AGRÁRIA
Revista Brasileira de Ciências Agrárias
ISSN (on line): 1981-0997
v.7, n.1, p.17-23, jan.-mar., 2012
Recife, PE, UFRPE. www.agraria.ufrpe.br
DOI:10.5039/agraria.v7i1a1121
Protocolo 1121 – 26/09/2010 *Aprovado em 08/04/2011

Claudine M. de Bona[1]

Gabriel D. Santos[2]

Luiz A. Biasi[2, 3]

Lavandula calli induction, growth curve and cell suspension formation

ABSTRACT

The cultivation of calli and cell suspension lines of aromatic plants is important because they are considered to be factories of active compounds such as, for instance, the rosmarinic acid found in *Lavandula* species. *L. angustifolia* is considered the most important species because it is regarded to be practically free from camphor, differently from other species in the genus, however, it is less productive than *L. dentata*, which is very productive and easily asexually propagated, but presents higher content of camphor. Information about calli and cell suspensions induction and maintenance for *Lavandula* species, especially the latter, is scarce. Not only that, but once callus formation is induced, it is necessary to identify the growth dynamic of the cells in order to maintain healthy, logarithmically phased growing cells for future elicitation. The goals of this work were to induce calli formation, evaluate growth curve and produce cell suspensions of *Lavandula angustifolia* and *Lavandula dentata*, two *Lavandula* species of great economical importance. Apical and lateral buds of *L. angustifolia* and *L. dentata* were placed on MS + 1 mg.L^{-1} 2.4-D or picloran + 0.5 mg.L^{-1} BAP or 1 mg.L^{-1} 2.4-D + 0.5 mg.L^{-1} GA_3, in the presence of light or in the dark. Calli were obtained only from *L. angustifolia*, under light, with the combination of 2.4-D + GA_3. The growth curve of *L. angusifolia* calli indicated the need for media exchange every three weeks. A blue pigment which may be interesting as a dye in the food industry was present in the media. Cells suspensions were formed.

Key words: Growth regulator, lavender, micropropagation, tissue culture.

Calogênese, curva de crescimento de calos e formação de suspensões celulares de *Lavandula*

RESUMO

O cultivo de calos e suspensões celulares de plantas aromáticas é importante porque estes são considerados fábricas de compostos ativos como, por exemplo, o ácido rosmarínico encontrado em espécies de *Lavandula*. *L. angustifolia* é considerada a espécie mais importante por ser praticamente livre de cânfora, todavia, é menos produtiva que *L. dentata*, que é facilmente propagada vegetativamente, mas apresenta alto teor de cânfora. Informação sobre indução e manutenção de calos e suspensões celulares de espécies de *Lavandula*, especialmente a última, é escassa. Não somente isto, mas uma vez que a formação de calos é induzida, é necessário identificar o crescimento dinâmico das células para mantê-las com crescimento saudável, em fase logarítmica para futuras elicitações. Os alvos deste trabalho foram indução de calos, avaliação de curva de crescimento e formação de suspensões celulares de *Lavandula angustifolia* e *Lavandula dentata*, duas espécies de *Lavandula* de grande importância econômica. Gemas de *L. angustifolia* e *L. dentata* foram colocadas em MS + 1 mg.L^{-1} 2,4-D ou picloran + 0,5 mg.L^{-1} BAP ou 1 mg.L^{-1} 2,4-D + 0,5 mg.L^{-1} GA_3, em condições de luz, ou no escuro. Foram obtidos calos de *L. angustifolia* em condições de luz, sendo 2,4-D + GA_3 a melhor combinação. A curva de crescimento de calos de *L. angustifolia* foi obtida e indicou a necessidade da troca de meio a cada três semanas. Pigmento azul que pode ser interessante para a indústria alimentícia estava presente no meio. Suspensões celulares foram produzidas.

Palavras-chave: Regulador de crescimento, alfazema, micropropagação, cultura de tecido.

[1] Instituto Agronômico do Paraná, Coordenação de Pesquisa, Rua Máximo João Kopp 274; Bloco 1 - Ala Sul; Centro Administrativo do Governo do Estado, Santa Cândida, CEP 82630-900, Curitiba-PR, Brasil. Fone: (41) 3351-7300 Ramal 41. Fax: (41) 3351-7301. E-mail: debona@iapar.br

[2] Universidade Federal do Paraná, Setor de Ciências Agrárias, Departamento de Fitotecnia e Fitossanitarismo, Rua dos Funcionários, 1540, Juvevê, CEP 81531-990, Curitiba-PR, Brasil. Caixa Postal 19061. Fone: (41) 3350-5682. Fax: (41) 3350-5601. E-mail: gds227@gmail.com; biasi@ufpr.br

[3] Bolsista de Pesquisa em Produtividade do CNPq

INTRODUCTION

Lavandula species (Labiaceae) are very valuable in the pharmaceutical industry, mainly because of the rosmarinic acid present in their cells (Georgiev et al., 2007). *L. angustifolia angustifolia* P. Mill. (English lavender), formerly known as *L. vera* or *L. officinallis*, is one of the most important aromatic plants and it has been used for rosmarinic acid production (Georgiev et al., 2007, Kovatcheva-Apostolova et al., 2008) because the species is regarded to be practically free from camphor, differently from other species in the genus, although less productive than other species of the genus (Cavanagh & Wilkinson, 2002). *L. dentata*, on the other hand, is very productive and easily asexually propagated (Bona et al., 2010a,b; Echeverrigaray et al., 2005), however its camphor content is higher than for *L. angustifolia*, while also being rich in 1.8-cineole and fenchol (Echeverrigaray et al., 2005).

Callus, roots and cell suspensions of aromatic and medicinal plants have been used to study the induction of secondary compounds by external elicitors (Rao & Ravishankar, 2002; Xu et al., 2008). Georgiev et al. (2007) studied plant lines of *Lavandula vera* DC. resistant to phenylalanine analogs from which high contents of rosmarinic acid were produced. Pavlov et al. (2000) induced the production of rosmarinic acid by manipulating nutrients in the medium and Pavlov et al. (2005) tri-folded the amount of produced rosmarinic acid by manipulating oxygen and temperature while agitating the flasks containing the suspended cells.

Cultivation and maintenance of *Lavandula* spp. calli and cell suspension lines is of great interest since cells in suspension tend to be very prolific and can reach logarithmic phases by culture media exchanges and stimulations with different agents (Grosser & Gmitter, 1990). Darkness and some growth regulators such as 4-**amino**-3,5,6-tricloropicolinic **acid** (Picloran), giberellic acid (GA_3), Kinetin, 2,4-dichlorophenoxyacetic acid (2,4-D), 2-isopenteniladenine (2iP), and benzyladenine (BA) have been used to induce calli formation in several species (Can et al., 2008; Costa, 2007; Mendonza & Kaeppler, 2002; Khaleghi et al., 2008; Nakajima et al., 1990; Sener et al., 2008; Sonyia et al., 2001).

Cell cultivation and manipulation, once initiated, offers the possibility of working with different research lines such as: performing manipulation tests with different media and substances; studying biochemical compounds, their pathways and biosynthesis; identifying, recognizing and monitoring the events which modulate the secondary metabolism (Bruni & Sacchetti, 2010); and eliciting and increasing the production of secondary metabolites and compounds of interest (Kraemer et al., 2002), having in mind that an important characteristic of the secondary compounds is that their synthesis is highly inducible (Bourgaud et al., 2001; Canter et al., 2005).

Nogueira et al. (2008) stated the importance of establishing a calli growth curve when working with medicinal and aromatic plants, not only to better determine the correct time for media exchange and the possibility of using such cells in suspensions but also when one is aiming the production of secondary metabolites.

The objective of this work was to induce calli formation and produce cell suspensions of *L. angustifolia* and *L. dentata*, two important *Lavandula* species, and to evaluate their growth curve, the first steps towards elicitation of secondary compounds experiments.

MATERIAL AND METHODS

The experiment was performed at the Tissue Culture and Plant Micropropagation Laboratory of the Plant Sciences Department in the Agrarian Sciences Sector of the Federal University of Parana. Buds of *L. angustifolia* and *L. dentata*, collected from greenhouse stock-plants, were rinsed three times with sterile de-ionized water, immersed in alcohol 70% for 1 min, transferred to sodium hypochlorite 20% plus two drops of commercial detergent for 20 min and soaked in sterile de-ionized water for 5 min three times to rinse the solution out.

Calli induction in light and dark room using 2,4-D and 6-benzylaminopurine (BAP)

L. angustifolia and *L. dentata* buds were aseptically introduced in 200 individual flasks (100 flasks per species; one explants per flask), containing 30 mL of MS media (Murashige & Skoog, 1962), with 30 $g.L^{-1}$ of sucrose, 6 $g.L^{-1}$ of agar, 1 $mg.L^{-1}$ 2,4-D and 0.5 $mg.L^{-1}$ BAP, pH 5.8. Half of the flasks of each species were placed inside a dark cabinet and the other half was submitted to 16 h photoperiod and irradiance of 10-20 $\mu mol.m^{-2}.s^{-1}$, at 25±2 °C, (Kintzios et al., 2002).

Calli induction in dark room followed by light conditions using picloran + BAP

Since *L. dentata* did not develop in vitro, even though it is easily asexually propagated (Bona et al., 2010a,b), *L. angustifolia* buds were placed (5 explants/flask) in 20 larger flasks containing basic MS media with 30 $g.L^{-1}$ of sucrose, 6 $g.L^{-1}$ of agar + 1 $mg.L^{-1}$ picloran + 0.5 $mg.L^{-1}$ BAP, at a pH 5.8, in the dark for 2 months and then placed inside a growth chamber at 25±2 °C, 16 h photoperiod and irradiance of 10-20 $\mu mol.m^{-2}.s^{-1}$.

Calli induction using 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3)

Since *L. dentata* did not develop in vitro, only *L. angustifolia* was further tested. *L. angustifolia* buds were aseptically introduced in 100 individual flasks, on MS media with 30 $g.L^{-1}$ of sucrose, 6 $g.L^{-1}$ of agar + 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ GA_3, at a pH 5.8, at 25±2 °C, 16 h photoperiod and irradiance of 10-20 $\mu mol.m^{-2}.s^{-1}$.

Cell suspension formation

Produced *L. angustifolia* friable calli were proliferated by sequential exchanges to fresh MS medium with 30 $g.L^{-1}$ of sucrose, 6 $g.L^{-1}$ of agar + 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ GA_3 in Petri dishes or taller glass flasks (approximately 9cm x 6cm) and were later transferred to flasks containing liquid MS medium with 30 $g.L^{-1}$ of sucrose + 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$

GA_3, at a 5.8 pH, and maintained at 27°C, under constant illumination and agitation (130 rpm) on a horizontal shaker in order to de-aggregate calli and to form the cell suspensions (Louzada et al., 2002; Miguel & Lima-Costa, 1998). Finally, they were submitted to a two-week medium exchange regime (Grosser & Gmitter, 1990).

Growth curve evaluation

One hundred milligrams of calli were weighted inside a sterile hood with a precision scale and placed inside twenty-eight (4 replications of 7 flasks) individual flasks containing 30 mL of MS media, 30 $g.L^{-1}$ of sucrose, 6 $g.L^{-1}$ of agar + 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ GA_3, pH 5.8, at 25±2 °C, 16 h photoperiod and irradiance of 10-20 $\mu mol.m^{-2}.s^{-1}$. The first calli weight evaluation was performed on the 10^{th} day after inoculation and each week after that, for one month.

Results and Discussion

Calli induction in light and dark room using 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP)

L. dentata explants did not form calli. *L. angustifolia* explants did not form calli in the dark and only 10 explants formed calli in light. Calli were healthy but the percentage of explants which formed calli may be considered low, indicating that the presence of 2,4-D and BAP in the media was not quite sufficient to induce a great percentage of calli. In tomato, calli was easily induced by Sonyia et al. (2001) using cytokine (BA), but combined to picloram. Herein, light seemed to be necessary for calli induction, differently from Nakajima et al. (1990), who produced *L. vera* calli in the dark, though calli were brown and showed oxidation. Light may have been a more important factor on calli formation than cytokine. Quisen (2007) obtained calli from *Eucalyptus globulus* in the dark after a 30-days exposure followed by transference to light for 30 more days, but different cytokines (BAP, TDZ, KIN) and auxins (NAA, 2,4-D, picloran) combinations did not influence calli formation. Rocha & Quoirin (2004), on the other hand, induced calli in mahohany explants with BAP + NAA.

Calli induction in dark room followed by light conditions using picloran + BAP

There was no calli formation in the dark and only two explants (2%) formed callus after being exposed to light. The presence of light appeared again to be beneficial for *in vitro* calli formation. Even though some authors have produced calli in dark, calli were brown showing levels of oxidation (Nakajima et al., 1990). Apparently 2,4-D was the main calli inducer in the first experiment, since the combination of cytokine with Picloran was not as good, at least on the doses tested herein. Nevertheless, information about picloran use in callus induction experiments has been controversial. Sener et al. (2008) affirmed that the picloran effect on calli induction is highly genotype dependant and produced only albino barley plantlets from picloran-induced callus. Mendonza & Kaeppler (2002) observed that picloran significantly enhanced callus growth from mature embryos of wheat; however, the embryogenic response and plant regeneration were low. On the other hand, Khaleghi et al. (2008), obtained 23% of compact callus induction of *Alstroemeria* cv. Fuego on Schenk and Hildebrant (SH) medium supplemented with 2 $mg.L^{-1}$ picloram from nodal segments. Can et al. (2008) obtained 18% of callus induction in crested wheatgrass (*Agropyron cristatum* (L.) Gaertn) when using 6 $mg.L^{-1}$, but the response was media dependant. Sonyia et al. (2001), on the other hand, easily induced regenerative calli in tomato by using a combination of picloram and benzyladenine (BA).

Calli induction in light using 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3)

Since *L. dentata* did not develop in vitro, only *L. angustifolia* was tested. There was 100% percent *L. dentata* calli formation when buds were placed on media containing 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ GA_3, at 25±2 °C, 16 h photoperiod and irradiance of 10-20 ìmol.$m^{-2}.s^{-1}$. White-cream colored friable calli were formed (Figure 1), similarly to calli obtained by Ghiorghiþã et al. (2009) who generated highly proliferative friable cream-colored calli of *L. angustifolia* when adding 2 $mg.L^{-1}$ of IAA in MS media, but improved calli growth on media containing 1 $mg.L^{-1}$ BAP and 0.5 $mg.L^{-1}$ GA_3. It demonstrated that gibberellins may help expedite the growth rate of *L. angustifolia* cells, while 2,4-D has been widely known and used as calli inducer (Mendonza & Kaeppler, 2002; Titon et al., 2007, Simões et al., 2010). Francis & Sorrel (2001), in a mini review about the interface between the cell cycle and plant growth regulators, suggest a link between auxins and the activation of cell divisions and that gibberellic acid (GA_3) treatment, which induces internode elongation, may have a role during cell multiplication. Xu et al. (2008) obtained a better callus induction rate (92%) and growth for *Agastache rugosa* (Labiaceae) in MS medium containing 2 $mg.L^{-1}$ 2,4-D.

Figure 1. Calli of *Lavandula angustifolia* and blue pigment on MS medium + 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ GA_3

Figura 1. Calo de Lavandula angustifolia e pigmento azul em meio de cultura MS + 1 $mg.L^{-1}$ 2,4-D + 0,5 $mg.L^{-1}$ GA_3

Blue pigmentation appeared in the media probably induced by calli exudates (Figure 1). Nakajima et al. (1990) observed some blue pigmentation in culture medium containing *L. angustifolia* cells when media was supplemented with L-cysteine under illumination, while brown pigments were formed in the dark to later become blue, and suggested that such pigments could be valuable coloring additives for food since they appeared to be inducible. Recently, Kovatcheva-Apostolova et al. (2008) observed that the addition of *L. vera* extract to minced chicken meat reduced lipid oxidation and the loss of á-tocopherol during cold storage after the meat was cooked.

The formation of the blue pigment was also found to occur under different conditions by Tsuro & Inorie (1996) who reported the production of such pigment in leaf derived callus of *L. angustifolia* and observed that pigmentation increased along with sucrose concentration. However, Trejo-Tapia et al. (2003) affirmed that high levels of sucrose (> 30 $g.L^{-1}$) did not stimulate the accumulation of the blue pigment and in these conditions, cell growth and cell viability were drastically affected.

It appears that rosmarinic acid yields a blue pigment upon reaction with $FeSO_4$ (López-Arnaldos et al., 1995a,b) and that the very presence of such pigment in the media may corroborate the elevated capacity of such cells to produce rosmarinic acid (Bauer et al., 2004).

Cell suspension formation

Most calli which were transferred to Petri dishes during the calli multiplication phase suffered oxidation and presented high mortality. The height of the flask might have been the reason why the calli died or oxidized since calli transferred to taller flasks were highly proliferated. The volumetric area for air seemed to matter in this case. At a certain point while cell division ceases, as oxygen in the media gets scarce, biomass concentration may continue, causing stress to the cells (Pépin et al., 2004).

On an extra attempt, some Petri dish surviving calli were transferred to liquid media to form cell suspensions but cell growth was slow and the media started to get dark, probably because of cell death due to pre oxidation suffered when they were in the Petri dishes. Such suspensions were discarded.

Calli from tall flasks also turned the media dark, but after some media changes, the cells presented fast growth and the media became clear, showing there was an adaptation period of the cells to the liquid media. Some adaptation period may be necessary in some cases because cells may suffer a hydrodynamic stress generated by aeration and agitation to which cells are exposed (Tanaka et al., 1988). However, after adaptation, very proliferative, health cells grew fast in the solution (Figure 2). It seemed that since healthy calli is submitted to liquid medium and agitation, even tough an adaptation period may be required, cells proliferate abundantly in suspensions. Similar observation was presented by Georgiev et al. (2007), who noticed that even older but healthy calli, whose initial culture had initiated 15 years before, were able to produce viable *L. vera* cell suspensions.

Figure 2. Cell suspension of *Lavandula angustifolia* on liquid MS medium + 1 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ GA_3 in 250 mL erlenmayers

Figura 2. Células em suspensão de Lavandula angustifolia em meio de cultura MS líquido + 1 $mg.L^{-1}$ 2,4-D + 0,5 $mg.L^{-1}$ GA_3 em erlenmayers de 250 mL

Growth curve evaluation

Calli grew following a logarithmic curve until the third week (Figure 3), then the stagnation tending to diminution in growth after that indicated a possible metabolic exigency from calli in order to growth, as suggested in Nogueira et al. (2008). Media exchange every 3 weeks seemed to be adequate to maintain the logarithmic phase growth. In Nogueira et al. (2008) a logarithmic growth of calli of murici-pequeno (*Byrsonima intermedia* A. Juss.) was observed for 90 days, after that, a diminution, coincident with the start of the deceleration process. In an experiment performed by Santos et al. (2003), the growth curve of *Coffea arabica* L. calli reached the maximum log phase with 70 days.

Knowledge about cell suspension growth curve is important for logarithmic growth maintenance and should be adjusted to the protocol used, as it may differ. Regardless, growth deceleration and diminution probably happened here in because calli started to exhaust nutrients of the medium; growth reduction due to cell signaling mechanism ; or cell death due to decrease in nutrient availability, smaller physical area and air available for growth inside the vessel as well as gases exchanges between cells and media inside the vessel's environment (Pépin et al., 2004). The stresses faced by cells in culture may include the cell growth rate, the maximum cell mass to a certain physical space and the size distribution of cell aggregates (Tanaka et al., 1988).

The *L. angustifolia* calli grew 40-fold in three weeks. Growth curve and average weight of calli is presented on Figure 3.

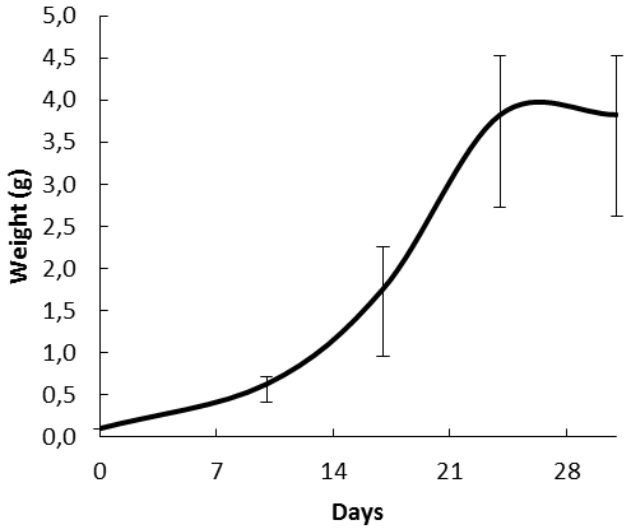

Figure 3. Growth curve of *Lavandula angustifolia* calli mean weight on MS medium + 1 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} GA_3

Figura 3. Curva de crescimento da massa média de calos de Lavandula angustifolia em meio de cultura MS + 1 mg.L^{-1} 2,4-D + 0,5 mg.L^{-1} GA_3

Conclusions

The presence of 2,4-D combined to GA_3 in the media was more efficient than the combinations of 2,4-D or picloran with BAP for calli induction of *L. angustifolia*.

Light seems to be essential for calli induction of *L. angustifolia*.

L. angustifolia calli reached lag (stationary) phase with three weeks, and started to decrease, showing the necessity of exchanging media every three weeks, approximately, in order to keep the cells growing logarithmically.

L. dentata did not form callus in the conditions tested herein.

Acknowledgements

To CNPq for granting a Post-Doc scholarship to the first author.

Literature Cited

Bauer, N.; Leljak-Levanic, D.; Jelaska, S. Rosmarinic acid synthesis in transformed callus culture of *Coleus blumei* Benth. Zeitschrift für Naturforschung, v.59c, n.7-8, p.554-560, 2004.

Bona, C.M. ; Biasi, L.A. Influence of leaf retention on cutting propagation of *Lavandula dentata* L.. Revista Ceres, v.57, n.4, p.526-529, 2010a. <http://www.ceres.ufv.br/ceres/revistas/V57N004P11409.pdf>. 05 Jul. 2010.

Bona, C.M.; Biasi, L.A.; Lipski, B.; Masetto, M.A.M.; Deschamps, C. Adventitious rooting of auxin-treated *Lavandula dentata* cuttings. Ciência Rural, v.40, n.5, p.1210-1213, 2010b. <http://www.scielo.br/pdf/cr/v40n5/a571cr2462.pdf>. doi:10.1590/S0103-84782010005000081. 05 Jul. 2010.

Bourgaud, F.; Gravot, A.; Milesi, S.; Gontier, E. Production of plant secondary metabolites: a historical perspective. Plant Sciences, v.161, n.5, p.839-851, 2001. <http://www.sciencedirect.com/science/article/pii/S0168945201004903>. doi:10.1016/S0168-9452(01)00490-3. 12 Jul. 2010.

Bruni, R.; Sacchetti, G. Micro-organism-plant interactions as influencers of secondary metabolism in medicinal plants. RedOrbit News. http://www.redorbit.com/news/science/309284/microorganismplant_interactions_as_influencers_of_secondary_metabolism_in_medicinal_plants/index.html. 24 Sep. 2010.

Can, E.; Çeliktaþ, N.; Hatipoðlu, R. Effect of auxin type and concentrations in different media on the callus induction and shoot formation of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn). Biotechnology & Biotechnological Equipment, v.22, n.3, p.782-786, 2008. <http://diagnosisp.com/dp/journals/view_pdf.php?journalid=1&archive=1&issue _id=19&article_id=503&PHPSESSID=5l74qqgkcjgp qfa8jqdbb3si52>. 24 Sep. 2010.

Canter, P.H.; Thomas, H.; Ernst, E. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends in Biotechnology, v.23, n.4, p.180-185. 2005. <http://www.sciencedirect.com/science/article/pii/S016777990500048X>. doi:10.1016/j.tibtech.2005.02.002. 24 Sep. 2010.

Cavanagh, H.M.A.; Wilkinson, J.M. Biological activities of lavender essential oil. Phytotherapy Research, v.16, n.4, p.301-318, 2002. <http://onlinelibrary.wiley.com/doi/10.1002/ptr.1103/pdf>. doi:10.1002/ptr.1103. 15 Sep. 2010.

Costa, D.A. Embriogênese somática em algodão (*Gossypium hirsutum* L. cv. BRS – 187 – 8H). Recife: Universidade Federal Rural de Pernambuco, 2007. 88p. Dissertação Mestrado.

Echeverrigaray, S.; Basso, R.; Andrade, L.B. Micropropagation of *Lavandula dentata* from axillary buds of field-grown adult plants. Biologia Plantarum, v.49, n.3, p.439-42, 2005.<http://www.springerlink.com/content/r609451266442l24/fulltext.pdf>. doi:10.1007/s10535-005-0024-7. 15 Sep. 2010.

Francis D.; Sorrell D.A. The interface between the cell cycle and plant growth regulators: a mini review. Plant growth regulation, v.33, n.1, p.1-12, 2001. <http://www.springerlink.com/content/g68276xmv612x545/fulltext.pdf>. doi:10.1023/A:1010762111585. 12 Jul. 2010.

Georgiev, M.I.; Kuzeva, S.L.; Pavlov, A.I.; Kovacheva, E.G.; Ilieva, M.P. Elicitation of rosmarinic acid by *Lavandula vera* MM cell suspension culture with abiotic elicitors. World Journal of Microbiology and Biotechnology, v.23, n. 2, p.301-304, 2007. <http://www.springerlink.com/content/1076640126176402/fulltext.pdf>. doi:10.1007/s11274-006-9214-5. 12 Jul. 2010.

Ghiorghiþã, G.; Maftei, D.; Nicuþã, D. Some aspects concerning the *in vitro* reaction of *Lavandula angustifolia*

L. Propagation of Ornamental Plants, v.9, n.1, p.47-49, 2009. <http://www.journal-pop.org/2009_9_1_47-49.html>. 17 Jul. 2010.

Grosser, J.W.; Gmitter Jr., F.G. Protoplast fusion and improvement. Plant Breeding Reviews, v.8, p.339-374, 1990.

Khaleghi, A.R.; Khalighi, A.; Azad, P.; Mii, M. Induction of embryogenic callus and plant regeneration from nodes of greenhouse grown plants of Alstroemeria cv. Fuego. Food, Agriculture & Environment, v.6, n.3-4, p.374-377, 2008. <http://www.isfae.org/scientficjournal/2008/issue3/pdf/agriculture/a36.pdf>. 17 Jul. 2010.

Kintzios, S.; Papanastasiou, I.; Tourgelis, I.; Papatellatos, P.; Georgopoulos, C.; Drossopoulos, J. The effect of light on callus growth and somatic embryogenesis from *Lavandula vera* and *Teucrium chamaedrys*: a preliminary study. Journal of Herbs, Spices & Medicinal Plants, v.9, n.2/3, p.223-227, 2002. <http://www.tandfonline.com/doi/pdf/10.1300/J044v09n02_32>. doi: 10.1300/J044v09n02_32. 12 Jul. 2010.

Kovatcheva-Apostolova, E.G.; Georgiev, M.I.; Ilieva, M.P.; Skibsted, L.H.; Redtjer, A.; Andersen, M.L. Extracts of plant cell cultures of *Lavandula vera* and *Rosa damascena* as sources of phenolic antioxidants for use in foods. European Food Research and Technology, v.227, n.4, p.1243-1249, 2008. <http://www.springerlink.com/content/86r8678h03h87357/fulltext.pdf>. doi:10.1007/s00217-008-0842-x. 11 Jul. 2010.

Kraemer, K.H.; Schenkel, E.P.; Verpoorte, R. *Ilex paraguariensis* cell suspension culture characterization and response against ethanol. Plant Cell, Tissue and Organ Culture, v.68, v. 3, p.257-263, 2002. <http://www.springerlink.com/content/h312661453383633/fulltext.pdf>. doi:10.1023/A:1013941826442. 15 Jul 2010.

López-Arnaldos, T.; Lopez"Serrano, M.; Ros, B.A. Calderon, A.A.; Zapata, J.M. Spectrophotometric determination of rosmarinic acid in plant cell cultures by complexation with Fe^{2+} ions. Analytical Chemistry, v.351, n. 2-3, p.311-314, 1995a. <http://www.springerlink.com/content/q780q46621whh5m1/>. doi:10.1007/BF00321655. 12 Jul. 2010.

López-Arnaldos, T.; López-Serrano, M; Barceló, A.R.; Calderón, A.A.; Zapata, J. M. A blue pigment derived from rosmarinic acid and $FeSO_4$ is responsible for the blue pigment secreted into the medium by *Lavandula x intermedia* cell cultures grown in the dark. Journal of Natural Product Research, v.7, n. 3, p.169-175, 1995b. <http://www.tandfonline.com/doi/abs/10.1080/10575639508043207>. doi:10.1080/10575639508043207. 15 Sep. 2010.

Louzada, E.S., Rio H.S.; Xia, D. Preparation and fusion of *Citrus* sp. microprotoplasts. Journal of the American Society for Horticultural Science, v.127, n. 4, p.484-488, 2002. <http://journal.ashspublications.org/content/127/4/484.full.pdf+html>. 15 Jul. 2010.

Mendonza, M.G.; Kaeppler, H.F. Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (*Triticum aestivum* L.). *In Vitro* Cellular and Developmental Biology - Plant, v.38, n.1, p.39-45, 2002. <http://www.springerlink.com/content/66810817766g312p/fulltext.pdf>. doi:10.1079/IVP2001250. 24 Sep. 2010.

Miguel, M.G.; Lima-Costa, M.E. Proteolytic and clotting activities of cell suspension cultures of *Ficus carica* under stress saline conditions. Acta Horticulturae, n.480, p.155-160, 1998. http://www.pubhort.org/members/showdocument?booknrarnr=480_26. 24 Sep. 2010.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, v.15, n. 3, p.473-479, 1962. <http://onlinelibrary.wiley.com/doi/10.1111/j.1399-3054.1962.tb08052.x/pdf>. doi:10.1111/j.1399-3054.1962.tb08052.x 10 Sep. 2010.

Nakajima, H.; Sonomoto, K.; Sato, F.; Yamada, Y.; Tanaka, A. Pigment synthesis by immobilized cultured cells of *Lavandula vera* and characterization of a component of the pigments. Agricultural and Biological Chemistry, v.54, n. 1, p.53-59, 1990. <http://www.journalarchive.jst.go.jp/jnlpdf.php?cdjournal=bbb1961&cdvol=54&noissue=1&startpage=53&lang=en& from=jnlabstract>. doi:10.1271/bbb1961.54.53. 10 Sep. 2010.

Nogueira, R.C.; Paiva, R.; Lima, E.C.; Soares, G.A.; Oliveira, L.M.; Santos, B.R.; Emrich, E.B.; Castro, A.H.F. Curva de crescimento e análises bioquímicas de calos de murici-pequeno (*Byrsonima intermedia* A. Juss.). Revista Brasileira de Plantas Medicinais, v.10, n.1, p.44-48, 2008. <http://www2.ibb.unesp.br/servicos/publicacoes/rbpm/pdf_v10_n1_2008/artigo7.pdf>. 08 Jul. 2010.

Pavlov, A.L.; Georgiev, M.L.; Panchev, I.N.; Ilieva, M.P. Optimization of rosmarinic acid production by *Lavandula vera* MM plant cell suspension in a laboratory bioreactor. Biotechnology Progress, v.21, n. 2, p.394-396, 2005. <http://onlinelibrary.wiley.com/doi/10.1021/bp049678z/full>. doi: 10.1021/bp049678z. 10 Sep. 2010.

Pavlov, A.L.; Ilieva, M.P.; Panchev, I.N. Nutrient medium optimization for rosmarinic acid production by *Lavandula vera* MM cell suspensions. Biotechnology Progress, v.16, n. 4, p.668-670, 2000. <http://onlinelibrary.wiley.com/doi/10.1021/bp000041z/full>. doi:10.1021/bp000041z. 10 Sep. 2010.

Pépin, M.; Archambault, J, Chavarie, C.; Cormier, F. Growth kinetics of *Vitis vinifera* cell suspension cultures: I. Shake flask cultures. Biotechnology and Bioengineering, v.47, v.2, p.131-138, 2004. <http://onlinelibrary.wiley.com/doi/10.1002/bit.260470203/pdf>. doi:10.1002/bit.260470203. 15 Sep. 2010.

Quisen, R.C. Transformação genética de *Eucalyptus calmdulensis* via co-cultivo com *Agrobacterium tumefasciens*. Curitiba: Universidade Federal do Paraná, 2007. 125p. Tese Doutorado.

Rao, S.R.; Ravishankar, G.A. Plant cell cultures: chemical factories of secondary metabolites. Biotechnology Advances, v.20, n.2, p.101-153, 2002. <http://www.sciencedirect.com/science/article/pii/S0734975002000071>. doi:10.1016S0734-9750(02)00007-1. 12 Set. 2010.

Rocha, S.C.; Quoirin, M. Calogênese e rizogênese em explantes de mogno (*Swietenia macrophylla* King) cultivados *in vitro*. Ciência Florestal, v.14, n. 1, p.91-101, 2004. <http://www.ufsm.br/cienciaflorestal/artigos/v14n1/A11V14N1.pdf>. 12 Jul. 2010.

Santos, C.G.; Paiva, R.; Paiva, P.D.O.; Paiva, E. Indução e análise bioquímica de calos obtidos de segmentos foliares

de *Coffea arabica* l., cultivar Rubi. Ciência e Agrotecnologia, v.27, n. 3, p.571-577, 2003. <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-70542003000300011&lng=pt&nrm=iso&tlng=pt>. doi:10.1590/S1413-70542003000300011. 10 Set. 2010.

Sener, O.; Can, E.; Arslan, M.; Celiktas, N. Effects of genotype and picloram concentrations on callus induction and plant regeneration from immature inflorescence of spring barley cultivars. Biotechnology and Biotechnological Equipment, v.22, n.4, p.915-920, 2008. <http://www.diagnosisp.com/dp/journals/view_pdf.php?journal_id=1&archive=0&issue_id=20&article_id=529 &PHPSESSID=mjkt1dbrjgfskrdf6ki41c9ne6>. 10 Sep. 2010.

Simões, C.; Albarello, N.; Callado, C.H.; Castro, T.C.; Mansur, E. Somatic embryogenesis and plant regeneration from callus cultures of *Cleome rosea* Vahl. Brazilian Archives of Biology and Biotechnology, v.53, n. 3, p.679-686, 2010. <http://www.scielo.br/pdf/babt/v53n3/a24v53n3.pdf>. doi:10.1590/S1516-89132010000300024. 10 Set. 2010.

Sonyia, E.V.; Banerjee, N.S.; Das, M.R. Genetic analysis of somaclonal variation among callus-derived plants of tomato. Current Science, v.80, n.9, p.1213-1215, 2001. <http://cs-test.ias.ac.in/cs/Downloads/article_34060.pdf>. 10 Set. 2010.

Tanaka, H.; Semba, H.; Jitsufuchi, T.; Harada, H. The effect of physical stress on plant cells in suspension cultures. Biotechnology Letters, v.10, n. 7, p.485-490, 1988. <http://www.springerlink.com/content/jg503650jl477423/>. doi:10.1007/BF01027061. 15 Sep. 2010.

Titon, M.; Xavier, A.; Otoni, W.O.; Motoike, S.Y. Efeito dos reguladores de crescimento dicamba e picloram na embriogênese somática em *Eucalyptus grandis*. Revista Árvore, v.31, n. 3, p.417-426, 2007. <http://www.scielo.br/pdf/rarv/v31n3/07.pdf>. doi:10.1590/S0100-67622007000300007. 12 Set. 2010.

Trejo-Tapia, G.; Arias-Castro, C.; Rodríguez-Mendiola, M. Influence of the culture medium constituents and inoculum size on the accumulation of blue pigment and cell growth of *Lavandula spica*. Plant Cell, Tissue and Organ Culture, v.72, n.1, p.7-12, 2003. <http://www.springerlink.com/content/gh85785h70xm0307/fulltext.pdf>. doi:10.1023/A:1021270907918. 08 Jul. 2010.

Tsuro, M.; Inorie, M. Production of blue pigment in leaf-derived callus of lavender (*Lavandula vera* DC). Japanese Journal of Breeding, v.46, n.4, p.361-366, 1996. <http://www.journalarchive.jst.go.jp/english//jnlabstract_en.php?cdjournal=jsbbs1951&cdvol=46&noissue=4&startpage=361>.doi:10.1270/jsbbs1951.46.361. 08 Jul. 2010

Xu, H.; Kim, Y.K.; Jin, X.; Lee, S.Y.; Park, S.U. Rosmarinic acid biosynthesis in callus and cell cultures of *Agastache rugosa* Kuntze. Journal of Medicinal Plants Research, v.2, n. 9, p.237-241, 2008. <http://www.academicjournals.org/jmpr/PDF/pdf2008/September/Xu%20et%20al.pdf>. 20 Sep. 2010.