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Scientific Article

Quality of light and indolbutyric acid *in vitro* rooting of lavender

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Abstract

Lavender, an aromatic and medicinal plant, used in the extraction of essential oil, as an ornamental and meliferous plant, can be successfully propagated as long as the light, the nutrient medium and the growth regulators are adequate. The aim of the study was to evaluate the effect of different concentrations of indolebutyric acid (IBA) associated to distinct light spectra, on the *in vitro* rooting of *Lavandula angustifolia*. The experiment consisted in the combination of two concentrations of indolebutyric acid in the growth medium (0 and 0.1 mg L^{-1}) and two light spectra, using cellulose acetate filters (blue and red), besides the control (without filter), totaling six treatments with four repetitions. For each repetition five explants were used. After 30 days, the variables evaluated were: survival percentage, number of buds and leaves, shoot length, number of roots, length of the longest root, shoot fresh and dry matter weight. There was a significant interaction among the indolebutyric acid concentrations and the light filters for the variables number of buds, leaves and roots and shoot length. Except for leaf number, promising results were obtained when the explants were held under red filters and with a concentration of 0 mg L^{-1} of indolebutyric acid in the growth medium. From these results, it can be concluded that the best *in vitro* rooting of *Lavandula angustifolia* is obtained when there is no indolebutyric acid in the growth medium associated with the use of the red filter.

Keywords: *Lavandula angustifolia*, *in vitro* rooting, natural light modifying filters, ornamental.

Resumo

Qualidade da luz e ácido indolbutírico no enraizamento *in vitro* de lavanda

A lavanda, planta aromática e medicinal, utilizada na extração de óleo essencial, como planta ornamental e melífera pode ser micropropagada com sucesso desde que a luz, o meio nutritivo e os reguladores de crescimento sejam adequados. O objetivo com o estudo foi avaliar o efeito de diferentes concentrações de ácido indolbutírico (AIB) associadas a espectros de luz distintos, no enraizamento *in vitro* de *Lavandula angustifolia.* O experimento consistiu na combinação de duas concentrações de ácido indolbutírico no meio de crescimento (0 e 0,1 mg L^{-1}) e dois espectros de luz, utilizando filtros de acetato de celulose (azul e vermelho), além do controle (sem filtro), totalizando seis tratamentos com quatro repetições. Para cada repetição foram utilizados cinco explantes. Após 30 dias, as variáveis avaliadas foram: porcentagem de sobrevivência, número de brotos e folhas, comprimento da parte aérea, número de raízes, comprimento da raiz, peso da matéria fresca e seca da parte aérea. Houve interação significativa entre as concentrações de ácido indolbutírico e os filtros de luz para as variáveis número de brotos, folhas e raízes e comprimento da parte aérea. Exceto pelo número de folhas, foram obtidos resultados promissores quando os explantes foram mantidos sob filtros vermelhos e com uma concentração de 0 mg L^{-1} de ácido indolbutírico no meio de crescimento. A partir desses resultados, pode-se concluir que o melhor enraizamento *in vitro* de *Lavandula angustifolia* é obtido quando não há ácido indolbutírico no meio de crescimento associado ao uso do filtro vermelho.

Palavras-chave: *Lavandula angustifolia*, enraizamento *in vitro*, filtros modificadores de luz natural, ornamentais.

Introduction

The lavender (*Lavandula angustifolia* Miller) belongs to the Lamiaceae family and is an aromatic and medicinal plant cultivated in various regions of temperate climate worldwide, and the plant is found natively in South Europe, Mediterranean countries of Northern Africa, France, Bulgaria, England, America and Austria (Gonçalves and Romano, 2013). The great potential of the specie lies in the production of essential oil, which may be extracted from the leaves and flowers, being used in the cosmetic, food, pharmaceutical and perfumery industries (Souza et al,

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2019), besides the usage as an ornamental plant. Lavender has a long history as a medicinal product. *In vitro* tests have demonstrated that lavender oil has analgesic (Silva et al. 2015). In Brazil there are few reports of cultivation of lavas in Brazil and some of the crops are related to tourism, since laundries also have great ornamental potential (Adamuchio et al., 2015).

The propagation of lavender may be performed by seed or by vegetative propagation. However, seedlings obtained from seeds present slow germination and variations in different characteristics of the genotype, such as the growth rate and essential oil composition (Panizza and Tognoni, 1991). Recalling that micropropagation enables pharmacological studies that contribute to the identification, increase and improvement of the use of active assets (Almeida et al., 2016).

The use of micropropagation enables the large-scale production of plants genetically identical to the parent plant, which is a key factor in the propagation of selected genotype. However, there are many factors involved in the regeneration of *in vitro* plants, among them, the type of the explant, the growth medium and light intensity (Zhang et al., 2003; Nogueira et al., 2017), therefore, the identification of adequate conditions for each genotype is necessary.

For the success of micropropagation, some factors such as light, the growth medium and growth regulators must be provided under adequate conditions in each step (Pasa et al., 2012; Ferreira et al., 2017). *In vitro* propagation has been applied for various species of *Lavandula*, in which plant regeneration is accomplished by direct and indirect organogenesis using different types of explants and growth media. Preliminary studies of somatic embryogenesis were performed for *L. angustifolia* by Kintzios et al. (2002). In this work, filters were used to modify the light commonly used.

The light source used in tissue culture is very important in the *in vitro* multiplication and rooting, being a fundamental factor for plants, by the direct or indirect action in the regulation of growth and development (Pasa et al., 2012). The light spectra affects the biological efficiency of the growth regulators added in the growth medium, as well as the hormone balance in the tissues, emerging as a tool in the manipulation of the physiological balance for the obtainment of specific responses on plant growth. It is relevant to explore research to understand more and better the parameters used to adapt the in vitro micropropagation protocol in genus *Lavandula* (Hernández, 2017).

Therefore, this work aims to check the effect of different concentrations of indolebutyric acid associated to the use of light filters, in the *in vitro* rooting of *Lavandula angustifolia*.

Materials and Methods

The study was carried out from November to January 2014 in the Laboratory of Fruit Plant Propagation, of the Faculdade de Agronomia Eliseu Maciel (FAEM), at the Universidade Federal de Pelotas (UFPel), Pelotas, Rio Grande do Sul State. The buds used were derived from plants micropropagated at the Faculdade de Agronomia Eliseu Maciel (FAEM). The explants (portions of newly formed buds) were transferred to the MS growth medium (Murashige and Skoog, 1962) containing three times the solution D, supplemented with 30 g L^{-1} of sucrose and 6 g L^{-1} of agar, two concentrations of indolebutyric acid (IBA), 0 and 0.1 mg L^{-1}, and pH was adjusted to 5.8. The growth medium was autoclaved at 120 ºC and a pressure of 1.5 atm for 20 minutes. Flasks of 250 mL with 20 mL of the growth medium and capped with cotton and aluminum foil were used. To obtain the treatments (light qualities), cellulose acetate filters were used (Lee Filters, Central Way, Walworth Ind. Estate, Andover, Hampshire SP10 5AN, England) red: number 106 – Primary red, with 9.32% transmittance; blue: number 724 - Ocean Blue, with 36.2% transmittance and control treatment (without light filters). Additionally, two concentrations of IBA were used, adding up to six treatments, with four repetitions each, using five explants per repetition. The explants were held in a plant growth room, with a photoperiod of 16 hours, a temperature of 25 $\pm$ 2 ºC and a light intensity of 27 μmol m^{-2} s^{-1}.

The size of the explants was around 2.5 cm from the second subculture (Figure 1). The colored filters as treatments were wrapped in the flasks to transform the light spectrum according to the color. The MS medium had a higher concentration of potassium nitrate and other necessary nutrients. The lamps used were fluorescent Tubular Y8 18W Cold White Empalux.

Figure 1. Explant extraction

At the 30 days after the experiment setup, the variables evaluated were shoot and longest root length, shoot fresh and dry matter weight, number of buds, leaves and roots and the survival percentage.

Shoot and longest root length – the explants which survived in each sample at the 30 days after the transplant to the growth medium were evaluated. The measurements were performed using a millimetric ruler and the result expressed in centimeters.

Shoot fresh matter weight – The explants were weighted in a precision balance of 0.001 g and the average results expressed in grams seedling^{-1}.

Shoot dry matter weight - the explants obtained at the determination of the fresh weight were used. The samples of each treatment were put into Kraft paper bags and placed in an air circulation kiln at 65 ºC, for a period of 72 hours. After drying, each repetition was weighted in a precision balance of 0.001 g and the results were expressed in grams seedling^{-1}.

Number of buds, leaves and roots – Aided by a magnifying glass, the number of buds, leaves and roots of each micropropagated seedling were counted, for number of roots; only roots > 2 mm were considered.

Survival percentage - the number of explants which survived in each sample at 30 days after the transplant were counted.

The experimental design used was the completely randomized and the averages, when significant, were compared between each other by the Tukey Test ($p < 0.05$).

Results and Discussion

According to Table 1, the average survival percentage of the explants did not statistically differ due to the use of light filters and IBA. The averages for all treatments were not lower than 77%, which indicates a good development of the explants. The survival percentage resembles the values observed by Machado (2013), on a study performed with *Lavandula angustifolia* (around 80% in treatments with IBA).

Table 1. Explant survival percentage of *Lavandula angustifolia*, in the step of *in vitro* rooting as a result of the concentration of indolebutyric acid (IBA) and colored filters.

Filters	Survival (%)
Control	77 a*
Blue	90 a
Red	85 a
IBA (mg L^{-1})	
0.0	81.66 a
0.1	86.66 a
CV (%)	30.8

*Means followed by the same letter, did not differ statistically between each other by the Tukey Test, at the 5% probability level of error.

There was interaction between the factors, according to Table 2. In respect to the number of buds, the red filter propitiated the greater performance in this variable, when associated or with the absence of IBA, contrasting with the results described by Ribeiro et al. (2009), which worked with the in vitro multiplication of calla lily (*Zantedeschia aethiopica*) under different light qualities, where the greater average for bud number was observed for the white light. However, in the concentration of 0.1 mg L^{-1}, the number of buds under the red filter presented a significantly lower result.

Table 2. Number of buds and leaves, shoot length (SL), root number of *Lavandula angustifolia*, in the step *in vitro* rooting as a result of the concentration of indolebutyric acid (IBA) and colored filters.

		Buds (Nº)		Leaves (Nº)		SL (cm)		Roots (Nº)	
		IBA (mg L^{-1})							
		0	0.1	0	0.1	0	0.1	0	0.1
Filters	**Control**	2.87 bA*	4.93 aA	31.60 aA	32.17 aA	4.19 aB	7.13 aA	0 bA	0.75 bA
	Blue	5.78 bA	2.71 aA	36.05 aA	17.20 bB	4.21 aB	10.13 aA	0.75 bA	0 bA
	Red	20.60 aA	5.80 aB	4.13 bA	8.67 bA	1.50 aA	2.58 bA	49.6 aA	21.9 aB
	CV (%)	49.32		29.17		34.31		49.23	

*Means followed by the same lowercase letter in the column and uppercase letter in the line did not differ between each other by the Tukey Test, at the 5% probability level of error.

For leaf number, the red filter was not favorable, since the greater averages were obtained under the white light and the blue filter, where under this filter the average was statistically lower in the concentration of 0.1 mg L^{-1} IBA (Table 2). Araújo et al. (2009) in a work performed with *Cattleya loddigesii* Lindl. also observed that the blue filter propitiated a greater average for this variable.

Yet, the number of leaves under the absence of light filters, independently of the concentrations of IBA used, was superior to the use of the red and blue filters. The use of IBA, when compared to the control treatment, suggested a stimulus on the shoot length. However, there was no significant effect of the use of filters on this variable. On the other hand, in the concentration of 0.1 mg L^{-1} IBA, the red filter was inferior to the blue filter and the control. Araújo et al. (2009) working with *Cattleya loddigesii* Lindl, observed that the cultivation in the growth room under red cellophane promotes the elongation of the shoot.

For the variable number of roots, a similar behavior was observed, with or without the use of IBA. Camargo et al. (2015) working with the *in vitro* multiplication of explants of *Oncidium baueri*, observed that in the rooting stage the obtainment of roots with adequate number and development is possible with the use of the MS growth medium with 0.1 mg L^{-1} IBA.

In respect to the light filters, both in the absence of filters or under the blue filter, the number of roots was inferior when compared to the use of the red filter, obtaining, on average, 36 roots. The absence of IBA associated with the red filter resulted in 50 roots, which is significantly superior to the other treatments.

The variables shoot fresh and dry matter weight and root length did not show interaction between the factors (Table 3); however, the averages of shoot fresh and dry matter weight were lower as a result of the application of IBA, which indicates that the presence of this growth regulator is not necessary for *in vitro* rooting.

Table 3. Shoot fresh matter weight (SFMW), Shoot dry matter weight (SDMW) and Root length (RL) of explants of *Lavandula angustifolia*, in the step of *in vitro* rooting as a result of the concentration of indolebutyric acid (IBA) and colored filters.

Filters	SFMW (g)	SDMW (g)	RL (cm)
Control	2.26 a*	0.12 a	0.51 b
Blue	1.69 a	0.10 a	0.38 b
Red	1.87 a	0.10 a	9.08 a
IBA (mg L^{-1})			
0.	3.25 a	0.17 a	3.95 a
0.1	0.63 b	0.04 b	2.70 a
CV(%)	71.18	50.84	90.61

*Means followed by the same letter, did not differ statistically between each other by the Tukey Test, at the 5% probability level of error.

The red filter propitiated a greater root length (9.08 cm) compared to the blue filter or white light, contradicting results obtained by Braga et al. (2008) which evaluated light quality in the micropropagation of Chrysanthemum [*Dendranthema grandiflorum* (Ramat) Kitam] and verified the greater average for this variable without the use of filters, on plants held under white light. The concentrations of IBA had no effect on root length (Table 3).

In general, the variables evaluated (except for the number of leaves) obtained good results in the explants held under the red filters and cultivated without the application of IBA in the growth medium, which is very favorable from an economic point of view, since the use of growth regulators increases the costs of *in vitro* cultivation. It is important to mention that the most important variables during the step of *in vitro* rooting (number of roots per plant and root length) expressed a better performance when the red filter was used.

Conclusions

The use of red filters associated with the MS growth medium and without IBA is the most promising for the *in vitro* rooting of *Lavandula angustifolia*.

Author Contribution

D.B.R.[0000-0003-3600-0320]: responsible for the execution, statistical analysis and writing, **A.K.R.**[0000-0001-5453-2713]: responsible for the execution and analysis, **L.R.S.**[0000-0002-7735-8188]: responsible for the execution, statistical analysis and writing, **D.S.B.R.**[0000-0003-4965-2635]: responsible for the execution and analysis, **M.W.S.**[0000-0001-5237-8302]: advisor and creator, **A.M.A.**[0000-0003-4230-1242]: advisor and creator.

References

ADAMUCHIO, L.G.; DESCHAMPS, C.; MACHADO, M.P. Aspectos gerais sobre a cultura da Lavanda (*Lavandula* spp.). **Revista Brasileira de Plantas Medicinais**, v.19, n.4, p.483-490, 2017. DOI: http://dx.doi.org/10.1590/1983-084X/0574

ALMEIDA, L.V.S.; OLIVEIRA, V.J.D.S.; JACOBI, C.C.B.; ALMEIDA, W.A.B. As plantas medicinais e a micropropagação como ferramenta para sua expansão e utilização. **Textura**, v.9, n.16, p.01-15, 2016. DOI: http://dx.doi.org/10.22479/244799342016v9n16p1-14

ARAÚJO, A.G; PASQUAL, M.; RODRIGUES, FA..; RODRIGUES, J.D.; CASTRO, E.M.; MATOS SANTOS, A. Crescimento *in vitro* de *Cattleya loddigesii* Lindl. em diferentes espectros luminosos associados com ácido giberélico. **Revista Ceres**, v.56, n.5, p. 542-546, 2009.

BRAGA, F.T.; PASQUAL, M.; CASTRO, E.D.; DIGNART, S.L.; BIAGIOTTI, G.; PORTO, J.M.P. Qualidade de luz no cultivo *in vitro* de *Dendranthema grandiflorum* cv. Rage: Características morfofisiológicas. **Ciência e Agrotecnologia**, v.33, n.2, 2009. DOI: http://dx.doi.org/10.1590/S1413-70542009000200022

CAMARGO, S.S.; RODRIGUES, D.B.; RODRIGUES, C.M.; ASSIS, A.M.; FARIA, R.T.; SCHUCH, M.W. Fitorreguladores e espectros de luz na micropropagação de *Oncidium baueri* Lindl. **Ciência Rural**, v.45, n.11, 2015. DOI: http://dx.doi.org/10.1590/0103-8478cr20141780

FERREIRA, L.T.; ARAÚJO SILVA, M.M.; MACÊDO, C.R.; WILLADINO, L. Fonte de luz e concentração de sacarose no cultivo *in vitro* da cana-de-açúcar (RB 867515). **Plant Cell Culture and Micropropagation**, v.12, n.2, p.46, 2017.

GONÇALVES, S.; ROMANO, A. Protocols for micropropagation of selected economically – important horticultural plants (Micropopagation of *Lavandula* spp.). In: LAMBARDI, M.; OZUDOGRU, E.; AYLIN, J.; MOHAN, S. **Protocols for Micropropagation of Selected Economically-Important Horticultural Plants**. New York: Humana Press, 2013. p. 189-198.

HERNÁNDEZ AMADOR, E. Estabelecimento de cultivo *in vitro* de *Lavandula dentata*. **Biologia**, p.1-28, 2017.

KINTZIOS, S.; PAPANASTASIOU, I.; TOURGELIS, P.; PAPASTELLATOS, C.; GEORGOPOULOS, V.; DROSSOPOULOS, J. The effects of light on callus growth on somatic embryogenesis from *Lavandula vera* and *Teucriu chamaedrys*: a preliminary study. **Journal of Herbs, Spices & Medicinal Plants**, v.9, n.2., p.223-227, 2002. DOI: http://dx.doi.org/10.1300/j044v09n02_32

MACHADO, M.P.; CIOTTA, M.N.; DESCHAMPS, C.; ZANETTE, F.; CÔCCO, L.C.; BIASI, L.A. Propagação in vitro e caracterização química do óleo essencial de *Lavandula angustifolia* cultivada no Sul do Brasil. **Ciência Rural**, v.43, n.2, p.283-289, 2013.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and biossay with tabacco tissue cultures. **Physiologia Plantarum**, v.15, n.3, p.473-497, 1962.

NOGUEIRA, J.S.; COSTA, F.D.S.; LUIS, Z.; SCHERWINSKI-PEREIRA, J.E. Micropropagação de bambu em larga escala: princípios, estratégias e desafios. In: DRUMOND, P.M.; WIEDMAN, G. **Bambus no Brasil: da biologia à tecnologia**. Rio Branco: Embrapa, 2017. pp.103-129.

PANIZZA, M.; TOGNONI, F. Micropropagation of Lavandin (*Lavandula officinalis* Chaix *Lavandula latifolia* Villars cv. Grosso). In: BAJAJ, Y.P.S. **High-Tech and Micropropagation III.** Berlin, Heidelberg: Springer, 1992. pp. 295-305.

PASA, M.S.; CARVALHO, G.L.; SCHUCH, M.W.; SCHMITZ, J.D.; TORCHELSEN, M.M.; NICKEL, G.K.; SOMMER, L.R.; LIMA, T.S.; CAMARGO, S.S. Qualidade de luz e fitorreguladores na multiplicação e enraizamento *in vitro* da amoreira-preta 'Xavante'. **Ciência Rural**, v.42, n.8, p.1392-1396, 2012. https://doi.org/10.1590/S0103-84782012000800010

RIBEIRO, M.D.N.O.; PASQUAL, M.; SILVA, A.B.; RODRIGUES, V.A. Multiplicação *in vitro* de copo-de-leite: espectros de luz e sacarose. **Ciência Rural**, v.39, n.8, p.2388-2393, 2009.

SILVA, G.L.; LUFT, C.; LUNARDELLI A.; AMARAL R.H.; MELO, D.A.S.; DONADIO M.V. Antioxidant, analgesic and anti-inflammatory effects of lavender essential oil. **Anais da Academia Brasileira de Ciências**, v.87, n.2, p.1397-1408, 2015.

SOUZA, E.R.L.; ARAÚJO CRUZ, J.H.; GOMES, N.M.L.; RAMOS, L.L.; OLIVEIRA FILHO, A.A. *Lavandula angustifolia* Miller e sua utilização na Odontologia: uma breve revisão. **Archives of Health Investigation**, v.7, n.12, 2019. DOI: http://dx.doi.org/10.21270/archi.v7i12.3125

ZHANG, L.; XU, T.; SUN, X.; ZHANG, H.; TANG, T.; TANG, K. Factors influencing shoot regeneration from cotyledons of tetraploid *Isatis indigotica* F. **In Vitro Cellular and Development Biology-Plant**, v.39, n.5, p.459-462, 2003. DOI: https://doi.org/10.1079/IVP2003447