2006814.
- HAQUE, S. M.; GHOSH, B. An improved micropropagation protocol for the recalcitrant plant Capsicum—a study with ten cultivars of Capsicum spp. (*C. annuum*, *C. chinense*, and *C. frutescens*) collected from diverse geographical regions of India and Mexico. **Journal of Horticultural Science and Biotechnology**, v.93, p.91–99. 2018. Available from: <<https://doi.org/10.1080/14620316.2017.1345331>>. Accessed: Mar. 06, 2021. doi: 10.1080/14620316.2017.1345331.
- HERRERA-MORENO, A. M. et al. Establishment of propagation methods for growing promising aromatic plant species of the *Lippia* (*Verbenaceae*) and *Tagetes* (*Asteraceae*) genera in Colombia. **Agronomía Colombiana**, v.31, p.27–37. 2013. Available from: <<https://revistas.unal.edu.co/index.php/agrocol/article/view/29494>>. Accessed: Mar. 09, 2021.

- JOSÉ, D. P. et al. Micropropagation and ploidy stability of *Lippia lacunosa* Mart. & Schauer: an endangered brazilian medicinal plant. **Journal of Neotropical Agriculture**, v.6, p.1–7. 2019. Available from: <<https://periodicosonline.uems.br/index.php/agrineo/article/view/3203>>. Accessed: Mar. 09, 2021. doi: 10.32404/rean.v6i1.3203.
- JULIANI, H. R. et al. Micropropagation of *Lippia junelliana* (Mold.) Tronc. **Plant Cell, Tissue and Organ Culture**, v.59, p.175–179. 1999. Available from: <<https://link.springer.com/article/10.1023/A:1006396531647>>. Accessed: Mar. 09, 2021. doi: 10.1023/A:1006396531647.
- LEYVA-JIMÉNEZ, F. J. et al. Functional ingredients based on nutritional phenolics. a case study against inflammation: *Lippia* genus. **Nutrients**, v.11, p.2–24. 2019. Available from: <<https://www.mdpi.com/2072-6643/11/7/1646>>. Accessed: Mar. 09, 2021. doi: 10.3390/nu11071646.
- LONE, S. M. et al. Plant propagation through tissue culture -a biotechnological intervention **Int.J.Curr.Microbiol.App.Sci**, v.9, p.2176–2190. 2020. Available from: <<https://www.ijcmas.com/abstractview.php?ID=18227&vol=9-7-2020&SNo=254>>. Accessed: Mar. 09, 2021. doi: 10.20546/ijcmas.2020.907.254.
- MANVI, S.; PARASHARAMI, V. *Garcinia indica* choisy: screening of location for donor female mother plants for micropropagation with effective protocol for establishment of sterile and healthy cultures. **Acta Scientific Agriculture**, 2019. v.3, p.39–48. 2019. Available from: <<https://actascientific.com/ASAG/pdf/ASAG-03-0514.pdf>>. Accessed: Mar. 09, 2021. doi: 10.31080/ASAG.2019.03.0514.
- MARINHO, M. J. et al. Establishment of protocol for *Lippia gracilis* Schauer micropropagation. **Revista Brasileira de Plantas Mediciniais**, v.13, p.246–252. 2011. Available from: <<http://dx.doi.org/10.1590/S1516-05722011000200019>>. Accessed: Mar. 09, 2021. doi: 10.1590/S1516-05722011000200019.
- MASUNDA, A. T. et al. Traditional uses, physical properties, phytochemistry and bioactivity of *Lippia multiflora* Moldenke (Verbenaceae): A Mini-review. **Discovery Phytomedicine**, v.7, p.19–26. 2020. Available from: <https://www.researchgate.net/publication/339460113_Traditional_uses_Physical_properties_Phytochemistry_and_Bioactivity_of_Lippia_multiflora_Moldenke_Verbenaceae_A_Mini-review>. Accessed: Mar. 09, 2021. doi: 10.15562/phytomedicine.2020.114.
- PASCUAL, M. E. et al. *Lippia*: Traditional uses, chemistry and pharmacology: A review. **Journal of ethnopharmacology**, v.76, p.201–214. 2001. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/11448540>>. Accessed: Mar. 09, 2021. doi: 10.1016/s0378-8741(01)00234-3.
- PEIXOTO, P. H. P. et al. *In vitro* propagation of endangered *Lippia filifolia* Mart. and Schauer ex Schauer. **In Vitro Cellular & Developmental Biology - Plant**, v. 42, p. 558–561. 2006. Available from: <<https://agris.fao.org/agris-search/search.do?recordID=US201500203677>>. Accessed: Mar. 09, 2021. doi: 10.1079/IVP2006814.
- PRIYA, E.; RAVINDHRAN, R. Micropropagation of *Lippia nodiflora* using shoot tip and nodal explants under *in vitro* conditions. **International Journal**, v.3, p.43–47. 2011. Available from: <<https://www.semanticscholar.org/paper/MICROPROPAGATION-OF-LIPPIA-NODIFLORA-USING-SHOOT-IN-Priya-Ravindhran/06e338e9ebcd7da24afe67bb9fa88d9865b4abd>>. Accessed: Mar. 09, 2021.
- RESENDE, C. F. et al. *In vitro* propagation and acclimatization of *Lippia rotundifolia*, an endemic species of Brazilian Campos Rupestres. **Revista Ciencia Agronomica**, v.46, p.582–589. 2015. Available from: <https://www.scielo.br/scielo.php?script=sci_arttext&pid=S1806-66902015000300582>. Accessed: Mar. 09, 2021. doi: 10.5935/1806-6690.20150041.
- RIBEIRO, A. F. et al. Circadian and seasonal study of the cinnamate chemotype from *Lippia origanoides* Kunth. **Biochemical Systematics and Ecology**, v.55, p.249–259. 2014. Available from: <<http://dx.doi.org/10.1016/j.bse.2014.03.014>>. Accessed: Mar. 09, 2021. doi: 10.1016/j.bse.2014.03.014.
- SILVA, G. C. et al. Propagação vegetativa e crescimento inicial de *Lippia origanoides* (Alecrim-de-tabuleiro). **Horticultura Brasileira**, v.33, p.236–240. 2015. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-053620150000200016>. Accessed: Mar. 09, 2021. doi: 10.1590/S0102-053620150000200016.
- SILVA, N. A. et al. Essential Oil composition and antioxidant capacity of *Lippia schomburgkiana*. **Natural Product Communications**, v.7, p.1281–1286. 2009. Available from: <<https://pubmed.ncbi.nlm.nih.gov/19831045/>>. Accessed: Mar. 09, 2021. doi: 10.1177/1934578x0900400925.
- URREA, A.; CASTRILLÓN, P.; MONSALVE, Z. Propagación *in vitro* y desdiferenciación tisular en *Lippia dulcis*. **Actualidades Biológicas**, v.31, p.21–29. 2010. Available from: <<https://revistas.udea.edu.co/index.php/actbio/article/view/4726>>. Accessed: Mar. 09, 2021.
- VICUÑA, G. C. et al. Chemical composition of the *Lippia origanoides* essential oils and their antigenotoxicity against bleomycin-induced DNA damage. **Fitoterapia**, v.81, p.343–349. 2010. Available from: <<https://pubmed.ncbi.nlm.nih.gov/19874875/>>. Accessed: Mar. 09, 2021. doi: 10.1016/j.fitote.2009.10.008.